ABSTRACT

Four antibiotics (enrofloxacin, sulfaquinoxaline, sulfadimethoxine, and sulfamethazine) were tested using the tube dilution method to determine the minimum inhibitory concentration (MIC) of each antibiotic against *E. coli* isolated from poultry. Enrofloxacin was the only antibiotic tested that had a maximum plasma level higher than the average MIC, which suggests the sulfonamides are inadequate to treat systemic *E. coli* infections in broiler chickens.

Thromboelastography values for the reaction rate (R), coagulation time (K), clot formation rate (angle) and maximum amplitude (MA) were determined for blood from broiler chickens after receiving one of the antibiotics. The MA of the enrofloxacin-treated chickens was significantly lower than the MA of the control chickens, suggesting a decrease in thrombocytes or decreased fibrinogen concentration. The R and K were prolonged and the angle and MA were increased in the sulfaquinoxaline-treated chickens, suggesting that sulfaquinoxaline affects thrombin generation, fibrin formation, clotting factors and thrombocyte number and function.

INDEX WORDS: Enrofloxacin, sulfonamides, MIC, thromboelastography, coagulation, broiler chickens, bioassay
INVESTIGATIONS ON FOUR ANTIBIOTICS FOR POSSIBLE USE AGAINST *E. COLI* IN
BROILER CHICKENS

by

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B.S.A., The University of Georgia, 2001

A Thesis Submitted to the Graduate Faculty of the University of Georgia in Partial Fulfillment of
the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2003
INVESTIGATIONS ON FOUR ANTIBIOTICS FOR POSSIBLE USE AGAINST *E. COLI* IN BROILER CHICKENS

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December 2003
ACKNOWLEDGMENTS

To my major professor, Dr. Roger Wyatt, thank you for challenging me and always encouraging me to think for myself. I wouldn’t be where I am today if you didn’t recruit me to be your graduate student. I want to thank you for the academic, financial, and emotional support you have given me over the past few years. You have truly been a teacher in laboratory and life lessons.

To Drs. Roger Broderson and Chuck Hofacre, thank you for agreeing to be on my graduate committee. I greatly appreciate your expertise in editing this thesis and guiding my research in the right direction.

To Deedra Bailey, thank you so much for helping me get started in the laboratory. Your efforts at the beginning of my research were what got this project going. Thank you for helping me feel comfortable and competent in a new environment.

To my parents, I will never be able to repay all that you have done for me. You have provided me with incredible amounts of emotional and financial support for the last 25 years. I want to thank you for believing in me and always encouraging me to pursue my education. I could never have completed this degree without the work ethic instilled in me by my dad or the sympathetic ear provided to me by my mom.

To my pets, Lexie, Lacey, Lucky and Georgia, thank you for being the most loyal friends a girl could have and making me forget about a bad day the second I walk in the door.

To my best friend and future husband, David, thank you for loving me during this stressful period of my life. There were times when I didn’t think I could complete this degree, but you always believed in me. Your friendship and encouragement mean the world to me and I look forward to having them for many years to come.
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INTRODUCTION

*Escherichia coli* is a gram-negative, rod shaped bacterium that causes infections throughout the poultry industry, resulting in significant economic losses each year (Gross, 1994). Antimicrobial therapy is an important tool in reducing the high incidence of morbidity and mortality caused by *E. coli* infections in poultry. However, the use of antibiotics in poultry has provided a selection pressure for antimicrobial resistance genes which has resulted in antibiotic-resistant bacterial strains associated with poultry meat (Quednau *et al.*, 1998, Turtura *et al.*, 1990).

Although many antibiotics have the potential to control *E. coli* infections in poultry, only a few are actually administered. The tetracyclines are efficacious, however, antibiotic residues impede the export of meat from the United States. Penicillin, such as ampicillin and amoxicillin, are also active against *E. coli*, but the expense and growing resistance to penicillin do not make it a feasible choice for producers. The fluoroquinolones, which includes enrofloxacin, are a relatively new class of antimicrobials that exhibit excellent activity against gram-negative bacilli and are currently being used to treat avian colibacillosis (Stahlmann, 1988). The FDA is questioning the safety of using these antibiotics, however, due to possible carcinogenicity and the fear of developing cross-resistance to the fluoroquinolones used for treating human enteric infections (Blanco *et al.*, 1997). The sulfonamides are an older class of antibiotics that are relatively inexpensive and effective against *E. coli*. Since the sulfonamides are not readily used in human medicine, cross-resistance is of little concern. Toxicities associated with
sulfaquinoxaline administration have been reported, however (Muskett & Seeler, 1947, Patterson & Grenn, 1975).

The purpose of the first experiment discussed in this thesis was to assess four antibiotics (enrofloxacin, sulfaquinoxaline, sulfadimethoxine and sulfamethazine) for their possible use in chickens to treat *E. coli* infections. Antibiotic sensitivity to representative strains of *E. coli* from poultry for each antibiotic was determined and the therapeutic concentration of each antibiotic in the plasma was also determined.

Numerous agents, including antibiotics, are known to affect the coagulation process. Outbreaks of a hemorrhagic syndrome in poultry have been reported as a result of exposure to sulfaquinoxaline (Cover *et al*., 1955). Muskett reported that sulfaquinoxaline administration resulted in hypoprothrombinemia and death in canines (1947). Other case studies reported the deaths of several dogs due to hemorrhage into the small bowel following the administration of sulfaquinoxaline (Patterson *et al*., 1975, Osweiler *et al*., 1978). Since sulfaquinoxaline causes a coagulopathy, then other related sulfamdrugs may also cause a coagulopathy. The second experiment discussed in this thesis was undertaken to characterize the effect of 3 sulfonamide antibiotics (sulfaquinoxaline, sulfadimethoxine, sulfamethazine) and enrofloxacin, a commonly used fluoroquinolone antibiotic in poultry, on the coagulation of blood from broiler chickens.

Thromboelastography was originally described in the late 1940’s by Hartert (1948) but has recently been gaining popularity because it provides a panoramic measure of blood clotting. Thromboelastography is unique because it allows for the use of an unmanipulated whole blood sample unlike other routine clotting tests which require alteration of the original blood sample. Routine tests examine only a portion of the coagulation process while thromboelastography
provides information on the interactions of the coagulation proteins and the blood borne cellular participants in coagulation.

Both experiments are designed to analyze the usefulness of enrofloxacin, sulfadimethoxine, sulfaquinoxaline, and sulfamethazine as treatments for systemic *E. coli* infections in broiler chickens. This information will increase our knowledge base on current sulfonamide and fluoroquinolone resistance rates in *E. coli* strains isolated from poultry and will also help us understand the complex nature of antibiotic-induced coagulopathies.
LITERATURE REVIEW

History and Use of Sulfonamides

A new era in the treatment of infections began in the early 1930’s with the discovery of the antibacterial activity of dyes containing sulfamyl. Although Fleming reported the inhibition of staphylococci by penicillin in 1929, the compound seemed too unstable to isolate and the problem wasn’t solved until 1940. Meanwhile, Prontosil, a sulfonamide radical containing azodye, was found to protect mice against streptococcal infections and rabbits against staphylococcal infections (Domagk, 1935). In 1939, the Nobel Prize in Medicine was awarded to Domagk for this discovery. It was later discovered that Prontosil breaks down into sulfanilamide \textit{in vivo} and the degradation product was as effective as the parent compound in treating bacterial infections (Northev, 1940). Woods (1940) and Fildes (1940) showed that sulfonamides are anti-metabolites of \(p\)-aminobenzoic acid (PABA). Sulfonamides competitively inhibit bacterial modification of PABA to dihydrofolate. A lack of dihydrofolate prevents the synthesis of bacterial DNA. Since mammalian cells do not synthesize folic acid, human purine synthesis is unaffected by sulfonamides.

