INFLUENCE OF SODIUM HYPOCHLORITE ON PHARMACOKINETICS OF ENROFLOXACIN IN BROILER CHICKEN

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This is to certify that the thesis entitled "**INFLUENCE OF SODIUM HYPOCHLORITE ON THE PHARMACOKINETICS OF ENROFLOXACIN IN BROILER CHICKEN"** submitted in partial fulfillment of the requirements for the degree of "**MASTER OF VETERINARY SCIENCE"** of **SRI VENKATESWARA VETERINARY UNIVERSITY, TIRUPATI**, is a record of the bonafide research work carried out by **Ms. CHELSIA YATHATI, ID NO: GVM/2017-039** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of the investigations have been duly acknowledged by the author of the thesis.

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CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

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DECLARATION

I, Ms. CHELSIA YATHATI, I.D.No. GVM/17-039 hereby declare that the thesis entitled **"INFLUENCE OF SODIUM HYPOCHLORITE ON THE PHARMACOKINETICS OF ENROFLOXACIN IN BROILER CHICKEN"** submitted to Sri Venkateswara Veterinary University, Tirupati for the degree of **MASTER OF VETERINARY SCIENCE** is the result of original research work done by me. I also declare that the materials contained in this thesis have not been published earlier in any manner.

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ABSTRACT

An experimental study was conducted on broiler chicken weighing around 2 kg to know the influence of sodium hypochlorite in drinking water on the oral pharmacokinetics of enrofloxacin. The birds were divided into three groups with eight birds in each. Group I birds that were on normal drinking water received enrofloxacin orally at the dose of 10 mg/kg body weight. Group II birds received sodium hypochlorite in drinking water (10 ml/100L) for seven days followed by enrofloxacin orally (10 mg/kg) on the seventh day. Group III birds also received sodium hypochlorite for seven days but enrofloxacin was given orally (10 mg/kg) 12 hours post withdrawal of sodium hypochlorite containing water. Blood samples from all the treated groups were collected from either left or right tarsal veins at 0 (blank), 0.16, 0.33, 0.5, 0.75, 1,

1.5, 2, 4, 6, 8, 12, 24 and 48 h post enrofloxacin dosing and plasma was separated and analyzed by HPLC method.

It was observed that $t_{1/2}$ and t_{max} did not differ significantly ($p<0.05$) in all the three groups under study. Elimination rate constant, β was observed significantly ($p<0.05$) increased in Group III (0.077±0.005 l/h) and Group II (0.071±0.009 l/h) birds compared to that in Group I (0.040±0.002 l/h) birds. There was a noticeable decline in C_{max} of enrofloxacin in Group II (1.793±0.160 µg/ml) and Group III (1.958±0.147µg/ml) birds over Group I (2.153±0.245 µg/ml) birds, although not statistically significant ($p<0.05$). The AUC_{0-t} was apparently decreased though statistically not significant ($p<0.05$) in both Groups II (31.587±4.241 µg/ml.h) and Group III (33.669±3.593 µg/ml.h) birds. $AUC_{0-\alpha}$ recorded in Group II (32.978±4.087 µg/ml.h) birds was significantly ($p<0.05$) low when compared to that in Group I (50.648±6.111 µg/ml.h) birds. Thus, the influence of sodium hypochlorite exposure on total exposure to enrofloxacin across time is evident. Likewise, administration of enrofloxacin, 12 hours post withdrawal of sodium hypochlorite (Group III) also resulted in reduced $AUC_{0-\alpha}$ (34.751±3.681 µg/ml.h) of enrofloxacin, though statistically not significant ($p<0.05$). The area under first moment curve (AUMC) and mean residence time (MRT) recorded in Group III (525.468±61.695 µg/ml.h^2 and 15.088±0.431 h) and Group II (538.396±61.064 µg/ml.h^2 and 16.484±0.748 h) birds were significantly ($p<0.05$) lower than those recorded in Group I (1407.185±216.254 µg/ml.h^2 and 27.076±1.375 h) birds. Sodium hypochlorite exposure or its exposure till 12 hours before the administration of enrofloxacin could not retain the enrofloxacin molecules for a period similar to that observed in control Group I. Although there was no influence of sodium hypochlorite on the volume of distribution (V_d/F), the total body clearance of enrofloxacin observed in Group III (0.310±0.032 l/kg/h) and Group II (0.329±0.031 l/kg/h) birds was significantly ($p<0.05$) higher when compared to that in Group I (0.216±0.022 l/kg/h)

birds. It can be attributable to altered environment in the renal mechanisms involved in elimination of enrofloxacin or it metabolite(s).

The study revealed that, sodium hypochlorite administration altered the pharmacokinetics of the enrofloxacin and the effect of sodium hypochlorite persisted even after its withdrawal 12 hours before the administration of enrofloxacin.

LIST OF SYMBOLS AND ABREVIATIONS

α	:	Distribution rate constant
β	:	Elimination rate constant
%	:	Per cent
@	:	At the rate of
µg	:	Microgram
µm	:	Micrometer
µl	:	Microlitre
µg/ml	:	Microgram per millilitre
µg .ml. h^2	:	Microgram per millilitre per square hour
µg.ml.h	:	Microgram per millilitre per hour
$^{\circ}C$	:	Degree centigrade
ANOVA	:	Analysis of variance
AUC_{0-t}	:	Area under the plasma concentration time curve
$AUC_{0-\infty}$	:	Area under the plasma concentration time from 0 h to infinity
AUMC	:	Area under the first moment curve
B.wt	:	Body weight
Cl_B	:	Total body clearance
C_{max}	:	Maximum (peak) plasma concentration
et al.,	:	And associates
F	:	Bioavailability

FDA	:	Food and Drug Administration
Fig.	:	Figure
GIT	:	Gastrointestinal tract
g	:	Gram
HPLC	:	High performance liquid chromatography
h	:	Hour(s)
HCl	:	Hydrochloric acid
i/m	:	Intramuscular
i/v	:	Intravenous
kg	:	Kilogram
l	:	Litre
Ltd.	:	Limited
MIC	:	Minimum inhibitory concentration
mg	:	Milligram(s)
min	:	Minute(s)
ml	:	Millilitre
MRT	:	Mean residence time
NaOH	:	Sodium hydroxide
NaOCl	:	Sodium hypochlorite
nm	:	Nanometer
$P<0.05$	:	Significant at 5% level
pH	:	Potential of hydrogen
Pk	:	Pharmacokinetic

ppm	:	Parts per million
Pvt.	:	Private
R^2	:	Regression correlation
rpm	:	Rotations per minute
SE	:	Standard error
SPSS	:	Statistical Package for the Social Sciences
t_{max}	:	Time of maximum concentration in plasma
$t_{0.5abs.}$	:	Absorption half-life
$t_{1/2\alpha}$	:	Distribution half life
$t_{1/2\beta}$	:	Elimination half life
$Vd_{(area)}$	:	Apparent volume of distribution
$Vd_{(ss)}$	:	Volume of distribution at steady state

INTRODUCTION

CHAPTER -I

INTRODUCTION

Poultry farming has evolved over the years to garner the major share of national agro based economy. It is also recognised as an industry with tremendous employment potential and effective means to combat malnutrition in weaker sections of the society. The worth of an industry to the economy and social well being of a community is reflected in accordance with certain factors, which may adversely affect the industry. One such factor influencing poultry industry is disease, which has devastating effects particularly on intensive production. Thus recognition, treatment or prevention of disease is of crucial importance and a subject of much research and investigation.

Maintaining drinking water quality for poultry is an important nutritional aspect as birds consume water at twice the level of feed. Hence, ensuring that the birds are provided with clean and quality water is a critical component in commercial poultry production. Multiple disinfectants and sanitizers such as those belonging to chlorides, iodides, quaternary ammonium compounds etc., are widely used in the poultry farms to optimize a good water quality program. The goal of these water disinfectants is to greatly reduce or eliminate the presence of microorganisms.

The antimicrobial properties of enrofloxacin prove it to be a more efficient drug for use in poultry farming. It is a synthetic bactericidal agent of fluroquinolones group. The properties of enrofloxacin that make it an important antimicrobial agent in poultry include broad spectrum of activity, bactericidal and mycoplasmicidal activity at low concentrations, efficacy against organisms that are resistant to antimicrobial substances such as beta-lactam antibiotics, aminoglycosides, tetracyclines, folic acid antagonists and macrolides, good tolerance and absorption after

parentral and oral administrations (Brown 1996). Similar to other quinolones the bactericidal activity of enrofloxacin is mediated by affecting bacterial DNA gyrase.

The most important factor that determines the clinical efficacy of an antimicrobial is its ability to reach the site of infection in desired concentration and its persistence within the tissues. Pharmacokinetic variables such as plasma concentration, half-life, bioavailability, and rate of elimination are to be considered for coherent use of antimicrobial agents. Thus pharmacokinetic studies of an antimicrobial drug provide valuable information for determining optimal dosage regimen in target animal species.

Enrofloxacin is administered usually through oral route in poultry. However, chlorine-based sanitizers like sodium hypochlorite possess high oxidation reactivity (Acero *et al.*, 2010) and hence the possible chemical interactions that may occur with simultaneously administered enrofloxacin will influence the absorption and bioavailability of the enrofloxacin.

In this context, the present study was designed in broiler chicken with the following objectives:

1. To study the pharmacokinetics of enrofloxacin in broiler chickens.
2. To know the influence of sodium hypochlorite on the oral pharmacokinetics of enrofloxacin.
3. To know if the withdrawal of sodium hypochlorite 12 hours before the administration of enrofloxacin will have any effect on oral pharmacokinetics of enrofloxacin or not.

REVIEW OF LITERATURE

CHAPTER –II

REVIEW OF LITERATURE

Water is the most important nutrient and a physiological requirement for all animals including poultry. Besides, the production perspective, providing adequate and good quality water is listed as a basic animal welfare criterion. Water is presumed safe if it has a zero microbial population, provided that mineral content is at safe levels and undesired contaminants are not present. However, presence of microbes in water is not always correlated with a disease in flocks unless it increases above a certain infectious level.

Sanitation and disinfection of water are the main part of an effective biosecurity program in poultry operations to prevent entry of disease agents and food borne pathogens in birds. Chemically, water sanitation and disinfection are carried out using powerful oxidizers such as chlorine and oxygen reactive species or by using heavy metal ions such as silver and copper or in synergism with the oxidizers and heavy metals. Physically, it is carried out by using ultraviolet rays and ultrasonic means.

These agents act specifically against microbes and their general biocidal activity can be explained by their ability to oxidize or rupture the cell wall of microorganisms or to diffuse into cells and interfere with the cellular metabolism (Cho *et al*., 2010; Denyer *et al*., 1998). Increased efficacy is attained by cleaning away organic matter and then applying the disinfectant (Stringfellow *et al*., 2009). At high concentrations most of these agents act in random and non specific ways against microbes (Maillard *et al*., 2002).

2.1 Commonly used sanitizers

Most commonly used chemical sanitizes in poultry for drinking water sanitation are halogens (iodophores, chlorine containing compounds), oxidizing agents (peroxyacetic acid, hydrogen peroxide) and quaternary ammonium compounds.

2.1.1 Halogens

Among the halogens the frequently used sanitizers are either iodophores or chlorine containing compounds.

2.1.1.1 Iodophores (iodine-polyvinylpyrrolidone)

They are often utilized to disinfect piping and waterers and to reduce bacterial load in water. Their efficacy is based on their high reactivity with chemical and organic entities. These compounds are less active than hypochlorites but are effective sanitizers and disinfectants. Iodophores attach to the sulfurs of proteins such as cysteine, causing inactivation and cell wall damage (Mc Donnell, 2007). Carriers with iodophores solutions allow sustained release effect resulting in continuous microbial mortality.

Iodophores fare better in situations in which the pH is slightly acidic, as less active forms exist above neutral pH. The common concentration for sanitization is 25 ppm for 1 minute. Unfortunately, iodine compounds easily stain many surfaces, particularly plastics. On the plus side, they are common sanitizers used on glass surfaces. The EPA has assessed iodophores as having no significant effect on the environment (EPA 2006).

2.1.1.2 Chlorine containing compounds

In poultry farms, the commonly used chlorine containing compounds for drinking sanitation are hypochlorites and chlorine dioxide.

2.1.1.2.1 Hypochlorites

Effectiveness, low cost and ease of manufacturing make hypochlorites the most widely used sanitizers. Hypochlorites cause broad microbial mortality by damaging the outer membrane, likely producing a loss of permeability control and eventual lysis of cell (Venkobachar *et al*., 1977; Vitro *et al*., 2005). These when dissolved in water produce hypochloric acid. Exposure of strains of *Escherichia coli, Pseudomonas* and *Staphylococcus* to lethal doses of hypochloric acid causes a decrease in ATP production (Barrette *et al*., 1989). Hypochlorites inhibit cellular enzymes and destroy DNA. Spores are resistant to hypochlorites, as the spore coat is not susceptible to oxidation except at high concentrations coupled with long contact times at elevated temperatures (Pfuntner, 2011). They are typically used at 0.02-0.2% levels of available chlorine for disinfection, and require 1-10 min contact time (Merianos, 1991). Commonly used hypochlorites are sodium hypochlorite and calcium hypochlorite. Sodium hypochlorite is most common compound and is an ideal sanitizer, as it is a strong oxidizer.

