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The Effects of Sulfadimethoxine Administered to Control *Campylobacter jejuni* in Small-Scale Broiler Operations

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Abstract

Campylobacter jejuni causes human foodborne gastroenteritis known as campylobacteriosis. Antimicrobial therapy could be a potentially important tool in reducing the prevalence of *C. jejuni* in poultry. The purpose of this study was to determine the effects of sulfadimethoxine antibiotic on the prevalence of *C. jejuni* in growing broilers. Day-old broilers (n= 600) were allotted to two treatments 1) control (drinking water only) and 2) antibiotic (drinking water + 0.05% (wt/vol) sulfadimethoxine) with two replications. Each week, fecal samples were collected from individual chickens (n=300). All samples were plated on modified charcoal-cefoperazone-deoxycholate agar (mCCDA) to determine the log CFU/g and prevalence (%) of *Campylobacter* spp. Isolation of *C. jejuni* was verified with latex agglutination and hippurate hydrolysis test. Over the six week period, the bacterial counts of *Campylobacter* spp. in the antibiotic treatment (5.12 log CFU/broiler) were significantly lower ($P<0.05$) than in the control treatment (6.05 log CFU/broiler). Additionally, the prevalence of *C. jejuni* in the antibiotic treatment (50.0%) was significantly lower ($P<0.05$) than in the control treatment (56.0%). Our findings suggest that the antibiotic sulfadimethoxine may aid in reducing *Campylobacter* spp. and the prevalence on both *Campylobacter* spp. and *C. jejuni* in growing broilers.

Keywords: *Campylobacter jejuni*; Sulfadimethoxine; broilers; Small-scale operation; Bacterial cross contaminant

Introduction

Campylobacter jejuni is a pathogenic bacterium that causes human foodborne gastroenteritis [1]. In the United States, *Campylobacter* is responsible for an estimated 2.1-2.4 million cases of foodborne illnesses each year [2-4] resulting in 13,000 hospitalizations, 100 deaths and an estimated cost of over $1 billion annually [3,5]. FoodNet [6] reported that the number of infections and incidence of *Campylobacter* per 100,000 persons were 6,621 and 13.82 respectively in 2013. Of these infected persons, 1,010 (15%) were hospitalized and 12 (0.2%) died from contaminated food [6].

Approximately 70% of human illnesses due to *Campylobacter* are caused by the consumption or handling of raw or undercooked poultry [2,7]. Additionally, *Campylobacter* can be transmitted via contact with infected animals or their feces. Many animals carry *Campylobacter* asymptomatically and shed the bacterium in their feces. Poultry, particularly broiler chickens, also frequently harbor the bacterium. Because of the threat to public health, serious efforts are being made to prevent the colonization and spread of *C. jejuni* in poultry production [8-10]. A reduction in numbers of *Campylobacter* in poultry, production can lead to a corresponding reduction in human infections. Quantitative risk assessment models have indicated that a reduction of 2 log units on a broiler carcass could result in 30 times less prevalence of campylobacteriosis [11]. Therefore, reduction or elimination of *C. jejuni* in the poultry reservoir is an essential consideration in the control of this food safety problem.

Although there are multiple levels at which *Campylobacter* contamination can be targeted, on-farm control of *Campylobacter* has the greatest impact because the living poultry intestine is the primary amplification point for *Campylobacter* throughout the food chain [12,13]. Therefore, the use of various antimicrobial therapies to control *Campylobacter* infection in poultry production is worthy of exploration.

Antimicrobial therapy is a potentially important tool in reducing the prevalence and enumeration of *C. jejuni* in poultry. Studies have addressed the use and efficacy of antibiotics on an array of poultry infections including *C. jejuni* producing varied results. An in vivo study [14] reported that a three-phage lytic cocktail administered to chickens resulted in a 2 log CFU/g reduction in *C. jejuni.* In another in *vivo* study using turkeys [15], the administration of enrofloxacin, neomycin and vancomycin resulted in a respective decrease of 1, 2 and 4 log CFU/g in *C. jejuni*. In a recent study [16] the live bacterium *Enterococcus faecalis* was administered to inhibit *C. jejuni* in chickens but was proved ineffective in preventing growth. Thus, more studies are needed to explore possible alternative antibiotics that can reduce the population of *C. jejuni* in poultry production.

Sulfonamides were first used to treat upper respiratory [17] and coccidial infections caused by *Eimeria tenella* and *Eimeria necatrix* in poultry [18,19]. The commonly used sulfonamide in poultry production is sulfadimethoxine and therefore is appropriate for in vivo testing [20]. Sulfadimethoxine has been used alone or in combination with other antibiotics and coccidiostats to improve weight gain and final body weight [21].