The general term “sulfonamide” refers to any derivative of sulfanilamide. In the past, many sulfonamides were approved for use in human medicine, but now there are relatively few due to toxicity and acquired resistance. The list of sulfonamides approved for use in veterinary medicine, however, is still quite long. The older sulfonamides are seldom used because of problems with crystallization of the antibiotic in the renal tubules. Recent sulfonamide
derivatives do not exhibit crystallization, are therefore less toxic and require less frequent administration for therapeutic efficacy.

Derivatives of sulfanilamide are made by substituting either the amide group (N1-substituents) or the p-amino nitrogen (N4-substituents). The amide group may be substituted with an acyclic derivative or a heterocyclic derivative with a five or six-membered ring. Blocking the free amino nitrogen by a group that is not removed in vivo destroys the antibacterial activity of the sulfonamide. Substitution at the N1 and N4 positions of sulfonamides will result in compounds that vary widely in their protein binding, kinetic behavior and chemotherapeutic effect (Vree and Hekster, 1987). There is no definitive relationship between chemical structure and therapeutic activity of the sulfonamides (Bell and Roblin, 1942). Each sulfonamide and its metabolites have unique pharmacokinetic properties that determine the suitability of the sulfonamide for clinical applications.

Sulfonamides have been used to treat a wide range of infectious diseases in humans and animals including pneumonia, meningitis, malaria, and urinary tract infections. Their broad spectrum of activity rivals that of the tetracyclines. They are used alone or in combination with trimethoprim. Trimethoprim inhibits the reduction of dihydrofolic acid (DHF) to tetrahydrofolic acid (THF) by binding to bacterial dihydrofolic acid reductase (DHFR). Sulfonamides decrease the de novo synthesis of DHF while trimethoprim decreases the conversion of new and recycled DHF to THF. Combination of the two drugs results in a synergistic sequential blockade.

In poultry, sulfonamides were first used to treat upper respiratory infections (Delaplane, 1945). They were later used to treat coccidial infections from *Eimeria tenella* and *Eimeria necatrix* infections (Delaplane et al., 1947, Grumbles et al., 1948, Waletzky et al., 1946). The
main veterinary sulfonamides currently administered are sulfadiazine-trimethoprim, sulfadimethoxine, sulfamethazine, and sulfathiazole.

In humans, adverse reactions to sulfonamides are relatively common and can occur from direct toxicity or hypersensitivity (Stowe, 1965). Although not a prominent effect, sulfonamides can produce disturbances in gastrointestinal flora due to their broad spectrum of activity. The most common contraindications are anorexia, nausea, and vomiting which occur in 1% to 2% of patients. Disturbances of the urinary tract, including renal crystalluria, can occur while taking sulfonamides if fluid intake is low. Sulfonamides can cause hypothyroidism by impairing thyroglobulin iodination and coupling of tyrosinases (Bevill, 1977). Hypersensitivity reactions including fever, itching, and skin rashes occur in up to 3% of patients. The Stevens-Johnson syndrome is a hypersensitivity reaction that occurs less frequently and is characterized by arthralgia, blistering of the skin, and weakness.

Certain sulfonamides can also cause disorders of the hematopoietic system. Acute hemolytic anemia is rare (0.05%) but can occur following sulfadiazine or sulfisoxazole therapy. The hemolysis could be due to a sensitization reaction or an erythorocytic deficiency of glucose-6-phosphate dehydrogenase activity (Bevill, 1977). Agranulocytosis occurs in about 0.01% of patients receiving sulfonamides, but granulocyte levels return to normal after the antibiotic therapy is halted. Hypoprothrombonemia can occur in animals undergoing sulfaquinoxaline therapy (Muskett et al., 1944, Osweiler & Green, 1978, Patterson & Grenn, 1975). This effect is most likely due to the inhibition of vitamin K epoxide reductase (Preusch et al., 1989). Complete suppression of bone-marrow activity with profound anemia, granulocytopenia, and thrombocytopenia is a rare occurrence with sulfonamide therapy and probably results from a direct myelotoxic effect (Stowe, 1965).
History and Use of Fluoroquinolones

The older quinolones, namely nalidixic acid, have been used to treat bacterial infections since the early 1960’s but never gained widespread use. This situation changed drastically in the late 1980’s with the development of fluorinated quinolones. The fluoroquinolone derivatives offer superior antibacterial and pharmacokinetic properties compared to the older quinolones (Stahlmann, 1988). The fluoroquinolones broad spectrum of activity consists of gram-negative and gram-positive aerobic bacteria including, staphylococci, *E. coli*, *Campylobacter*, *Salmonella*, *Listeria*, and *Shigella*. Fluoroquinolones are also active against mycoplasmas, mycobacteria and rickettsias. Fluoroquinolones are bactericidal and act by inhibiting DNA replication. Fluoroquinolones inhibit the A subunit of DNA gyrase, the enzyme responsible for the unwinding and supercoiling of bacterial DNA (Hoshino *et al*., 1989).

The fluoroquinolones have been used to treat a wide range of infections in humans. These include, but are not limited to, animal bites, bone and joint infections, gastro-enteritis, gonorrhea, meningitis, otitis, lower-respiratory tract infections, spotted fevers and urinary-tract infections (von Rosenstiel *et al*., 1994). Fluoroquinolones recently gained national attention when ciprofloxacin was prescribed to treat victims exposed to anthrax. Fluoroquinolones are also used to treat *E. coli* and Pasteurella infections in animals.

Fluoroquinolones have a wide variety of adverse effects. The most common side effects involve the gastro-intestinal tract, central nervous system, and skin. Gastro-intestinal disturbances include nausea, vomiting, diarrhea, and abdominal pain. The most common effects on the central nervous system are headache, dizziness and restlessness. Although rare, more serious side effects including hallucinations, psychotic reactions, depression, and catatonia have been associated with ciprofloxacin (Jay *et al*., 1991, Reeves, 1992, Akhtar *et al*., 1993).
Hypersensitivity and photosensitivity reactions have occurred including rashes, vasculitis, toxic epidermal necrolysis, and even fatal anaphylaxis (Choe, 1989, Yerasi, 1996, Assouad, 1995). Disturbances in the hematopoietic system have occurred following ciprofloxacin therapy. These include eosinophilia, leukopenia, thrombocytopenia and very rarely, hemolytic anemia and agranulocytosis (Paton et al., 1992). A coagulopathy resulting from transient reductions in factor VIII and von Willebrand’s factor has also been reported (Castaman, et al., 1994). Effects on the musculoskeletal system can occur in humans, but are more commonly seen in immature animals. Quinolones induce cartilage alterations and arthropathy in juvenile animals of several species including rats, dogs, and non-human primates (Stahlmann et al., 1988b). Tendon damage and arthropathy have also occurred in humans undergoing fluoroquinolone therapy (Huston, 1994, Ribard et al., 1992, Chevalier et al., 1992). For this reason, a voluntary moratorium on the use of fluoroquinolones in children and adolescents has existed for the last 20 years (Hayem, 1995).