2.1.1.2.2 Chlorine dioxide

This is another successfully used water disinfectant for sanitizing poultry drinking water. This inorganic compound is a broad sanitizer effective against bacteria, fungi and viruses. Chlorine dioxide is an oxidizer that reacts with the proteins and fatty acids within the cell membrane, resulting in loss of permeability control and disruption of protein synthesis (Berg *et al*., 1986 and EPA 1999). It acts as a selective oxidant as it has a single electron transfer mechanism and reduces to form chlorite ion which exists as the dominant species in water. Chlorine dioxide is produced on-site as it can’t be compressed or stored commercially in gaseous form. Most chlorine dioxide generation is accomplished with complex systems. However, recent advances in formulation procedures allow the production of solutions of chlorine dioxide on-site without the use

of expensive equipment. Compared with hypochlorites, chlorine dioxide requires much lower concentrations to achieve microbial mortality. Disinfection can be achieved with 100 ppm using contact time of 10 minutes (Pfuntner, 2011).

Chlorine dioxide reacts more selectively with compounds present in microbial cells as opposed to reacting with organic compounds in general. This ability allows chlorine dioxide to function in more organically loaded solutions, though as organic load increases, efficacy does decrease. Chlorine dioxide functions well over a pH range of about 6 to 10, thus allowing increased mortality of some microbes at higher values. Another advantage is that chlorine dioxide does not form chlorinated organic compounds, making it more environmental friendly (Berg *et al*., 1986).

2.1.2 Oxidizing agents

Most commonly used oxidizing agents in poultry are peroxyacetic acid and hydrogen peroxide.

2.1.2.1 Peroxyacetic acid

Peroxyacetic acid is an effective sanitizer that is active against many microorganisms and their spores. Peroxyacetic acid-based sanitizers function well under cold conditions (4°C). Peroxyacetic acid is also effective in removing biofilms and is more active than hypochlorites (Schmidt, 2003). It can be attenuated by the organic load and will begin to lose activity as the pH approaches neutral. These sanitizers are also less corrosive to equipment than hypochlorites. Microbial mortality is produced by the disruption of chemical bonds within the cell membrane (Block, 2001). Peroxyacetic acid oxidises and denatures proteins and lipids of microorganisms, leading to disorganization of the membrane. Swelling of microbial cell may take place due to saturation of H^+ ions, which attract water (Russell, 1991).

2.1.2.2 Hydrogen peroxide

Recent field experiences have shown that poor performing farms are greatly benefitted from water sanitation programs using hydrogen peroxide which is an alternative disinfectant to chlorine. Hydrogen peroxide produces mortality in microbes by the disruption of chemical bonds within the cell membrane (Block, 2001). These sanitizers function well under cold conditions (4°C), thus producing acceptable microbial mortality on equipment normally held below ambient temperature. It is also effective in removing biofilms and is more active than hypochlorites (Schmidt, 2003). Hydrogen peroxide has a rapid bactericidal action and is an effective sanitizer that is active against many microorganisms like viruses, yeast, and fungi including their spores. These solutions can be attenuated by the organic load and will begin to lose activity as the pH approaches neutral. These solutions are applied at concentrations ranging from about 80 ppm to 600 ppm. They are environmental friendly and are less corrosive to equipment than hypochlorites (Maharjan and Watkins, 2016).

2.1.3 Quaternary ammonium compounds

Quaternary ammonium compounds are surface-active agents, which on account of their particularly desirable attributes, are finding greatly increased usage as sanitizing agents, disinfectants and germicides. Two types of quaternary ammonium compounds mostly used as disinfectants are alkyldimethylbenzylammonium chloride and dialkyldimethylammonium chloride. Quaternary ammonium compounds are fairly complex chemicals in which nitrogen is bound to four organic groups. The positively charged cations in the compounds bind with the acidic phospholipids in the microbial cell wall (Mc Bain *et al*., 2004). This action blocks the uptake of nutrients into the microbial cell and prevents the discharge of waste. They are effective against a wide range of microbes, although the spore phase is unaffected. The efficacy of quaternary

ammonium compounds against specific bacteria varies with their hydrocarbon chain length (Merianos, 1991).

At low concentrations Gram positive organisms are more sensitive than Gram negative organisms. They may be applied at concentrations varying from about 100 ppm to 400 ppm. As sanitizers, these are commonly applied at 200 ppm to food contact surfaces, and the solution is allowed to dry. Once dry, residues of these compounds remain and provide germicidal activity until degradation occurs. They also can function as detergents when present in high concentration because the compounds possess both hydrophilic and lipophilic chemical groups (Maharjan and Watkins, 2016)

Those are usually odorless, non-staining, noncorrosive and relatively nontoxic to users. They function well over a broad temperature range and a wide pH range, although activity is greater at warmer temperatures and in alkaline situations. They tolerate light organic load and heavy soil will significantly decrease their activity. Some may not function adequately in hard water, but others are formulated with added chelating agents that allow such use (Schmidt, 2003).

2.2 Sodium hypochlorite

Sodium hypochlorite is most widely used disinfectant in poultry despite the increasing availability of other disinfectants. The major benefits of using sodium hypochlorite are that it fulfills many requirements of the ideal disinfectant (Rutala and Weber, 1997) and has an excellent cleaning action. Furthermore, it can be easily combined with other cleaning elements and detergents.

2.2.1 Physico chemical properties

Chemical names: Sodium hypochlorite, liquid bleach or bleach, Dakin's solution, chloride of soda, antiformin

Chemical formula: NaOCl

Molecular weight: 74.442 g/mol

Color: Pale greenish or yellowish solution

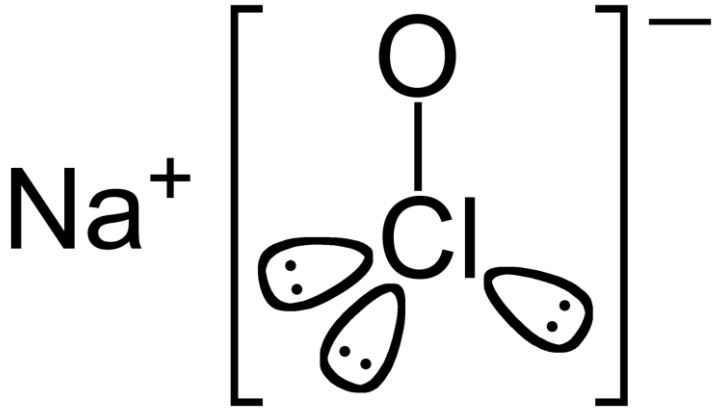


Fig. 1 Structure of sodium hypochlorite

2.2.2 Mechanism of action

Sodium hypochlorite has broad antimicrobial spectrum. The germicidal activity of a diluted sodium hypochlorite solution depends on the concentration of hypochlorous acid but not on the total available chlorine concentration (Brazis *et al*., 1958; Charlton and Levine, 1935; Le Dantec *et al*., 2002; Sagripanti and Bonifacino, 1996). This is attributed to the penetration of hypochlorous acid into the microbial cell across the cell wall and membrane. It is believed that the mechanism of germicidal activity of hypochlorous acid or hypochlorite ion is due to the inhibition of enzyme activity essential for the growth. Other mechanisms include damage to the membrane and DNA and perhaps interference with the membrane transport capacity. Hypochlorous acid stress is also suggested to generate common deleterious oxidative species which can damage cellular components (Dunkan and Touati, 1996; Dukan *et al*., 1999). Sodium hypochlorite, elemental chlorine gas and calcium hypochlorite presence in the optimal pH range will create hypochlorous acid on hydrolysis. Chlorination is more effective at lower pH levels. On the other hand the germicidal activity of a concentrated sodium hypochlorite solution is based on its pH and hypochlorite ion oxidation (Estrela *et al*., 2002).

The germicidal action of hypochlorous acid and hypochlorite ion depend on their ability to penetrate the membrane into the microbial cell. Ionized hypochlorite ion has poor germicidal activity because of its inability to diffuse through microbial plasma membrane and it exerts an oxidizing action only from outside of the cell. Hypochlorous acid can attack the microbial cell both from the outside and inside the cell which is responsible for the potent germicidal activity (Fukuzaki, 2006). Drinking water is acidified for improving chlorine disinfectant efficacy which supports better bird performance. When using chlorine and acidifiers together in water, they should be mixed and injected separately to avoid poisonous gas formation. An appropriate application strategy of chlorine in drinking water on poultry farms helps to reduce the microbial load and also minimize the biofilm buildup in water systems.

2.2.3 Sodium hypochlorite as disinfectant

Hypochlorites are most widely used chlorine disinfectants with fast action and broad spectrum of antimicrobial activity. They are unaffected by water hardness and have low incidence of serious toxicity (Chima *et al*. 2012).

Commercial chlorine bleach contains 5.25% sodium hypochlorite in aqueous solution and 50,000 ppm available chlorine. Low concentrations (2 to 500 ppm) are active against vegetative bacteria, fungi and most viruses. Rapid sporicidal action can be obtained around 2500 ppm, however this concentration is very corrosive so should be limited in its use. High concentrations are also irritating to the mucous membranes, eyes and skin. Chlorine compounds are rapidly inactivated by light and some metals so fresh solutions should always be used. Hypochlorites should never be mixed with acids or ammonia as this will result in the release of toxic chlorine gas (Dvorak, 2005).

Hulan and Proudfoot (1982) studied the effect of sodium hypochlorite on mortality and biological performance in chicks. It was observed that administration of

1200 ppm available chlorine significantly increased the mortality, lowered the feed efficiency, reduced the water consumption and lowered the weights of liver, kidney and testes. Administration of 300 ppm available chlorine resulted in lowered body weights in chicks.

Williams *et al*. (1985) compared the bactericidal efficacy of N-chloramine compound 3-chloro-4,4-dimethyl-2-oxazolidinone with calcium hypochlorite. The study revealed that for highly pure, demand-free water, calcium hypochlorite was more rapid disinfectant at given total chlorine concentration. While 3-chloro-4,4-dimethyl-2-oxazolidinone was the better disinfectant for water containing a controlled amount of organic load. The difference in efficacy of each of the two disinfectants was attributed primarily to their different stabilities in water at various controlled conditions.

2.2.3.1 Adverse effects of sodium hypochlorite

Daniel *et al*. (1990) studied the comparative sub-chronic toxicity studies of three disinfectants chlorine, monochloramine and chlorine dioxide in Sprague-Dawley rats. It was observed that highest dose of chlorine (250 mg/ml) produced no observable adverse effect in rats. Monochloramine (200 mg/ml) produced decreased body and organ weights, reduced erythrocyte count and serum calcium levels in rats. Similarly, chlorine dioxide (250 mg/ml) decreased the body and organ weights in rats.

A study was conducted by Damron and Flunker (1993) to know the tolerance of broiler chick and laying hen to sodium hypochlorite in drinking water. The results revealed that the water intake of chicks was reduced by 100 ppm chloride. Body weight of chicks was reduced by 300 ppm of chloride in drinking water. In hens the water intake was affected by 40 ppm chloride and egg production was affected by 60 ppm chloride in drinking water. In older hens, the water consumption was affected by 50 ppm chloride in drinking water.

Pathological effects of sodium hypochlorite administered through drinking water in male Japanese quails (*Coturnix japonica*) were reported by Khan *et al*. (2008). The results showed that there was decreased weight and volume of testes in birds that received high levels of sodium hypochlorite (50 mg/ml). Birds that received 400 mg/ml of chlorine had smaller but functional testes. It was concluded by authors that sodum hypochlorite at higher levels in drinking water was toxic to quails.

In vitro efficacies of different disinfectants of drinking water used in commercial poultry farms were compared by Gehan *et al*. (2009). It was observed that most of the tested disinfectants were effective at recommended levels within 30 min contact time when tested in absence of organic load. However, in the presence of organic load, longer contact times were needed to demonstrate the effectiveness of disinfectants.

2.2.3.2 Effect of water quality on sodium hypochlorite

Pangloli and Hung (2013) studied the effect of water hardness and pH on the efficacy of chlorine-based sanitizers for inactivating *Escherichia coli* and *Listeria monoctyogenes* in water. It was observed that increase in water hardness and pH increased the free chlorine levels and oxidation-reduction potential of chlorine. Water hardness and pH had minor effects on pH of sodium hypochlorite solutions. Sodium hypochlorite solutions prepared from hard water had lowered the efficacy of sodium hypochlorite in inactivating *E.coli* but had no effect on inactivation of *Listeria monocytogenes*. It was concluded from the study that increasing the hardness and pH of water decreased the bactericidal activity of sodium hypochlorite.

Swanson and Fu (2017) studied the effect of water hardness on the efficacy of sodium hypochlorite in inactivating *Escherichia coli* in water. The results showed that in absence of sodium hypochlorite, no reduction in counts of *E.coli* was observed regardless of degree of water hardness. In the presence of hard water, the

inactivation of *E.coli* by sodium hypochlorite was lowered. It was concluded by authors that water hardness could affect the germicidal efficacy of sodium hypochlorite.

2.3 Enrofloxacin

Enrofloxacin, a fluorinated quinolone carboxylic acid derivative, is a chemotherapeutic agent with extensive use in veterinary medicine in view of its broad spectrum of activity (Brown, 1996). The favorable pharmacokinetic properties of enrofloxacin make this drug a suitable antibacterial in poultry. Enrofloxacin has been indicated in poultry for the treatment of respiratory and intestinal tract infections including collibacillosis, pasteurellosis, and mycoplasmal infection (Devreese *et al*., 2014).

