Pharmacology of tetracycline water medication in swine

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ABSTRACT: Medicating drinking water with tetracycline is commonly used in swine production systems to treat and prevent disease outbreaks. However, little information is known of the pharmacokinetics of this medication in water formulations. Twenty-four barrows, divided into 1 control group (of nontreated animals) and 3 equally sized treatments groups (n = 6/group), were treated with tetracycline water medication for 5 d at 125, 250, and 500 mg/L. Blood samples were collected at 0 (prestudy), 4, 8, 12, 24, 32, 48, 56, 72, 80, 96, and 104 h after exposure. Data analyses consisted of a noncompartmental pharmacokinetic analysis and statistical analysis of steady state concentrations with repeated measures ANOVA and multiple-comparison testing to determine whether plasma concentrations differed among groups. Derived pharmacokinetic parameters were consistent with previously published feed and intravenous data. Plasma tetracycline concentrations at steady state were 0, 0.33, 0.47, and 0.77 µg/mL for 0-, 125-, 250-, and 500-mg/L exposures, respectively. Treatment group steady-state plasma concentrations were significantly different from plasma concentrations in control animals (P < 0.0001); however, whereas the 125- and 250-mg/L groups were significantly different from the 500-mg/L group (P < 0.0001), their mean plasma tetracycline concentrations did not differ from one another. Furthermore, the study showed that tetracycline oral bioavailability is very small. The dose response curve also shows that concentrations of plasma tetracycline increase linearly, yet not in a 1 to 1 ratio, to the direct increase in water medication dose.

Key words: pharmacokinetics, plasma concentration, swine, tetracycline, water medication

INTRODUCTION

In commercial swine production, antimicrobial medication often is used after weaning, transport, or during disease outbreaks. Concern has grown over the last decade between increased antimicrobial resistance in human pathogens and food animal antimicrobial use (FDA, 2003). In Europe, this policy led to the ban of feed additives (EU, 2003) and a concurrent increase in the use of water medications (personal observation in European countries). Currently, there is only limited published pharmacokinetic (PK) data on water medication formulations, including amoxicillin (Agerso et al., 1998), sulfamethazine (Mason et al., 2008), and tetracycline (Nielsen and Gyrd-Hansen, 1996).

Although a ban on feed additives is not in effect in the United States, PK studies to evaluate antibiotic treatment and its effects should be conducted before adopting blanket policies prohibiting drug use. Pharmacokinetic studies would characterize therapeutic potential and PK parameters unique to water antimicrobials. In addition to traditional PK parameters, PK-pharmacodynamic studies can potentially relate the impact of antimicrobial use on the food supply and public health (CDC, 2006).

Tetracycline, an antibiotic labeled for the control and treatment of salmonellosis, some enteric bacteria, and...
susceptible respiratory diseases in pigs, was selected as a test compound because of its common use (Aar-
estrup, 2005) and lack of PK data from observation-
al studies (Luthman et al., 1989; Pijpers et al., 1989; 
Reeve-Johnson, 1998). An experiment was designed to 
measure tetracycline concentrations in swine plasma. 
This study design allowed for group comparisons of 4 
doses, including control, and to determine if the dose 
response of tetracycline is linear. These studies also as-
essed whether tetracycline concentrations in plasma 
consistently reached minimum inhibitory concentra-
tions of quality control (sensitive) bacteria (Isenberg, 
2004; CLSI, 2007; Qaiyumi, 2007).

MATERIALS AND METHODS

All animals used in this study were housed and 
treated in accordance with the North Carolina State 
University Internal Animal Care and Use Committee 
standards.