Although fluoroquinolones are potent and possess excellent pharmacokinetic properties, problems with fluoroquinolone-resistant strains of bacteria threaten their continued usefulness. Fluoroquinolone resistance is most often seen in hospital environments. Acquired resistance to ciprofloxacin and other fluoroquinolones has been reported in staphylococci, campylobacter, salmonella, E. coli and others (Trucksis, 1991, McDermott et al., 2002, Mitchell, 1997, Threlfall, 1997). Cross-resistance between the fluoroquinolones is possible and is the main reason behind the push to ban enrofloxacin from use in the poultry industry. In 1999, the European Union placed a ban on five growth promoting antibiotics in animal agriculture, however, resistance to human analogues of these antibiotics has continued to increase (Casewell et al., 2002). A ban is still under consideration in America by the Food and Drug Administration, but a recent study suggests banning enrofloxacin would increase human health risks of campylobacteriosis by
increasing the variance of microbial loads reaching people (Cox and Popken, 2003). These researchers also estimated the economic loss from increased chicken mortality if enrofloxacin was banned at $11,692,000 a year (Cox and Popken, 2003). This figure does not include the loss from smaller average bird weights and chickens that survive but don’t pass inspection. More research is needed to understand the development of fluoroquinolone-resistant bacteria, the impact it has on human and animal health, and the steps necessary to control this emerging problem.
Thromboelastography and Its Application in Detection of Coagulopathies

Many chemicals, including sulfonamide and fluoroquinolone antibiotics, can cause a coagulopathy. Prior to thromboelastography, disturbances in the hematopoietic system were studied by using routine clotting tests such as prothrombin time, activated partial thromboplastin time, and platelet count. Each of these tests results in an assessment of an isolated component of the coagulation system without giving any information on the interplay of each of these components with one another. Thromboelastography is unique because it allows for the use of an unmanipulated whole blood sample unlike other routine clotting tests which alter the original sample. Routine tests examine only a portion of the coagulation process while thromboelastography provides information on the totality of the interactions of the coagulation proteins and the blood borne cellular participants in the entire coagulation process.

Thromboelastography was originally described in the late 1940’s by Hartert (1948). His “new and easy to learn” method involved continuously measuring fibrin elasticity in whole blood. It involves the placement of a measured sample of whole blood into a sample cup. A plastic pin, suspended on a calibrated torsion wire, is then lowered into the sample. The cup oscillates through a $4^\circ 45'$ angle over a 10 second interval including a one second rest period at the end of each rotation in each direction. As fibers of the blood clot begin to form between the cup wall and the pin, the rotation of the cup is transmitted to the pin. This transfer of the rotational characteristics is converted into digital data that is displayed on a computer screen in real-time. This graphic representation, from which qualitative assessment and quantitative measurements can be made, is referred to as a thromboelastogram (TEG). The TEG begins as a straight line which diverges into symmetrical branches as clot formation begins and progresses through maximum clot strength.
Four measurements (R value, K, angle, & MA) are standard in defining the TEG. The R value or reaction time is measured in minutes from the beginning of the TEG until the tracing is 1 mm wide. The reaction time provides information on thrombin generation (Chandler, 1995). The R value is prolonged by anticoagulants and clotting factor deficiencies or inhibitors (Traverso et al., 1995).

The coagulation time (K) is measured in minutes from the end of R to the point of the tracing where the two branches of the curve are 20 mm wide. The coagulation time is a measurement of the rate at which a clot is formed to a defined strength and was arbitrarily defined by Hartert (Traverso et al., 1995). Traverso et al. (1995) described K as defining both fibrin formation and thrombin activity.

The angle is measured between the midline of the tracing and a line drawn from the 1 mm wide point tangential to the curve (Chandler, 1995). The angle relates the clot formation rate with clot strength and primarily reflects fibrinogen function (Traverso et al., 1995). The K and angle are influenced by thrombocyte concentration and clotting factor activity.

The maximum amplitude (MA), measured in millimeters, is the maximum width between the two branches of the tracing. The MA measures maximum clot strength and is influenced by thrombocyte and fibrinogen concentrations. Chandler (1995) reported that platelet number affected the MA more than fibrinogen.

De Nicola (1957) demonstrated that thromboelastography could be used for the comprehensive assessment of blood clotting. However, until the mid 1980’s, thromboelastography was used mainly for research as opposed to clinical purposes (Zuckerman et al., 1981; Kang et al., 1985). The use of thromboelastography to monitor coagulation in cancer patients undergoing surgery was raised at the 48th Congress of the International
Anesthesia Research Society (Howland, 1974). The Department of Hematology at the Mayo Clinic later expanded research into the diagnosis of bleeding patients using thromboelastography.

In the past two decades, thromboelastography has proven to be an effective monitor of coagulation in research and clinical applications. Thromboelastography has been used during liver transplantation to monitor preoperative coagulation profiles and intraoperative changes in coagulation (Kang et al., 1985). Using thromboelastography instead of prothrombin time, activated partial thromboplastin time, or platelet count, resulted in a 33% reduction in the required volume of fluid infusion. Thromboelastography has also been used to monitor a large number of cardiopulmonary bypass (CPB) patients (Spiess and Ivankovich, 1988). Routine coagulation tests had a false positive rate of 73% while thromboelastography gave a significantly less false positive rate (15%) for predicting abnormal bleeding after CPB (Tuman et al., 1989).

Thromboelastography has also been used to evaluate plasminogen activators as thrombolytic agents in the treatment of thromboembolic diseases (Summaria et al., 1986). Using induced clotting in the jugular vein of the dog, these investigators found agreement between fibrinolytic response in vivo and thromboelastographic analysis of fibrinolysis. The use of thromboelastography in animal coagulation research has been limited, however several experiments assessing normal blood coagulation and mycotoxin-induced and drug-induced coagulopathies in broiler chickens have also been performed (Miller et al., 1999).

The above stated observations demonstrate that thromboelastography can, and should, be used to characterize blood clotting. Thromboelastography has proven to be a superior method of analyzing blood clotting in research and clinical applications as opposed to routine blood clotting tests. Using thromboelastography will add to our knowledge of avian coagulation and abnormalities that can occur within the coagulation system.
References


STUDIES ON FOUR ANTIBIOTICS FOR POSSIBLE USE AGAINST *E. coli* IN BROILER CHICKENS

1 Manning, K.L. and R.D. Wyatt. To be submitted to Poultry Science.
ABSTRACT

The tube dilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC) for enrofloxacin, sulfaquinoxaline (SQ), sulfadimethoxine (SDM), and sulfamethazine (SMZ) against 40 strains of *E. coli* isolated from poultry. The average MIC for enrofloxacin was 0.288µg/mL and the average MBC was 3.95µg/mL. The MIC for all sulfonamides was >2200µg/mL and the average MBC for all sulfonamides was >8000µg/mL.

An antibiotic dosing experiment was performed to determine the maximum plasma concentration of each antibiotic in 7 week old broiler chickens. The chickens were orally dosed with an antibiotic and plasma samples were collected 4, 6, 8, 10 and 12 hours post-dosing. A bioassay was developed to detect the level of antibiotics in the plasma samples. The average maximum plasma level of enrofloxacin was 0.58µg/mL. The sulfonamides had average maximum plasma levels of 100µg/mL (SMZ), 320µg/mL (SDM) and 390µg/mL (SQ).

Enrofloxacin was the only antibiotic tested that had a maximum plasma level higher than the MIC. The sulfonamides all had high MIC levels and the maximum plasma levels were much lower which suggests the sulfonamides are inadequate as therapeutic agents for *E. coli* infections in broiler chickens.