2.3.1 Mechanism of action

Enrofloxacin exhibits a concentration-dependent bactericidal effect on a range of bacteria, including *Escherichia coli, Pasteurella multocida, Mycoplasma gallisepticum, M. synoviae and Ornithobacterium rhinotracheale* (Trouchon and Lefebvre, 2016). In multiple species of bacteria, early biochemical evidence indicated that fluoroquinolones damage bacterial DNA and lead to defect in negative super coiling (Gellert *et al.,* 1976). This effect was linked to inhibition of DNA gyrase activity, an enzyme found in all bacteria. In concert with other proteins, gyrase catalyzes changes in the degree of double-stranded DNA super coiling. In this capacity, it plays a vital role in DNA packing, replication and transcription. The active holoenzyme is a heterotetramer composed of two subunits each of *gyrA* and *gyrB* (A2B2). *GyrA* binds to DNA and mediates strand breakage and rejoining activity, whereas *gyrB* contains the ATP binding site. *GyrA* activity involves cleavage of both DNA strands (mediated by an enzyme-DNA covalent intermediate), passage of DNA through the break and relegation of the strand. *In vivo*, this process results in two negative supercoils and hydrolysis of ATP.

The topoisomerase IV enzyme, encoded by *parC/parE*, is a secondary

fluoroquinolone target. This enzyme is also a multimeric protein composed of two *parC* subunits and two *parE* subunits, which exhibit sequence homology to *gyrA* and *gyrB*, respectively. This enzyme mediates relaxation of duplex DNA and the unlinking of daughter chromosomes following replication (Zechiedrich and Cozzarelli, 1995). When susceptible DNA gyrase is exposed to a quinolone, the drug interacts at the surface of an alpha-helical domain of the enzyme involved in DNA cleavage and re-ligation, termed the quinolone resistance determining region. This prevents progression of replication forks and transcription complexes (Willmott *et al.,* 1994), leading to fragmentation of the chromosome and cell death. Because quinolones mediate DNA damage by binding to susceptible enzymes, fluoroquinolone-resistance mutations are recessive. The result of this is that for topoisomerase mediated fluoroquinolone resistance to be transferred horizontally, an acquired mutated gene would have to supplant the wild-type gene (Guthrie *et al.,* 2004).

2.3.2 Pharmacokinetics of enrofloxacin

Pharmacokinetic studies of enrofloxacin performed in different animal species by various routes of administration show that it exhibits good absorption, high bioavailability, large volume of distribution and low protein binding (Lopez-Cadenas *et al*., 2013).

The disposition and tissue distribution of enrofloxacin and of its metabolite ciprofloxacin in ducks were investigated by Intorre *et al.* (1997) following oral or intramuscular administration of single dose of enrofloxacin (10 mg/kg). The pharmacokinetic parameters recorded following intramuscular administration were AUC 10.11±0.87 μg.ml.h, MRT 8.35±0.65 h, t_{max} 0.94±0.18 h, C_{max} 1.67±0.29 μg/ml, $V_{d(area)}$ 7.45±1.76 l/kg and Cl_B 1.03±0.07 l/kg/h. In case of oral administration the values were AUC 6.65±0.44 μg.ml.h, MRT 6.01±0.54 h, t_{max} 1.38±0.18 h, C_{max} 0.99±0.08 μg/ml, $V_{d(area)}$ 8.89±1.04 l/kg and Cl_B 1.56±0.14 l/kg/h.

Helmick *et al*. (1997) reported the disposition of single-dose intravenously administered enrofloxacin (2.2±0.03 mg/kg) in emu birds. Enrofloxacin levels were measured using high-performance liquid chromatography and the resulting concentration *vs*. time curve was analyzed using nonlinear regression. The data were represented by a two-compartment model with $t_{1/2}$ 3.33 h, AUC 8.26 µg.ml.h, MRT 4.40 h, V_d 1.49 l/kg and Cl_B 0.36 l/h/kg and C_{max} 2.0 µg/ml.

The pharmacokinetic parameters recorded after intravenous and oral administration of enrofloxacin at dose rate of 5mg/kg body weight in broiler birds were studied by Bugyei *et al.* (1999). Following intravenous administration the findings were: $t_{1/2\beta}$ of 10.96 h, V_d of 3.82 ± 0.24 l/kg, AUC of 21.7±1.59 µg.ml.h and MRT of 12.5±0.77 h. The findings for oral administration were: C_{max} of 2.10 ±0.21 µg/ml, t_{max} of 0.79 ± 0.10 h, AUC of 17.4±2.04 µg.ml.h, MRT of 13.7±1.97 h and F of 80.1 %.

The pharmacokinetic characteristics of enrofloxacin and its metabolite ciprofloxacin were studied in healthy broilers by Gongzheng *et al*. (1999). The results showed that the enrofloxacin in plasma after intravenous administration and oral administration in healthy broilers could fit to a two compartment open model. The pharmacokinetic parameters after intravenous administration were $t_{1/2\alpha}$ (0.25±0.04) h, $t_{1/2\beta}$ (5.26 ±0.81) h, V_d (4.53±1.07) l/kg, Cl_B (0.61±0.11) l/(kg·h) and AUC (17.39±3.92) mg/ (l·h). While the pharmacokinetic parameters after oral administration were $t_{1/2k\alpha}$ (0.44±0.11) h, $t_{1/2\alpha}$ (1.15±0.38) h, $t_{1/2\beta}$ (9.14±1.45) h, AUC (12.48±2.36) mg/ (l·h), t_{max} (1.77±0.21) h and C_{max} (1.44±0.31) µg/ml.

Garacia-Ovando *et al.* (1999) studied the pharmacokinetics of enrofloxacin and ciprofloxacin (5 mg/kg body weight) given intravenously in broiler chickens. The $t_{1/2\beta}$ (h), AUC (µg.ml.h), AUMC (µg.ml. h^2), MRT (h), Cl_B (ml/min/kg), $V_{d\beta}$ (l/kg) and (V_{dss}) (l/kg) for enrofloxacin were 6.99±0.48, 26.76±2.55, 280.1±41.9, 10.24±0.73, 3.30 ± 0.35, 1.94 ±0.14, 1.98 ±0.18 respectively. The $t_{1/2\beta}$ (h), AUC

(µg.ml.h), AUMC (µg.ml.h^2), MRT (h), Cl_B (ml/min/kg), $V_{d\beta}$ (l/kg) and V_{dss} (l/kg) for ciprofloxacin were 3.11±0.25, 5.67±0.52, 24.66±2.83, 4.44±0.46, 15.45±1.63, 4.22±0.58, 4.04±0.69 respectively.

The single dose (10 mg/kg) pharmacokinetics of enrofloxacin in male broiler chickens was studied by Da Silva *et al*. (2006). The plasma concentration time graph was characteristic of a two-compartment open model. C_{max} was 1.5±0.2 mg/ml, t_{max} was 9±2 h, $t_{1/2\beta}$ was 1.5±0.2 h, and MRT was 15.64 h, AUC is 35±4 mg.ml.h.

Pharmacokinetics of enrofloxacin (10 mg/kg) following intravenous and oral route in turkeys was reported by Dimitrova *et al.* (2007). The $t_{1/2\beta}$ (h) and MRT (h) of enrofloxacin after intravenous administration were 6.64±0.90, 8.96±1.18 and 6.92±0.97 respectively. Following oral administration, enrofloxacin was absorbed slowly (MRT 2.76±0.48 h) with (6.33±2.54 h) time to reach maximum serum concentrations of (1.23±0.30 µg/ml). Bioavailability for enrofloxacin after oral administration was found to be 69.20 ± 1.49%.

Ibrahim and Yarsan (2009) investigated the pharmacokinetics of enrofloxacin in broilers after oral administration at a dose of 10 mg/kg. The concentration of enrofloxacin at various time intervals in plasma was determined by microbiological assay, agar gel diffusion analysis; with two compartmental open model method. The pharmacokinetic parameters of enrofloxacin recorded were $t_{1/2\beta}$ 2.06±0.61 h, β 0.36±0.09 l.h^{-1}, AUC_{0-24} 21.74±1.52 µg.ml.h, the C_{max} 3.91±0.60 µg.ml^{-1} and t_{max} 1.50±0.85 h, MRT 33.91±6.32 h.

Jakubowskip *et al*. (2010) studied the pharmacokinetic parameters of enrofloxacin in chicken plasma by HPLC after a single intravenous administration at a dose rate of 10mg.kg^{-1}. The values were $t_{1/2\beta}$ 9.14 (h), $AUC_{(0-24)}$ 25.09 (µg.ml.h), the C_{max} 4.62 (µg.ml^{-1}), V_{dss} 2.7 (l.kg^{-1}) and MRT 6.99 (h).

Comparative pharmacokinetics of enrofloxacin, danofloxacin and marbofloxacin after intravenous and oral administration in Japanese quail (*Cotrunix cotrunix japonica*) was reported by Haritova *et al*. (2013). The birds received enrofloxacin, danofloxacin and marbofloxacin at the rate of 10, 10 and 5 mg/kg body weight respectively. The pharmacokinetic parameters following intravenous administration analyzed for enrofloxacin were V_d 5.36±0.68 l/kg, Cl_B 1.52±0.13 l/h kg^{-1}, MRT 3.53 h and AUC 6.59 μg.ml.h. For danofloxacin the values were V_d 9.52±0.53 l/kg, Cl_B 1.48±0.17 l/h/kg, MRT 5.8 h and AUC 6.09 μg.ml.h. For marbofloxacin the results were V_d 1.18±0.05 l/kg, Cl_B 0.47±0.03 l/h kg^{-1}, MRT 2.90 h and AUC 11.55 μg.ml.h. In case of oral administration the values of C_{max} for enrofloxacin, danofloxacin and marbofloxacin were 0.29 μg/ml, 0.79 μg/ml and 6.25 μg/ml, respectively. The values of t_{max} for enrofloxacin, danofloxacin and marbofloxacin were 0.35 h, 2.08 h and 1.36 h, respectively.

Mekala *et al.* (2014) studied the pharmacokinetics and bioavailability of enrofloxacin in broiler chicken dosed at the rate of 10 mg/kg body weight through intravenous and oral route. The concentration of enrofloxacin at various time intervals in plasma was determined by HPLC method with non-compartmental model analysis. Authors found that the elimination half-life ($t_{1/2\beta}$) was 6.84±0.15 h for intravenous and 10.57±0.35 h for oral routes, elimination rate constant (β) ($l.h^{-1}$) of 0.101±0.02 (intravenous) and 0.065±0.02 (oral), $AUC_{0-\infty,}$ (μg.ml.h) value 32.72±1.15 high for intravenous compared to oral route value 25.35±1.92. The high AUC observed in the study was attributed to longer stay of drug in the body in both routes and longer absorption phase in case of oral route. Highly significant increase in mean residence time (MRT, h) and volume of distribution were observed for oral route when compared to intravenous route. The MRT (h) and V_d (l/kg) values after intravenous and oral administration were 8.86±0.23, 15.81±0.54 and 3.04±0.09, 4.69±0.16 respectively. The

C_{max} (μg.ml^{-1}) of 1.63±0.12 was attained at t_{max} (h) of 3.58±0.61 and absolute bioavailability was 77.47±5.86 after oral administration.

Pharmacokinetics of enrofloxacin after single oral bolus and pulse dose administration in broiler chicken (six weeks of age) at a dose of 10 mg/kg was reported by Mekala *et al*. (2015). The concentration of enrofloxacin at various time intervals in plasma was determined by HPLC method. With non-compartmental analysis, there was significant differences in the AUC_{0-24}, (μg/ml.h) of 25.35±1.92 for oral bolus dose and 19.66±1.68 for pulse dose, $t_{1/2\ \beta}$ (h) of 10.63±0.35 for oral bolus dose and 8.70±0.74 h for pulse dose but not in other pharmacokinetic parameters such as MRT, MAT, V_d, C_{max}, t_{max}. The β (l.h^{-1}) of 0.080±0.008 (pulse dose) and 0.065±0.02 (oral dose), C_{max} (μg/ml) 1.63±0.12 for oral bolus dose and 1.38±0.04 for pulse dose and the t_{max} (h) of 3.58±0.61 for oral bolus dose and 4.33±0.61 for pulse dose, V_d (l/kg) of 6.17±0.38 for oral bolus dose and 6.39±0.13 for pulse dose, MRT (h) of 15.81±0.54 oral bolus dose and 14.09±1.10 for pulse dose. MRT (h) of 6.95±0.54 for oral bolus, 5.23±1.10 for pulse dose were reported.

Kumar *et al*. (2015) determined pharmacokinetics of enrofloxacin in healthy emu birds (*Dromaius novaehollandiae*) aged between 18-24 months after intravenous and oral bolus administration at a dose of 10 mg.kg^{-1}. The concentration of enrofloxacin and its active metabolite ciprofloxacin in plasma was determined by HPLC method. With non-compartmental analysis, the values recorded for enrofloxacin were: $t_{1/2\beta}$ of 4.364±0.179 h for intravenous and 4.125±0.361 h for oral, β (l.h^{-1}) of 0.159±0.007 for intravenous and 0.162±0.015 for oral, $AUC_{0-\infty}$ (μg.ml.h) of 20.085±3.493 for intravenous and 16.056±1.436 for oral routes, with the oral bioavailability of 80%. The C_{max} (μg.ml^{-1}) was 2.39±0.052 and t_{max} was (h) 2.167±0.279. MRT (h) of 5.105±0.216 for intravenous and 6.616±0.475 for oral routes, V_d (l.kg^{-1}) of 3.921±1.005 for intravenous and 3.171±0.269 for oral routes, Cl_B, (l/kg/h)

of 0.629±0.164 for intravenous and 0.507±0.003 for oral routes. Pharmacokinetic parameters for ciprofloxacin such as $t_{1/2\ \beta}$ (h) of 4.595±0.163 for intravenous and 5.393±0.186 for oral routes, MRT (h) of 7.454±0.223 for intravenous and 8.625±0.173 for oral routes were higher when compared to enrofloxacin.

