

Antimicrobial Effects of Sulfadimethoxine on *Salmonella*, *Escherichia coli* and Aerobic plate count (APC) in Small-Scale Broiler Operations

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Abstract

Poultry is a source of *Salmonella* and *Escherichia coli*. Antibiotics can be used to reduce the enumeration and prevalence of these bacteria. The objective of this study was to determine the effects of sulfadimethoxine antibiotic on the enumeration and prevalence of *Salmonella*, *Escherichia coli* and aerobic plate count in broilers. Broilers (n = 600) were allotted to two treatments, each with twelve replications. The treatments were control (drinking water without antibiotic) and with antibiotic at 0.05% (wt/vol) sulfadimethoxine. After a six-week period, the enumeration of *Salmonella* in the experimental treatment was detected at 2.55 log CFU/g. This value is not different (P>0.05) than that detected in the control at 2.81 log CFU/g. With respect to prevalence, there was a difference (P<0.05) between the experimental treatment at 90.0% and the control at 100%. The enumeration of *E. coli* in the experimental treatment was detected at 3.97 log CFU/g. This value is lower (P<0.05) than that detected in the control treatment at 4.37 log CFU/g. With respect to prevalence, there was no difference (P>0.05) between the experimental treatment at 100% and the control at 100%. The enumeration of aerobic plate count in the antibiotic treatment were detected at 6.62 log CFU/g. This value is lower (P<0.05) than that detected in the control at 7.50 log CFU/g. With respect to prevalence, there was no difference (P>0.05) between treatments. Our overall findings suggest that the use of the antibiotic sulfadimethoxine can reduce the number of *E. coli*, *Salmonella* and aerobic plate count in the small-scale broiler operations.

Keyword: sulfadimethoxine, *Salmonella*, *E. coli*, aerobic plate count, small-scale

1. Introduction

Poultry is a host for the bacteria *Salmonella* (Lee, Runyon, Herrman, Phillips, & Hsieh, 2015) and *Escherichia coli* (Nolan, 2019) which can be a source of human illness (Haleem, Al-bakri, & Al-Hiyaly, 2013). Increased attention has been given to reducing the level of these bacteria in pre- and post-harvest poultry with the aim to reduce the level and incidence of raw product contamination.

Antimicrobial therapy is an important tool in reducing microorganisms in poultry production (Aarestrup, 2015) and enhances growth productivity (Page & Gautier, 2012). However, the use of antibiotics in animal production may produce resistant bacteria which could limit the effectiveness of antibiotics in humans (Simonsen et al., 1998; Klare et al., 1999; Van, London, Driessen, & Stobberingh, 2001). Salmonellosis is one of the most common foodborne diseases caused by *Salmonella* in poultry and can be transmitted to humans (Authority, 2016; Antunes, Mourão, Campos, & Peixe, 2016). Colibacillosis is an infectious disease caused by *E. coli* which may produce morbidity and mortality in poultry (Piercy & West, 1976; DeRosa, Ficken, & Barnes, 1992; Lutful, 2010). Aerobic plate count (APC) is commonly used to assess the microbial load of poultry and the counts can be used to determine the quality, safety and shelf life of poultry products (Haleem et al., 2013; Rouger, Tresse, & Zagorec, 2017). Growth of spoilage bacteria lead to defects in meat products and can be responsible for unwanted taste, color, odor, and texture. An APC count at 7 log CFU/g or greater is used to define food spoilage (Zhang et al., 2012; Höl, Behr, & Vogel, 2016) which is associated with food-borne illness (Rouger et al., 2017). Therefore, there is a need to find an alternative antibiotic for prevention of *Salmonella* and *E. coli* infections and reducing the number of APC in poultry production.

Sulfonamide is commonly used to treat upper respiratory (Delaplane, 1945), coccidial infections caused by

Eimeria tenella and *Eimeria necatrix* (Waletzky & Hughes, 1946; Grumbles & Delaplane, 1948) and promote growth in poultry (Whitehill, Oleson, & Hutchings, 1950; Aarestrup, 2000). The commonly used sulfonamide in poultry production is sulfadimethoxine and therefore is appropriate for in vivo testing (FDA, 2013). Sulfadimethoxine can be used to treat coccidiosis (Orton & Hambly, 1971), fowl cholera, and coryza in poultry (Vree & Hekster, 1987; Wang, MacNeil, & Kay, 2012). In addition, sulfadimethoxine improves weight gain and final body weight (Davami, Peterson, Jones, & Ilardi, 1987). Previous studies showed that sulfadimethoxine can reduce the number of *Campylobacter* spp. and *C. jejuni* in growing broilers (Tangkham, Janes, & LeMieux, 2016a) and turkeys (Alexandra, 2009). Therefore, most previous studies have concentrated on the transmission routes from commercial flock farm to carcasses after slaughter and retail products with limited information on the effects of production practices within small-scale poultry operations. The purpose of this study is to use antimicrobial therapy techniques to control bacterial contamination in poultry. Specifically, this study examines the effects of sulfadimethoxine antibiotic on the enumeration of *Salmonella*, *E. coli* and aerobic plate count in growing broilers.