Animals, Treatment, and Facility

Twenty-four Yorkshire-Landrace cross barrows were 
housed at North Carolina State University’s Swine 
Unit Facility in individual pens on concrete. Barrows, 
approximately 8 wk of age and weighing 16 to 18 kg at 
the initiation of the study, were assigned to 1 of 4 treat-
ment groups (n = 6/group). One group received 0.5 
times the label dose (125 mg/L) of tetracycline hydro-
chloride; the second, a label dose (250 mg/L); the third, 
2 times the label dose (500 mg/L); and the control 
group received water without tetracycline. Treatments 
of 125, 250, and 500 mg/L were achieved by dissolving 
a preweighed amount of an approved tetracycline 
water medication (AmTech, IVX Animal Health Inc., 
Saint Joseph, MO) into 19.2 L of water in individual 
carboys for each animal. All animals were given 5 d 
to acclimate to the facility and adapt to the carboy 
drinking system before the start of the trial. The Nal-
gene carboys (Thermo Fisher Scientific, Waltham, MA) 
were suspended from the ceiling and attached to the 
barn plumbing via 0.9 to 1.4 m of 1.27-cm plastic tub-
ing and plumbers fittings. Arato80 drinkers (Aratowerk 
GmbH & Co., Koln, Germany) were mounted in the 
individual pens to minimize waste and spillage of wa-
ter used. Water flow rates varied between 500 and 800 
ml/min, which is consistent with flow rates needed to 
prevent dehydration in adult pigs (Leibbrandt et al., 
2001). Daily maximum and minimum temperatures 
within in the barn were collected with a thermometer 
and recorded daily.

Feed and Water Composition

Water was assessed the month before the trial by the 
North Carolina Agronomics department to determine 
water quality on the farm. A 17% protein antibiotic-
free feed, ground on site, was fed to all animals ad
libitum. Animals did not receive any antimicrobials or 
growth promotants at least 10 d before the start of the 
study. Carbadox, which did not interfere with HPLC, 
was the only medication these pigs received in the nurs-
ery. Water was changed and tetracycline was remixed 
every other day (i.e., d 1, 3, and 5) except for 2 pigs. 
One pig drank excessively, using most of 1 carboy every 
day; therefore, the water was replaced daily. One other 
carboy fell on d 2, and the water was replaced. Water 
use by each pig was measured with a graduated cylin-
der (to within 10 mL) on 2 separate days, the day be-
fore the study began, and on d 2, which was consistent 
with daily water use. Water samples, randomly chosen, 
were collected daily except for on d 2 and 5 when all 
carboys were collected.

Other Measurements

Free catch urine samples were attempted daily (AM 
only). Urine specific gravities were determined using a 
refractometer, and tetracycline concentrations were de-
termined. All animals were weighed at the start of the 
acclimation period and on the final day of the study.

Plasma Sample Preparations 
and Chemistries

Blood was collected from each pig via venipuncture 
of the anterior vena cava into 10-mL sodium heparin 
tubes before administration of the tetracycline water 
medication (time 0, 800 h) and then at 4, 8, 12, 24, 32, 
48, 56, 72, 80, 96, and 104 h after dosing. Whole blood 
(before centrifugation) was assayed for hematocrit with 
microcapillary tubes and total protein (via specific 
gravity) on d 1. Antech Diagnostic Labs (Ithaca, NY) 
performed urine creatinine (daily as collected) and se-
rum creatinine on d 1 and 5 for all pigs. Plasma was 
harvested from all blood samples after centrifugation 
(1,110 × g; 15 min; 4°C) within 1 h of collection and 
split into 2 samples, for processing that week and for 
storage at −80°C.

HPLC Analysis

A Waters Alliance 2695 HPLC with vacuum pump 
and autosampler (Milford, MA) was used for tetracy-
cline quantification. The assay was a modified version of 
Cheng et al. (1997) and Santosa et al. (1996). Two hun-
dred microliters of plasma were added to an equivalent 
volume of releasing agent (78% water, 20% acetonitrile, 
2% α-phosphoric acid) in a YM 10,000 Ultracel kit (Mil-
lipore Corporation, Milford, MA). The Ultracel apparatu-
sus was placed in an Eppendorf tube and centrifuged at 
7,840 × g for 30 min at 22°C. Tetracycline was quanti-
fied using a Photodiode Array at 354.4-nm wavelength, 
with peak integration by Empower software (Waters 
Corporation). Samples were held away from light at 
4°C for up to 7 d before a 4-min run on a Waters Atlan-
tis 4.6- × 150-mm C18 column in 28% acetonitrile, 72%
Pharmacokinetic Analysis

Pharmacokinetic analysis was performed on each group with WinNonLin (Pharsight, Mountain View, CA) using a noncompartmental analysis to calculate mean residence time (MRT), area under the moment curve (AUMC), area under the curve (AUC), and the AUC between 32 and 48 h \((\text{AUC}_{32-48})\). Due to animal use limitations and the availability of previously published data, an apparent oral bioavailability \((\text{Foral})\) was estimated using experimental oral data and intravenous content/vol87/issue11/), animal (Supplemental Table 1 in http://jas.fass.org/). Considered concentrations of the water medication multiplied by the amount of water consumed over 24 h for the hog farm spiked with tetracycline from 80 to 800 mg/L was adequate for our water samples analyzed by HPLC. All values were within the accepted CV of 20\%, based on daily standard curves.