Ehmeza *et al*. (2016) determined the comparative pharmacokinetics and absolute bioavailabilities of two enrofloxacin generic preparations after single intracrop bolus administration in broiler chicken at a dose of 10 $mg.kg^{-1}$. The two tested formulations were ENROL® given as ENRO-A by intravenous and ENRO-B by oral routes. The concentration of enrofloxacin at various time intervals in plasma was determined by HPLC method. With non-compartmental analysis, $t_{1/2\beta}$ of 8.391±0.312 h for intravenous and 8.4583±0.004 h for oral, β ($l.h^{-1}$) of 0.0826±0.005 for intravenous and 0.0819±0.004 for oral route, AUC (0-24) (μg.ml.h) of 12.744±2.951 for intravenous and 14.354±2.851 for oral. The C_{max} ($\mu g.ml^{-1}$) of 1.6100±0.203 for intravenous and 1.7900±0.283 for oral and the t_{max} (h) of 2 h for intravenous and 2 h for oral, V_d, (l/kg) of 7.5006±0.781 for intravenous and 7.5609±0.995 for oral, MRT (h) of 10.306±0.805 for intravenous and 10.430±1.935 for oral. It was concluded that despite the superior pharmacokinetic profile of ENRO-B over ENRO-A, both generics were within the FDA and EMA bioequivalence range of 80 -125% and thus can be used as interchangeable therapeutic agents in chickens.

2.4 Oral administration of quinolones and their interactions in gut

Sumano *et al*. (2004) reported the influence of hard water on the bioavailability of enrofloxacin in broilers. There were statistically significant differences in the pharmacokinetic variables like maximum serum concentration, area under curve *vs*. concentration curves, and half-lives of the elimination phases in birds that received different kinds of water. The means of these values showed a linear decay of maximum serum concentration from one group to next as water hardness increased.

Pavitra *et al*. (2009) studied the influence of curcumin (60 mg/kg) pre-treatment on pharmacokinetic disposition of norfloxacin (100 mg/kg) given orally in rabbits. The pharmacokinetic data revealed that curcumin-treated animals had significantly higher AUC and AUMC. Prior treatment of curcumin significantly increased the elimination half-life and volume of distribution of norfloxacin.

Patel *et al*. (2011) studied the co-administration of piperine (15 mg/kg) on pharmacokinetic profile of single oral dose of gatifloxacin (10 mg/kg) in layer birds. Co-administration of piperine with gatifloxacin enhanced the $t_{1/2}$, C_{max} and AUC of gatifloxacin when compared to gatifloxacin administered alone. Piperine significantly enhanced the bioavailability of gatifloxacin from 74.52% to 85.74%.

A study was conducted by Lim *et al*. (2012) to evaluate the oral absorption of enrofloxacin in rats when co-administered with orange oils or its main component limonene. It was observed that there was significant decrease in rate and extent of absorption of enrofloxacin in group that were co-administered with limonene when compared with the group treated with enrofloxacin alone. In addition, the $t_{1/2}$ and MRT of enrofloxacin were prolonged by concomitant administration of limonene.

Clinical relevance of pharmacokinetic interactions of enrofloxacin and diclofenac were studied by Kumar *et al.* (2013) in female buffalo calves that received enrofloxacin (4 mg/kg) intravenously alone or in combination with diclofenac. It was observed that all kinetic parameters of enrofloxacin when given alone or in combination with diclofenac did not differ significantly indicating that diclofenac had no influence on distribution, metabolism and excretion of enrofloxacin.

A study was conducted by Mekala *et al*. (2015) to explore the influence of hydrated sodium calcium alumino silicate (HSCAS) supplemented (0.5%) feed on pharmacokinetic behavior of enrofloxacin in broiler chicken. Enrofloxacin was administered at dose rate of 10 mg/kg body weight through drinking water. It was

observed that the HSCAS supplementation significantly lowered the C_{max} and delayed the T_{max}. There was significant increase in V_{dss} in HSCAS group than in control group. Bioavailability of HSCAS group was lower than control group.

A study was conducted by Pavlova *et al*. (2015) to evaluate the effect of probiotics (belonging to genus *Lactobacillus brevis, L. plantarum and L. bulgaricus*) on pharmacokinetics of enrofloxacin in healthy chickens. Absorption of enrofloxacin was significantly faster (k_{ab} of 0.16±0.54 h^{-1}, t_{max} 7.81±3.52 h and C_{max} of 1.34±0.15 µg/ml) in probiotic treated group. Compared to probiotic treated group the absorption rate for the enrofloxacin treated group was slower (k_{ab} 0.10±0.065 h^{-1}, t_{max} 15.42±3.07 h and C_{max} 1.61±0.24 µg/ml respectively). The authors attributed the findings that probiotic administration produced higher degree of metabolism of enrofloxacin to ciprofloxacin in liver in chicken.

Potential pharmacokinetic effect of rifampicin on enrofloxacin in broilers was studied by Guo *et al*. (2016). Co-administration of rifampicin significantly changed the pharmacokinetics of enrofloxacin administered orally by showing lowered $AUC_{0-\alpha}$, AUC_{0-t}, and C_{max} as well as longer T_{max}. The bioavailability of enrofloxacin was decreased fom 72.5% to 24.8% by rifampicin.

Pharmacokinetic interaction between ciprofloxacin (10 mg/kg) and quercetin was investigated in male rats by Devi *et al*. (2016). It was observed that plasma concentrations of ciprofloxacin were detected upto 6 hrs in rats that received ciprofloxacin alone, while plasma concentrations of ciprofloxacin were detectable upto 8 hrs in quercetin pretreated rats. The study also revealed that prior administration of quercetin modified the kinetic profile of ciprofloxacin which was evident through higher AUC, AUMC and lower V_{dss} and Cl_B values.

Role of quercetin in modulation of P-gp expression and subsequent effect on pharmacokinetics of enrofloxacin in broilers was studied by Bhutto *et al.*

(2018). It was observed that quercetin altered the pharmacokinetics of enrofloxacin by decreasing the area under curve, peak concentration, time to reach peak concentration and increasing the clearance rate. Quercetin induced the P-gp expression in tissues, by interaction with chicken xenobiotic receptor. It reduced the bioavailability of orally administered enrofloxacin by restricting its intestinal absorption and liver/kidney clearance in broilers.

Ledesma *et al*. (2018) studied the influence of chlorine, iodine and citrate based water sanitizers on the oral bioavailability of enrofloxacin in broiler chickens. The study revealed that sodium hypochlorite and iodine-polyvinylpyrrolidone decreased the *in-vitro* antimicrobial activity as well as maximum serum concentration and bioavailability of enrofloxacin in chickens. In contrast citrate based sanitizer increased the *in-vitro* antimicrobial activity as well as maximum serum concentration and bioavailability of enrofloxacin. The authors concluded that water sanitizers influence the orally administered enrofloxacin and interfere with its absorption.

MATERIALS AND METHODS

CHAPTER – III

MATERIAL AND METHODS

The present study was carried out to know the influence of sodium hypochlorite on the pharmacokinetics of enrofloxacin in broiler chickens. The study was conducted in the Department of Veterinary Pharmacology and Toxicology, NTR college of veterinary science, Gannavaram.

3.1 MATERIALS

3.1.1 Chemicals and reagents

- Acetic acid glacial (Merck Life Science Pvt. Ltd.,Mumbai)
- Ortho-phosphoric acid (Merck Life Science Pvt. Ltd.,Mumbai)
- Acetonitrile (Merck Life Science Pvt. Ltd.,Mumbai)
- Potassium dihydrogen phosphate (Merck Specialities Pvt.Ltd.,Mumbai)
- Enrofloxacin (Sigma Aldrich,USA)
- Triethylamine (Merck Specialities Pvt. Ltd.,Mumbai)
- Water (Ultra-pure Type1)

(All the chemicals used in the present study were of analytical grade)

3.1.2 Equipment

1. Cliklok micro-centrifuge tubes (Tarsons Products Pvt. Ltd.,Kolkata)
2. Digital pH meter (Cyberscan – Eutech instruments pH-510)
3. Disposable syringes (Dispovan, Hindustan Syringes and Medical Devices Ltd., Haryana)
4. Electronic balance (AND GR-200)
5. HPLC (Shimadzu Corporation, Kyoto, Japan) consisted of LC-20AD quaternary gradient pump, a rheodyne manual injector with 20 µl loop, a SPD-20 AV UV-Vis detector, a column oven CTO-10ASVP. The

analytical column was a 5µ C_{18} (RP- C_{18}) column (4.6 x 250 mm, 5 µm particle size).

6. Microcentrifuge (Spinwin)
7. Microliter (25µl) syringe (Hamilton,Romania)
8. Micropipettes (Accupipet)
9. Microtips (TarsonsPvt. Ltd.,Kolkata)
10. Nylon syringe filter (0.22 µm)
11. Refrigerated centrifuge (Thermo Scientific ST8R)
12. Ultra pure Type 1 Water (Millex, Simplicity®UV)
13. Vortex mixer (Spinix)

3.1.3 Experimental animals

Four week old birds were procured from M/s Srinivasa Hatcheries, Vijayawada, India and were maintained under standard conditions in Livestock Farm Complex, NTR College of Veterinary Science, Gannavaram. Acclimatization period of two weeks was allowed prior to the commencement of the experiment. Thus at the time of experiment the birds were aged 6 weeks and weighed about 2 kgs. No antibiotic medication was given to the birds during the two weeks acclimatization period.

3.2 METHODS

3.2.1 Experimental design

This work has been approved by IAEC with reference No. 14/IAEC/NTR CVSc/2019 Dated: 29/06/19. A total of 24 chickens were randomly divided into three groups with eight birds in each and were kept off feed for 12 hours prior to the experimentation and water was withheld 2 hours prior the collection of blood .Access to feed (free of anticoccidiostats and toxin binders) and water was provided after 6 hours and 4 hours of post enrofloxacin dosing respectively. Body weights were taken just before the experiment and the treatment was given as follows:

1. Group I: Enrofloxacin alone at the dose rate of 10 mg.kg^{-1} body weight orally
2. Group II: Sodium hypochlorite at dose rate of 10 ml/100L of drinking water for seven days followed by enrofloxacin at the dose rate of 10 mg.kg^{-1} body weight in sodium hypochlorite containing water on seventh day.
3. Group III: Sodium hypochlorite at the dose rate of 10 ml/100L of drinking water for seven days followed by enrofloxacin at the dose rate of 10 mg.kg^{-1} body weight in normal drinking water 12 hours post withdrawal of sanitizer containing water.

Table. 1 Treatment protocol in different groups

GROUP	TREATMENT
I	Enrofloxacin orally @ 10 mg kg^{-1}
II	Sodium hypochlorite @ 10 ml/100L of drinking water for seven days followed by enrofloxacin @ 10 mg kg^{-1} in sodium hypochlorite containing water on seventh day.
III	Sodium hypochlorite @ 10 ml /100L of drinking water for seven days followed by enrofloxacin @ 10 mg kg^{-1} in normal drinking water 12 hours post withdrawal of sanitizer containing water.

3.2.2 Collection of blood samples

Blood samples of 1 ml from all the birds were collected with fresh disposable needles and syringes from either left (or) right tarsal vein into heparinized micro centrifuge tubes (Cliklok tubes). The collection timings were at 0 (blank), 0.16, 0.33, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 24 and 48 h post dosing. The sample tubes were centrifuged at 4779.45 g for 5 min to separate the plasma. The plasma samples were stored at -20°C until further use.

3.2.3 Preparation of standard solution

3.2.3.1 Enrofloxacin standard solution

Enrofloxacin stock solution was prepared by dissolving 200 mg of enrofloxacin in 20 ml of 0.1N NaOH solution. Working standard solutions were prepared in the concentration ranges of 100 µg/ml to 0.3125 µg/ml by diluting the stock solution with HPLC water.

3.2.3.2 Standard solutions in plasma

Working plasma standards were prepared by adding 100 µl of respective working standard solution in 900 µl of pooled untreated poultry plasma samples to get the final concentration of 10 µg/ml to 0.3125 µg/ml.

3.2.4 Standard calibration curve

Standard calibration curve for enrofloxacin was constructed from the peak areas of the chromatogram verses concentration.

3.2.5 Quantification

Concentration of enrofloxacin in plasma samples was determined by using linear regression formula obtained from the standard curve.

$$Y=a+bx$$

Where ‘y’ is concentration; ‘x’ is peak area of the chromatogram; ‘a’ is Y intercept of the regression line (standard curve) and ‘b’ is slope of the regression line (standard curve). By substituting the respective peak area of the chromatogram (x value in the above equation) the concentration of enrofloxacin in the test plasma sample was calculated.

3.2.6 Analytical recovery and precision

Analytical recovery was determined by using two known concentrations of 0.3125 µg/ml and 2.5 µg/ml of enrofloxacin in pooled untreated bird plasma with the

help of standard curve prepared for enrofloxacin in solvent. Each one of the above concentrations was determined eight times.

