BioOne COMPLETE

Pharmacokinetics and Safety of Sulfamethoxazole-Trimethoprim After Oral Administration of Single and Multiple Doses to Rhode Island Red Chickens (Gallus gallus domesticus)

Authors: Petritz, Olivia A., Enomoto, Hiroko, Meyer, Emma G., Thomson, Andrea, Baynes, Ronald E., et al.

Original Study

Pharmacokinetics and Safety of Sulfamethoxazole-Trimethoprim After Oral Administration of Single and Multiple Doses to Rhode Island Red Chickens (*Gallus gallus domesticus*)

Olivia A. Petritz, Hiroko Enomoto, Emma G. Meyer, Andrea Thomson, Ronald E. Baynes, and Keven Flammer

Abstract: Sulfamethoxazole-trimethoprim (SMZ-TMP), a commonly prescribed antibiotic for backyard hens, is neither Food and Drug Administration approved nor prohibited in laying hens in the United States. The aim of this study was to determine whether plasma concentrations above targeted minimum inhibitory concentration breakpoint values for Enterobacteriaceae could be achieved with oral dosing. Five Rhode Island red hens (*Gallus gallus domesticus*) were administered a single dose of 96 mg/kg SMZ-TMP (80 mg/kg SMZ and 16 mg/kg TMP) IV followed by the same dose orally after a washout period. Following oral dosing, mean SMZ concentrations exceeded the target breakpoint for approximately 12 hours; however, TMP only briefly exceeded the target breakpoint. Bioavailability was 60.5% for SMZ and 82.0% for TMP. Ten naïve birds were allocated into control (n = 4) and treatment (n = 6) groups for a 7-day multi-dose study. Treatment birds received an oral suspension dosed at 16 mg/kg TMP and 80 mg/kg SMZ every 48 hours (on days 1, 3, 5, and 7); TMP tablets were additionally dosed at 25 mg/bird on days 1, 3, 5, and 7, and 50 mg/bird on days 2, 4, and 6. Plasma SMZ-TMP concentrations were measured on a multiple time interval by ultraperformance liquid chromatography–mass spectrometry, and pharmacokinetic analyses were performed using a noncompartmental model. No accumulation for either drug was noted following repeated dosing, and no statistical differences in biochemical values, packed cell volumes, or weight were found between pre- and posttreatment in either the treatment or control groups. Sulfamethoxazole (80 mg/kg q48h PO) and TMP (24.1–28.0 mg/kg q24h PO) maintained therapeutic plasma concentrations at or exceeding the minimum inhibitory concentration breakpoint of Enterobacteriaceae for 72 and 24 hours for TMP and SMZ, respectively, without evidence of adverse effects or drug accumulation. Further studies are needed to refine this dosage regimen and evaluate adverse effects in ill birds.

Key words: pharmacokinetics, sulfamethoxazole-trimethoprim, safety, avian, chicken, *Gallus gallus domesticus*

INTRODUCTION

Backyard poultry flocks are increasing in popularity in urban and suburban areas across the United States.[1] According to a 2013 US Department of Agriculture survey of 4 metropolitan cities, 1% of households owned chickens and another 4% planned to own chickens in the next 5 years.[2] These flocks are often comprised of heritage breeds, and Rhode Island red hens (*Gallus gallus domesticus*) were the breed most favored by backyard hobbyists according to a recent survey.[3]

From the Department of Clinical Sciences, College of Veterinary Medicine (Petritz, Thomson, Flammer) and the Comparative Medicine Institute (Enomoto, Baynes), North Carolina State University, Raleigh, NC 27607, USA; School of Pharmacy, University of Missouri–Kansas City, Kansas City, MO 64108, USA (Meyer); and the Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606, USA (Enomoto, Baynes).
Corresponding author: Olivia Petritz, oapetrit@ncsu.edu

1

Consequently, they were selected as study subjects for this study, rather than a breed commonly used in commercial flocks, such as the white leghorn.

A majority of backyard hens are being kept for either egg production or as companion pets,[3] and owners are seeking veterinary care for these birds, similar to other household pets. However, the United States Food and Drug Administration (FDA) prohibits the use of many classes of antibiotics in chickens within country, as they are considered major food-producing animals, regardless of their intended purpose—meat production, egg production, or as companion pets. According to the FDA, drugs administered to laying hens must have a 0-day egg withdrawal time; therefore, there are very few drugs, including antibiotics, that meet these criteria. Veterinarians who treat small flocks or individual birds are often forced to rely on extralabel drug use, as culling ill birds is often not an acceptable option for backyard flock owners. Veterinarians are allowed to prescribe certain extralabel medications for use in backyard chickens; however, there is minimal pharmacokinetic information to guide appropriate drug dosing. Judicious use of antimicrobial medications is extremely important in backyard chickens because inappropriate use not only leads to treatment failure and antibiotic resistance but also potentially exposes an uninformed public to drug residues in eggs, an important human health concern.[4] Highlighting the need for appropriate drug use to avoid violative drug residues, the United States Food Animal Residue Avoidance Databank (FARAD) has identified extralabel treatment of backyard chickens as an area of concern.[5]

While sulfamethoxazole-trimethoprim (SMZ-TMP) is not approved for use in laying hens in the United States, its use is also not forbidden in this species pursuant to the FDA Code of Federal Regulations, title 21 (21CFR 530.41). Trimethoprim-sulfamethoxazole is commonly prescribed to backyard poultry, and it was one of the top 5 drugs that FARAD received as egg withdrawal requests from prescribing veterinarians.[5] Both sulfonamides and diaminopyrimidines, such as TMP, interfere with the production of folic acid in bacterial cells.[6] This drug combination is commercially available in a 1:5 fixed ratio of diaminopyrimidine to sulfonamide, as this has been shown to provide the maximum synergy in humans following oral or parenteral administration[7] and is likely bacteriostatic at the concentrations achieved with routine dosing.[8] The lipid-soluble diaminopyrimidines are much more active than the sulfonamides because they concentrate in the tissues, while weak organic acids remain mainly in extracellular fluids. The ideal drug ratio and resultant bactericidal effect is likely variable across host and bacterial species, making dosage extrapolation across species challenging.[7] Further complicating dosing, SMZ is commercially available only in combination with TMP and TMP is only commercially available in 100-mg and 200-mg tablets.

The pharmacokinetics of SMZ-TMP have been previously evaluated in a variety of mammals,[9–13] invertebrates,[14,15] pigeons,[16] and egg-laying hens.[17] In a previous study in chickens, a single oral and intravenous dose of a compounded SMZ-TMP product with a different SMZ-TMP ratio than the commercially available product was administered; however, safety and the effects of repeated dosing were not evaluated.[17] The published dose range for oral administration of SMZ-TMP in avian species, including chickens, is wide and mostly empirically derived: 10–50 mg/kg PO once to twice daily.[18,19] According to FARAD, the most common dose of SMZ-TMP used by veterinarians when inquiring about egg residues and withdrawal recommendations is 30 mg/kg SMZ-TMP (5 mg/kg TMP and 25 mg/kg SMZ).