(Key words: broiler chickens, sulfonamides, enrofloxacin, MIC, MBC, antibiotic bioassay)
INTRODUCTION

*Escherichia coli* infections are widespread in the poultry industry, resulting in significant economic losses each year (Gross, 1994). Although *E. coli* is commonly found in the gastrointestinal tract of animals, only certain pathogenic serotypes cause disease conditions. Avian colibacillosis generally begins with an infection of the upper respiratory tract, followed by septicemia (Gross, 1991). Antimicrobial therapy is an important tool in reducing the high incidence of morbidity and mortality caused by *E. coli* infections in poultry. However, the use of antibiotics in poultry has provided a selection pressure for antimicrobial resistance genes which has resulted in antibiotic-resistant bacterial strains associated with poultry meat (Quednau *et al*., 1998, Turtura *et al*., 1990).

Although many antibiotics have the potential to control *E. coli* infections in poultry, only a few are actually administered. The tetracyclines are efficacious, however, concern for antibiotic residues impede the export of meat from the United States. Penicillin, such as ampicillin and amoxicillin, are also active against *E. coli*, but the expense and growing resistance to these compounds do not make it a feasible choice for producers. The fluoroquinolones are a relatively new class of antimicrobials that exhibit excellent activity against gram-negative bacilli and are currently being used to treat avian colibacillosis (Stahlmann, 1988). The FDA is questioning the safety of using these antibiotics, however, due to possible carcinogenicity and the fear of developing cross-resistance to the fluoroquinolones used for treating human enteric infections (Blanco *et al*., 1997). The sulfonamides are an older class of antibiotics that are relatively inexpensive and effective against *E. coli*. Since the sulfonamides are not readily used in human medicine, cross-resistance is of little concern. Toxicities associated with
sulfaquinoxaline administration have been reported, however (Muskett & Seeler, 1947, Patterson & Grenn, 1975).

The purpose of this experiment was to assess four antibiotics (enrofloxacin, sulfaquinoxaline, sulfadimethoxine and sulfamethazine) for their possible use in chickens to treat *E. coli* infections. Antibiotic sensitivity to representative strains of *E. coli* from poultry for each antibiotic was determined and the therapeutic concentration of each antibiotic in the plasma was also determined.

**MATERIALS AND METHODS**

**MIC Determination**

*E. coli* isolates collected from clinical cases of airsacculitis in commercial poultry were acquired from the Oakwood Poultry Diagnostic Laboratory (Oakwood, GA) and the Poultry Diagnostic Research Center (University of Georgia, Athens, GA). The 40 *E. coli* isolates were maintained on nutrient agar slants at 5°C and transferred every two months. The tube dilution method (NCCLS, 1993) was used to determine the minimum inhibitory concentration (MIC) for enrofloxacin², sulfaquinoxaline³ (SQ), sulfadimethoxine³ (SDM), and sulfamethazine³ (SMZ) against all 40 *E. coli* isolates.

To determine the MIC for enrofloxacin, a two-fold serial dilution was prepared using test tubes, nutrient broth⁴ and enrofloxacin. The dilution series ranged from 0.0156 µg/mL to 4.0 µg/mL and a tube containing no enrofloxacin was also included as a control. A similar procedure was followed for the sulfonamides, except the dilution series was prepared with Mueller-Hinton broth⁴ and ranged from 31.25µg/mL to 8000µg/mL. The tubes were incubated

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² Bayer Corp., Shawnee Mission, KS 66201  
³ Sigma Chemical Co., St. Louis, MO 63103
for 24 hours at 37°C and then checked to ensure freedom from contamination. An inoculum for each isolate was prepared by transferring cells from the appropriate agar slant, inoculating a tube of nutrient broth, and allowing the inoculated tubes to incubate at 37°C for 24 hours. Each dilution series was then inoculated with one drop from the appropriate inoculum tube and incubated at 37°C for 24 hours. The tubes were then checked for visible growth of the organism. The MIC was defined as the minimum concentration of the antibiotic that inhibits visible growth of *E. coli*.

**MBC Determination**

The minimum bacteriocidal concentration (MBC) was also determined for the 4 antibiotics. The MBC was defined as the minimum concentration of an antibiotic that kills the bacteria. This was determined by plating samples of broth from the tubes used in the MIC determination which exhibited no visible growth, on nutrient agar. The nutrient agar plates were incubated for 24 hours at 37°C and then checked for visible growth of *E. coli*.

**Bioassay Development**

A bioassay to detect the concentration of enrofloxacin, SQ, SDM and SMZ in broiler chicken plasma was developed. Nutrient agar plates were prepared with *Bacillus subtilis* (American Type Culture Collection 6633) and 1.0ppm trimethoprim\(^5\) (for the sulfonamide plates only) added after autoclaving and cooling to 50°C. A vacuum pump with a stainless steel tube attached was used to create five 50µL wells in each plate via suction. A two-fold serial dilution of the antibiotic to be tested in deionized water was prepared. A 50µL sample of each dilution was placed in the appropriately labeled well and the plates were incubated at 37°C for 24 hours. The plates were checked the next day and all zones of inhibition (ZI) were measured with an

\(^4\) Difco, Sparks, MD 21152  
\(^5\) Sigma Chemical Co., St. Louis, MO 63103
electronic caliper. A standard curve was then constructed by transforming the data \( (y=y^2) \) and performing a linear regression for each antibiotic.

The plasma samples collected from the antibiotic dosing experiment were also analyzed using the above procedure except 50µL of plasma was pipetted into the wells. The ZI results were compared to the standard curve to determine the maximum plasma level for each antibiotic.

**Antibiotic Dosing Experiment**

An antibiotic dosing experiment was performed to determine the maximum plasma level of each antibiotic in broiler chickens. Day old chicks (*Gallus domesticus*) were obtained from a commercial source and fed the standard University of Georgia broiler starter ration *ad libitum* until 7 weeks of age. On the day of the experiment, the chickens were taken off feed two hours prior to dosing. The 20 chickens were weighed, banded, and placed in 4 pens (5 chickens per pen) with each pen designating a separate antibiotic. The chickens were orally dosed at time 0 with enrofloxacin (15mg/kg) or SQ, SDM, or SMZ (50mg/kg). A blood sample was taken from the brachial vein of each bird at time 0 and 4, 6, 8, 10 and 12 hours post dosing. The blood samples were immediately transferred to 1.8 mL heparinized polypropylene micro-centrifuge tubes. The samples were centrifuged and the plasma was collected and stored at 0°C until analysis. After all the samples had been collected, the plasma was thawed in a water bath and analyzed using the bioassay described above.

**Statistical Analysis**

Statistical analysis was performed on all data using Instat®6. An F-value was calculated using ANOVA, and means were compared using Tukey-Kramer multiple comparisons test for all means having equal standard deviations as determined by Bartlett’s test for homogeneity of variances. Kruskal-Wallis nonparametric ANOVA test was used to test for significance and
Dunn’s multiple comparisons test was used to compare means not having equal standard deviations.

RESULTS AND DISCUSSION

The average MIC results for each antibiotic are summarized in Table 3.1. The lowest concentration required to inhibit growth of *E. coli* isolates was achieved with enrofloxacin. The average MIC for enrofloxacin was 0.288µg/mL. However, this did not include 4 isolates that had an MIC <0.0156µg/ml or one isolate that had an MIC >4.0µg/mL. The MIC’s for the sulfonamides were all similar to each other. Sulfaquinoxaline was the most effective of the sulfonamides against the 40 *E. coli* isolates with an average MIC of 2250µg/mL. Sulfadimethoxine had an average MIC of 2750µg/mL and sulfamethazine was the least effective sulfonamide with an average MIC of 2900µg/mL.

