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Research Note

Concentrations of Antibiotic Residues Vary between Different Edible Muscle Tissues in Poultry

IXCHEL REYES-HERRERA,[1] MARILYN J. SCHNEIDER,[2] KIMBERLY COLE,[1] MORGAN B. FARNELL,[3] PAMELA J. BLORE,[1] AND DAN J. DONOGHUE[1]*

[1]*Department of Poultry Science, University of Arkansas, Fayetteville, Arkansas 72701, USA;*
[2]*U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, Pennsylvania 19038; and*
[3]*U.S. Department of Agriculture, Agricultural Research Service, Poultry Production and Product Safety Research Unit, Fayetteville, Arkansas 72701*

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ABSTRACT

Antibiotics are used by veterinarians and producers to treat disease and improve animal production. The federal government, to ensure the safety of the food supply, establishes antibiotic residue tolerances in edible animal tissues and determines the target tissues (e.g., muscle) for residue monitoring. However, when muscle is selected as the target tissue, the federal government does not specify which type of muscle tissue is used for monitoring (e.g., breast versus thigh). If specific muscle tissues incorporate residues at higher concentrations, these tissues should be selected for residue monitoring. To evaluate this possibility in poultry, chickens were divided into four groups and at 33 days of age were dosed with enrofloxacin (Baytril), as per label directions, at either 25 ppm for 3 days, 25 ppm for 7 days, 50 ppm for 3 days, or 50 ppm for 7 days. Breast and thigh muscle tissues were collected from each bird ($n = 5$ birds per day per group) during the dosing and withdrawal period, and fluoroquinolone concentrations were determined. The results indicate higher overall enrofloxacin concentrations in breast versus thigh muscle for each treatment group ($P < 0.05$). These data indicate, at least for enrofloxacin, that not all muscle tissues incorporate antibiotics at the same concentrations. These results may be helpful to regulatory agencies as they determine what tissues are to be monitored to ensure that the established residue safety tolerance levels are not exceeded.

Antibiotics are used by veterinarians and poultry producers to treat disease and enhance production in poultry *(2)*. Prior to their approval for use, the U.S. Food and Drug Administration (FDA) requires extensive toxicological and pharmacokinetics testing to ensure that antibiotic residues in edible tissues do not pose harm to consumers *(6, 9)*. As part of this approval process, the FDA establishes the safe concentration of residues in edible tissues (tolerance) and the type of tissue (e.g., muscle tissue) that should be monitored for residues *(1, 7)*. This information is published in the Code of Federal Regulations (CFR *(3)*). Depending on the type of poultry tissue, either the FDA or the U.S. Department of Agriculture (USDA) monitors edible poultry products to ensure tolerances are not exceeded *(1, 4, 6, 8)*. If violative residues are identified, corrective regulatory action is taken.

To ensure consumer confidence in our food supply, the FDA or USDA has historically updated their pre- and post-drug approval and monitoring procedures to provide the most accurate assessment of the safety of foods *(1, 7)*. Recently, Schneider and Donoghue *(12)* observed apparently higher concentrations of enrofloxacin residues in breast versus thigh muscle tissues in pooled samples collected from treated birds. The FDA, to our knowledge, does not delineate what type of edible muscle tissues (e.g., breast or thigh) should be tested for residue determination *(3)*. It may be that specific edible muscle tissues would be a better indicator of the highest residue concentrations that may exceed established safety tolerances. Although Schneider and Donoghue *(12)* observed differences between breast and thigh tissues, the purpose of their study was to compare analytical methods; it was not designed to address tissue residue differences. Therefore, the purpose of the present study is to determine whether breast or thigh tissues incorporate residues at higher concentrations in poultry dosed with the fluoroquinolone antibiotic, enrofloxacin.

MATERIAL AND METHODS

A total of 160 day-old male meat-type chickens (broilers) were obtained from a local hatchery and were randomly assigned to four separate pens. During this study, birds had ad libitum access to a standard nonmedicated broiler diet (starter and grower) prepared at the university poultry feed mill and nonmedicated (predosing period) or medicated water (dosing period). At 33 days of age, each pen received one of four enrofloxacin (Baytril) treatments. Enrofloxacin was dosed in the water according to the FDA-approved label directions at either 25 ppm for 3 days ($n = 30$ birds), 25 ppm for 7 days ($n = 50$ birds), 50 ppm for 3 days ($n = 30$ birds), or 50 ppm for 7 days ($n = 50$ birds). Medicated water was prepared daily.