Escherichia coli is considered a primary cause of disease-related loss in the poultry industry worldwide.[20] According to a recent review of mortalities in backyard poultry in the United States, bacterial infections were present in 42% of necropsied birds, and *E coli* was the most common bacterial isolate.[21] Ideal dosing of antimicrobial drugs is achieved when the minimum inhibitory concentration (MIC) of the target bacteria is known. When this is not known, breakpoint concentrations established by Clinical and Laboratory Standards Institute (CLSI) are often used. These breakpoints are derived from a large database of bacteria isolated from a number of animal species. Therapeutic targets for this study were the CLSI breakpoints for Enterobacteriaceae of 2 μg/mL for TMP and 38 μg/mL of SMZ.[22] Trimethoprim is the primary driver of treatment efficacy,[8] so TMP concentrations were emphasized in selecting a multiple-dose regimen.

The goals of the current study were to determine the pharmacokinetics of SMZ-TMP orally following single and multiple doses to Rhode Island red hens, and to assess safety of this medication during oral administration for 7 consecutive days. We hypothesized that we could determine an effective dose of SMZ-TMP in hens that maintained plasma concentrations for both drugs above the published

CLSI MIC breakpoints for Enterobacteriaceae in hens.

MATERIALS AND METHODS

Birds

Twenty-five 3-year-old Rhode Island red hens were obtained from a commercial source (North Carolina State University, Poultry Department, Raleigh, NC, USA), and group-housed in a climate-controlled facility within a 3.6 × 3.6-m (12 × 12-foot) floor pen with wooden shavings as substrate. To reduce egg laying in this flock, a molt diet (9% protein; North Carolina State University Feed Mill, Raleigh, NC, USA) and water, via automatic waterers with nipple attachments, were provided ad libitum, and the birds were provided a 8:16-hour light : dark cycle. They were housed in this environment for 4 weeks prior to undergoing any procedures. The hens were determined to be healthy based on serial physical examinations every 2 to 4 weeks, fecal flotation, serial packed cell volumes (PCVs) via microhematocrit tube and centrifugation, serial total solids via refractometer, and serial biochemical panels (VetScan Avian/ Reptile Profile Plus, Abaxis Inc, Union City, CA, USA). All study procedures were approved by the North Carolina State University Institutional Animal Care and Use Committee (IACUC no. 18-046-O).

Preliminary experiments

Fifteen birds were used for 3 initial dose ranging studies, with 5 birds in each study, prior to the main experiments. The body weights of these birds ranged from 1.8 to 2.51 kg. In the first trial, each hen was administered a single oral dose of 96 mg/ kg SMZ-TMP (80 mg/kg SMZ and 16 mg/kg TMP; Aurobindo Pharma USA Inc, Dayton, NY, USA). In the second trial, each hen received a single oral dose of 30 mg/kg SMZ-TMP (25 mg/kg SMZ and 5 mg/kg TMP). Blood samples (0.3–0.7 mL) were collected at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 16, and 24 hours after dose administration for both trials. To better characterize drug elimination at the higher dose, a third trial was performed with the same dose as in the first trial, 96 mg/kg SMZ-TMP (80 mg/kg SMZ and 16 mg/kg TMP), and blood samples (0.3–0.7mL) were collected at 1, 4, 8, 12, 24, 48, 72, 120, 144, and 168 hours after dose administration. Blood samples were each placed into a lithium heparin microtainer tube (BD Microtainer, Thermo Fisher Scientific Inc, Waltham, MA, USA), placed on wet ice for ≤1 hour,

and then centrifuged at 3800g for 3 minutes. Plasma was harvested and stored at –80°C (–112°F) until analysis.

Single-dose primary crossover experiment

Five hens were used for the single-dose primary experiment. The body weights of these birds ranged from 2.11 to 2.62 kg. The washout period for these birds from their use in the preliminary experiments ranged from 12 to 14 weeks. Food and water were withheld for 1–2 hours prior to the morning dosing, just after the room lights were turned on for the day. For intravenous administration, the birds were manually restrained, and a 24-gauge over-the-needle intravenous catheter was placed in the right ulnar vein. A single 96-mg/kg (80 mg/kg SMZ and 16 mg/kg TMP) dose of SMZ-TMP (Teva Parenteral Medicines, Inc, Irvine, CA, USA) was diluted 4:1 with D5W (Baxter Healthcare Corp, Deerfield, IL, USA) and administered intravenously by hand with a 12-mL syringe and a microbore T-connector (Henry Schein Animal Health, Dublin, OH, USA) over a period of 3–4 minutes. The SMZ-TMP solution was diluted within 1 hour prior to administration to all birds. Following the infusion, the intravenous catheter was removed and brief manual pressure was placed over the vessel to prevent extravasation of the drug. A blood sample (0.3–0.6 mL) was collected at 0 (predose), 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 32, and 48 hours after dose administration. Blood samples were collected from either the jugular, left ulnar, or medial metatarsal veins with a 26-gauge needle and either a 1- or 3-mL syringe. Food and water were withheld from the birds immediately prior to dosing and were reintroduced 2 hours following dose administration. All treated birds were temporarily housed in a large metal transport cart (61 × 76 × 107 cm [24 × 30 × 42 inches]) for ≤2 hours immediately after dose administration and then were placed back in the large group pen. These birds were monitored for 72 hours following drug administration for signs of regurgitation, diarrhea, inappetence, and phlebitis of the right ulnar vein. Blood samples were processed as previously described. After a 17-day washout period, these birds were administered the same dose of SMZ-TMP orally into the crop with a 12-French red rubber feeding tube (Covidien LLC, Mansfield, MA, USA) and 6-mL syringe in a nonrandomized crossover design. A blood sample (0.3–0.6mL) was collected at 0 (predose), 0.5, 1, 2, 4, 6, 8, 12, 24, 32, 48, and 75 hours after dose administration.

Sampling sites, sample handling, and bird monitoring were identical to the previous trials.

Multiple-dose primary experiment

Ten naïve birds were randomly selected and allocated into control (n = 4) and treatment (n = 6) groups by drawing their identification numbers out of a container. Another health assessment for these naïve birds was performed the day before drug administration, which included a physical examination, body weight, and blood collection for measuring PCV, total solids via refractometer, and a plasma biochemical panel. The body weights of these birds ranged from 1.7 to 2.6 kg. For this portion of the experiment, the original indoor floor pen was divided into 3 sections—1 for the main flock not used in this experiment, 1 for the treatment birds, and 1 for the control birds. The treatment and control birds were provided the same diet and water ad libitum similar to the remainder of the flock. The remainder of their environment and husbandry was unchanged. Two additional naïve birds (termed cohoused controls) were housed with the 6 treatment birds to monitor for SMZ-TMP concentrations from possible ingestion of the treated birds' feces. To maintain identical housing conditions, 4 additional hens were also housed with the 4 control birds for a total of 8 birds each in the treatment and control pens. Those 4 additional birds housed in the control pen received no treatments.