2. Method

2.1 Broiler Production

The research experiment was approved by the McNeese State University Institutional Animal Care and Use Committee prior to data collection. Broilers were obtained from the McNeese State University Research Farm in Lake Charles, Louisiana. Birds (n = 600 Ross x Ross) were allotted to one of two treatments: 1) control (drinking water without antibiotic) and 2) drinking water + 0.05% (wt/vol) sulfadimethoxine (Durvet Inc., Blue Springs, Missouri). Drinking water was refreshed every day in both treatment groups. Feces was collected to determine *Salmonella*, *Escherichia coli* and aerobic plate counts from January 2014 to May 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 of 74.7 cm × 99.1 cm × 24.13 cm (Tangkham et al., 2016a; Tangkham, Janes, LeMieux, 2016b). Each pen housed twenty-five birds. Individual water and feed troughs were provided for each pen and supplied ad libitum. Birds were provided a commercial 18% protein chick grower crumbles with no antibiotics. The housing system was emptied of birds, feed, and litter and cleaned with hot water wash and disinfected. Animal care givers monitored feed and water and removed litter trays daily. Normal pest and rodent control were maintained throughout the experiment. The temperature and % RH during time period was 32 °C and 58%, respectively.

2.2 Bacterial Isolation and Identification

The microorganisms were determined following the standards of the Association of Official Analytical Chemists (AOAC, 2000). Each week, fecal samples via swabbing were randomly collected from individual broilers (n = 600). To determine the enumeration (log CFU/g) and prevalence (%) of *E. coli* and *Salmonella*, samples were plated on brilliant green agar. For aerobic plate count, samples were plated on nutrient agar.

Samples were plated on 3M[™] Petrifilm to determine the enumeration (log CFU/g) of *E. coli* and APC. *Salmonella* was isolated with brilliant green agar. Plates were incubated in a horizontal position, clear side up in stacks of no more than 20 plates at 37 °C for 24-48 h. Results were obtained by selecting a countable plate (30-300 colonies) and the colonies were counted and reported as CFU/g.

2.3 Statistical Analysis

Statistical analysis was performed using SAS windows (SAS, 2003). The Proc GLM procedures were used to evaluate the significance differences of the obtained data. The PDIFF option of LSMEANS was employed to determine significance (P<0.05) among treatments. All data are presented as means with standard deviation (SD) and a significance level of was used for statistical analysis of means from treatments.

3. Results and Discussion

3.1 Enumeration of *Salmonella*

Feces was collected and plated to determine the enumeration of *Salmonella*. The enumeration of *Salmonella* ranged from 0-4.25 log CFU/g. *Salmonella* increased from week 1 through week 3 in both the control and antibiotic treatments (Figure 1). Specifically, the counts of *Salmonella* in the control treatment increased from an initial value of 1.22 log CFU/g in week one to a maximum value of 4.25 log CFU/g in week three (Figure 1). In the antibiotic treatment, the initial value was not detected in week one but increased to 4.02 log CFU/g in week three (Figure 1). Our study supported previous studies that poultry is a source of *Salmonella*, which leads to contamination of diverse foodstuffs (Barrow, Jones, Smith, & Wigley, 2012; Mazengia et al., 2014; Crump, Sjolund, Gordon, & Parry, 2015; Cosby et al., 2015).

For the overall experiment, there was no difference ($P>0.05$) in the enumeration of *Salmonella* in the antibiotic treatment and the control treatment in weeks 1 through 6. However, the counts of *Salmonella* in the antibiotic treatment of 2.55 log CFU/g was lower than in the control treatment at 2.81 log CFU/g. Similar, to previous studies (Seiffert, Hilty, Perreten, & Endimiani, 2013; Mazengia et al., 2014; Aarestrup, 2015) which indicated that the use of antibiotics had significantly lower rates of recovery of *Salmonella*. These results suggest that the antibiotic sulfadimethoxine, as applied in this study reduces the enumeration of *Salmonella* in small-scale poultry farming.