The percent recovery was calculated according to the following formula

$$\% \text{ recovery} = \frac{xy(\sum x)\ (\sum y)}{N\sum x^2 - (\sum x)2} \times 100$$

Where, x=known amount of drug added

y=amount of drug found by the assay method

N= number of observations.

Intra-day variation was determined by assaying two standard plasma samples (0.3125 and 2.5 µg/ml) eight times each in a day. Inter-day variation was determined by assaying two standard plasma samples (0.3125 and 2.5 µg/ml) on eight occasions at least 24 h apart.

3.2.7 Plasma sample preparation procedure for HPLC injection

To 0.2 ml of plasma sample, 0.2 ml of de-proteinising solution (acetonitrile: acetic acid = 95:5) was added and mixed with high-speed vortex shaker for 15 seconds. The tubes were centrifuged for 30 min at 13276.25 g. From this, 0.2 ml of supernatant was taken and mixed with 0.2 ml of 1% acetic acid (distilled water: acetic acid = 99:1) and filtered through a 0.22 µm nylon membrane syringe filter and 20 µl of filtrate was injected in to the HPLC system.

3.2.8 Detection of enrofloxacin in plasma samples

Enrofloxacin assay was carried out as per the methods described by Mekala *et al*. (2014) and Nielsen *et al*. (1997) with slight modifications in the extraction procedure as mentioned above by using HPLC.

3.2.8.1 Standardization of HPLC conditions for enrofloxacin

HPLC with C_{18} reversed-phase column (particle size 5 µm, 4.6 mm x 250 mm) as the stationary phase and photo diode array detector was used for detection. The solution consisting of potassium dihydrogen phosphate buffer (70%), acetonitrile

(20%) and methanol (10%) was used as mobile phase at a flow rate of 0.8 ml/min with 7 minutes run time. Buffer pH was adjusted to 2.37 with 0.3% ortho-phosphoric acid and 0.3% of triethylamine. Column oven temperature was set to 40°C and photo diode array detector (SPD-20A) wave length was set at 277 nm.

3.2.9 Pharmacokinetic analysis

3.2.9.1 Non-compartmental analysis

Plasma concentration versus time data of enrofloxacin obtained for three groups in the present study were utilized for calculating various pharmacokinetic parameters given below in broilers with an interactive programme for personal computer software (PK Solver Version.2.0, by Zhang *et al*., 2010).

a) AUC_{0-t} and AUMC: The total area under the plasma drug/metabolite concentration time curve was calculated by using trapezoid rule.

b) $AUC_{0-\alpha}$: $AUC_{0-t} + C_{last}/\beta$

c) V_d/F: volume of distribution of drug at steady state is calculated according to

$V_d/F = Dose \times AUMC/(AUC)^2$, Where F is undetermined bioavailability

d) MRT: The mean residence time was determined by

$MRT = AUMC/AUC$

e) Cl_B/F: The total body clearance was obtained by following equation

$Cl_B/F = Dose/AUC$

f) C_{max}: The maximum plasma drug concentration and t_{max}, the time to reach C_{max} were taken directly from observed values.

g) F: $AUC_{test}/AUC_{control}$, where F is relative bioavailability

3.2.10 Statistical analysis

All the data were expressed as Mean ± Standard error. The differences in pharmacokinetic data of enrofloxacin among three groups were analysed for statistical

significance by applying one-way ANOVA with Tukey's post hoc test by using SPSS statistics version 17.0. The level of significance was kept at $p<0.05$.

RESULTS

CHAPTER –IV

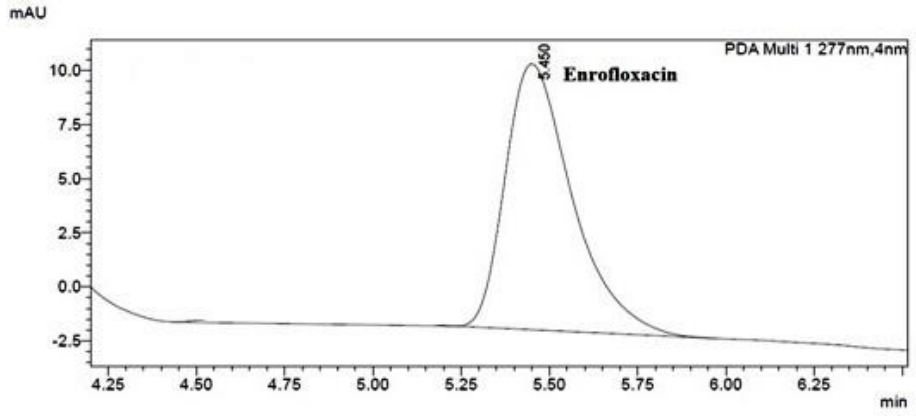
RESULTS

The results of the experimental study conducted to know the influence of sodium hypochlorite on the pharmacokinetics of enrofloxacin in broiler chickens are presented here under.

4.1 Detection of enrofloxacin by HPLC

Chromatograms (Fig. 2) without noise were obtained with mobile phase of potassium dihydrogen phosphate buffer (70%), acetonitrile (20%) and methanol (10%) with pH of the buffer adjusted to 2.37. Detection was carried at wave length of 277 nm for enrofloxacin for concentrations from 0.3125 to 10µg/ml in plasma. The response linearity graph (Fig. 2) showed a linear pattern in increase of area with increase in concentration.

Fig. 2 Chromatogram of enrofloxacin



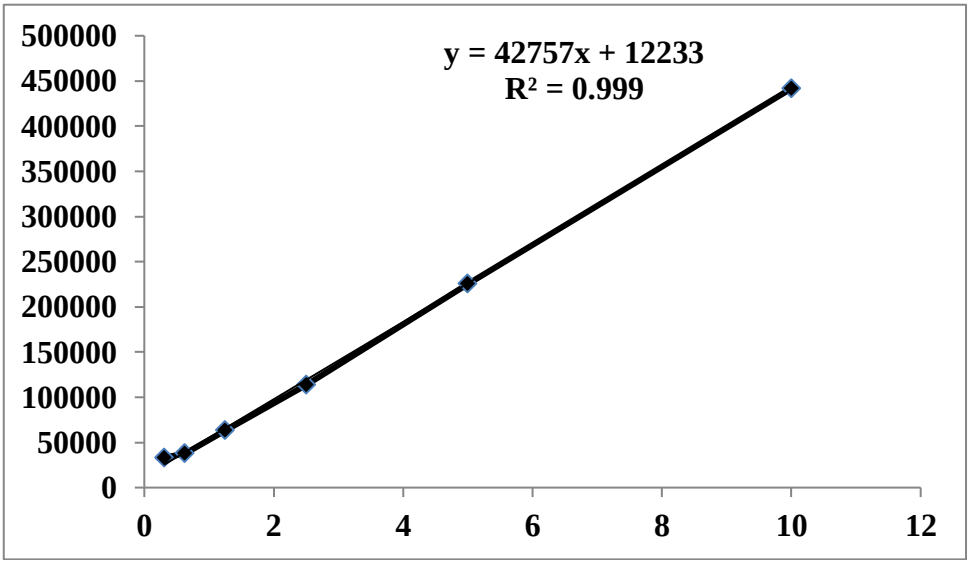
4.2 Standard calibration curve for enrofloxacin

The peak areas obtained from chromatograms against different concentrations of enrofloxacin are presented in Table. 2. Standard calibration curve for enrofloxacin was constructed from the peak areas of chromatograms (Fig. 3). The curves were linear from 0.3125 to 10 µg/ml with regression coefficient of 0.999.

Table. 2 Different concentrations of enrofloxacin and their chromatogram areas obtained by HPLC

Concentration (µg/ml)	Area of chromatogram (mAU)
0.3125	33318
0.625	37929
1.25	63243
2.5	113708
5	225365.5
10	441609

Fig. 3 Standard calibration curve for enrofloxacin



4.3 Analytical recovery and precision

Analytical recovery values for enrofloxacin, spiked in pooled untreated bird plasma of two known concentrations of 0.3125 and 2.5 µg/ml with the help of

standard curve prepared from solvent standards are shown in Table. 3. As per these values the recovery for enrofloxacin was 96.56 %.

Table. 3 Recovery of enrofloxacin from plasma

Enrofloxacin		
Known concentration (µg/ml)	0.3125	2.5
Concentration found (range- µg/ml)	0.268-0.314	2.172-2.501
Mean ±SE (µg/ml)	0.306±0.09	2.417±0.057
Coefficient of variation %	8.6	6.6
Recovery %	**96.56**	

The concentrations of enrofloxacin estimated eight times in a day (intra-day variation) and on eight occasions with at least 24 hours gap (inter-day variation) for two standard concentrations of 0.3125 and 2.5 µg/ml in broiler chicken plasma are shown in Table. 4.

Table. 4 Intra and inter-day estimated values of enrofloxacin for precision of the HPLC assay

	Known concentration (µg/ml)	**Concentration found (µg/ml) (Mean±SE)**	**Coefficient of variation (%)**	**Accuracy (%) on nominal concentration**
Intra-day (n=8)	0.3125	0.299±0.008	7.5	95.83
	2.5	2.404±0.05	6.6	96.16
Inter-day (n=8)	0.3125	0.298±0.008	7.4	95.36
	2.5	2.414±0.03	6.3	96.56

The intra-day coefficients of variation for two concentrations were below 10% (7.5% and 6.6%) with accuracy of 95.83 to 96.16%. Likewise, the inter-day

coefficients of variation for two concentrations were below 10% (7.4% and 6.3%) with accuracy of 95.36 to 96.56%. This indicated that the analytical accuracy and precision of the HPLC equipment used in the present study was optimum.

4.4 Plasma concentration of enrofloxacin in birds following single oral dose of enrofloxacin (Group I)

The peak concentration of enrofloxacin in Group I birds was in the range of 1.361 to 3.576 µg/ml with a mean of 1.92±0.27 µg/ml and the time to reach the peak ranged from 1.5 to 6 h (Table. 5; Fig. 4)

4.5 Pharmacokinetics of enrofloxacin in birds following single oral dose of enrofloxacin (Group I)

The plasma concentration-time data (Table. 5) were analyzed by non-compartmental method and the values were presented in Table. 6. The elimination rate constant (β) ranged from 0.032-0.050 l/h with a mean value of 0.040±0.002 l/h; the elimination half-life ranged from 10.819-13.992 h with a mean value of 11.668±0.352 h; the value of C_{max} ranged from 1.361-3.576 µg/ml with a mean of 2.153±0.245 µg/ml. The time to achieve peak plasma concentration, t_{max} was observed in the time range of 4.000-6.000 h with a mean value of 5.250±0.366 h. The area under plasma concentration time curve (AUC_{0-t}) was observed in the range of 28.395-63.893 µg/ml.h with a mean of 40.873±4.496 µg/ml.h. The area under plasma concentration time curve from 0 h to infinity ($AUC_{0-\infty}$) ranged from 32.619-80.712 µg/ml.h with a mean of 50.648±6.111 µg/ml.h. The area under the first moment curve (AUMC) ranged from 684.096-2313.317 µg/ml.h^2 with a mean of 1407.185±216.254 µg/ml.h^2. The mean residence time (MRT) ranged from 20.972-33.233 h with a mean of 27.07±1.375 h. The volume of distribution (V_d/F) ranged from 3.396-6.756 l/kg with a mean of 5.381±0.418 l/kg. The total body clearance (Cl_B/F) ranged from 0.124-0.307 l/kg/h with a mean of 0.216±0.022 l/kg/h.