\[ \text{Foral} = \frac{\text{Dose}_\text{oral} \times \text{F}_{\text{oral}}}{\text{AUC}_{\text{oral}}} = \frac{\text{Dose}_{\text{IV}}}{\text{AUC}_{\text{IV}}} \]

where \(\text{F}_{\text{oral}}\) is the apparent oral bioavailability of the tetracycline, \(\text{Dose}_\text{oral}\) is the dose from the water (measured concentrations of the water medication multiplied by the amount of water consumed over 24 h) for the animal (Supplemental Table 1 in http://jas.fass.org/content/vol87/issue11/), \(\text{AUC}_{\text{oral}}\) is the AUC\(_{32-48}\) from WinNonLin for that animal, and \(\text{AUC}_{\text{IV}}\) and \(\text{Dose}_{\text{IV}}\) are the AUC and corresponding intravenous dose from the Kniffen et al. (1989) study. The calculated \(\text{F}_{\text{oral}}\) was then used to estimate clearance and volume of distribution at steady state for each group based on the following equations:

\[ \text{Vd}_\text{ss} = \frac{\text{F}_{\text{oral}} \times \text{Dose}_\text{oral}}{\text{C}_{\text{ss}}}, \quad \text{Cl}_\text{ss} = \frac{\text{Dose}_\text{oral} \times \text{F}_{\text{oral}}}{\text{C}_{\text{ss}} \times 24} \]

where \(\text{Vd}_\text{ss}\) is the volume of distribution at steady state, \(\text{Cl}_\text{ss}\) is clearance for the group, \(\text{F}_{\text{oral}}\) is apparent bioavailability of the group, \(\text{Dose}_\text{oral}\) is the dose (water consumption multiplied by the concentration of water) over a 24-h period for the group, and \(\text{C}_{\text{ss}}\) is the concentration of the group at steady state. For each group, the individual animal peak plasma concentration \((\text{C}_{\text{max}})\) is reported with its corresponding time \((\text{T}_{\text{max}})\). Statistical Analysis

A randomized block design was employed to test the hypotheses that tetracycline concentrations in plasma differed among 4 treatment groups and if steady-state concentrations of any group reached 1 µg/mL. Daily BW gain and group BW pre- and poststudy were calculated and compared via 1-way ANOVA. Daily maximum and minimum temperatures were used to calculate a mean maximum and minimum temperature during the study. Two-way repeated ANOVA was performed (SAS Inst. Inc., Cary, NC) using PROC MIXED with dose and time as independent variables for concentrations at steady state (from 32 to 104 h). Multiple comparison testing was performed with Scheffe to determine if treatment of tetracycline groups 125, 250, and 500 µg/L were different. The data were then graphed to determine if treatment groups displayed a linear exposure-concentration curve. One-way ANOVA, using dose as the independent variable with PROC MIXED, was also performed on group PK parameters. All errors were calculated as per error analysis methods by Taylor (1982).

The ANOVA compared plasma steady state concentration (32 to 104 h) points across all groups. Steady state is considered the time at which at least 5 half-lives have transpired. Previous data (Kniffen et al., 1989; Nielsen and Gyrd-Hansen, 1996) and our current analysis indicate that the half-life for tetracycline in swine ranges from 4.5 to 6 h. Therefore, steady state should be reached between 20 and 30 h; and the first time point after steady state was reached, 32 h, was used as a conservative initial time for steady-state PK analysis.

RESULTS

All animals remained healthy throughout the study irrespective of treatment group as evidenced by values for BW, hematocrit, serum creatinine, and urine specific gravity (except pig 13 discussed below) typical for pigs of this age (Supplemental Table 1 available online at http://jas.fass.org/content/vol87/issue11/). Daily peak temperatures were elevated at the beginning of the week, were less in the middle of the week, and then increased to above 32°C by the end of the week (Table 1). Plasma concentrations of tetracycline from each group increased from below the limit of detection to steady-state concentrations within a 32-h period (Figure 1). Steady-state concentrations between groups given 125 and 250 mg/L were not different from each other, but were different from control \((P < 0.0001)\) and the 500 mg/L \((P < 0.0001)\) treatment (Table 2). The AUC were also different between the control \((P < 0.0001)\) and dosed groups, 125 and 500 mg/L \((P < 0.0001)\) treatments, 250 and 500 mg/L \((P = 0.0025)\) groups. A linear but indirect exposure-concentration relationship of tetracycline water concentration and plasma concentration is presented in Figure 2. This is consistent with reports in human literature and resources that indicate decreased absorption of larger doses of tetracycline (Chambers, 2006).