The plasma concentrations from the single dose experiments suggested that administering a dose of 96 mg/kg TMP-SMZ (16 mg/kg TMP and 80 mg/kg SMZ) would maintain SMZ plasma concentrations above target concentrations for approximately 24 hours; however, this regimen would underdose TMP according to the CLSI MIC breakpoints for Enterobacteriaceae. Dosing SMZ every 24 hours would likely result in accumulation due to prolonged elimination of this drug. Constrained by available drug formulations, we supplemented the every 48-hour administration of the oral TMP/SMZ suspension at 96 mg/kg with an additional dose of TMP (TMP 100-mg tablets, Actavis, Barnstaple, UK) at 25 mg/bird PO (total TMP dose 26.7–29.4 mg/kg, mean 28 mg/kg). On days when the birds did not receive TMP-SMZ (days 2, 4, and 6), 25 mg/kg TMP/bird PO was administered (total TMP dose 21.4–26.7 mg/kg, mean 24.1 mg/kg). The control birds received an equal volume of tap water via oral dosing syringe and a small pellet of food, equivalent in size to the TMP tablet, using a corresponding dosing schedule. All birds were dosed at the same time each morning.

Blood samples (0.3–0.6 mL) were collected from both treatment and control birds at 0 (predose), 1, 4, 8, 12, 24, 48, and 96 hours after administration of the first dose. In addition, blood samples (0.3–0.6mL) were also collected from both treatment and control birds at 0 (predose), 1, 4, 8, 12, 24, 32, 48, and 72 hours after administration of the last dose administered on day 7 to assess depletion in plasma after the multiple-dose regimen. Sampling sites, sample handling, and bird monitoring were identical to the previous trials. Another general health assessment was performed for the treatment and control birds on day 7 of the trial, which included a physical examination, body weight, PCV, total solids via refractometer, and a plasma biochemical panel. Body weights for all treatment and control birds were obtained on days 1, 3, 5, 7, and 9. Appetite and a subjective behavioral assessment (overall activity level, interactions with conspecifics) were evaluated once daily for both treatment and control birds for the duration of the experiment. Blood samples (0.3–0.6 mL) were obtained from the 2 cohoused control birds at 12, 24, and 48 hours after the first dose and at 12, 24, 32, 48, and 72 hours after the last dose on day 7.

Analysis of plasma samples

In preparing the calibration standard, a 5:1 ratio of SMZ to TMP was chosen to mimic the commercially available formulation. If not otherwise provided, concentrations of TMP are described, and the concentration of SMZ at any step is simply 5 times as much. Trimethoprim (0.004 g) was weighed directly into a glass vial while SMZ (0.02 g) was weighed onto wax paper and transferred into the glass vial containing the TMP. Next, 2 mL of methanol were added to the glass vial to give final concentrations of 10 000 μg/mL SMZ and 2000 μg/mL TMP. This was then serially diluted to create working solutions of 10, 50, 100, 500, and 1000 μg/mL TMP in methanol. The plasma calibration curve was prepared by diluting the working solutions with chicken plasma (obtained from study chickens prior to study start) for a final calibration curve of 0.2, 1, 2, 10, 20, and 40 μg/mL TMP (1, 5, 10, 50, 100, and 200 μg/mL SMZ). The plasma calibration curve was prepared fresh each day of sample analysis.

Thawed plasma samples were pretreated by diluting 50 μL of sample with 950 μL of ultrapure water and vortexing for 15 seconds. Diluted samples (1000 μL) were then added onto solid-

phase cartridge (HLB PRIME 1mL, Oasis; Waters Corp, Milford, MA, USA) and pushed through slowly with nitrogen. The sample was then washed with 1 mL of 95:5 water : methanol and dried with nitrogen at 30 psi for 1 minute. Samples were then eluted into clean 16 × 100-mm borosilicate glass tubes with 1 mL 90:10 acetonitrile : methanol and dried with nitrogen at 55°C (131°F) for 10 minutes. Samples were reconstituted with 80:20 0.1% acetic acid in water : acetonitrile before being vortexed for 30 seconds. Finally, samples were filtered using 0.2-μm Whatman PVDF mini-uni prep filter vials with pre-slit caps (Global Life Sciences Solutions Operations UK Ltd, Amersham Place, Little Chalfont, Buckinghamshire, UK) before being analyzed by ultraperformance liquid chromatography-mass spectrometry (UPLC-MS).

An Acquity UPLC Classic system (Waters Corp) was used for solvent and sample delivery. Chromatographic separation was performed on an Acquity UPLC BEH C18 1.7-μm (2.1 × 50 mm) column with a Vanguard precolumn (BEH C18, 1.7 μm, 2.1 × 5 mm). A gradient, using mobile phases A: 0.1% acetic acid in water and B: acetonitrile, was used with the initial mobile phase (95:5 A : B) for the first 2.5 minutes. The mobile phase was then switched to 20:80 A : B from minute 2.5 to 3.51. The last 1.49 minutes of the run, the mobile phase was returned to 95:5 A : B. During gradient, flow rate was maintained at 0.4 mL/min. Volume injected was 5 μL, and column and sample temperatures were maintained at 35°C (95°F) and 25°C (77°F), respectively. The SMZ and TMP were detected in a UPLC-MS operated with positive electrospray ionization in selected ion recording mode. The cone voltage was 20 V and capillary voltage was 0.8 V.

Pure analytical reference standards (Sigma Aldrich, Burlington, MA, USA) were used for both SMZ and TMP. The standard curve was linear from 0.2 to 40 μg/mL TMP (1 μg/mL to 200 μg/mL SMZ), with a coefficient of determination (R^2) greater than or equal to 0.99. A minimum of 5 replicates of 0.2, 1, and 20 μg/mL TMP were used to calculate intraday precision (%) of 3.99, 5.73, and 4.89, with accuracies (%) of 85.0, 114.1, and 92.5, respectively. A minimum of 5 replicates of 1, 5, and 100 μg/mL SMZ were used to calculate intraday precision (%) of 12.68, 3.49, and 12.16, with accuracies (%) of 86.0, 112.5, and 95.7, respectively. The interday precision and accuracy for TMP and SMZ were also calculated. The precision range and accuracy for TMP were 0.6–4.9% and 86.8–111.5%, while the precision range and accuracy for SMZ were 1.1–12.4% and

between 86.2% and 110.5%. The limit of quantification was determined to be 0.2 μg/mL TMP (1 μg/mL SMZ), as it was the lowest concentration in the standard curve with acceptable accuracy (100 ± 15%) and precision (<15%). The limit of detection was not determined.