Table. 5 Plasma concentration (µg/ml) of enrofloxacin following single oral administration (10 mg/kg) in Group I birds (n=8)

Time(h)	1	2	3	4	5	6	7	8	Mean±SE
0	0	0	0	0	0	0	0	0	**0**
0.16	0.209	0.746	0.193	0.063	0.087	0.064	0.634	0.173	**0.27± 0.09**
0.33	0.331	1.325	0.306	0.617	0.237	0.396	0.669	0.462	**0.54±0.12**
0.5	0.517	1.886	0.482	1.058	0.297	0.644	0.549	0.844	**0.78±0.18**
0.75	0.649	1.989	0.619	1.277	0.403	0.982	0.505	1.117	**0.94±0.18**
1	0.777	2.541	0.743	1.552	0.525	1.293	0.516	1.334	**1.16±0.24**
1.5	1.117	3.052	1.063	2.137	0.864	1.628	0.725	1.702	**1.54±0.27**
2	1.378	3.288	1.254	1.998	0.854	1.810	0.997	1.983	**1.70±0.27**
4	1.786	3.576	1.713	1.721	1.361	1.990	1.026	2.196	**1.92±0.27**
6	1.989	2.647	1.990	1.622	1.238	1.621	1.535	2.648	**1.91±0.18**
8	1.356	1.989	1.708	1.446	1.213	1.173	1.197	1.937	**1.50±0.12**
12	1.095	1.621	1.015	0.955	1.022	0.641	1.117	1.498	**1.12±0.11**
24	0.532	0.962	0.619	0.587	0.437	0.333	0.606	1.014	**0.64±0.08**
48	0.299	0.614	0.333	0.331	0.231	0.209	0.374	0.559	**0.37±0.05**

Note: No. 1 to 8 represent the individual number given to each bird in the group

Fig. 4 Plasma concentration-time curve of enrofloxacin (10 mg/kg) following single oral administration in Group I birds. Each point represents mean of eight birds

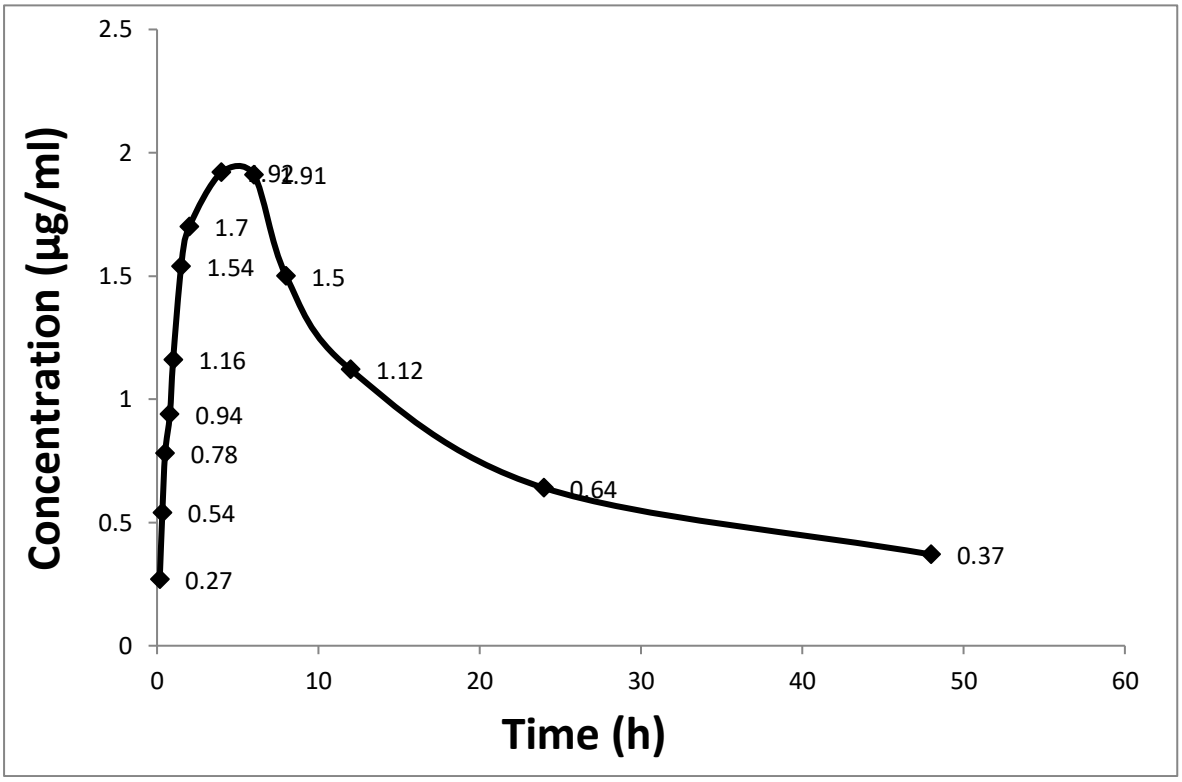


Table. 6 Pharmacokinetics of enrofloxacin following single oral administration (10 mg/kg) in Group I birds (n=8)

Parameter	Unit	1	2	3	4	5	6	7	8	Mean±SE
β	l/h	0.042	0.036	0.041	0.041	0.042	0.050	0.032	0.034	**0.040±0.002**
$t_{1/2}$	h	11.057	11.768	11.136	11.421	10.819	13.992	11.411	11.743	**11.668±0.352**
t_{max}	h	6.000	4.000	6.000	6.000	4.000	4.000	6.000	6.000	**5.250±0.366**
C_{max}	µg/ml	1.989	3.576	1.990	2.137	1.361	1.990	1.535	2.648	**2.153±0.245**
AUC_{0-t}	µg/ml.h	36.473	63.893	38.504	38.010	29.570	28.395	35.315	56.823	**40.873±4.496**
$AUC_{0-\infty}$	µg/ml.h	43.569	80.712	46.694	46.187	35.052	32.619	46.906	73.443	**50.648±6.111**
AUMC	µg/ml.h^2	1091.712	2313.317	1233.992	1205.982	871.015	684.096	1558.852	2298.515	**1407.185±216.254**
MRT	h	25.057	28.662	26.427	26.111	24.850	20.972	33.233	31.297	**27.076±1.375**
V_d/F	l/kg	5.451	3.396	5.260	5.341	6.756	6.189	6.601	4.050	**5.381±0.418**
Cl_BF	l/kg/h	0.230	0.124	0.214	0.217	0.285	0.307	0.213	0.136	**0.216±0.022**

Note: No. 1 to 8 represent the individual number given to each bird in the group

4.6 Plasma concentration of enrofloxacin in birds following single oral dose of enrofloxacin co-administered with sodium hypochlorite (Group II).

The peak concentration of enrofloxacin in Group II birds that received enrofloxacin along with sodium hypochlorite was in the range of 1.336 to 2.574 µg/ml with a mean of 1.26±0.25 µg/ml and achieved at a time of 2 to 8 h (Table. 7 and Fig. 5).

4.7 Pharmacokinetics of enrofloxacin in birds following single oral dose of enrofloxacin co-administered with sodium hypochlorite (Group II).

The plasma concentration-time data values (Table. 7) were analyzed by non compartmental method and the obtained pharmacokinetic parameters are presented in Table. 8. The elimination rate constant (β) ranged from 0.044-0.131 1/h with a mean value of 0.071±0.009 1/h, elimination half-life ranged from 5.282-15.656 h with a mean of 10.629±1.023 h, the C_{max} ranged from 1.336-2.574 ug/ml with a mean of 1.793±0.160 µg/ml, and the time required to achieve peak plasma concentration, t_{max} was observed in the time range of 2.000-8.000 h with a mean value of 5.250±0.750 h. The area under plasma concentration time curve (AUC_{0-t}) was observed in the range of 20.089-58.471 µg/ml.h with a mean of 31.587±4.241 µg/ml.h. The area under the plasma concentration time from 0 h to infinity ($AUC_{0-\infty}$) ranged from 22.327-58.578 µg/ml.h with a mean of 32.978±4.087 µg/ml.h. The area under the first moment curve (AUMC) ranged from 360.40-875.666 µg/ml.h^2 with a mean of 538.396±61.064 µg/ml.h^2. The mean residence time (MRT) ranged from 14.278-20.201 h with a mean value of 16.484±0.748 h. The volume of distribution (V_d/F) ranged from 1.301-8.130 l/kg with a mean of 5.218±0.739 l/kg. The total body clearance (Cl_B/F) ranged from 0.171-0.448 l/kg/h with a mean of 0.329±0.031 l/kg/h.

Table. 7 Plasma concentration (µg/ml) of enrofloxacin following single oral administration of enrofloxacin (10 mg/kg) co-administered with sodium hypochlorite (10 ml/100L) in Group II birds (n=8)

Time(h)	1	2	3	4	5	6	7	8	Mean±SE
0	0	0	0	0	0	0	0	0	**0**
0.16	0.156	0.188	0.052	0.083	0.067	0.223	0.634	0.174	**0.20±0.07**
0.33	0.225	0.293	0.605	0.235	0.306	0.365	0.665	0.454	**0.39±0.06**
0.5	0.357	0.408	0.875	0.292	0.595	0.534	0.549	0.820	**0.55±0.07**
0.75	0.425	0.654	1.024	0.409	0.893	0.695	0.505	0.995	**0.70±0.09**
1	0.532	0.765	1.231	0.523	1.036	0.823	0.515	1.012	**0.80±0.10**
1.5	0.646	0.984	1.527	0.775	1.433	1.213	0.725	1.321	**1.08±0.12**
2	0.913	1.065	2.321	0.854	1.709	1.477	0.996	1.675	**1.38±0.18**
4	1.145	1.235	1.924	1.364	1.879	1.502	1.028	1.898	**1.50±0.13**
6	1.336	1.612	1.765	1.228	1.547	1.227	1.535	2.014	**1.53±0.10**
8	1.086	1.828	1.433	1.124	1.013	0.875	0.179	2.574	**1.26±0.25**
12	0.548	1.012	0.924	1.022	0.643	0.652	1.012	1.914	**0.97±0.15**
24	0.228	0.585	0.578	0.432	0.332	0.321	0.667	1.321	**0.56±0.12**
48	0.123	0.115	0.108	0.068	0.076	0.070	0.099	0.014	**0.08±0.01**

Note: No. 1 to 8 represent the individual number given to each bird in the group

Fig. 5 Plasma concentration-time curve of enrofloxacin following single oral administration of enrofloxacin (10 mg/kg) co-administered with sodium hypochlorite (10 ml/100L) in Group II birds. Each point represents mean of eight birds

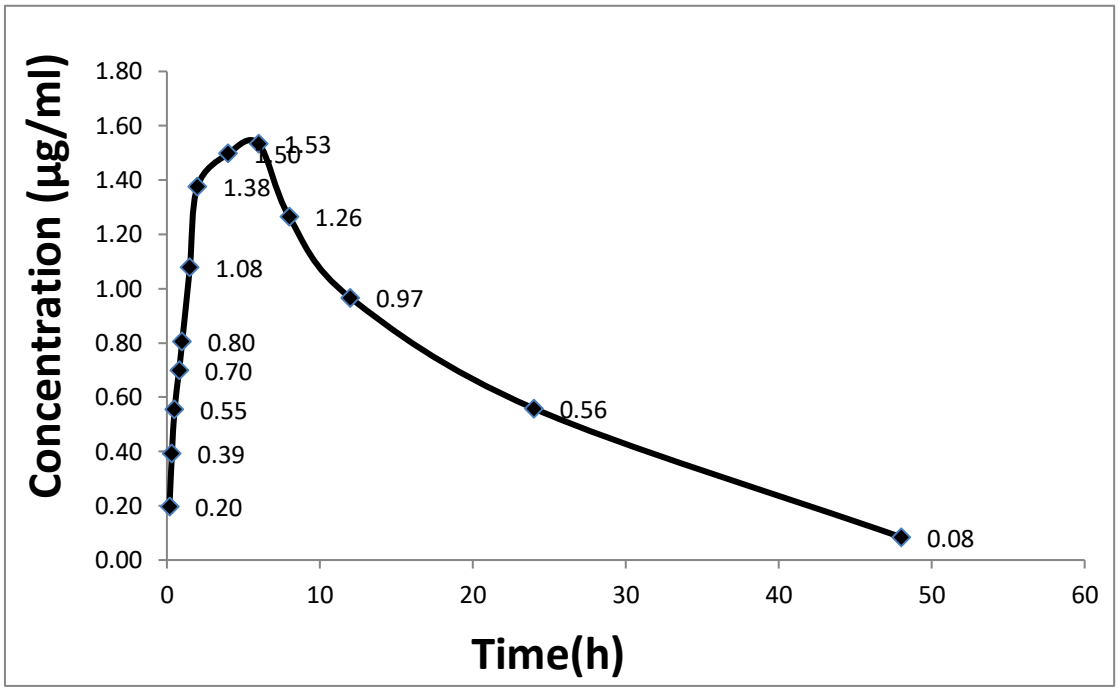


Table. 8 Pharmacokinetics of enrofloxacin following single oral administration of enrofloxacin (10 mg/kg) co-administered with sodium hypochlorite (10 ml/100L) in Group II birds (n=8)

Parameter	Unit	1	2	3	4	5	6	7	8	Mean±SE
β	l/h	0.055	0.066	0.065	0.069	0.070	0.067	0.044	0.131	**0.071±0.009**
$t_{1/2}$	h	12.582	10.577	10.661	10.064	9.900	10.313	15.656	5.282	**10.629±1.023**
t_{max}	h	6.000	8.000	2.000	4.000	4.000	4.000	6.000	8.000	**5.250±0.750**
C_{max}	µg/ml	1.336	1.828	2.321	1.364	1.879	1.502	1.535	2.574	**1.793±0.160**
AUC_{0-t}	µg/ml.h	20.089	33.625	35.444	27.190	25.583	23.073	29.223	58.471	**31.587±4.241**
$AUC_{0-\infty}$	µg/ml.h	22.327	35.386	37.106	28.172	26.670	24.117	31.470	58.578	**32.978±4.087**
AUMC	µg/ml.h^2	424.360	604.659	584.757	440.787	380.807	360.406	635.729	875.666	**538.396±61.064**
MRT	h	19.007	17.088	15.759	15.646	14.278	14.944	20.201	14.949	**16.484±0.748**
V_d/F	l/kg	8.130	4.312	4.145	5.154	5.355	6.169	7.177	1.301	**5.218±0.739**
Cl_B/F	l/kg/h	0.448	0.283	0.269	0.355	0.375	0.415	0.318	0.171	**0.329±0.031**

Note: No. 1 to 8 represent the individual number given to each bird in the group

4.8 Plasma concentration of enrofloxacin following single oral dose of enrofloxacin in birds pre-treated with sodium hypochlorite (Group III)

The peak plasma concentration of enrofloxacin in Group III birds was in the range of 1.366 to 2.643 μg/ml with a mean of 1.521±0.209 μg/ml and it was achieved in the time range of 4 to 8 hrs (Table. 9 and Fig. 6).