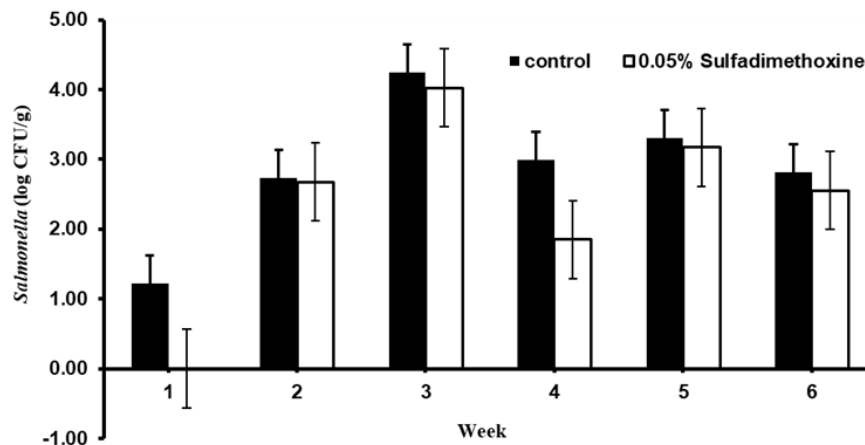


Figure 1. *Salmonella* counts in live broilers from the control and antibiotic treatments from weeks 1 through 6. Data are means from two replications. SEM=3.590

3.2 Enumeration of *E. coli*

The number of *E. coli* ranged from 3.67-4.55 log CFU/g. There was a small decrease in the enumeration of *E. coli* in the control treatment in weeks 1 through 6 from 4.39 log CFU/g to 4.37 log CFU/g. At week 6, the counts of *E. coli* decreased in the antibiotic treatment from 4.35 log CFU/g to 3.97 log CFU/g (Figure 2). These results were similar to the previous study (Tessi, Salsi, Caffer, & Moguilevsky, 1997) which found that the use of sulfamethoxazole-trimethoprim as an antibiotic can inhibit the growth of *E. coli*. Other studies by Huff, Huff, Rath, Balog, & Donoghue (2002) used a bacteriophage aerosol spray as an antibiotic to reduce the mortality rate of *E. coli* and their results showed that there was a significant decrease in mortality when the birds were challenged with *E. coli* immediately after bacteriophage administration. Other researchers (Al-Ghamdi, El-Morsy, Al-Mustafa, Al-Ramadhan, & Hanif, 1999) also found that ampicillin and tetracycline can be used to inhibit the growth of *E. coli* from chickens. For the overall experiment, the enumeration of *E. coli* in the antibiotic treatment was lower ($P<0.05$) 3.97 log CFU/g than the control treatment at 4.37 log CFU/g. These results suggest that the antibiotic sulfadimethoxine, as applied in this study reduces the enumeration of *E. coli* in small-scale poultry farming.

3.3 Enumeration of Aerobic Plate Count

After a six-week period, the enumeration of aerobic plate count ranged 6.46-7.93 log CFU/g. These data were related to the previous study by Haleem et al. (2013) who tested the counts of microflora at 6.55 log CFU/g in poultry meat. Our study showed that the counts of APC steadily increased from week 1 through week 6 in the control treatment from an initial value of 6.72 log CFU/g to a maximum value of 7.5 log CFU/g (Figure 3). This may be due to the elevated initial viable count of APC (Haleem et al., 2013) and microbial spoilage occurs because of the growth and metabolic activities of spoiling bacteria (Zhang, et al., 2012; Höl et al., 2016; Rouger et al., 2017). For the overall experiment, the enumeration of aerobic plate count in the antibiotic treatment was significantly lower (6.62 log CFU/g) than in the control treatment (7.50 log CFU/g). Therefore, our results found that the antibiotic sulfadimethoxine, as applied in this study reduces the enumeration of aerobic plate count in small-scale poultry farming.

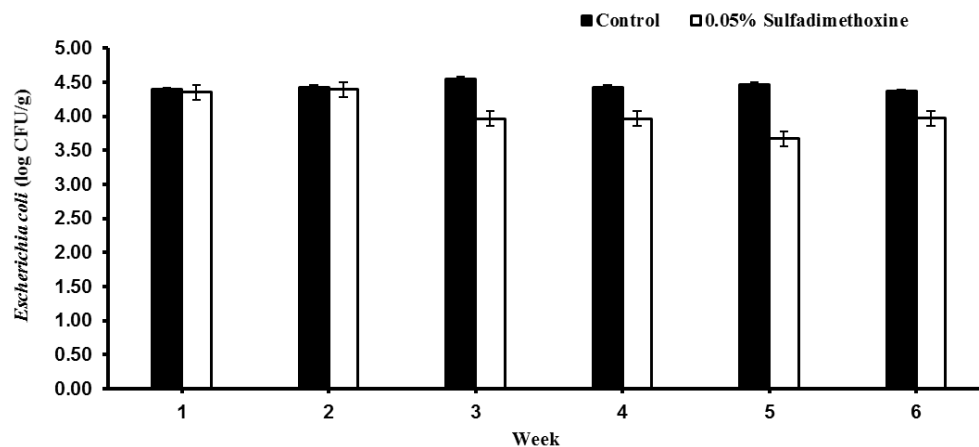


Figure 2. *E. coli* counts in live broilers from the control and antibiotic treatments from weeks 1 through 6. Data are means from two replications. SEM=3.410