Pharmacokinetic parameters (Table 2) were calculated based on the HPLC data as explained in the Materials and Methods section. A water analysis on the farm
Table 1. Daily maximum and minimum ambient temperatures during the study

<table>
<thead>
<tr>
<th>Day</th>
<th>Daily maximum, °C</th>
<th>Daily minimum, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>—</td>
<td>21.5</td>
</tr>
<tr>
<td>2</td>
<td>31.4</td>
<td>20.1</td>
</tr>
<tr>
<td>3</td>
<td>28.4</td>
<td>20.7</td>
</tr>
<tr>
<td>4</td>
<td>29.1</td>
<td>21.7</td>
</tr>
<tr>
<td>5</td>
<td>32.9</td>
<td>23.3</td>
</tr>
<tr>
<td>6</td>
<td>33.2</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>31.0 ± 0.4</td>
<td>21.4 ± 0.2</td>
</tr>
</tbody>
</table>

1 Daily maximum temperature is based on the peak temperatures reached within the pig facility each afternoon or early evening.
2 Daily minimum temperature is the temperature reached within the pig facility in the early morning, listed as the minimum temperature from the previous day.

(Results) shows that the water quality was appropriate for the study and did not result in elevated levels of tetracycline cation-complexing. Water tetracycline concentrations were measured for each animal on 3 d or more, and freshly mixed tetracycline water concentrations were within the assay variability (20%) of the expected concentration (Table 4), which further supports water quality. Water consumption was not different across all groups of pigs. Based on linear scatter plots, there was no observable relationship between the water consumption and flow rates on tetracycline steady state or average plasma concentrations.

DISCUSSION

Pharmacokinetic parameters of MRT, AUC, and C_{max} were consistent with previously collected data (Kniffen et al., 1989; Nielsen and Gyrd-Hansen, 1996) from pigs given tetracycline orally. The main differences and intended test variable among the treatment groups are the concentration at steady state (C_{ss}). This is significant because the other parameters of half-life, MRT, F_{oral} and V_d{ss} are all similar to those previously reported. Based on statistical analysis, parameters with at least one difference were plasma concentrations at steady state, as discussed above, and the corresponding AUC_{total}. Calculated clearances are similar to previously reported values but should be interpreted carefully based on the age of the pigs in our study and our calculation of apparent bioavailability. An apparent bioavailability, or more correctly the ratio between the amount of tetracycline in water that was used by the pigs compared with the amount of an intravenous dose, was calculated at approximately 6% across all treatment groups. This ratio may be used in the future to help determine how much tetracycline in water would be needed to reach known concentrations in the blood. Essentially, this ratio theoretically means that a dose of tetracycline in water would have to be approximately 20 times the dose given intravenously to approach the AUC of that intravenous dose. This is based only on this study and may not hold for other scenarios; however, it provides an estimate of the magnitude of the difference between an oral dose in a production setting and an intravenous dose. This may not be a true bioavailability as is observed by the potential discrepancy in the clearances. Clearance rates were similar among the groups but slightly less (0.10 µg × mL⁻¹ × h⁻¹) than those previously reported between 0.16 and 0.22 µg × mL⁻¹ × h⁻¹ (Kniffen et al., 1989; Nielsen and Gyrd-Hansen, 1996), which adds credence to the apparent bioavailability not being a true bioavailability.

Based on this F_{oral} value, volumes of distribution at steady state (V_d{ss}) also were calculated but cannot be directly compared with previous PK studies values of V_d{area} or V_d{initial} because they may not be equivalent (Williams, 1999).

It is interesting to note that a few of the animals appeared to overshoot steady state at 12 h, as seen by the plasma concentrations, but steady state is not reached for another day. Furthermore, the peak concentration for the 500 mg/L group is at 12 h. However, for some of these animals, the peak at 12 h was subsequently followed by a drop to steady-state concentrations by 32 h. This may be explained by the fact that some animals drank very little overnight and that the first day of the study was warm.