4.9 Pharmacokinetics of enrofloxacin following single oral dose of enrofloxacin in birds pre-treated with sodium hypochlorite (Group III)

The plasma concentration-time data (Table. 9) were analyzed by non-compartmental method and the values are showed in Table. 10. The elimination rate constant (β) ranged from 0.064-0.115 l/h with a mean value of 0.077±0.005 l/h. The elimination half-life ranged from 6.029-10.805 h with a mean value of 9.300±0.538 h, the C_{max} value ranged from 1.366-2.643 μg/ml with a mean value of 1.958±0.147 μg/ml. The time to achieve peak plasma concentration, t_{max} was observed in the time range of 4.000-8.000 h with a mean value of 5.750±0.453 h. The area under plasma concentration time curve (AUC_{0-t}) was observed in the range of 20.656-53.237 μg/ml.h with a mean of 33.669±3.593 μg/ml.h. The area under plasma concentration time curve ($AUC_{0-\alpha}$) ranged from 21.627-55.201 μg/ml.h with a mean of 34.751±3.681 μg/ml.h. The area under the first moment curve (AUMC) ranged from 333.615-891.457 μg/ml.h^2 with a mean of 525.468±61.695 μg/ml.h^2. The mean resident time (MRT) ranged from 13.356-17.066 h with a mean of 15.088±0.431 h. The volume of distribution (V_d/F) ranged from 2.432-6.890 l/kg with a mean of 4.226±0.587 l/kg. The total body clearance (Cl_B/F) ranged from 0.181-0.462 l/kg/h with a mean of 0.310±0.032 l/kg/h.

Table. 9 Plasma concentration (µg/ml) of enrofloxacin following single oral administration of enrofloxacin (10 mg/kg) in Group III birds pre-treated with sodium hypochlorite (10 ml/100L) (n=8).

Time(h)	1	2	3	4	5	6	7	8	Mean±SE
0	0	0	0	0	0	0	0	0	**0**
0.16	0.279	0.221	0.063	0.083	0.192	0.082	0.172	0.175	**0.158±0.027**
0.33	0.468	0.338	0.395	0.262	0.274	0.247	0.466	0.270	**0.340±0.033**
0.5	0.540	0.568	0.670	0.292	0.407	0.428	0.840	0.455	**0.525±0.061**
0.75	0.710	0.819	0.975	0.406	0.605	0.646	0.913	0.751	**0.728±0.064**
1	0.947	1.040	1.021	0.534	0.713	0.723	1.012	0.923	**0.864±0.065**
1.5	1.212	1.568	1.290	0.868	1.063	0.988	1.332	1.013	**1.167±0.080**
2	1.404	2.226	1.680	0.988	1.213	1.052	1.712	1.198	**1.434±0.147**
4	1.572	2.412	1.801	1.317	1.713	1.213	1.912	1.534	**1.684±0.133**
6	1.232	2.351	1.983	1.765	1.944	1.366	2.214	1.977	**1.854±0.137**
8	0.845	1.913	1.621	1.231	1.773	1.022	2.643	1.118	**1.521±0.209**
12	0.601	0.985	1.171	1.023	0.988	0.436	1.904	0.906	**1.002±0.154**
24	0.331	0.453	0.643	0.431	0.684	0.321	0.912	0.674	**0.556±0.073**
48	0.077	0.083	0.077	0.062	0.014	0.065	0.142	0.099	**0.077±0.013**

Note: No. 1 to 8 represent the individual number given to each bird in the group

Fig. 6 Plasma concentration-time curve of enrofloxacin (10 mg/kg) following single oral administration of enrofloxacin in Group III birds pre-treated with sodium hypochlorite (10 ml/100L). Each point represents mean of eight birds

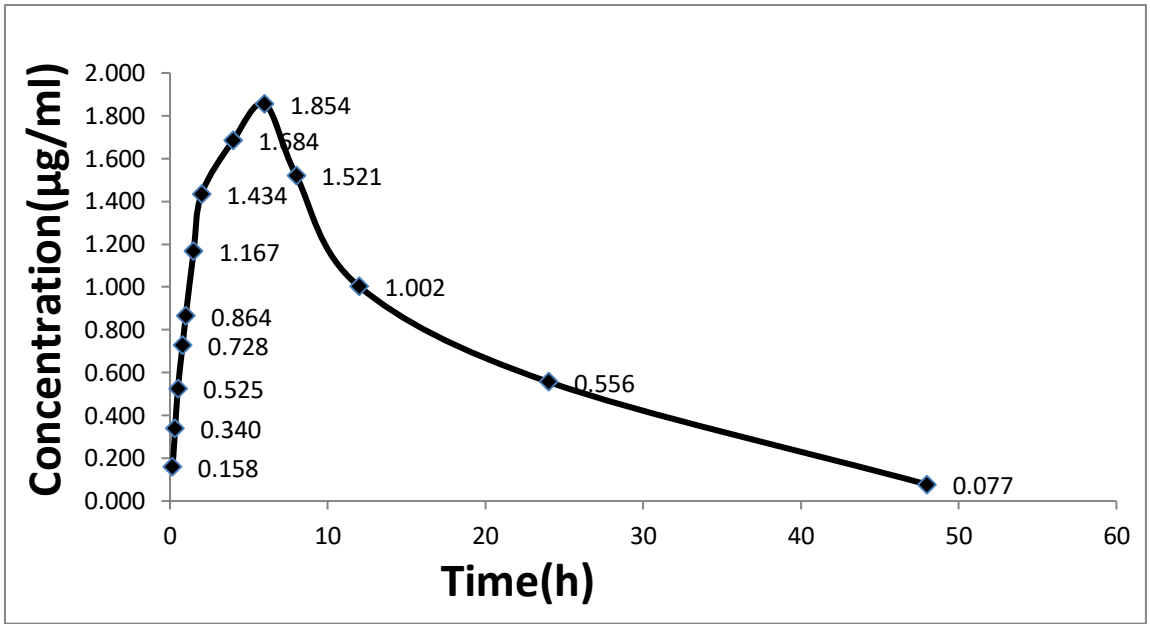


Table. 10 Pharmacokinetics (µg/ml) of enrofloxacin after single oral administration of enrofloxacin (10 mg/kg) in Group III birds pre-treated with sodium hypochlorite (10 ml/100L) (n=8).

Parameter	Unit	1	2	3	4	5	6	7	8	Mean±SE
β	1/h	0.065	0.078	0.076	0.077	0.115	0.067	0.073	0.064	**0.077± 0.005**
$t_{1/2}$	h	10.647	8.919	9.160	8.954	6.029	10.329	9.559	10.805	**9.300±0.538**
t_{max}	h	4.000	4.000	6.000	6.000	6.000	6.000	8.000	6.000	**5.750±0.453**
C_{max}	µg/ml	1.572	2.412	1.983	1.765	1.944	1.366	2.643	1.977	**1.958±0.147**
AUC_{0-t}	µg/ml.h	22.966	36.674	37.889	28.629	35.646	20.656	53.237	33.655	**33.669±3.593**
$AUC_{0-\infty}$	µg/ml.h	24.141	37.743	38.900	29.425	35.769	21.627	55.201	35.200	**34.751±3.681**
AUMC	µg/ml.h^2	371.985	504.104	579.307	433.216	489.327	333.615	891.457	600.730	**525.468±61.695**
MRT	h	15.409	13.356	14.892	14.723	13.680	15.426	16.149	17.066	**15.088±0.431**
V_d/F	l/kg	6.363	3.409	3.397	4.390	2.432	6.890	2.498	4.429	**4.226±0.587**
Cl_B/F	l/kg/h	0.414	0.265	0.257	0.340	0.280	0.462	0.181	0.284	**0.310±0.032**

Note: No. 1 to 8 represent the individual number given to each bird in the group

4.10 Pharmacokinetics of enrofloxacin in different groups of birds

Various pharmacokinetic parameters of enrofloxacin estimated after its oral administration to different groups of birds are presented in Table. 11. The salient findings of the study are:

- Enrofloxacin was detected in all the three groups of birds at all tested time points.
- Elimination rate constant (β) observed in Group II (0.071±0.009 $l.h^{-1}$) and Group III (0.077±0.005 $l.h^{-1}$) birds was significantly ($p<0.05$) higher when compared to that of Group I (0.040±0.002 $l.h^{-1}$) birds.
- The $t_{1/2}$ and t_{max} did not differ significantly ($p<0.05$) among the three groups.
- There was a noticeable decline in C_{max} of enrofloxacin in Group II (1.793±0.160 μg/ml) and Group III (1.958±0.147 μg/ml) birds over Group I (2.153±0.245 μg/ml) birds, although not statistically significant ($p<0.05$).
- The area under plasma concentration curve (AUC_{0-t}) apparently declined in both Groups II (31.587±4.241 μg/ml.h) and Group III (33.669±3.593 μg/ml.h) birds when compared to that of Group I (40.873±4.496 μg/ml.h) birds, though not significant ($p<0.05$) statistically.
- Group II (32.978±4.087 μg/ml.h) birds showed significantly ($p<0.05$) lower area under plasma concentration curve ($AUC_{0-\alpha}$) compared to that of Group I (50.648±6.111 μg/ml.h) birds. Reduced $AUC_{0-\alpha}$ was also observed in Group III (34.751±3.681 μg/ml.h) birds though statistically not significant ($p<0.05$).

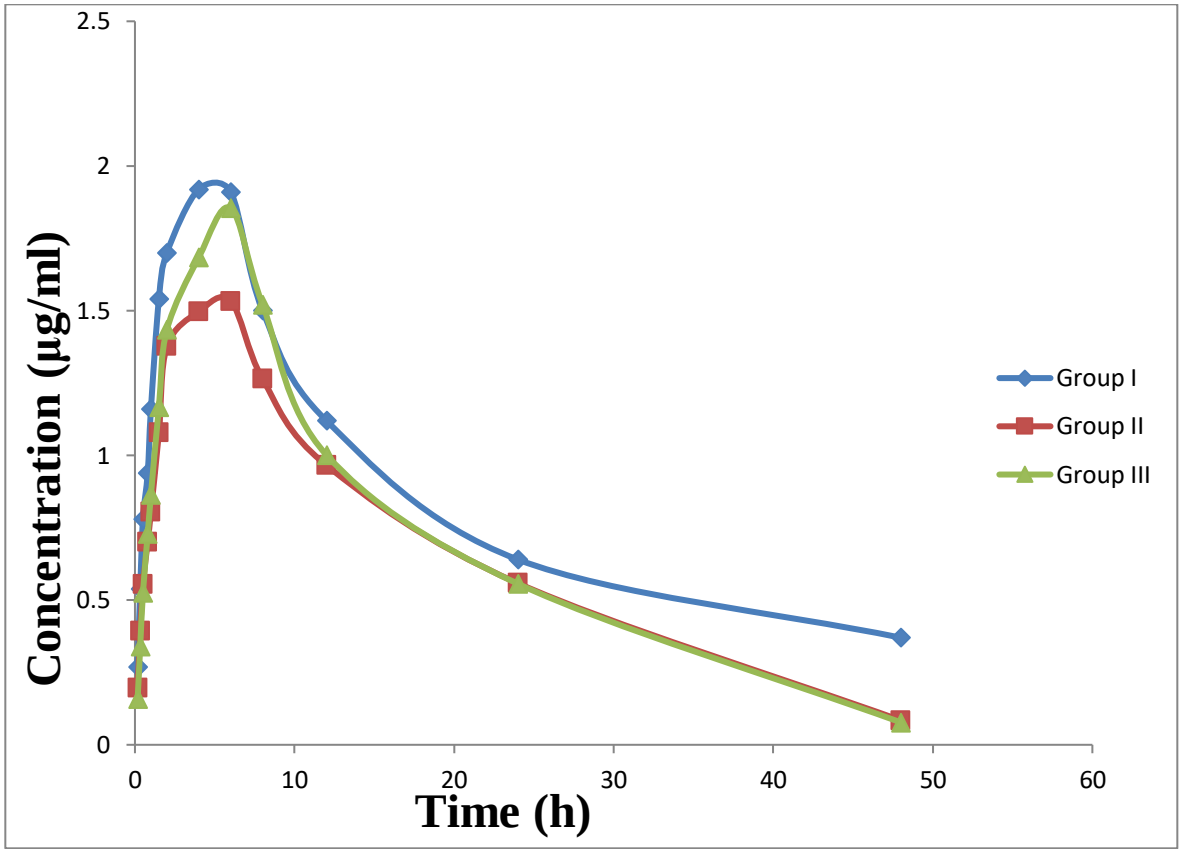
- The area under the first moment curve (AUMC) observed in Group II (538.396±61.064 μg/ml.h^2) and Group III (525.468±61.695 μg/ml.h^2) birds was significantly ($p<0.05$) lower when compared to that in Group I (1407.185±216.254 μg/ml.h^2) birds.
- The mean residence time (MRT) observed in Group II (16.484±0.748 h) and Group III (15.088±0.431 h) birds was significantly ($p<0.05$) lower when compared to that in Group I (27.076±1.375 h) birds.
- There was no significant difference in volume of distribution (V_d/F) in the birds of three groups.
- The total body clearance (Cl_B/F) observed in Group II (0.329±0.031 l/kg) and Group III (0.310±0.032 l/kg) birds was significantly ($p<0.05$) higher when compared to that in Group I (0.216±0.022 l/kg) birds.
- The relative bioavailability (F) observed in Group II birds (65%) and that of Group III birds (68%) was decreased when compared to that of Group I birds (100%).