The average MBC results for each antibiotic are summarized in Table 3.2. Enrofloxacin had the lowest concentration necessary to kill all growth of the *E. coli* isolates with an MBC of 3.95µg/mL. The sulfonamides all had high MBC’s of >8000µg/mL.

The average maximum plasma concentrations for each antibiotic are summarized in Table 3.3. The average time post-dosing that the maximum plasma level was achieved is also included for each antibiotic. Enrofloxacin had an average maximum plasma concentration of 0.58µg/mL. The average time post-dosing that this plasma level was attained was 6.0 hours. Sulfaquinoxaline had an average maximum plasma concentration of 390µg/mL. Sulfadimethoxine had an average maximum plasma concentration of 320µg/mL. Sulfamethazine was the lowest of the sulfonamides with an average maximum plasma concentration of

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6 GraphPad Software, Inc., San Diego, CA 92121
100µg/mL. The average time post-dosing that the maximum plasma levels were achieved was 7.2 hours for all three sulfonamides.

The effectiveness of four antibiotics against *E. coli* in broiler chickens was examined in this study. Enrofloxacin was the most effective antibiotic, however, it also had the most variation of the antibiotics. The plasma levels of enrofloxacin ranged from 0.54µg/mL to 0.65µg/mL with the average being 0.58µg/mL. The MIC for enrofloxacin ranged from 0.0313µg/mL to 4.0µg/mL. One isolate was considered resistant with an MIC >4.0µg/mL and four isolates had an MIC <0.0156µg/mL. The variation in this data suggests that a portion of the *E. coli* isolates tested may be acquiring resistance to enrofloxacin. However, at this time enrofloxacin is still the most effective antimicrobial used in this study to treat *E. coli* in chickens because the plasma levels achieved are still higher than 90% of the MIC’s measured.

The sulfonamides all had very similar MIC’s and MBC’s. The MIC for sulfaquinoxaline ranged from 1000µg/mL to 4000µg/mL while the MIC’s for sulfadimethoxine and sulfamethazine ranged from 2000µg/mL to 4000µg/mL. The average MIC’s for sulfaquinoxaline, sulfadimethoxine, and sulfamethazine were 2250µg/mL, 2750µg/mL, and 2900µg/mL, respectively. The most variation for the sulfonamides was the maximum plasma concentration achieved. The plasma concentration of sulfaquinoxaline ranged from 320µg/mL to 450µg/mL with the average being 390µg/mL. The plasma concentration of sulfadimethoxine ranged from 210µg/mL to 400µg/mL with the average being 320µg/mL. Sulfamethazine had a much lower plasma concentration that ranged from 80µg/mL to 160µg/mL with an average of 100µg/mL. All three sulfonamides would fall well short of inhibiting the *E. coli* isolates tested in vivo. Five times as much sulfaquinoxaline, 8 times as much sulfadimethoxine, and 29 times as much sulfamethazine would be needed in the plasma to inhibit these *E. coli* isolates in vivo.
Our results suggest that the sulfonamides tested are inadequate to control systemic *E. coli* infections in chickens. The similarities in the MIC’s of all three sulfonamides suggest that none of the sulfonamides possess the antimicrobial activity necessary to inhibit *E. coli*. Enrofloxacin was effective in inhibiting 36 out of the 40 *E. coli* isolates tested. Enrofloxacin is an adequate antibiotic to treat *E. coli* infections in chickens, however, increasingly resistant *E. coli* strains and cross-resistance with fluoroquinolones used in human medicine could limit its continued use in the poultry industry. Further research is necessary to find an alternative antibiotic that is effective against *E. coli* and is also prudent for use in animals.
REFERENCES


Table 3.1 Average Minimum Inhibitory Concentration (MIC) against E. coli using the tube dilution method.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>N</th>
<th>Average MIC (µg/mL)</th>
<th>Range (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrofloxacin</td>
<td>35*</td>
<td>0.288(^c)</td>
<td>&lt;0.0156 - &gt;4.0</td>
</tr>
<tr>
<td>Sulfaquinoxaline</td>
<td>40</td>
<td>2250(^b)</td>
<td>1000 - 4000</td>
</tr>
<tr>
<td>Sulfadimethoxine</td>
<td>40</td>
<td>2750(^a)</td>
<td>2000 - 4000</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>40</td>
<td>2900(^a)</td>
<td>2000 - 4000</td>
</tr>
</tbody>
</table>

*4 isolates had an MIC <0.0156 µg/mL and 1 isolate had an MIC >4.0 µg/mL. These isolates were not included in the average MIC calculation.

\(^a,b,c\)Means with the same superscript are not significantly different (p<0.05)
Table 3.2  Average Minimum Bacteriocidal Concentrations (MBC) of four antibiotics against *E. coli*.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>N</th>
<th>Average MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrofloxacin</td>
<td>40</td>
<td>3.95 µg/mL$^b$</td>
</tr>
<tr>
<td>Sulfaquinoxaline</td>
<td>40</td>
<td>&gt;8000 µg/mL$^a$</td>
</tr>
<tr>
<td>Sulfadimethoxine</td>
<td>40</td>
<td>&gt;8000 µg/mL$^a$</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>40</td>
<td>&gt;8000 µg/mL$^a$</td>
</tr>
</tbody>
</table>

$^{a, b}$Means with the same superscript are not significantly different (p<0.05).
Table 3.3  Average maximum plasma concentrations from 7 week old broiler chickens orally dosed with an antibiotic.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Average Maximum Plasma Level (µg/mL)</th>
<th>Average Time Post-Dosing (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrofloxacin</td>
<td>0.58&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sulfaquinoxaline</td>
<td>390&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sulfadimethoxine</td>
<td>320&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>100&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c,d</sup>Means with the same superscript in the same column are not significantly different (p<0.05).
ANTIBIOTIC-INDUCED COAGULOPATHY IN BROILER CHICKENS AS CHARACTERIZED BY THROMBOELASTOGRAPHY \(^1\)

\(^1\) Manning, K.L. and R.D. Wyatt. To be submitted to Poultry Science.
ABSTRACT

Broiler chickens were orally dosed with enrofloxacin (15mg/kg) once per day for 5 days (21-25 days of age). A blood sample was taken from the brachial vein of each bird and individually analyzed by thromboelastography. The R, K, and angle values were similar for the treatment compared to the control group, however the MA of the treatment chickens was significantly lower than the MA of the control chickens, suggesting a decrease in the number of thrombocytes or decreased fibrinogen concentration in the chickens treated with enrofloxacin.

Broiler chickens received feed with either sulfaquinoxaline (SQ), sulfadimethoxine (SDM), or sulfamethazine (SMZ) at a concentration of 1000ppm for 5 days (21-25 days of age). A blood sample was collected and analyzed using a thromboelastograph (TEG). The TEG values from chickens treated with SMZ were no different from control values. The K and angle values were significantly different from the control group in the chickens treated with SDM but the changes were not indicative of a severe coagulopathy. All TEG values were significantly different for the SQ-treated chickens compared to control values. The R and K values were prolonged and the angle and MA values were increased. This data suggests that SQ affects multiple components of the hemostatic mechanism, such as thrombin generation, fibrin formation, multiple clotting factors and thrombocyte number and function.