Furthermore, 2 animals on the dose response curve had greater than anticipated concentrations (greater than 1.5 SD from the average, with each animal included in the average). The first outlier, within the 125 mg/L group, used approximately 12 L of water within 1 d. The second outlier did not exhibit any measurable difference in BW, water consumption, or other factors that explain the high plasma concentrations. Exclusion of both animals increases the coefficient of determination for mean concentrations at steady-state from 0.75 to 0.91 for the dose-response relationship.

The high temperatures ranged from 28.4 to 33.2°C for the whole week. According to previous studies, the maximal estimated water consumption of 1 L of water per 10 kg of BW is expected each day for pigs up to 50 d of age (Harvey, 1994). Based on this estimated water consumption for pig BW of 15 to 20 kg, the expected water consumption for the pigs in our study was 1.5 to 2 L of water daily. However, the pigs in our study consumed a larger amount of water relative to their size. The increased water consumption for the animal size could be explained by increased ambient temperatures as compared with other studies. On average, the water consumption was 3 to 4 L per pig per day (Supplemental Table 1 available online at http://jass.fass.org/content/vol87/issue11/). Due to the warm ambient temperatures, it is anticipated that plasma concentrations of these animals are close to peak attainable concentrations based on the amount of water consumed (Harvey, 1998, 1994).

Only 1 animal appeared to use water excessively, up to 12 L over one 24-h period. This may be due to either psychogenic water drinking, which can occur in a small proportion of animals from multiple species or from the pig playing with the drinker. The urine-specific grav-
Figure 1. Individual animal plasma concentrations over time by treatment group. A) Plasma concentrations from pigs (n = 6) exposed to 125 mg/L of tetracycline in water. B) Plasma concentrations from pigs (n = 6) exposed to 250 mg/L of tetracycline in water. C) Plasma concentrations from pigs (n = 6) exposed to 500 mg/L. Note that for many animals, there was a slight overshoot of steady-state concentrations at 12 h on d 1 and a decline in plasma concentrations when the temperatures were less in the middle of the week. Then, at the end of the week as water consumption likely increased with increased ambient temperatures, the plasma concentrations rose again. All axes use the same units for easier comparison.
ity of that pig on that same day was 1.003, which is consistent with medullary washout and excessive consumption of water. Regardless of this particular animal, increased water consumption is the only variable that appears correlated to increased temperatures.

It was also noted that the tetracycline from the 250-mg/L group began darkening in color by the late afternoon of d 2. This color change was also seen in the 125- and 500-mg/L-treated carboys at later times. The control water never changed color. This color change was presumed to be related to degradation from tetracycline exposure to light; however, a study by Wu and Fassihi (2005) suggested that increased temperatures and increased humidity cause degradation of tetracycline and not light exposure. The HPLC assay continued to detect the tetracycline present in the water even after the color change began. Follow-up studies are being performed to better characterize the antimicrobial

### Table 2. Pharmacokinetic parameters for tetracycline water medication for each treatment group

<table>
<thead>
<tr>
<th>Parameter/group</th>
<th>125, mg/L</th>
<th>250, mg/L</th>
<th>500, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;, µg/mL</td>
<td>0.80</td>
<td>1.26</td>
<td>1.29</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;, h</td>
<td>80</td>
<td>56</td>
<td>12</td>
</tr>
<tr>
<td>C&lt;sub&gt;ss&lt;/sub&gt;, µg/mL</td>
<td>0.33 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.47 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.77 ± 0.04&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;max&lt;/sub&gt;, µg·h/mL</td>
<td>30.71 ± 6.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.93 ± 8.26&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>73.74 ± 4.88&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;32–48&lt;/sub&gt;, µg·h/mL</td>
<td>3.54 ± 0.82</td>
<td>6.55 ± 1.24</td>
<td>10.46 ± 1.31</td>
</tr>
<tr>
<td>MRT&lt;sup&gt;e&lt;/sup&gt;, h</td>
<td>6.64 ± 0.25</td>
<td>6.61 ± 0.21</td>
<td>6.44 ± 0.15</td>
</tr>
<tr>
<td>Half-life, h</td>
<td>4.60 ± 0.25</td>
<td>4.58 ± 0.21</td>
<td>4.46 ± 0.15</td>
</tr>
<tr>
<td>Cl&lt;sup&gt;f&lt;/sup&gt;, L·kg⁻¹·h⁻¹</td>
<td>0.083 ± 0.008</td>
<td>0.107 ± 0.006</td>
<td>0.106 ± 0.008</td>
</tr>
<tr>
<td>V&lt;sub&gt;dss&lt;/sub&gt;, L/kg</td>
<td>0.554 ± 0.169</td>
<td>0.646 ± 0.252</td>
<td>0.769 ± 0.209</td>
</tr>
<tr>
<td>F&lt;sub&gt;oral&lt;/sub&gt;,%</td>
<td>0.059 ± 0.009</td>
<td>0.061 ± 0.014</td>
<td>0.057 ± 0.015</td>
</tr>
</tbody>
</table>