Table. 11 Pharmacokinetic parameters of enrofloxacin (Mean±SE) in different groups

Parameter	Unit	Group I	Group II	Group III	F value	P value
β	l/h	**0.040±0.002^{a}**	**0.071±0.009^{b}**	**0.077± 0.005 ^{b}**	**9.894**	**0.001**
$t_{1/2}$	h	**11.668±0.352**	**10.629±1.023**	**9.300±0.538**	**2.896**	**0.077**
t_{max}	h	**5.250±0.366**	**5.250±0.750**	**5.750±0.453**	**0.772**	**0.475**
C_{max}	µg/ml	**2.153±0.245**	**1.793±0.160**	**1.958±0.147**	**0.911**	**0.418**
AUC_{0-t}	µg/ml.h	**40.873±4.496**	**31.587±4.241**	**33.669±3.593**	**1.394**	**0.270**
$AUC_{0-\infty}$	µg/ml.h	**50.648±6.111^{b}**	**32.978±4.087^{a}**	**34.751±3.681^{ab}**	**4.202**	**0.029**
AUMC	µg/ml.h^2	**1407.185±216.254^{b}**	**538.396±61.064^{a}**	**525.468±61.695^{a}**	**14.110**	**0.000**
MRT	h	**27.076±1.375^{b}**	**16.484±0.748^{a}**	**15.088±0.431^{a}**	**48.896**	**0.000**
V_d/F	l/kg	**5.381±0.418**	**5.218±0.739**	**4.226±0.587**	**1.101**	**0.351**
Cl_B/F	l/kg/h	**0.216±0.022^{a}**	**0.329±0.031^{b}**	**0.310±0.032^{ab}**	**4.403**	**0.025**

Values are expressed as Mean±SE.
F= variance of the group means
*Significantly different ($p<0.05$) from respective normal values.

Fig. 7 Comparison of plasma concentration-time curves of enrofloxacin (10 mg/kg) in three groups



DISCUSSION

CHAPTER-V

DISCUSSION

Providing *ad libitum* access to clean and safe drinking water to poultry is a basic requirement for optimizing production. One prime factor that determines wholesomeness of drinking water is its microbial quality. Microbial contamination above acceptable levels in drinking water can directly affect the health and performance of birds. The practice of regular water sanitation and line cleaning between flocks can solve much of microbial problem in water systems. Poultry operations like performing daily water sanitation and line cleaning between flocks have exhibited improved performance of birds.

Sodium hypochlorite is a commonly used chemical sanitizer. It is effective against a variety of bacteria, fungi and viruses. It has excellent cleaning action and is unaffected by water impurities. It can be easily combined with other cleaning agents and leaves minimal residue or film on surfaces.

Enrofloxacin, a fluoroquinolone antibacterial developed exclusively for veterinary use is still one of the highly used antibacterials in poultry. Its favorable pharmacokinetic properties like good absorption, high bioavailability, large volume of distribution and low protein binding makes this drug more suitable antibacterial for use in poultry. It exhibits concentration-dependent bactericidal effect on a range of bacteria. Good solubility in water yielding stable solutions makes it suitable for economic water medication for chicken

The clinical efficacy of an antimicrobial is determined not only by its activity against infective organisms but also by its pharmacokinetic variables such as plasma concentration, half-life, bioavailability, and rate of elimination. Many a times these pharmacokinetic variables are likely to be influenced by the concomitantly administered

drugs. Sodium hypochlorite decreased the *in-vitro* antimicrobial activity as well as maximum serum concentration and bioavailability of enrofloxacin in chickens (Ledesma *et al*., 2018). Keeping the above in view, present study was conducted to know the influence of sodium hypochlorite on the oral pharmacokinetics of enrofloxacin in broiler chicken.

Twenty four broiler chickens weighing around 2.0 kg were randomly divided into three groups with eight birds in each. The dose of enrofloxacin at 10 mg/kg body weight was uniform in all the three groups. Group I birds that received normal drinking water were administered with enrofloxacin orally. Group II birds received sodium hypochlorite in drinking water at the dose rate of 10 ml/100L for seven days and on the seventh day enrofloxacin was given in sodium hypochlorite containing water. Group III birds were also given with sodium hypochlorite similar to group II birds, but enrofloxacin was given 12 hours post withdrawal of sodium hypochlorite in drinking water. In all the birds blood samples were collected at 0 (blank), 0.16, 0.33, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 24 and 48 h post enrofloxacin dosing and plasma was separated and stored at -20˚C until further analysis by HPLC.

The detection method adopted for enrofloxacin by using HPLC was accurate and precise and exhibited acceptable recovery from the plasma samples for enrofloxacin which was 96.5%.

The pharmacokinetic parameters observed in Group I birds, that received enrofloxacin alone, *i.e.* in normal drinking water were in accordance with the previous observations of pharmacokinetics of enrofloxacin in broiler chicken in which nearly similar values of $t_{1/2}$, AUC, MRT, etc. were reported (Mekala *et al*., 2015, Garacia-Ovando *et al*., 1999).

Elimination rate constant (β) of enrofloxacin was significantly ($p<0.05$) higher in Group II and Group III birds when compared to Group I birds. The elimination half-life ($t_{1/2}$) and t_{max} of enrofloxacin did not differ statistically among the three groups.

There was a noticeable decline in peak concentration (C_{max}) of enrofloxacin in Group II and Group III birds over Group I birds, although not statistically significant ($p<0.05$). Sodium hypochlorite being an alkaline solution the disturbance in the absorption of enrofloxacin might be due to altered gut pH (Ledesma *et al*. 2018) in both Group II and as well as in Group III birds due to persistence of its influence even 12 hours after post withdrawal. Decreased C_{max} might be on account of decreased bioavailability.

It was evident from the data that AUC_{0-t} was apparently declined though not statistically significant ($p<0.05$) in both Group II and Group III birds. Assuming linear pharmacokinetics of enrofloxacin in broiler chicken, it can be concluded that AUC_{0-t} is proportional to the total drug absorbed from the gut. There was significant ($p<0.05$) decline in the $AUC_{0-\alpha}$ in Group II birds compared to that in Group I birds. Thus, the influence of sodium hypochlorite exposure on total exposure to enrofloxacin across time is evident. Likewise, administration of enrofloxacin 12 hours post withdrawal of sodium hypochlorite in Group III birds also resulted in reduced $AUC_{0-\alpha}$ of enrofloxacin, though statistically not significant ($p<0.05$).

The area under first moment curve (AUMC) and mean residence time (MRT) values were found significantly ($p<0.05$) decreased in Group II and Group III birds compared to those of Group I birds. MRT is specific for the average total time the molecules (enrofloxacin or its metabolites) spend in the body. Thus, sodium hypochlorite exposure or its exposure till 12 hours before the administration of enrofloxacin could not retain the enrofloxacin molecules for a period similar to that of control Group I.

Although there was no influence of sodium hypochlorite on volume of distribution (V_d/F), the total clearance from the body (Cl_B/F) in Groups II and Group III birds was significantly ($p<0.05$) increased when compared with that of Group I birds, and it can be attributable to altered environment in the renal mechanisms involved in elimination of enrofloxacin or its metabolite(s). Further, sodium hypochlorite exposure might have played a role in tissue binding pattern of enrofloxacin, since it is a highly lipid soluble agent among fluroquinolones.

The two major predictors of effectiveness of fluoroquinolone antibacterials are the C_{max}/MIC and AUC_{24h}/MIC ratios (Kang *et al*., 2019). The C_{max}/MIC is the ratio of the peak concentration of the drug in serum quantified *in vivo* (C_{max}) to the minimum inhibitory concentration (MIC) obtained *in vitro*. AUC_{24h}/MIC is the ratio of the area under the curve (AUC) over 24 h to the MIC. The C_{max}/MIC values ≥ 8–12 h and $AUC_{0–24}$/MIC values ≥ 100–125 are generally utilized as threshold levels for an effective healing response of fluoroquinolone drugs against Gram-negative bacterial strains and are usually recognized as the best measures of activity for an antibacterial that kills bacteria concentration-dependently (Marin *et al*., 2009). Similarly, Rodvold and Neuhauser (2001) reported that the break points determining efficacy of fluoroquinolone are a C_{max}/MIC ≥ 10 or an AUC_{24h}/MIC≥ 125.

Taking into consideration of previously reported minimum inhibitory concentration (MIC) of enrofloxacin for specific strains of *E. coli* as 0.25 µg/ml (Sang *et al*., 2015 and Wang *et al*., 2016), the above two indices were calculated. The C_{max}/MIC values for Group I, Group II and Group III were 8.612, 7.172 and 7.832, respectively and the AUC/MIC values were 163.49, 126.35 and 134.58, respectively. As the bactericidal

action of enrofloxacin is concentration dependant, it appears that, its clinical efficacy will be reduced by the presence of sodium hypochlorite.

Overall, it is evident that sodium hypochlorite exposure altered the pharmacokinetic profile of enrofloxacin and the effect of sodium hypochlorite persisted even after its withdrawal 12 hours before the administration of enrofloxacin. Similar reports of altered pharmacokinetics of enrofloxacin were reported when it was given along with hard water (Sumano *et al*. 2004). Ledesma *et al*. (2018) reported that administration of sodium hypochlorite along with enrofloxacin in broiler chicken showed alteration in the pH of the gastrointestinal contents caused by chlorinated water and opined that alteration in the pH might change the ratio of protonated to non protonated enrofloxacin.

SUMMARY

CHAPTER-VI

SUMMARY

An experimental study was conducted on 24 broiler chicken weighing around 2 kg to know the influence of sodium hypochlorite in drinking water on the oral pharmacokinetics of enrofloxacin. The birds were divided into three groups with eight birds each. Group I birds on normal drinking water were administered with enrofloxacin (10mg/kg) alone. Group II birds received sodium hypochlorite (10 ml/100L) in drinking water for seven days followed by enrofloxacin (10 mg/kg) on seventh day. Group III birds received sodium hypochlorite (10 ml/100L) in drinking water for seven days and received enrofloxacin (10 mg/kg) 12 hours post withdrawal of sodium hypochlorite containing water. Blood samples were collected from either left or right tarsal veins at 0 (blank), 0.16, 0.33, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 24 and 48 h post enrofloxacin dosing and plasma was separated and analyzed by HPLC method.

The HPLC method applied was accurate and precise and exhibited acceptable statistics from plasma spiked samples. Enrofloxacin was detected in the birds of all three groups at all tested time points.

The elimination rate constant (β) of enrofloxacin observed in Group II (0.071±0.009 l/h) and Group III (0.077±0.005 l/h) birds was significantly ($p<0.05$) higher compared to that in Group I (0.040±0.002 l/h) birds. There was no significant difference in elimination half-life ($t_{1/2}$) and in t_{max} of enrofloxacin among the three groups.

There was a noticeable decline in C_{max} of enrofloxacin in Group II (1.793±0.160 µg/ml) and Group III (1.958±0.147 µg/ml) birds over Group I (2.153±0.245 µg/ml) birds, although no statistical significance was observed. Sodium hypochlorite being an alkaline solution, the decline in peak concentration can be attributable to disturbances in

the absorption of enrofloxacin due to altered gut pH in both sodium hypochlorite administered groups (Group II and Group III) and due to persistence of its influence even 12 hours after post withdrawal.

It was evident from the data that AUC_{0-t} apparently declined in both the sodium hypochlorite administered groups (Group II and III). Thus, assuming linear pharmacokinetics of enrofloxacin in birds, it can be concluded that AUC_{0-t} is proportional to the total drug absorbed from the gut. There was significant ($p<0.05$) decline in $AUC_{0-\alpha}$ in Group II (32.978±4.087 µg/ml.h) compared to that in Group I (50.648±6.111 µg/ml.h) birds. Thus, the influence of sodium hypochlorite on total exposure to enrofloxacin across time is evident. Likewise, administration of enrofloxacin 12 hours post withdrawal of sodium hypochlorite (Group III) also reduced $AUC_{0-\alpha}$ (34.751±3.681 µg/ml.h) of enrofloxacin, though not significant statistically.

The area under first moment curve (AUMC) and mean resident time (MRT) values were found decreased significantly ($p<0.05$) in Group II (538.396±61.064 µg/ml.h^2 and 16.484±0.748 h) and Group III (525.468±61.695 µg/ml.h^2 and 15.088±0.431 h) birds compared with those of Group I (1407.185±216.254 µg/ml.h^2 and 27.076±1.375 h) birds. MRT is specific for the average total time the molecules (enrofloxacin or its metabolites) spend in the body. Thus, sodium hypochlorite exposure or its exposure till 12 hours before the administration of enrofloxacin could not retain the enrofloxacin molecules for a period similar to that of control Group I.

Although there was no influence of sodium hypochlorite on volume of distribution (V_d/F), the total clearance from the body (Cl_B/F) in Group II (0.329±0.031 l/kg/h) and Group III (0.310±0.032 l/kg/h) birds was significantly ($p<0.05$) higher when compared to that in Group I (0.216±0.022 l/kg/h) birds, and it can be attributable to altered

environment in the renal mechanisms involved in elimination of enrofloxacin or it metabolite(s). Further, sodium hypochlorite exposure might have played a role in tissue binding pattern of enrofloxacin, since it is a highly lipid soluble agent among fluroquinolones.

It can be concluded that sodium hypochlorite administration altered the pharmacokinetics of enrofloxacin and the effect of sodium hypochlorite persisted even after its withdrawal 12 hours before administration of enrofloxacin.

LITERATURE CITED

CHAPTER-VII

LITERATURE CITED

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