Pharmacokinetic analysis

Noncompartmental pharmacokinetic analyses of TMP-SMZ in the chicken plasma were performed using commercially available software (Phoenix WinNonlin Software version 8.3, Certara, Princeton, NJ, USA). The pharmacokinetic parameters estimated for TMP-SMZ in plasma after oral or intravenous administration included the elimination rate constant (λz), elimination half-life ($HL_{\lambda z}$), the area under the curve from time 0 to the last time point (AUC_{last}), the area under the curve from time 0 to the infinity ($AUC_{infinity}$), the area under the curve from time 0 to 24-hour time points (AUC_{0-24}), the maximum concentration (C_{max}), time to maximum concentration (T_{max}), the mean drug residence time from time 0 to the infinity ($MRT_{infinity}$), clearance (CL_F) and volume of distribution (Vz_F), and the extrapolated AUC, which was calculated using the linear log trapezoidal method. The bioavailability of orally administered TMP-SMZ was calculated by using the following equations: Bioavailability $(F)_{PO} = (AUC_{infinity\ PO}/AUC_{infinity\ IV}) \times (dose_{IV}/dose_{PO})$, and Bioavailability $_{PO}$ (%) $= (AUC_{infinityPO} / AUC_{infinityIV}) \times (dose_{IV}/dose_{PO}) \times 100$. The Vz_F and CL_F of orally administered TMP-SMZ were adjusted using bioavailability with the following equation: $Vz\_F_{PO} = dose/C_{max} \times F : CL\_F_{PO} = dose/AUC \times F$.

Statistical analysis

Mean ± SD values were reported for all pharmacokinetic variables in the single dose study. Eleven variables were analyzed with individual alternative hypotheses: weight (decrease), PCV (decrease), total solids (2-sided), aspartate aminotransferase (increase), bile acids (2-sided), creatinine kinase (increase), uric acid (increase), glucose (2-sided), calcium (decrease), phosphorus (increase), and total protein (2-sided). A series of 2-sample Wilcoxon rank-sum tests were utilized to evaluate pre- and posttreatment values. Three machine-censored calcium values were removed from the analysis as they were too high to read and no numerical value was provided. A Bonferroni correction to the family-wise error rate was
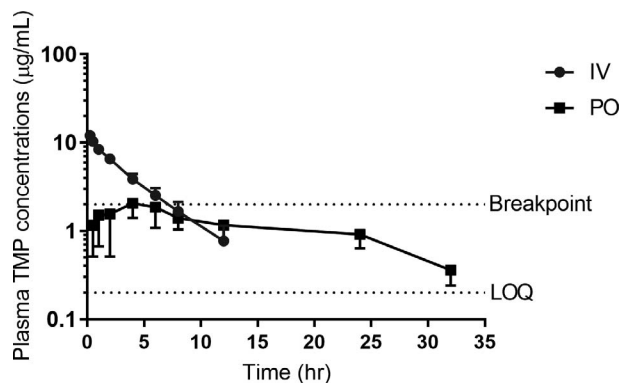
Figure 1. Mean ± SD concentrations of trimethoprim (TMP) in plasma samples obtained from 5 Rhode Island red hens (*Gallus gallus domesticus*) that received a single dose of 16 mg/kg TMP in combination with 80 mg/kg sulfamethoxazole IV (circles) and PO (squares). The Clinical and Laboratory Standards Institute breakpoint published for Enterobacteriaceae for TMP is 2 µg/mL, and the limit of quantification (LOQ) is 0.2 µg/mL.

Figure 2. Mean ± SD concentrations of sulfamethoxazole (SMZ) in plasma samples obtained from 5 Rhode Island red hens (*Gallus gallus domesticus*) that received a single dose of 80 mg/kg SMZ in combination with 16 mg/kg trimethoprim IV (circles) and PO (squares). The Clinical and Laboratory Standards Institute breakpoint published for Enterobacteriaceae for SMZ is 38 µg/mL, and the limit of quantification (LOQ) is 1 µg/mL.

applied. Values of $P \leq 0.05$ were considered statistically significant.

RESULTS

Preliminary experiments

All birds remained clinically healthy during the 3 dosing trials, and all birds exhibited normal behavior and appetite. The chickens' urofeces were within normal limits, and there was no evidence of diarrhea or regurgitation noted in any bird. The maximum concentration (C_{max}) of TMP (Supplemental Figure S1) was significantly below the published MIC breakpoint for Enterobacteriaceae when administered at a dose of 30 mg/kg SMZ-TMP (5 mg/kg TMP and 25 mg/kg SMZ). Sulfamethoxazole remained above the target SMZ-TMP breakpoints published for Enterobacteriaceae (≤ 38 µg/mL) in 80% (4/5) of birds at that dose (Supplemental Figure S2). The data for the preliminary studies of a single dose of 96 mg/kg SMZ-TMP are not shown.

Single-dose primary experiment

All birds maintained a normal appetite and displayed normal behaviors during the study period. No evidence of diarrhea or spontaneous regurgitation was noted in any bird. One bird had a large fluid-filled crop first noted 4 hours after oral SMZ-TMP administration, but this resolved without additional intervention within 12 hours of initial dosing. In addition, all birds exhibited lacrimation, ptyalism, and increased swallowing
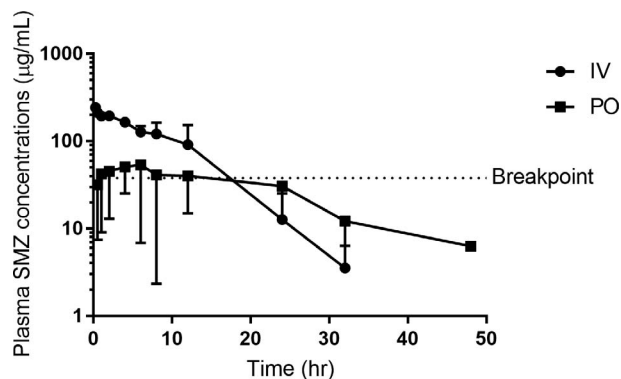
immediately after starting the intravenous infusion of SMZ-TMP. These signs resolved within 10 minutes following the end of the infusion. No evidence of phlebitis was noted at the catheter placement site at any point following the infusion. Plasma concentration of SMZ-TMP-versus-time curves for both intravenous and oral administration of both TMP and SMZ at a dose of 96 mg/kg (16 mg/kg TMP and 80 mg/kg SMZ) were plotted (Figs 1 and 2). Pharmacokinetic parameters were calculated for both routes for both drugs and summarized in Table 1.