(Key words: thromboelastography, broiler chickens, sulfonamides, enrofloxacin, blood coagulation)
INTRODUCTION

Thromboelastography was originally described in the late 1940’s by Hartert (1948) but has recently been gaining popularity because it provides a panoramic measure of blood clotting. Thromboelastography is unique because it allows for the use of an unmanipulated whole blood sample unlike other routine clotting tests which require alteration of the original blood sample. Routine tests examine only a portion of the coagulation process while thromboelastography provides information on the interactions of the coagulation proteins and the blood borne cellular participants in coagulation.

Thromboelastography involves the placement of a measured sample of whole blood into a sample cup. A plastic pin, suspended on a calibrated torsion wire, is then lowered into the sample. The cup oscillates through a 4° 45’ angle over a 10 second interval including a one second rest period at the end of each rotation in each direction. As fibers of the blood clot begin to form between the cup wall and the pin, the rotation of the cup is transmitted to the pin. This transfer of the rotational characteristics is converted into digital data that is displayed on a computer screen in real-time. This tracing, from which qualitative assessment and quantitative measurements can be made, is referred to as a thromboelastogram (TEG). The TEG begins as a straight line which diverges into symmetrical branches as clot formation begins and progresses through maximum clot strength.

This technique has been used in human medicine to predict potential bleeding in adults after cardiopulmonary bypass (Tuman et al., 1989), to monitor the changes in blood coagulation during liver transplantation (Kang et al., 1985), and to detect hypercoagulability in patients with breast and colorectal cancer (Francis et al., 1994). The use of thromboelastography in animal coagulation research has been limited, however several experiments assessing normal blood
coagulation and mycotoxin-induced and drug-induced coagulopathies in broiler chickens have been performed (Miller et al., 1999).

Numerous agents, including antibiotics, are known to affect the coagulation process. Outbreaks of a hemorrhagic syndrome in poultry have been reported as a result of exposure to sulfaquinoxaline (Cover et al., 1955). Muskett reported that sulfaquinoxaline administration resulted in hypoprothrombinemia and death in canines (1947). Other case studies reported the deaths of several dogs due to hemorrhage into the small bowel following the administration of sulfaquinoxaline (Patterson et al., 1975, Osweiler et al., 1978). Since sulfaquinoxaline causes a coagulopathy, then other related sulfa drugs may also cause a coagulopathy. This experiment was undertaken to characterize the effect of 3 sulfonamide antibiotics (sulfaquinoxaline, sulfadimethoxine, sulfamethazine) and enrofloxacin, a commonly used fluoroquinolone antibiotic in poultry, on the coagulation of blood from broiler chickens.

MATERIALS AND METHODS

Thromboelastography

A 0.36 mL blood sample was pipetted into a pre-warmed (37°C) cup of the Thromboelastograph® (TEG™) Coagulation Analyzer² immediately after collection from the brachial vein. The TEG pin was lowered into the blood and raised three (3) times to mix the sample. After lowering the pin into the cup the third time, the sample was covered with a thin layer of heavy mineral oil to prevent drying of the sample during analysis. The TEG analysis was started 1.0 min from the time the blood sample was drawn into the syringe. Each TEG was allowed to run until maximum clot strength (MA) was established.

² Haemoscope Corporation, Skokie, IL 60077
Four measurements (R, K, Angle, & MA) are standard in defining the TEG (Figure 1). The R value or reaction time is measured in minutes from the beginning of the TEG until the tracing is 1 mm wide. The reaction time provides information on thrombin generation (Chandler, 1995). The R value is prolonged by anticoagulants and clotting factor deficiencies or inhibitors (Traverso et al., 1995).

The coagulation time (K) is measured in minutes from the end of R to the point of the tracing where the two branches of the curve are 20 mm wide. The coagulation time is a measurement of the rate at which a clot is formed to a defined strength and was arbitrarily defined by Hartert (Traverso et al., 1995). Traverso et al. (1995) defined K as describing both fibrin formation and thrombin activity.

The angle is measured between the midline of the tracing and a line drawn from the 1 mm wide point tangential to the curve (Chandler, 1995). The angle relates the clot formation rate with clot strength and primarily reflects fibrinogen function (Traverso et al., 1995). The K and angle values are influenced by thrombocyte concentration and clotting factors.

The maximum amplitude (MA), measured in millimeters, is the maximum width between the two branches of the tracing. The MA measures maximum clot strength and is influenced by thrombocyte and fibrinogen concentration. Chandler (1995) reported that platelet number affected the MA more than fibrin.

Sulfonamide Feeding Experiment

Broiler chickens (*Gallus domesticus*) were obtained from a local commercial broiler source at day of age. Thirty chickens were randomly placed in each of 6 pens of an electrically heated battery brooder for each antibiotic to be tested. The chickens were given standard University of Georgia broiler starter ration and water *ad libitum* for 3 weeks. At 21 days of age,
3 pens of chickens, designated as the treatment, were given feed containing 1000ppm of the appropriate sulfonamide, sulfaquinoxaline\(^3\) (SQ), sulfadimethoxine\(^3\) (SDM) or sulfamethazine\(^3\) (SMZ), for 5 days. Three pens were designated as control pens and continued receiving the original broiler starter ration. On day 26, a 0.5 mL blood sample was collected from the brachial wing vein of each treatment bird using a 0.5 inch, 26 gauge needle and a 1.0 mL syringe. After collection, the blood sample was transferred immediately to a 1.8 mL polypropylene micro-centrifuge tube. A 0.36 mL sample of this blood was placed immediately in the cup of the TEG and analyzed as described above. The control chickens underwent the same procedure on Day 27.

**Enrofloxacin Dosing Experiment**

Broiler chickens (\textit{Gallus domesticus}) were obtained from a local commercial broiler source at day of age. Thirty chickens were randomly placed in each of 6 pens of an electrically heated battery brooder. The chickens were given standard University of Georgia broiler starter ration and water \textit{ad libitum} for three weeks. Three pens of chickens, designated as the treatment, were weighed and orally dosed with 15mg/kg enrofloxacin (Baytril®)\(^4\), once per day, on days 21-25. The dosing regime for enrofloxacin exceeded the labeled dose for Baytril®, however, the intent was to exceed the highest therapeutic level to determine any impact on the hemostatic mechanism. On day 26, a 0.5 mL blood sample was collected from the brachial wing vein of each treatment bird using a 0.5 inch, 26 gauge needle and a 1.0 mL syringe. After collection, the blood sample was transferred immediately to a 1.8 mL polypropylene micro-centrifuge tube. A 0.36 mL sample of this blood was placed immediately in the cup of the TEG and analyzed as described above. The 3 pens of control chickens underwent the same procedure on day 27.

\(^3\) Sigma Chemical Co., St. Louis, MO 63103
\(^4\) Bayer Corp., Shawnee Mission, KS 66201
Statistical Analysis

Statistical analysis was performed on all data using Instat®. An unpaired t test was performed for each parameter and a p-value was calculated. Welch’s approximate t-test was used to calculate a p-value when standard deviations were considered significantly different (p<0.05).

RESULTS

The average TEG values for the sulfaquinoxaline (SQ) feeding experiment are summarized in Table 4.1. There was a significant difference between treatment and control chickens for all four TEG values. The R and K times were significantly increased, while the angle and MA values were significantly decreased when compared to the control group. All four TEG values were significantly different from the control group (p<0.01).

The average TEG values for the sulfadimethoxine (SDM) feeding experiment are summarized in Table 4.2. The R and MA values for the treatment group were not considered significantly different than those in the control group. The K and angle values in the treatment group were significantly different (p<0.05) however, the K time decreased and the angle increased which suggests a hypercoagulability.

