DIETARY EFFECT OF DIFFERENT ANTIBIOTICS AND DETECTION OF ANTIBIOTICS RESIDUE IN THE MEAT OF SONALI CHICKEN IN BANGLADESH

A Thesis

By

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Registration No. 1705455
Semester: July-December, 2018

MASTER OF SCIENCE (M.S.)
IN
POULTRY SCIENCE

DEPARTMENT OF DAIRY AND POULTRY SCIENCE
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

DECEMBER, 2018

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DEPARTMENT OF DAIRY AND POULTRY SCIENCE
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DECEMBER, 2018

DEDICATED

TO MY

BELOVED PARENTS

AND

FAMILY

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The author

ABSTRACT

This study was conducted to evaluate the efficacy of various antibiotics on production performance, dressing yield and detection of antibiotic residue in the meat of Sonali Chicken in Bangladesh. For this purpose, a total of 120 day old chicks were randomly assigned into four treatment groups namely T_0, T_1, T_2 and T_3 having three replications in each treatment group. Chicks were brooded upto 7 days then randomly separate into replication wise in separate pen for rearing 9 weeks. Each treatment group contains 30 birds whereas each replication contains 10 birds. Experimental birds in T_1, T_2 and T_3 were provided ciprofloxacin @ 1ml, oxytetracycline @ 1gm and amoxicillin @ 1gm per liter respectively drinking water while T_0 was provided only plain water and T_0 was maintained as control group. The results of this study indicated that final live weight gain and feed efficiency of birds were insignificantly ($P>0.05$) higher in T_2 group and T_1 group compared to T_0 group. In case of meat yield parameters, there were no significant difference among the treatment groups. Antibiotic residue was the highest in T_3 group in meat. But antibiotic residue was nil in liver in T_3 group. On the other hand, T_2 group found with antibiotic residue in both meat and liver. In T_1 group, there were no antibiotic residue in both meat and liver. Based on the result it could be concluded that the supplementation of different antibiotics, oxytetracycline @ 1gm/L drinking water has potential effect on growth performance and kept as antibiotic residue in both meat and liver of Sonali chicken in Bangladesh.

Keywords: Ciprofloxacin, Oxytetracycline, Amoxicillin, Sonali chicken, Production performance and Antibiotic residue.

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LIST OF ABBREVIATIONS

^{0}C	Temperature
ADI	Allowed Daily Intake
AGP	Antibiotic Growth Promotor
BSDA	*B. stearothermophilus* Disc Assay
CAC	Codex Alimentarius Commission
CAST	The Calf Antibiotic and Sulfonamide Test
CRD	Complete Randomized Design
CTC	Chlortetracycline
DAD	Diode Array Detector
DC	Doxycycline
DOCs	Day Old Chicks
DOS	Dextran Oligosaccharide
DW	Drinking Water
FAO	Food and Agriculture Organization
FAST	Fast Antimicrobial Screening Test
FCR	Feed Conversion Ratio
FDA	Food and Drug Administration
FPT	Four Plate Test
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
HSTU	Hajee Mohammad Danesh Science and Technology University
LC	Liquid Chromatography
MOS	Mannan Oligosaccharide
MRLs	Maximum Limits of Drug Residues
OTC	Oxytetracycline
PLDP	Participatory Livestock Development Project
PRTC	Poultry Research & Training Centre
RIR	Rhode Island Red
SC	Subcutaneously
SLDP	Smallholder Livestock Development Project
STAR	Screening Test for Antibiotic Residues

TCA	Trichloro Acetic Acid
TCs	Tetracyclines
TLC	Thin Layer Chromatography
UHPLC	Ultra High Performance Liquid Chromatography
UV	Ultraviolet Detection
WHO	World Health Organization
WP	Withdrawal Period
ZI	Zone of Inhibition

CHAPTER - 1

INTRODUCTION

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INTRODUCTION

1.1 General Background

Bangladesh is an agriculture-based developing country in south-east Asia where natural disasters are frequent. Poultry is one of the most important agricultural subsectors in the country and about 87 percent of rural house-holds rear poultry, with an average flock size of 6.9 birds (Apu and Saleque, 2012).

The contribution of the poultry sector is an important tool in global efforts to overcome malnutrition and poverty in developing countries is widely recognized. Poultry often represents a farmer's first investment in the livestock ladder (followed by goats/sheep and then cattle) as a way of increasing income and emerging from the poverty trap. The share of commercial poultry production by the private sector is expanding rapidly in Bangladesh, and now accounts for 50 percent of egg production and 60 percent of meat production (Bhuiyan, 2011).

The poultry sector in Bangladesh is dynamic and has potential for rapid poverty reduction through income generation and employment creation. As commercial poultry farming gains in popularity, employment opportunities are created for rural farmers, retailers, traders, service providers, entrepreneurs, etc. The current poultry production system in Bangladesh can be divided into four main categories: i) traditional rural backyard scavenging systems; ii) semi-scavenging systems; iii) commercial farming systems; and iv) contract farming or integrated systems (Saleque, 2009; Dolberg, 2008).