Multiple-dose primary experiment

All treatment and control birds maintained a normal appetite and displayed normal behaviors throughout the study. No evidence of diarrhea or regurgitation was noted in any bird from either group. Three birds in the treatment group had large, fluid-filled crops first noted 4 hours after oral SMZ-TMP administration, but all resolved without additional intervention within 12 hours of initial dosing, similar to the single-dose experiment. No statistical differences were found in the biochemical values, PCV, or weight between pre- and posttreatment control and treatment groups, with all P values being > 0.05 after Bonferroni correction. No SMZ-TMP was detected in any plasma samples collected from the 2 cohoused control birds at any time point. Mean plasma concentration of SMZ-TMP-versus-time curves after oral administration of both TMP and SMZ

Table 1. Mean $\pm$ SD for pharmacokinetic parameters after single oral and intravenous doses of TMP (16 mg/kg) and SMZ (80 mg/kg) to 5 Rhode Island red hens (*Gallus gallus domesticus*).

Parameters	Units	TMP PO	TMP IV	SMZ PO	SMZ IV
λz	1/h	0.09 ± 0.08	0.19 ± 0.04	0.12 ± 0.05	0.19 ± 0.02
$HL_{\lambda z}$	hours	11.6 ± 5.5	3.9 ± 0.7	7.7 ± 4.1	3.6 ± 0.4
T_{max}	hours	3.6 ± 0.8		5.3 ± 4.3	
C_{max}	µg/mL	2.1 ± 0.7		74.5 ± 36.2	
AUC_{last}	h $\times$ µg/mL	32.1 ± 5.1	45.0 ± 6.3	1265.7 ± 437.9	2249.1 ± 659.8
$AUC_{infinity}$	h $\times$ µg/mL	38.7 ± 6.2	47.5 ± 6.8	1298.1 ± 414.3	2271.7 ± 667.1
AUC_{0-24}	h $\times$ µg/mL	28.8 ± 4.1			
Vz_F	L/kg	6.8 ± 2	1.9 ± 0.6	0.8 ± 0.4	0.2 ± 0.04
CL_F	L/h per kilogram	0.34 ± 0.07	0.34 ± 0.05	0.03 ± 0.01	0.04 ± 0.01
Extrapolated AUC	%	16.5 ± 7.8	5.2 ± 3.8	3.5 ± 3.2	1.0 ± 0.6
$MRT_{infinity}$	hours	18.0 ± 7.2	4.7 ± 0.6	16.7 ± 4.6	7.5 ± 1.8
Bioavailability	%	82.0 ± 12		60.5 ± 20.7	

Abbreviations: TMP, trimethoprim; SMZ, sulfamethoxazole; PO, per os; IV, intravenous; λz, elimination rate constant; $HL_{\lambda z}$, elimination half-life; T_{max}, time to the maximum concentration; C_{max}, maximum concentration; AUC_{last}, area under the curve from time 0 to the last time point; $AUC_{infinity}$, area under the curve from time 0 to infinity; AUC_{0-24}, area under the curve from time 0 to 24 hour time point; Vz_F, volume of distribution; CL_F, total clearance; and $MRT_{infinity}$, mean residence time from time 0 to infinity.

were plotted (Figs 3 and 4, respectively). Pharmacokinetic parameters were calculated for both drugs and summarized in Tables 2 and 3. One bird was excluded from estimation of pharmacokinetic parameters because of high extrapolated AUC (52% for TMP and 74% for SMZ).

DISCUSSION

The pharmacokinetics of SMZ-TMP, a commonly used antibiotic in backyard poultry, were evaluated in Rhode Island red hens in 3 single-dose ranging trials as a preliminary pilot study, 1 single-dose crossover trial, and a multi-dose trial. Results from the single-dose experiments indicated a dose of 96 mg/kg SMZ-TMP (16 mg/kg TMP and 80 mg/kg SMZ) could exceed plasma concentrations above the MIC for Enterobacteriaceae for SMZ in this species (38 µg/mL); however, TMP only briefly exceeded the MIC for Enterobacteriaceae (2 µg/mL) at this dosage. Due to the prolonged elimination of SMZ, and concerns for toxicity after doses that were increased and/or repeated at 24 hours, supplemental TMP was added to the oral
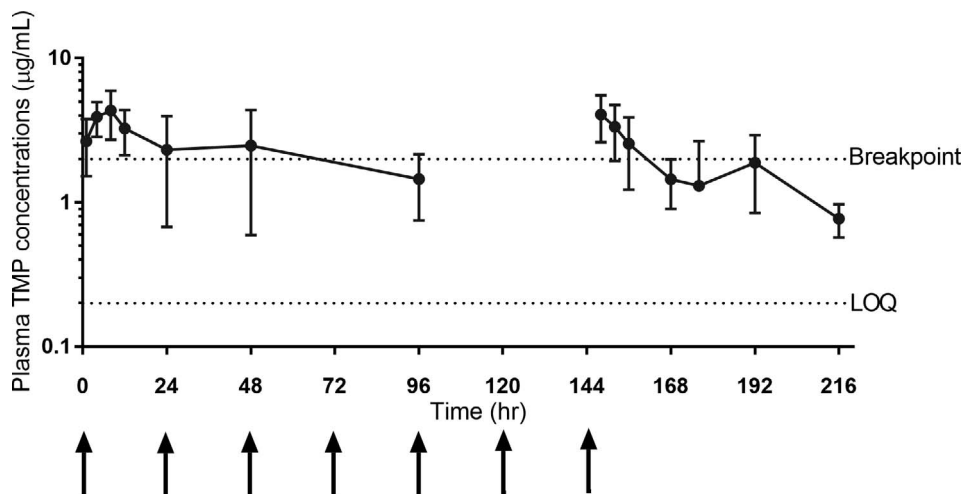

Figure 3. Mean $\pm$ SD concentration-time curve of trimethoprim (TMP) in plasma samples from 6 Rhode Island red hens (*Gallus gallus domesticus*) after repeated oral administration once daily for 7 days. A mean total dose of 28 mg/kg of TMP was administered orally on days 1, 3, 5, and 7, and 50 mg per chicken (mean total dose of 24.1 mg/kg) of TMP was administered orally on days 2, 4, and 6 during this multiple dose experiment. The arrows represent the days of drug administration. The Clinical and Laboratory Standards Institute breakpoint published for Enterobacteriaceae for trimethoprim is 2 µg/mL, and the limit of quantification (LOQ) is 0.2 µg/mL.
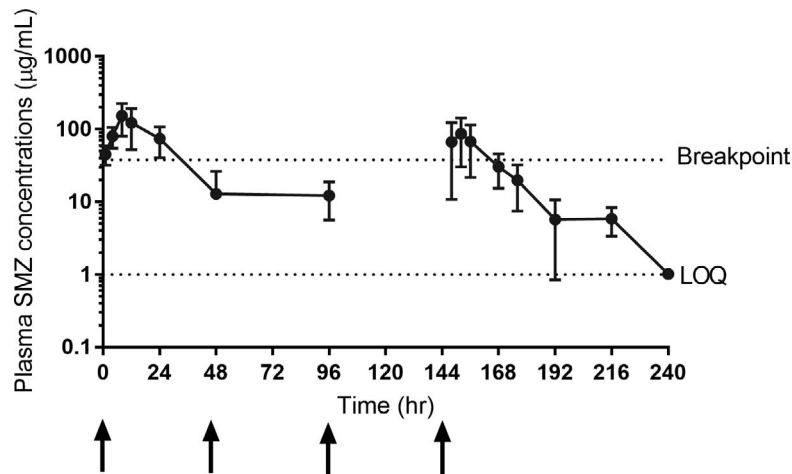
Figure 4, Mean ± SD concentration-time curve of sulfamethoxazole (SMZ) in plasma from 6 Rhode Island red hens (*Gallus gallus domesticus*) after repeated oral administration of 80 mg/kg SMZ every 48 hours for 7 days. The arrows represent the days of drug administration. The Clinical and Laboratory Standards Institute breakpoint published for Enterobacteriaceae for SMZ is 38 μg/mL, and the limit of quantification is 1 μg/mL.