The average TEG values for the sulfamethazine (SMZ) feeding trial are summarized in Table 4.3. There was no significant difference found between the treatment and control group for any of the TEG values. The R, K, angle and MA values are similar for all chickens tested with the least amount of variability observed among the different antibiotics.

The average TEG values for the enrofloxacin feeding experiment are summarized in Table 4.4. There was no significant difference found between the control and treatment groups.
for the R, K, and angle values. There was, however, a significant difference between the MA value of the controls and treatment chickens (p<0.01). The enrofloxacin treated chickens had a lower MA which correlates with decreased clot strength.

DISCUSSION

Thromboelastography was used in this study to characterize the coagulation of broiler chicken blood after receiving either sulfaquinoxaline, sulfadimethoxine, sulfamethazine, or enrofloxacin. The chickens receiving sulfamethazine did not show any significant changes in blood clotting as compared to the control group. A significant difference was seen in the K and angle values for the group of chickens receiving sulfadimethoxine, however this difference from the control group indicates a mild hypercoagulation which is the opposite reaction of sulfaquinoxaline and enrofloxacin. The sulfaquinoxaline treatment group displayed a disruption in coagulation with all four TEG values differing significantly from the control group (p<0.01). Finally, the chickens receiving enrofloxacin had a decreased MA value that was significantly different from the control group (p<0.01) but the other three TEG values were within normal range.

The R value is similar to the whole blood clotting time in that it is a measure of the time required for the first fibrin strands to form. Except for the chickens in the SQ treatment group, the average values of R reported in this study are similar to some whole blood clotting times reported for poultry. Stopforth (1970) found average Lee White whole blood clotting times of 5.1 min in cockerels, 9.2 min in pullets, and 4.5 min in capons when blood samples were obtained via cardiac puncture. However, Bigland and Starr (1965) obtained average whole blood clotting times in 8 and 24 week old chickens of 59 and 46 min, respectively, when samples were allowed

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5 GraphPad Software, Inc., San Diego, CA 92121
to flow freely from the alar vein using 18 to 21 gauge needle tubing. Each of the investigators
above found very wide ranges of values for whole blood clotting times in all groups tested. The
range of R measured in this study was 1.0 – 32.0 min for all control groups, 3.5 – 30.5 min for
the enrofloxacin treated group, 3.0 – 53.0 min for the SQ treated group, 1.0 – 9.5 min for the
SDM treated group, and 1.5 – 4.0 min for the SMZ treated group. Even though the R varies
greatly, the average R value for the SQ treated group was found to be significantly different than
the control group. Four out of the 15 SQ treated chickens had R times of 3.0, 5.0, 7.0 and 12.5
min, however, the 11 remaining chickens all had R times of greater than 25.0 minutes with three
times being greater than 50.0 min. Of the four antibiotics tested, sulfaquinoxaline was the only
one that significantly affected clot initiation time in 6 week old broiler chickens.

The K and angle values measure the rate at which the clot structure is formed (Chandler,
1995; Traverso et al., 1995). Increased values of K and decreased values of angle have been
reported with clotting factor deficiencies, platelet dysfunction and hypofibrinogenemia in
humans (Mallet and Cox, 1992). The K describes both thrombin activity and fibrin formation and
the angle is primarily a reflection of the fibrinogen concentration in the blood (Traverso et al.,
1995). The K values measured in this experiment for all control groups ranged from 1 – 9 min
but only one measurement was over 5 min. The K value for the enrofloxacin, SDM, and SMZ
groups ranged from 1 – 6 min and one measurement was over 2 min. The K value for the SDM
treated group was significantly different from the control group (p<0.05). The average K value
for the SDM group was 1.1 min and it was 1.6 min for the control group. Increased values of K
are indicative of a hypocoagulopathy so the decrease in K in the SDM treatment group indicates
a mild hypercoagulopathy. The increase in K for the SQ treatment group is significantly
different from the control group (p<0.01). The average value of K for the control group was 2.5
min and the average value for the SQ treatment group was 7.0 min. This suggests that sulfaquinoxaline may affect both thrombin activity and fibrin formation.

The angle values in this experiment for the control groups ranged from 27.5 – 82.5° with most values between 65.0 – 75.0°. The angle values for the treatment groups ranged from 8.0 – 82.5°. The angle value for the SDM treated group was significantly different from the control group (p<0.05). The angle value for the SDM treated group was higher than the angle value for the controls so, once again, this should be considered hypercoagulation. The decrease in the angle value for the SQ treatment group is an indication of a coagulopathy. The average angle value for the SQ group was 39° which is significantly different from the control group (p<0.01). This data suggests a decrease in the fibrinogen concentration in the blood of the chickens treated with sulfaquinoxaline.

The MA is a measure of the maximum strength of the clot formed. Significant correlations have been found between the MA and platelet number and function and fibrinogen concentration in humans (Tuman et al., 1991; Mallett and Cox, 1992; Chandler, 1995). The MA values for the control groups in this experiment ranged from 48.0 – 91.0mm with the average being 68.0mm. The MA range for all the treatment groups was 43.5 – 76.5mm. The average MA values for the SDM and SMZ treatment groups were 68.0mm and 66.0mm, respectively. These values were not significantly different from the controls. However, the MA values for the SQ and enrofloxacin treated groups were found to be significantly different from the controls (p<0.01). The MA value for the sulfaquinoxaline treatment group ranged from 43.5mm to 72.5mm with the average being 59.0mm. The MA value for the enrofloxacin treatment group ranged from 56.5mm to 68.5mm with the average being 63.5mm. The decrease in the MA for
the SQ and enrofloxacin treated groups indicates a decrease in the final strength of the clot formed and is most likely due to decreased thrombocyte number and/or function.

Thromboelastography has been used to demonstrate a coagulopathy in broiler chickens receiving sulfaquinoxaline and enrofloxacin for 5 days. The TEG abnormalities in the sulfaquinoxaline-fed chickens revealed a decrease in the rate of clot formation coupled with a decrease in final clot strength, whereas the TEG abnormalities in the enrofloxacin-dosed chickens revealed only a decrease in final clot strength. Thus, thromboelastography is capable of providing more descriptive information about a coagulopathy than are the isolated coagulation tests which measure only one aspect of blood clotting. The decrease in the rate of clot formation for the sulfaquinoxaline treated chickens is due to a deficiency in Factor X (Stuart-Prower factor), which is a vitamin K dependent clotting factor synthesized in the liver (Patterson and Grenn, 1975). The decrease in the final clot strength for both antibiotics is most likely due to a decrease in thrombocyte number and/or function but may also be due to a decrease in fibrinogen concentration (Chandler, 1995). Further study is required to completely understand the mechanism of action of sulfaquinoxaline and enrofloxacin on thrombocytes and fibrinogen in broiler chickens.
REFERENCES


Table 4.1  TEG parameters\(^1\) of native whole blood from 3 wk old broiler chickens fed sulfaquinoxaline at 1000ppm for 5 days\(^2\)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>R (min)</th>
<th>K (min)</th>
<th>MA (mm)</th>
<th>Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.8</td>
<td>2.5</td>
<td>69.6</td>
<td>64.0</td>
</tr>
<tr>
<td></td>
<td>±8.2</td>
<td>±2.3</td>
<td>±9.4</td>
<td>±15.0</td>
</tr>
<tr>
<td>SQ</td>
<td>29.1</td>
<td>7.2</td>
<td>59.3</td>
<td>39.0</td>
</tr>
<tr>
<td></td>
<td>±16.9</td>
<td>±4.5</td>
<td>±8.4</td>
<td>±22.2</td>
</tr>
<tr>
<td>Difference</td>
<td>18.3**</td>
<td>4.7**</td>
<td>10.3**</td>
<td>25.0**</td>
</tr>
</tbody>
</table>

\(^1\)TEG parameters (R, K, MA, & Angle) are explained in the text.