Birds were collected for tissue analysis immediately before dosing (controls, $n = 5$ birds in each of 4 pens), daily after dosing

* Author for correspondence. Tel: 479-575-2913; Fax: 476-575-7139; E-mail: ddonogh@uark.edu.

for the 3- or 7-day dosing groups, and the first 2 days after drug withdrawal ($n = 5$ birds per day per dosing group). Individual breast and thigh samples collected from each bird were prepared and analyzed for fluoroquinolone residues according to the method of Schneider and Donoghue *(12).* In brief, after collection, each sample was kept on ice and homogenized fresh daily (commercial food processor) and diluted 1:3 (wt/vol) with 1% phosphate buffer, pH 9.0, and centrifuged at 1,500 $\times$ *g* for 15 min at 5°C. The supernatant was decanted and stored at −80°C until assayed by an agar diffusion microbiological method *(12).* On the day of the assay, petri dishes (100 mm in diameter) were filled with 8 ml of Mueller-Hinton agar (Difco, Becton Dickinson, Sparks, Md.) inoculated with approximately 1.0×10^6 CFU/ml of *Klebsiella pneumoniae* (ATCC 10031) as the indicator organism, and then six penicylinders (8 by 10 mm) were evenly placed on the agar. A standard curve was constructed by addition of the known amount of enrofloxacin in buffer, with each standard concentration pipetted onto three plates; three alternate cylinders were filled with standard concentration (200 μl each), and the other three cylinders were filled with a reference concentration (200 μl each). The reference concentration on each plate corrects for any potential plate-to-plate variation (internal plate standard). The reference concentration is a standard with enrofloxacin concentrations in the mid range of the standard curve. Individual breast or thigh samples were assayed in a similar manner to standards (200 μl per cylinder), except samples were assayed on only one plate. Plates were incubated at 37°C for approximately 16 h. Plate averages for the standards and breast or thigh samples were corrected to the overall reference concentrations. The overall reference concentration is determined by averaging the reference concentrations from all the standard curve plates. A best-fit regression line, with the diameter of the inhibition zones (millimeters) measured by a zone reader (Fisher-Lilly, Pittsburgh, Pa.), was calculated by the method of least squares. To evaluate the ratio of enrofloxacin to ciprofloxacin in the breast or thigh samples, samples from day 3 or 7 were evaluated from the 3-day, 25-ppm or 7-day, 50-ppm treatment groups ($n = 5$ birds per day per dosing group), respectively, by the liquid chromatography–fluorescence–mass spectrometry method as described by Schneider and Donoghue *(12).*

Statistical analysis was accomplished by analysis of variance using the Statistical Analysis System *(11)* general linear models program. Treatment means were partitioned by least-squares means analysis *(11).* A probability of $P < 0.05$ was required for statistical significance.

RESULTS

During the dosing period, the number of days chickens were dosed did not affect differences between breast and thigh fluoroquinolone concentrations ($P > 0.05$); therefore, the data were combined and are presented in Figure 1. All samples had fluoroquinolone concentrations below the 300 ppb tolerance by the end of the first day of drug withdrawal. These withdrawal period concentrations were 154.2 ± 40, 217.0 ± 34, 58.8 ± 8, and 118.1 ± 12 ppb for the 25- and 50-ppm 3-day dosing period and the 25- and 50-ppm 7-day dosing period breast samples, respectively (mean ± SEM) ($n = 20$ birds). The thigh samples collected from the same birds during the corresponding withdrawal period contained fluoroquinolone amounts of 120.7 ± 16, 169.2 ± 22, 34.5 ± 4, and 49.7 ± 5 ppb ($n = 20$ birds). When samples were analyzed by liquid chromatography–fluorescence–mass spectrometry, the ratio of enrofloxacin to the enrofloxacin metabolite, ciprofloxacin, in the breast or thigh samples was 97:3 or 98:2 (enrofloxacin:ciprofloxacin) from either the 3-day, 25-ppm group or the 7-day, 50-ppm group, respectively.