Sulfadimethoxine is also commonly used as an antimicrobial for the treatment and/or prevention of coccidiosis, fowl cholera, and coryza in poultry [22,23]. The previous study [24] supported that the use of sulfadimethoxine can reduce the load of *C. jejuni* in turkey.

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To date, there have been no in *vivo* studies on the efficacy of sulfadimethoxine in the control of *C. jejuni* in broilers in small-scale poultry operations. Although largely unknown, the positive potential effects of sulfadimethoxine antibiotic treatment to control *C. jejuni* infection in chickens should be explored.

The purpose of this study is to determine the effects of sulfadimethoxine antibiotic on the enumeration of *Campylobacter* spp. and the prevalence on both *Campylobacter* spp. And *C. jejuni* in growing broilers.

Materials and Methods

Broiler production

The McNeese State University Animal Care and Use Committee approved the methods related to animal care that were used in this experiment.

Two treatments with two replications each using 300 broiler chickens (Ross × Ross) obtained from a commercial hatchery were used. These experiments were conducted from January 2014 to May 2014. Replication I was initiated on January, 2014 and replication II on March, 2014. Birds were housed in a controlled environment and maintained in Petersime®Battery Cages (32°C) with raised wire flooring (Petersime Incubator Co., Gettysburg, OH). Each cage was divided into 12 pens of equal size (74.7 cm × 99.1 cm × 24.13 cm). Each pen housed twenty-five birds. Individual open water and feed troughs were provided for each pen. Individual water and feed troughs were provided for each pen and feed was supplied ad libitum. Feed was procured from the Texas Farm Products Company. This feed contains 18% protein chick grower crumbles and no antibiotics.

The housing system was emptied of birds, feed, and litter and cleaned with hot water wash and disinfect. Animal care givers monitored feed and water and removed litter trays daily. Normal pest and rodent control was maintained throughout the experiment. The temperature and % RH during time period was 32°C and 58%, respectively.

Birds were allotted to one of two treatments: 1) control (drinking water) and 2) drinking water + 0.05% (wt/vol) sulfadimethoxine (Durvet Inc., Blue Springs, MO). Drinking water was refreshed every day in both treatment groups. Each week, 150 individual broilers were randomly tested via cloacal sterile rayon tipped swabs and assayed for the presence of *Campylobacter* spp. [25] and *C. jejuni* [26]. The swabs were placed in a tube containing 3 ml of sterile tryptone soy broth (TSB) for further analysis.

Bacterial isolation and identification

Immediately upon arrival in the laboratory, the swab samples were whirl-mixed in a shaker incubator (Excella E24/E24R Temperature-Controlled Benchtop Shaker, New Brunswick Scientific, Edison, NJ) for approximately 1 h at 37°C and then mixed with a vortexer for 2 min to release the bacteria. Each 0.1 ml of swab sample was aseptically transferred and directly spread onto modified charcoal cefoperazone deoxycholate agar (mCDDA). The inoculated plates were then incubated at 42°C for 48 h in a microaerophilic environment (5% O_2, 10% CO_2, 85%N_2) [27]. *Campylobacter* spp. was verified via latex agglutination [28,29] with a Microgen M46 Campylobacter Assay Kit (Microgen Bioproducts Ltd., Camberley, Surrey, UK) by manufactures instructions and *C. jejuni* was confirmed via hippurate hydrolysis [26].

Statistical analysis

Statistical procedures were performed using SAS Windows [30]. Day old broilers were randomly allotted to two treatments (with and without the antibiotic sulfadimethoxine administration in chicken production) with two replications. All calculations were performed with Proc GLM procedures (SAS 2003) using P=0.05 for significance of Least Squares Means with a model of the antibiotic sulfadimethoxine administration in chicken production and week of testing. When treatment difference is detected, specific comparisons between treatment means at that time point were made with the PDIFF option of LSMEANS.

Results

Enumeration of *Campylobacter* spp.

Our results showed that the counts of *Campylobacter* spp. steadily increased from week 1 through week 6 in both the control and antibiotic treatments (Figure 1). The counts of *Campylobacter* spp. in the control treatment increased from an initial value of 3.58 log CFU/broiler in week one to a maximum value of 6.05 log CFU/broiler in week six. This represents a total increase of 2.47 log CFU/broiler during the course of the experiment. In the antibiotic treatment the initial value of 3.44 log CFU/broiler in week one increased to a maximum value of 5.12 log CFU/broiler in week six (Figure 1). This represents a total increase of 1.68 log CFU/broiler.