\(^2\)Values are reported as mean ± standard deviation.

*denotes significant difference between control and treatment group (p<0.05)

**denotes significant difference between control and treatment group (p<0.01)
Table 4.2  TEG parameters\(^1\) of native whole blood from 3 wk old broiler chickens fed sulfadimethoxine at 1000ppm for 5 days\(^2\)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>R</th>
<th>K</th>
<th>MA</th>
<th>Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(min)</td>
<td>(min)</td>
<td>(mm)</td>
<td>(°)</td>
</tr>
<tr>
<td>Control</td>
<td>5.2</td>
<td>1.6</td>
<td>69.3</td>
<td>64.9</td>
</tr>
<tr>
<td></td>
<td>±3.4</td>
<td>±0.7</td>
<td>±3.5</td>
<td>±15.3</td>
</tr>
<tr>
<td>SDM</td>
<td>3.3</td>
<td>1.1</td>
<td>68.0</td>
<td>76.0</td>
</tr>
<tr>
<td></td>
<td>±2.1</td>
<td>±0.5</td>
<td>±4.7</td>
<td>±5.6</td>
</tr>
<tr>
<td>Difference</td>
<td>1.9</td>
<td>0.5*</td>
<td>1.3</td>
<td>11.1*</td>
</tr>
</tbody>
</table>

\(^1\)TEG parameters (R, K, MA, & Angle) are explained in the text.

\(^2\)Values are reported as mean ± standard deviation.

*denotes significant difference between control and treatment group (p<0.05)

**denotes significant difference between control and treatment group (p<0.01)
Table 4.3  TEG parameters\(^1\) of native whole blood from 3 wk old broiler chickens fed sulfamethazine at 1000ppm for 5 days\(^2\)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>R</th>
<th>K</th>
<th>MA</th>
<th>Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(min)</td>
<td>(min)</td>
<td>(mm)</td>
<td>(°)</td>
</tr>
<tr>
<td>Control</td>
<td>3.0</td>
<td>1.0</td>
<td>67.4</td>
<td>76.0</td>
</tr>
<tr>
<td></td>
<td>±1.6</td>
<td>±0.3</td>
<td>±3.3</td>
<td>±6.5</td>
</tr>
<tr>
<td>SMZ</td>
<td>2.6</td>
<td>1.1</td>
<td>66.3</td>
<td>75.0</td>
</tr>
<tr>
<td></td>
<td>±1.0</td>
<td>±0.3</td>
<td>±5.4</td>
<td>±5.5</td>
</tr>
<tr>
<td>Difference</td>
<td>0.4</td>
<td>0.1</td>
<td>1.1</td>
<td>1.0</td>
</tr>
</tbody>
</table>

\(^1\)TEG parameters (R, K, MA, & Angle) are explained in the text.

\(^2\)Values are reported as mean ± standard deviation.

*denotes significant difference between control and treatment group (p<0.05)

**denotes significant difference between control and treatment group (p<0.01)
Table 4.4  TEG parameters\(^1\) of native whole blood from 3 wk old broiler chickens orally dosed with 15mg/kg enrofloxacin once per day for 5 days\(^2\)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>R (min)</th>
<th>K (min)</th>
<th>MA (mm)</th>
<th>Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.4 ± 5.3</td>
<td>1.8 ± 1.1</td>
<td>67.9 ± 4.6</td>
<td>68.0 ± 10.0</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>9.2 ± 6.9</td>
<td>1.9 ± 1.5</td>
<td>63.5 ± 3.6</td>
<td>62.0 ± 17.9</td>
</tr>
<tr>
<td>Difference</td>
<td>1.2 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>4.4** ± 3.6</td>
<td>6.0 ± 17.9</td>
</tr>
</tbody>
</table>

\(^1\)TEG parameters (R, K, MA, & Angle) are explained in the text.

\(^2\)Values are reported as mean ± standard deviation.

* denotes significant difference between control and treatment group (p<0.05)

** denotes significant difference between control and treatment group (p<0.01)
CONCLUSION

The overall objective of this research was to assess the use of four antibiotics in the treatment of *E. coli* infections in broiler chickens. The four antibiotics tested were enrofloxacin, sulfaquinoxaline (SQ), sulfadimethoxine (SDM), and sulfamethazine (SMZ). The effectiveness of the antibiotics against *E. coli* was tested by determining the minimum inhibitory concentration (MIC) for each antibiotic and coagulopathies caused by these antibiotics were assessed using thromboelastography.

The tube dilution method was used to determine the MIC for the four antibiotics against 40 strains of *E. coli* isolated from poultry. The average MIC for enrofloxacin was 0.288µg/mL and the average MIC’s for the sulfonamides ranged from 2250µg/mL to 2900µg/mL. The minimum bacteriocidal concentration (MBC) was also determined for the four antibiotics.

An antibiotic dosing trial was performed to determine the maximum plasma level of each antibiotic in 7 week old broiler chickens. The chickens were orally dosed with an antibiotic and plasma samples were collected 4, 6, 8, 10 and 12 hours post-dosing. A bioassay was developed to detect the level of antibiotics in the plasma samples. This was used to determine the maximum plasma level of each antibiotic in broiler chickens.

Enrofloxacin was the only antibiotic tested that had a maximum plasma level higher than the average MIC. The sulfonamides all had high MIC levels but the maximum plasma levels were much lower suggesting that the sulfonamides are not an adequate choice to treat systemic *E. coli* infections in broiler chickens.
Thomboelastography (TEG) values for the reaction rate (R), coagulation time (K), clot formation rate (angle) and maximum amplitude (MA) were determined for native whole blood from 3 wk old broiler chickens after receiving either SQ, SDM, SMZ or enrofloxacin. Broiler chickens were orally dosed with enrofloxacin (15mg/kg) once per day for 5 days (21-25 days of age). A blood sample was then drawn from the brachial vein of each bird and individually analyzed by thromboelastography. The R, K, and angle values were all similar for the treatment versus the control group, however the MA of the treatment chickens was significantly lower than the MA of the control chickens, suggesting a decrease in the number of thrombocytes or decreased fibrinogen concentration in the chickens treated with enrofloxacin.

Broiler chickens received feed with either SQ, SDM, or SMZ at a concentration of 1000ppm. The chickens were allowed to consume this feed ad libitum for 5 days (21-25 days of age) before a blood sample was drawn and analyzed using a thromboelastograph. The chickens treated with SMZ had no significant differences in TEG values. The K and angle values were significantly different from the control group in the chickens treated with SDM but the levels were not indicative of a severe coagulopathy. All TEG values were significantly different for the SQ-treated chickens. The R and K values were prolonged and the angle and MA values were increased. This data implies that SQ affects thrombin generation, fibrin formation, clotting factors and thrombocyte number and function and should not be administered to broiler chickens.

This research suggests enrofloxacin is an effective antibiotic against systemic E. coli in broiler chickens. However, enrofloxacin may cause a coagulopathy in broiler chickens involving decreased thrombocytes and/or decreased fibrinogen concentration. More research is needed to evaluate the risks versus the benefits of administering enrofloxacin to control E. coli infections in broiler chickens.