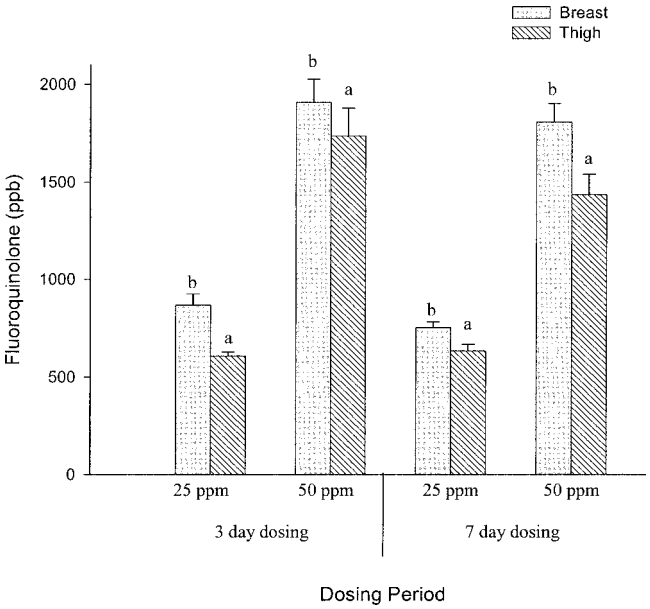

FIGURE 1. *Fluoroquinolone antibiotic residues (ppb) in breast or thigh muscle from chickens dosed with either 25 or 50 ppm enrofloxacin in the water for 3 or 7 days (mean ± SEM). Breast and thigh samples were collected at the end of each day of dosing (*n *= 5 birds per day per dosing group). During the dosing period, the number of days chickens were dosed did not affect differences between breast versus thigh (*P *> 0.05) and, therefore, the data were combined (*n *= 100 birds). Means with different superscripts indicate statistical differences (*P *< 0.05).*

DISCUSSION

Enrofloxacin (Baytril) is a fluoroquinolone class of antibiotics that is presently approved by the FDA for therapeutic use in poultry *(3).* Before marketing enrofloxacin-treated poultry, producers are required to observe a 2-day withdrawal period. The FDA has established muscle as the target tissue for residue monitoring in chickens and turkeys and set the tolerance at 300 ppb *(3).* Results from our study indicate that fluoroquinolone residues exceeded the tolerance of 300 ppb in all treated groups during the dosing period (Fig. 1) but were below the tolerance by the end of the first day of drug withdrawal. Therefore, observing the FDA mandatory 2-day withdrawal period would protect consumers from exposure to violative enrofloxacin residues from treated birds.

Unfortunately, it is possible that misuse of some antibiotics, including enrofloxacin, could result in food products containing residues exceeding established safety tolerances. Examples of misuse could include the intentional, illegal shipment of animals to market without observing a required withdrawal period or the simple misreading of the product label and dosing with too much antibiotic or not understanding the requirements for a withdrawal period. To determine the extent of violative antibiotic residues in the edible animal tissues, the FDA or USDA tests specific tar-

get tissues for residues, depending on the antibiotic and animal species, as specified in the CFR *(3).*

To detect any potential violative enrofloxacin residues, our results suggest that breast tissue may be preferable to thigh tissue for determining the highest tissue residue concentrations (Fig. 1). When we used both the lowest and highest FDA-approved enrofloxacin doses (25 ppm for 3 days or 50 ppm for 7 days) and two intermediate doses (50 ppm for 3 days or 25 ppm for 7 days), breast tissues had consistently higher incurred fluoroquinolone concentrations than thigh tissues during the dosing period (Fig. 1). Thus, this effect appears to be dose independent for the treatments tested.

The published information on residue deposition between muscle tissues in poultry is limited. Parks and Doerr *(10)* reported differences in the percentage recovery of zoalene between the breast and thigh muscle. However, that study evaluated differences in only three chickens. De Vos and coworkers *(5)* evaluated polychlorinated biphenyl residues in poultry and reported higher concentrations in thigh versus breast tissues. These authors suggest the higher concentrations detected in thigh tissue is due to the lipophilic nature of polychlorinated biphenyls and the greater intramuscular fat content of thighs determined during that study (fat in thigh tissue ranged from 3.1 to 11.9% and in breast from 0.8 to 1.5%). Although these two studies support our results, additional residue studies should be conducted to determine the prevalence of this effect.

Even though the present study found greater residue concentrations in breast versus thigh muscle tissues, the possibility also exists that other edible muscle tissues (e.g., legs or wings) could have even higher levels. Furthermore, the site of preferential deposition could vary between different antibiotics. In other words, although fluoroquinolone residue concentrations were higher in breast versus thigh tissues, another antibiotic may produce higher concentrations in thigh muscle. Therefore, it may be of interest to determine which edible tissues contain the highest residue content for each antibiotic when muscle is the target tissue. This information could then be helpful in selection of the appropriate tissues for postapproval monitoring to identify any potential residue problem so regulatory action could be taken to protect the safety of the food supply.

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