<sup>a</sup>125-mg/L and 250-mg/L group concentrations were different from control and 500-mg/L group (P < 0.0001).
<sup>b</sup>500-mg/L group concentrations differed from all other groups (P < 0.0001).
<sup>c</sup>125-mg/L and 250-mg/L group area under the curve (AUC) differed from control (P < 0.0001) group AUC.
<sup>d</sup>500-mg/L group AUC differed from control and 125-mg/L group AUC (P < 0.0001) and 250-mg/L group AUC (P = 0.0025).
<sup>e</sup>The C<sub>max</sub> is the peak value reported for one animal; no comparisons were performed for this value.
<sup>f</sup>The T<sub>max</sub> is reported for the greatest individual value for an individual animal; no comparisons were performed for this value.
<sup>g</sup>C<sub>ss</sub> is the mean concentration at steady state for the treatment group.
<sup>h</sup>AUC<sub>total</sub> refers to the area under the concentration time curve from 0 to 104 h.
<sup>i</sup>AUC<sub>32–48</sub> is the area under the concentration time curve from 32 to 48 h and was used to calculate clearance.
<sup>j</sup>MRT refers to mean residence time.
<sup>k</sup>Cl refers to clearance at steady state.
<sup>l</sup>V<sub>dss</sub> is the volume of distribution at steady state.
<sup>m</sup>F<sub>oral</sub> refers to the apparent oral bioavailability of tetracycline compared with a typical 10-mg/kg intravenous dose.

![Figure 2](image-url)  
Exposure response relationship between tetracycline water medication concentration and individual animal steady-state plasma concentrations of tetracycline. The line represents the relationship, based on least squares best fit, between the average individual animal steady-state concentrations and the treatment exposure concentrations. Removal of 2 data points because each steady-state concentration was greater than 1.5 SD from the calculated mean, using all data points, increases R² to 0.91.
Tetracycline water medication in swine

Table 3. Solution analysis report from the North Carolina Agronomics Department, Raleigh

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.45</td>
</tr>
<tr>
<td>Electrical conductivity</td>
<td>0.00016</td>
</tr>
<tr>
<td>Sodium adsorption ratio</td>
<td>0.60</td>
</tr>
<tr>
<td>Total alkalinity (CaCO₃), mg/L</td>
<td>55</td>
</tr>
<tr>
<td>Hardness, mg/L</td>
<td>63</td>
</tr>
<tr>
<td>B, mg/L</td>
<td>0.02</td>
</tr>
<tr>
<td>Ca, mg/L</td>
<td>17.70</td>
</tr>
<tr>
<td>Cl, mg/L</td>
<td>4.53</td>
</tr>
<tr>
<td>Cu, mg/L</td>
<td>0.02</td>
</tr>
<tr>
<td>Mg, mg/L</td>
<td>4.63</td>
</tr>
<tr>
<td>Mn, mg/L</td>
<td>0.09</td>
</tr>
<tr>
<td>Inorganic N, mg/L</td>
<td>0.72</td>
</tr>
<tr>
<td>P, mg/L</td>
<td>0.18</td>
</tr>
<tr>
<td>K, mg/L</td>
<td>8.90</td>
</tr>
<tr>
<td>Na, mg/L</td>
<td>10.80</td>
</tr>
<tr>
<td>S, mg/L</td>
<td>4.58</td>
</tr>
<tr>
<td>Zn, mg/L</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Water from the farm well was assayed before the study. This assay tested for the suitability of the water for its intended purpose. Physical properties and concentrations of divalent and trivalent cations which could bind tetracycline were also reported. Recommendations from the report: “The general water quality... from this farm well looks good. Mineral nutrient and soluble salt levels are low and alkalinity is moderate.”