dosing regimen for the 7-day multiple dose trial. We were constrained by TMP only being commercially available in 100-mg and 200-mg tablets, so the supplemental TMP dose was given per bird versus on a mg/kg basis. The actual TMP dose, based on the bird's body weight, is reported in the "Methods" section. It might be possible to compound TMP tablets into an oral solution for more precise mg/kg dosing; however, we chose the tablet method to simplify dosing for bird owners and due to legal concerns with administration of compounded medications to major food-producing animals in the United States.

No evidence of toxicity was noted clinically or on plasma biochemical parameters for any bird in the multiple-dose trial. The pharmacokinetics of a single dose of SMZ and TMP orally or intravenously have been previously evaluated in adult Warren hens;[17] however, the formulations admin-

Table 2. Mean ± SD for pharmacokinetic parameters in plasma samples from 5 Rhode Island red hens (*Gallus gallus domesticus*) after repeated oral administration of trimethoprim (TMP). A mean total dose of 28 mg/kg of TMP was administered orally on days 1, 3, 5, and 7, and 50 mg per chicken (mean total dose of 24.1 mg/kg) of TMP was administered orally on days 2, 4, and 6 during this multiple-dose experiment.

Parameters	Units	Values
λz	1/h	0.061 ± 0.03
$HL_{\lambda z}$	hours	14.3 ± 9.1
T_{max}	hours	149 ± 3.0
C_{max}	μg/mL	4.3 ± 2.3
AUC_{last}	h × μg/mL	97.1 ± 84.5
$AUC_{infinity}$	h × μg/mL	111.3 ± 91.7
Vz_F	L/kg	6.6 ± 2.6
CL_F	L/h per kilogram	0.41 ± 0.26
Extrapolated AUC	%	13.0 ± 7.3
$MRT_{infinity}$	hours	24.7 ± 13.3

Abbreviations: λz, elimination rate constant; $HL_{\lambda z}$, elimination half-life; T_{max}, time to the maximum concentration; C_{max}, maximum concentration; AUC_{last}, area under the curve from time 0 to the last time point; $AUC_{infinity}$, area under the curve from time 0 to infinity; Vz_F, volume of distribution; CL_F, total clearance; and $MRT_{infinity}$, mean residence time from time 0 to infinity.

Table 3. Mean ± SD for pharmacokinetic parameters in plasma samples from 5 Rhode Island red hens (*Gallus gallus domesticus*) after repeated oral administration of 80 mg/kg sulfamethoxazole every 48 hours for 7 days.

Parameters	Units	Values
λz	1/h	0.07 ± 0.03
$HL_{\lambda z}$	hours	11.6 ± 5.3
T_{max}	hours	151.2 ± 1.8
C_{max}	μg/mL	100.8 ± 68.6
AUC_{last}	h × μg/mL	1943.6 ± 1302.7
$AUC_{infinity}$	h × μg/mL	2013.3 ± 1254.6
Vz_F	L/kg	1 ± 0.9
CL_F	L/h per kilogram	0.06 ± 0.05
Extrapolated AUC	%	6.7 ± 12
$MRT_{infinity}$	hours	19.8 ± 5.4

Abbreviations: λz, elimination rate constant; $HL_{\lambda z}$, elimination half-life; T_{max}, time to the maximum concentration; C_{max}, maximum concentration; AUC_{last}, area under the curve from time 0 to the last time point; $AUC_{infinity}$, area under the curve from time 0 to infinity; Vz_F, volume of distribution; CL_F, total clearance; and $MRT_{infinity}$, mean residence time from time 0 to infinity.

istered were different from the current study because they were compounded from tablets for both intravenous and oral administration and represented different ratios of SMZ : TMP than the current commercially available injectable and liquid formulations. Additionally, compounded drugs are not allowed by the FDA in major food-producing animals, such as chickens, in the United States.

The average absolute bioavailability of TMP and SMZ were 82% and 60.5%, respectively, in the current study. Queralt et al[17] reported a bioavailability of 36% and 46% for TMP and SMZ, respectively, in hens. Since the Queralt study utilized a compounded product, this discrepancy may be due to the differences in formulation between that and the current study. Löscher et al[23] found a bioavailability of 100% for sulfadiazine and 60% for TMP in broiler chickens that received the drugs suspended in a 1% methylcellulose and water compounded solution. Baert et al[24] reported a bioavailability of 80% for sulfadiazine (33.34 mg/kg) and 79% for TMP (6.67 mg/kg) administered via oral bolus using a crop tube in nonfasted broiler chickens. Although multiple factors, including route of administration, dosage, population or breed, and fasting status, can influence the pharmacokinetics of a drug (eg, absorption of the drug and estimation of the bioavailability), our data were comparable to previous reports. The elimination half-life after intravenous administration was 3.9 hours for TMP and 3.6 hours for SMZ in the current study. Queralt et al[17] reported an elimination half-life of 2.4 hours for TMP and 8.25 hours for SMZ after intravenous injection of a compounded SMZ and TMP solution (16 mg/kg TMP and 64 mg/kg SMZ).