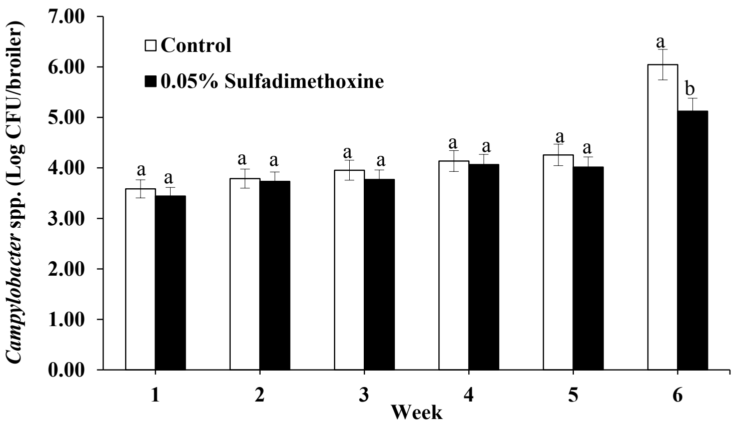

Figure 1: *Campylobacter* species bacterial counts in live broilers from the control and antibiotic treatments from weeks 1 through 6. Data are means from two replications. SEM=4.9738. Superscript letters a and b show treatment means with different superscripts for the same week are significantly different (P<0.05).

There was no significant difference in the enumeration of *Campylobacter* spp. in the antibiotic treatment ($P>0.05$) and the control treatment in weeks 1 through 5. However, the *Campylobacter* spp. counts were significantly higher in the control treatment than the antibiotic treatment ($P<0.05$) at week 6 (Figure 1).

For the overall experiment, the *Campylobacter* spp. counts in the antibiotic treatment were lower than in the control treatment (Figure 1). These results suggest that the antibiotic sulfadimethoxine, as applied in this experiment can reduce the counts of *Campylobacter* spp. in the broilers.

Prevalence of *Campylobacter* spp. and *C. jejuni*

For each of the test weeks, the prevalence of *Campylobacter* spp. in individual broilers from the control treatment ranged from 37.3% (112 of 300) to 66.7% (200 of 300). From the antibiotic treatment, the prevalence ranged from 13.3% (40 of 300) to 65.3% (196 of 300) (Table 1). At week 1, the prevalence of *Campylobacter* spp. was significantly lower in the antibiotic treatment ($P<0.05$) than in the control treatment (Table 1). In weeks 2 and 3, the prevalence of *Campylobacter* spp. declined by 63.0% in the antibiotic treatment but increased by 43.0% in the control treatment.

In week 3, the prevalence of *Campylobacter* spp. was significantly higher in the control treatment than the antibiotic treatment ($P<0.05$) (Table 1). Specifically, there was a 13.3% (40 of 300) incidence in the antibiotic treatment and a 53.3% (160 of 300) incidence in the control treatment. These finding showed that the antibiotic sulfadimethoxine can reduce the prevalence of *Campylobacter* spp. in broilers especially in week 3 (Table 1).

In week 4, the prevalence of *Campylobacter* spp. increased to 52.0%, (156 of 300) in the antibiotic treatment but it was unchanged at 53.3% (160 of 300) in the control treatment. In week 5, the prevalence of *Campylobacter* spp. declined somewhat in both treatments. Specifically, the prevalence was measured at 41.3% (124 of 300) in the antibiotic treatment and at 42.7% (128 of 300) in the control treatment (Table 1). In week 6, the prevalence of *Campylobacter* spp. in the control treatment was 66.7% (200 of 300) and in the antibiotic treatment was 65.3% (196 of 300) (Table 1). Overall, for the six-week period of testing, the prevalence of *Campylobacter* spp. in the antibiotic treatment was lower ($P<0.05$) than in the control treatment (Table 1).

Additionally, the overall prevalence of *C. jejuni* in the control treatment ranged from 33.3% (100 of 300) to 56.0% (168 of 300) and from 13.3% (40 of 300) to 50.0% (150 of 300) in the antibiotic treatment (Table 2). In week 1, the prevalence of *C. jejuni* was significantly higher ($P<0.05$) in the control treatment at 41.3% (124 of 300) than in the antibiotic treatment at 25.3% (76 of 300) (Table 1). In week 2, the prevalence of *C. jejuni* was the same in both control and antibiotic treatments at 33.3% (100 of 300) (Table 2). In week 3, the prevalence of *C. jejuni* declined in the antibiotic treatment to 13.3% (40 of 300) whereas, it increased to 45.3% (136 of 300) in the control treatment. These values represent a significant difference ($P<0.05$) (Table 2).