Electrical conductivity is moderate. Mineral nutrient and soluble salt levels are low and alkalinity is moderate.

Total alkalinity (CaCO₃), mg/L

Sodium adsorption ratio

B, mg/L

Ca, mg/L

Cl, mg/L

Cu, mg/L

Mg, mg/L

Mn, mg/L

Inorganic N, mg/L

P, mg/L

K, mg/L

Na, mg/L

S, mg/L

Zn, mg/L

2This equation relates the concentration of tetracycline measured by HPLC to the expected concentration of tetracycline and incorporates all errors, including measurement error in water volume and weighing of tetracycline, and variability in the HPLC method. A ratio of 1.00 shows that the measured water concentration matches the expected concentration.

Table 4. Tetracycline HPLC assay quantitation compared with the expected amount of tetracycline mixed into carboy water on d 3 of the study

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>True dose/expected dose²</th>
</tr>
</thead>
<tbody>
<tr>
<td>125</td>
<td>1.11</td>
</tr>
<tr>
<td>125</td>
<td>1.19</td>
</tr>
<tr>
<td>125</td>
<td>1.15</td>
</tr>
<tr>
<td>250</td>
<td>0.93</td>
</tr>
<tr>
<td>250</td>
<td>0.90</td>
</tr>
<tr>
<td>250</td>
<td>0.85</td>
</tr>
<tr>
<td>250</td>
<td>0.88</td>
</tr>
<tr>
<td>250</td>
<td>0.88</td>
</tr>
<tr>
<td>500</td>
<td>1.11</td>
</tr>
<tr>
<td>500</td>
<td>0.85</td>
</tr>
<tr>
<td>Average proportion of water dose to expected value</td>
<td>0.99 ± 0.01</td>
</tr>
</tbody>
</table>

1Each carboy was emptied, rinsed, and refilled with 19.2 L of water from the farm, and then preweighed tetracycline powder was added to the carboy. These samples were randomly collected on d 3.

2This data was collected to see how much tetracycline was in the water after it was added to the carboy. This data was then compared to the expected amount of tetracycline that was added to the carboy. The results showed that the measured amount of tetracycline in the water was consistent with the expected amount. This information indicates that water medication values are in line with feed additive concentrations and will likely have similar efficacy. Furthermore, based on the Clinical and Laboratory Standards Institute guidelines, sensitive control bacteria used in antibiotic susceptibility assays need at least 1 µg/mL for growth inhibition (CLSI, 2007). Only in a few pigs is 1 µg/mL reached or exceeded, and even this level is not consistently met by any animal or group. Based on treatment group PK analyses, it is evident that all steady state plasma concentrations are adequate for treatment of highly susceptible bacteria. Furthermore, tetracycline has been used more than any other medication in actual tons in Europe and the United States (Aarestrup, 2005). Bunner et al. (2007) showed that Escherichia coli are highly resistant to tetracycline with minimum inhibitory concentrations above 16 µg/mL, which is often considered the breakpoint of tetracycline.

As a reference for in vivo tetracycline concentrations, the Food and Drug Administration has set a tolerance of tetracycline at 2 µg/mL for muscle, 12 µg/mL for kidney and fat, and 6 µg/mL in liver (FDA, 2005).
Concentrations within the plasma from our study are not even 50% of the least muscle tolerance concentration for any time points. At this time no published tetracycline residue studies are available; therefore, the true tissue concentrations of tetracycline in vivo are unknown. However, based on oxytetracycline tissue concentrations in pigs and ruminants, the concentrations of tetracycline will likely be a maximum of 6 to 7 times the plasma concentrations in the kidney (Mercer et al., 1978). The lung, which is often the target of tetracycline treatment, only reaches or slightly exceeds the concentration in the plasma. Other tissues such as fat reach less than one-half of peak plasma concentrations (Mercer et al., 1978; Craigmill, 2003).

Injectable antibiotics are still used in livestock production; however, due to labor constraints, the possibility of needle breakage, and the expense of injectable medications, other methods of medicating animals are used first. Food producers need to be aware of the medications available and what they actually can treat. Based on this study and corroboration with previous work on tetracycline oral medications in pigs, tetracyclines should not be used enterally to treat salmonellosis and respiratory disease. There may still be positive gastrointestinal effects from using oral tetracycline for prevention of scours and other enteric disease, but this needs to be assessed further.

LITERATURE CITED