The volume of distribution after a single dose administered intravenously indicated that the tissue distribution of TMP (1.9 ± 0.6 L/kg) was more extensive than that of SMZ (0.2 ± 0.04 L/kg). The volume of distribution for TMP was similar to a previous study in this species.[23] The absorption of a single dose of SMZ administered orally was slightly slower but more variable (T_{max} 5.3 ± 4.3 hours) compared with TMP (T_{max} 3.6 ± 0.8 hours). Sulfonamides have variable and species-dependent plasma protein binding, which can significantly impact the plasma half-life of these drugs.[7] Protein binding was not measured for either drug, as it was outside the scope of the current study. The clearance of TMP administered intravenously (0.34 ± 0.05 L/hr per kilogram) was almost 10 times that of SMZ (0.04 ± 0.01 L/hr per kilogram), and this may explain the extended

elimination phase of SMZ in this study. Additionally, the volume of distribution of TMP was almost 10 times that of SMZ, which accounts for the similar half-lives of both drugs. This difference in drug clearance of TMP compared to SMZ is also found in dogs.[7]

The average concentration over time of a single dose of SMZ administered orally at 80 mg/kg exceeded the breakpoint of Enterobacteriaceae (38 µg/mL) for up to 12 hours after dosing (Fig 2), which suggested good gastrointestinal absorption. In contrast, the average concentration of TMP administered orally as a single dose of 16 mg/kg did not exceed the published MIC breakpoint of Enterobacteriaceae (2 µg/mL) for any clinically significant time (Fig 1). The limited absorption of TMP (pKa = 7.12, logP [logarithm of the partition coefficient] = 0.9) from the gastrointestinal membrane, combined with a low lipophilicity and permeability of intestinal membranes for this drug, has been hypothesized as a cause for reduced gastrointestinal absorption and short elimination half-life in chickens.[25–28] Goren et al[25] reported only small differences in plasma concentrations in poultry administered TMP at doses of 42, 60, and 120 mg/kg via drinking water; therefore, dramatic increases in oral doses of TMP may also not yield significant increased plasma concentrations in this species.

In the multiple-dose experiment, the plasma concentrations of TMP exceeded the MIC breakpoint of Enterobacteriaceae (2 µg/mL) for approximately 70 hours after the first dose and up to 10 hours on the seventh day after administration of a mean total dose of 28 mg/kg PO on days 1, 3, 5, and 7 and mean total dose of 24.1 mg/kg of TMP PO on days 2, 4, and 6 (Fig 3). This confirms that supplemental TMP would likely be required in addition to the liquid formulation (ie, >16 mg/kg of TMP PO) to exceed the published MIC breakpoint of Enterobacteriaceae. Plasma concentration of SMZ exceeded the breakpoint of Enterobacteriaceae (38 µg/mL) for approximately 30 hours after the first dose and approximately 15 hours after the seventh dose after administration of SMZ 80 mg/kg PO every 48 hours (Fig 4). No significant increase in plasma concentrations of TMP or SMZ were noted on the seventh day compared with the first dosing day, suggesting that no significant accumulation occurred during that time frame. Since the 48-hour dosing interval was >3 times the half-life (11.6 ± 5.3 hours) for SMZ, the drug was eliminated from the body before the next dosing, and accumulation of SMZ did not occur.[28] Repeated dosing of TMP did not result in

accumulation after 7 days, which may be related to limited TMP absorption, limited rate of drug dissolution, limited tissue perfusion, and an increase in clearance. Trimethoprim is primarily eliminated via the kidneys and is largely unchanged in humans;[29] however, the primary route of excretion and hepatic metabolism of this drug in chickens is unknown.

Toxicity of sulfonamides in poultry has been previous reported, and includes bone marrow suppression and decreased egg production.[30] No significant changes to the plasma biochemical panels or PCV were noted following the administration of multiple doses of SMZ-TMP for 7 days in this population of hens compared with control birds. The only clinically adverse effect noted during both the single- and multiple-dose trials was a transient enlarged, fluid-filled crop in several birds. As this was only noted in the treatment birds, the authors hypothesize this could have been from transient crop stasis secondary to SMZ-TMP administration, but ultimately this was deemed clinically insignificant and resolved without additional intervention. The undiluted SMZ-TMP intravenous solution has a pH of 10, and is recommended to be administered at a rate of approximately 2 mL/min IV in humans. Precautions were taken in this study to prevent extravasation of this drug during intravenous administration, and no evidence of phlebitis or tissue necrosis was noted at the catheter placement sites in those hens. Transient lacrimation and ptyalism were noted during intravenous administration, despite slow administration and dilution of the drug. These signs resolved without additional intervention.

In addition to appropriate dosing for antimicrobial efficacy, egg residues and drug depletion are also important considerations when treating egg-laying hens with any antibiotic due to the risks to human health. Drug residues in food, including eggs, can cause toxicity or immune reactions, and can potentially contribute to antibiotic resistance.[4] There are no FDA-approved tolerances for any sulfonamide in poultry in the United States, which effectively makes the acceptable level zero for eggs and other tissues. The egg residues and drug depletion of SMZ-TMP was recently evaluated in a flock of 14 3-year-old Rhode Island red hens.[27] Residues for both drugs in albumen and yolk were analyzed via UPLC-MS. Based on those results, the recommended egg withdrawal interval using the FDA tolerance method was 43 days for SMZ and 17 days for TMP.

There are several limitations to the current study. Additional animals for each of the trials performed could have helped to offset the individual variability noted. The analytical assays for TMP-SMZ were not sensitive enough to quantify all samples collected in this study, which affected the ability to estimate $AUC_{infinity}$, bioavailability, and to identify the terminal slope for some pharmacokinetic analyses. This could lead to greater variability of pharmacokinetic parameters including the elimination rate constant, elimination half-life, volume of distribution, and clearance. The 7-day duration of the multiple-dose trial may not have been long enough to detect drug toxicity, and a transient leukopenia could have gone unnoticed as complete blood counts were not performed. Healthy animals were used in this study and adverse effects may be more common in ill chickens.

The clinical efficacy of SMZ-TMP was not evaluated in this study. Instead, the effectiveness of the drug was evaluated based on the CLSI MIC breakpoints for Enterobacteriaceae, the most common type of bacterial infection reported in backyard poultry.[21] However, individual bacterial strains may have MIC values that are lower than the breakpoint and there are several non-Enterobacteriaceae bacteria with lower breakpoints for SMZ-TMP, such as *Streptococcus* species and *Haemophilus influenza*. This, and potentially the specific site of infection, could explain apparent clinical success following administration of lower doses such as 30–50 mg/kg SMZ-TMP (a range of 25 mg/kg SMZ and 5 mg/kg TMP to 40 mg/kg SMZ and 10 mg/kg TMP) in poultry, and emphasizes that antibiotic dosing should ideally be based on a confirmed etiologic agent and site of infection to help ensure successful treatment.[31]

This study evaluated single and multiple oral doses of SMZ-TMP in Rhode Island red hens, a common breed kept as pets and for egg production in small backyard flocks. A multiple-dose regimen was developed by combining the commercially available TMP-SMZ oral solution with TMP tablets. This regimen maintained plasma concentrations at or above the CLSI breakpoints for TMP for Enterobacteriaceae for half of the whole entire study period, with no adverse effects detected after 7 days of treatment. Significant advantages of this regimen are once-daily dosing and the commercial availability of generic drugs used in the study. Lower doses may be efficacious for bacterial infections with lower MIC values. Additional studies are needed to determine the effects of this regimen in unhealthy birds and to

determine if a lower drug dosage can achieve similar plasma concentrations.