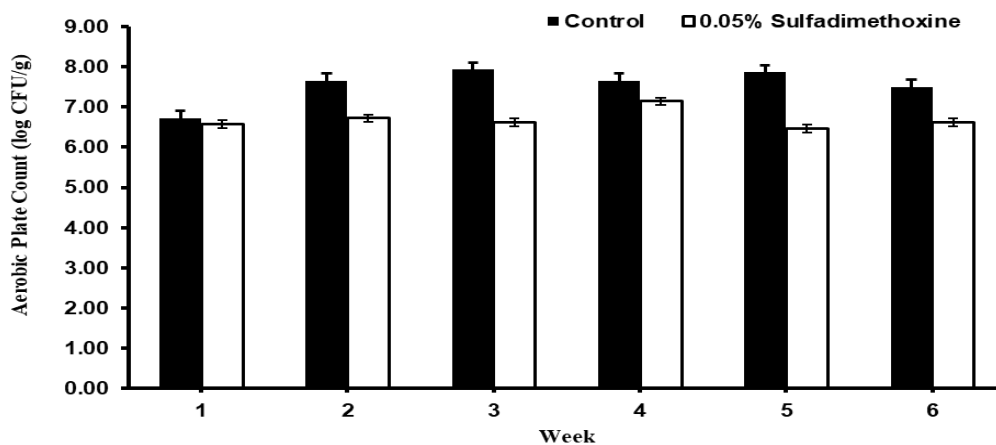


Figure 3. APC counts in live broilers from the control and antibiotic treatments from weeks 1 through 6. Data are means from two replications. SEM= 6.820

3.4 Prevalence of *Salmonella*, *E. coli* and Aerobic Plate Count

The prevalence of *Salmonella*, *E. coli* and aerobic plate counts were randomly tested from individual broilers ($n = 300$) in both treatments. At week 1, the prevalence of aerobic plate count was detected from both treatments at 100%. For *Salmonella* and *E. coli* were found at 96.7% in the control treatment (Table 1). In week 2, the prevalence of *Salmonella*, *E. coli* and aerobic plate count were detected 100% in both control and experimental treatments. In week 3, the prevalence of *Salmonella*, *E. coli* and aerobic plate count were declined in the control and the antibiotic treatment ($P < 0.05$) (Table 1). Specifically, they were lower ($P < 0.05$) in the antibiotic treatment at 83.3%, 76.7%, and 93.3% of *Salmonella*, *E. coli* and aerobic plate count, respectively. These finding showed that the antibiotic sulfadimethoxine can reduce the prevalence of these microorganisms in broilers especially in week 3 (Table 1). Overall, for the six-week period of testing, the prevalence of *Salmonella* in the antibiotic treatment was lower ($P < 0.05$) than in the control treatment (Table 1). Similar, to the studies of Hanson, Kaneene, Paduangtod, Hirokawa, & Zeno (2002) who concluded that tetracycline, nalidixic acid, florfenicol, ampicillin, and ceftiofur were able to decrease the prevalence of *Salmonella*. No difference ($P > 0.05$) was found in both treatments on the prevalence of *E. coli* and aerobic plate count.

Table 1. The prevalence of *Salmonella*, *Escherichia coli* and aerobic plate count in live broilers (n = 600) from the control and antibiotic treatments from weeks 1 through 6

| Week | Control | | | 0.05% sulfadimethoxine | | |
|------|-------------------|---------------|---------|------------------------|----------------|---------|
| | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) |
| | <i>Salmonella</i> | <i>E.coli</i> | APC | <i>Salmonella</i> | <i>E. coli</i> | APC |
| 1 | 96.7 | 96.7 | 100 | 100 | 100 | 100 |
| 2 | 100 | 100 | 100 | 100 | 100 | 100 |
| 3 | 86.7 | 80.0 | 100 | 83.3 | 76.7 | 93.3 |
| 4 | 96.7 | 96.7 | 100 | 93.3 | 93.3 | 100 |
| 5 | 100 | 100 | 100 | 96.7 | 100 | 100 |
| 6 | 100 | 100 | 100 | 90.0 | 100 | 100 |

4. Conclusions

This study revealed that poultry is a source of *Salmonella* and *E. coli* in small-scale poultry farming. This may contribute to cross-contamination of meat carcasses after slaughter and retail products. Therefore, the use of sulfadimethoxine as an antibiotic can reduce the enumeration of *Salmonella*, *Escherichia coli* and aerobic plate count in small-scale broiler operations.

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