Week	No. (%) of broilers testing positive for Campylobacter spp.	
	Control	0.05% Sulfadimethoxine
1	124/300 (41.3)^a	92/300 (30.7)^b
2	112/300 (37.3)^a	108/300 (36.0)^a
3	160/300 (53.3)^a	40/300 (13.3)^b
4	160/300 (53.3)^a	156/300 (52.0)^a
5	128/300 (42.7)^a	124/300 (41.3)^a
6	200/300 (66.7)^a	196/300 (65.3)^a
Data are sum totals from two replications. SEM for Campylobacter spp.=0.0344. Letters a and b show treatment totals with different superscripts for the same week that are significantly different (P<0.05).		

Table 1: The prevalence of *Campylobacter* spp. in live broilers from the control and antibiotic treatments from weeks 1 through 6.

Week	No. (%) of broilers testing positive for C. jejuni	
	Control	0.05% Sulfadimethoxine
1	124/300 (41.3)^a	76/300 (25.3)^b
2	100/300 (33.3)^a	100/300 (33.3)^a
3	136/300 (45.3)^a	40/300 (13.3)^b
4	144/300 (48.0)^a	136/300 (45.3)^a
5	128/300 (42.7)^a	124/300 (41.3)^a
6	168/300 (56.0)^a	150/300 (50.0)^b
Data are sum totals from two replications. SEM for *C. jejuni*=0.0366. Letter a and b show treatment totals with different superscripts for the same week that are significantly different (*P*<0.05).		

Table 2: The prevalence of *C. jejuni* in live broilers from the control and antibiotic treatments from weeks 1 through 6.

In week 4, the prevalence of *C. jejuni* was 45.3% (136 of 300) in the antibiotic treatment and 48.0% (144 of 300) in the control treatment (Table 2). These values are not significantly different ($P>0.05$) (Table 2). In week 5, the prevalence of *C. jejuni* was at 41.3% (124 of 300) in the antibiotic treatment and at 42.7% (128 of 300) in the control treatment (Table 2). These values are not significantly different ($P>0.05$) (Table 2). In week 6, the prevalence of *C. jejuni* was significantly higher ($P<0.05$) in the control treatment at 56.0% (168 of 300) than in the antibiotic treatment at 50.0% (150 of 300) (Table 2). For the overall experiment, the prevalence of *C. jejuni* in the antibiotic treatment was significantly lower ($P<0.05$) than in the control treatment (Table 2). These results suggest that the antibiotic sulfadimethoxine, as applied in this experiment can reduce the prevalence of *C. jejuni* in the broilers.

Discussion

This study suggests that the antibiotic sulfadimethoxine can reduce the bacterial counts of *Campylobacter* spp. and the prevalence of *Campylobacter* spp. and *C. jejuni* in small-scale poultry farming. This is the first study in which sulfadimethoxine has been used in an in vivo setting to control *Campylobacter*.

Results from previous studies whose aim was to control *C. jejuni* in poultry are mixed. For example, the previous research [16] attempted to inhibit *C. jejuni* in chickens through the administration of the live bacterium *Enterococcus faecalis*. As they reported, this bacterium failed to inhibit the growth of *C. jejuni*. In a second study using a bacteriophage lytic cocktail administered to chickens resulted in a 2 log CFU/ml reduction in the counts of *C. jejuni* [14]. In a third study using turkeys, the administration of the antibiotics enrofloxacin, neomycin and vancomycin resulted in a respective decrease of 1, 2 and 4 log CFU/ml of *C. jejuni* [15]. For purposes of comparison, the present study found that the use of sulfadimethoxine resulted in a decrease of 0.93 log CFU/broiler in the experimental group as compared to the control group. Therefore, the positive results from this study compare favorably with the previously mentioned second and third studies.

The quantitative risk assessment model [31] suggests that reducing *Campylobacter* spp. levels by 1, 2 and 3 log CFU/ml could result in a reduction in the prevalence of *Campylobacter* spp. by 55%, 81% and 94% respectively. However, this model is not supported by the present study. Specifically, the enumeration of *Campylobacter* spp. in the experimental group decreased by 0.93 log CFU/ml as compared to the control group but the prevalence of *Campylobacter* spp. was only reduced by 1.34%. Subsequent studies may investigate the addition of sulfadimethoxine at varied levels or in combination with other antimicrobials to control or prevent *C. jejuni* during the growing phase of broilers.

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