REFERENCES

1. Brinkley C, Kingsley JS, Mench J. A method for guarding animal welfare and public health: tracking the rise of backyard poultry ordinances. *J Community Health*. 2018;43(4):639–646.

2. American Veterinary Medical Association. *AVMA Pet Ownership and Demographics Sourcebook*. American Veterinary Medical Association; 2018.

3. Elkhoraibi C, Blatchford RA, Pitesky ME, et al. Backyard chickens in the United States: a survey of flock owners. *Poult Sci*. 2014;93(11):2920–2931.

4. Goetting V, Lee KA, Tell LA. Pharmacokinetics of veterinary drugs in laying hens and residues in eggs: a review of the literature. *J Vet Pharmacol Ther*. 2011;34(6):521–556.

5. Marmulak T, Tell LA, Gehring R, et al. Egg residue considerations during the treatment of backyard poultry. *J Am Vet Med Assoc*. 2015;247(12):1388–1395.

6. Bushby SRM. Sulfonamide and trimethoprim combinations. *J Am Vet Med Assoc*. 1980;176(10):1049–1053.

7. Prescott JF. Sulfonamides, diaminopyrimidines, and their combinations In: Giguere S, Prescott JF, Dowling PM, eds. *Antimicrobial Therapy in Veterinary Medicine*. 5th ed. Ames, IA: Wiley Blackwell; 2013:279–294.

8. Brown GR. Cotrimoxazole-optimal dosing in the critically ill. *Ann Intensive Care*. 2014;4(1):1–9.

9. Chakwenya J, Lakritz J, Tyler J, et al. Pharmacokinetics and bioavailability of trimethoprim-sulfamethoxazole in alpacas. *J Vet Pharmacol Ther*. 2002;25(5):321–327.

10. Peck KE, Matthews NS, Taylor TS, et al. Pharmacokinetics of sulfamethoxazole and trimethoprim in donkeys, mules, and horses. *Am J Vet Res*. 2002;63(3):349–353.

11. Duijkeren EV, Vulto A, Oldruitenborgh-Oosterbaan MSV, et al. A comparative study of the pharmacokinetics of intravenous and oral trimethoprim/sulfadiazine formulations in the horse. *J Vet Pharmacol Ther*. 1994;17(6):440–446.

12. Page CD, Mautino M, Derendorf HD, et al. Comparative pharmacokinetics of trimethoprim-sulfamethoxazole administered intravenously and orally to captive elephants. *J Zoo Anim Med*. 1991;22(4):409–416.

13. Mengelers MJ, van Gogh ER, Huveneers MB, et al. Pharmacokinetics of sulfadimethoxine and sulfamethoxazole in combination with trimethoprim after oral single-and multiple-dose administration to healthy pigs. *Vet Res Commun*. 2001;25(6):461–481.

14. Ma R, Wang Y, Zou X, et al. Pharmacokinetics of sulfamethoxazole and trimethoprim in Pacific white shrimp, *Litopenaeus vannamei*, after oral administration of single-dose and multiple-dose. *Environ Toxicol Pharmacol*. 2017;52:90–98.

15. Fu G, Peng J, Wang Y, et al. Pharmacokinetics and pharmacodynamics of sulfamethoxazole and trimethoprim in swimming crabs (*Portunus trituberculatus*) and in vitro antibacterial activity against Vibrio: PK/PD of SMZ-TMP in crabs and antibacterial activity against Vibrio. *Environ Toxicol Pharmacol*. 2016;46:45–54.

16. Dorrestein GM. *Studies on Pharmacokinetics of Some Antibacterial Agents in Homing Pigeons (Columba livia)* [dissertation]. Utrecht, Netherlands: Utrecht University; 1986. 17. Queralt J, Castells I. Pharmacokinetics of sulfamethoxazole and trimethoprim association in hens. *Poult Sci* 1985;64(12):2362–2367.

17. Hawkins M, Guzman DS, Beaufrere H, et al. Birds. In: Carpenter JW, ed. *Exotic Animal Formulary*. 5th ed. St Louis, MO: Elsevier; 2018:167–375.

18. Greenacre C, Luna G, Morishita T. Backyard poutry and waterfowl. In: Carpenter JW, ed. *Exotic Animal Formulary*. 5th ed. St Louis, MO: Elsevier; 2018:376–431.

19. Barnes HJ. Colibacillosis. In: Saif Y, ed. *Diseases of Poultry*. 12th ed. Ames, IA: Blackwell; 2008:691–732.

20. Cadmus KJ, Mete A, Harris M, et al. Causes of mortality in backyard poultry in eight states in the United States. *J Vet Diagn Invest*. 2019;31(3):318–326.

21. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals. CLSI Guideline VET01S*. 5th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.

22. Löscher W, Fabbender CP, Weissing M, et al. Drug plasma levels following administration of trimethoprim and sulphonamide combinations to broilers. *J Vet Pharmacol Ther*. 1990;13(3):309–319.

23. Baert K, De Baere S, Croubels S, et al. Pharmacokinetics and oral bioavailability of sulfadiazine and trimethoprim in broiler chickens. *Vet Res Commun*. 2003;27(4):301–309.

24. Goren E, De Jong W, Doornenbal P. Some pharmacokinetic aspects of four sulphonamides and trimethoprim, and their therapeutic efficacy in experimental *Escherichia coli* infection in poultry. *Vet Q*. 1984;6(3):134–140.

25. White G, Williams RB. Evaluation of a mixture of trimethoprim and sulphaquinoxaline for the treatment of bacterial and coccidial diseases of poultry. *Vet Rec*. 1983;113(26–27):608–612.

26. Enomoto H, Petritz OA, Thomson AE, et al. Egg residue and depletion in Rhode Island red hens (*Gallus gallus domesticus*) following multiple oral doses of trimethoprim-sulfamethoxazole. *Regul Toxicol Pharmacol*. 2021;123:104941.

27. Derendorf H, Schmidt S. *Rowland and Tozer's Clinical Pharmacokinetics and Pharmacodynamics: Concepts and Applications*. 5th ed. Philidelphia, PA: Lippincott Williams & Wilkins; 2019.

28. Grose WE, Bodey GP, Loo TL. Clinical pharmacology of intravenously administered trimethoprim-sulfamethoxazole. *Antimicrob Agents Chemother*. 1979;15(3):447–451.

29. Hofacre CL, Fricke JA, Inglis T. Antimicrobial drug use in poultry In: Giguere S, Prescott JF, Dowling PM, eds. *Antimicrobial Therapy in Veterinary Medicine*. 5th ed. Ames, IA: Wiley Blackwell; 2013;569–587.

30. Onufrak NJ, Forrest A, Gonzalez D. Pharmacokinetic and pharmacodynamic principles of anti-infective dosing. *Clin Ther*. 2016;38(9):1930–1947.