The poultry industry creates numerous employment opportunities (Shamsuddoha and Sohel, 2003). Peoples in our country reared deshi chicken for egg and meat purpose and consumer have high demand on its, but the production performance of deshi chicken could not fulfill consumer demand. As like deshi, Sonali chicken are reared recent year. The Sonali is a cross-breed of Rhode Island Red (RIR) cocks and Fayoumi hens and has a similar phenotypic appearance to that of local chickens; it was introduced in 1996–2000 in northern parts of Bangladesh, through SLDP and PLDP. Sonali birds are well adapted to the country's environmental conditions and require less care and attention than other breeds, making them easier for women and children to rear (Saleque and Saha, 2013). Traders can sell Sonali at higher prices than broilers. The Sonali population

has been increasing and in 2010 about 150.9 million Sonali Day Old Chicks (DOCs) were produced, representing about 35 percent of the country's total commercial broiler and layer production (Huque, 2011).

Poultry farmers are interested in Sonali chicken production due to its high market price, smaller marketing age, less space requirement, less feed requirement, high quality meat production and lower mortality. Many antibiotic drugs and growth promoters are supplemented to the Sonali for rapid growth, but their use have shown many disadvantages like high cost, adverse side-effect on health of birds and long residual properties etc. In the past few decades, antibiotics have been extensively used in livestock animals as food additive for growth promotion, prevention or treatment of infectious diseases.

According to the Food and Drug Administration (FDA), about 87% of antibiotics used in livestock animals are for treatment, control or prevention of infectious diseases and 13% as food supplement for nutritional purposes to increase growth and productivity (Meredith *et al.*, 1965; Dipeolu and Alonge, 2002; Donoghue, 2003; Mahgoub *et al.*, 2006). Due to the extensive nontherapeutic use of antibiotics and lack of adequate control of administration, the risk of drug accumulation and their residuals in animal tissues and their product will increase (Meredith *et al.*, 1965; Lemus, 2008).

Antibiotic residues and their metabolites in poultry meat may cause several adverse effects on consumers. They can cause direct toxicity, developing resistant bacteria, and allergies, even when used at very low doses (Kirbis *et al.*, 2007). In addition to direct toxicity, normal micro flora in digestive system can adapt and acquire resistance to the antibiotic by long term over exposure to trace amounts of antibiotics through contaminated foods (Javadi *et al.*, 2011; Myllyniemi, 2004).

Concerns about the risk of antibiotic residues resulted in establishing the maximum limits of drug residues (MRLs), which are the maximum amount of residues that could legally permitted to be in the food product without causing adverse effects to the consumers (Myllyniemi, 2004; Reyes-Herrera, 2005).

Several methods have been developed for determining the residues of antibiotics in poultry tissues, including microbiological methods, chromatography methods, ELISA and immunochemical methods (Kaya and Filazi, 2010). The microbiological methods are

the methods of choice since they are cheap, easy to perform and able to measure a large number of samples (Karraouan *et al.*, 2009; Kirbis *et al.*, 2007; Javadi *et al.*, 2011). Due to the high sensitivity and specificity of the Four Plate Test (FPT), it has been used by several researchers and also has been accepted by the European Union as a standard method for determining antibiotic residues. The aim of this study was to investigate the possible presence of main groups of antibiotic in Sonali carcasses, including penicillin, fluoroquinolones, and tetracyclines family.

1.2 Research objectives

From the view of point the research program had been undertaken with the following objectives:

- To evaluate the growth performance and carcass characteristics of Sonali chicken.
- To detect the antibiotics residue in the meat and liver of Sonali Chicken.

CHAPTER - 2

REVIEW OF LITERATURE

CHAPTER - 2

REVIEW OF LITERATURE

Antibiotics are commonly used for various purposes in livestock and poultry industry such as for treatment of infections/diseases, growth promoting agents etc. If these antibiotics when used in excess dosage, use of abnormal route of administration, increase rate of frequency, substandard drugs, extra labeled use of drug may leads to development of residues in different tissues of body. Exposure of individual to such kind of animal origin food containing these antimicrobial drug residues may pose public health problem (Thompson *et al.*, 1976). The common health related problems observed in human being includes allergic reactions in sensitized individuals, development of bacterial resistance and in case of more chronic condition it can lead to toxic effects like teratogenicity, mutagenicity, carcinogenicity etc. Therefore it is imperative to survey the various foods of animal origin for presence of antimicrobial residue.

2.1 Antibiotics

2.1.1 Definition of antibiotics

Antibiotics are chemical compounds that kill or inhibit the growth of microorganisms but cause little or no damage to the host. They are naturally produced by microorganisms such as fungi (e.g. penicillin) and bacteria (e.g. tetracycline) or can be semi-synthetically produced (e.g. amoxicillin) or totally synthetically produced (e.g. sulfonamides) (Guardabassi *et al.*, 2008).

2.1.2 Classification of antibiotics

According to Wang *et al.* (2012), antibiotics can be classified by many ways;

1. According to the spectrum of activity:

    - Broad spectrum antibiotics.
    - Narrow spectrum antibiotics.

2. According to the mode of action:

    - Inhibiting cell wall synthesis.
    - Inhibiting protein synthesis.

- Inhibiting nucleic acid synthesis.
- Inhibiting the synthesis of essential metabolites.
- Injuring the plasma membrane.

3. According to their effects on microorganisms:

- Bactericidal antibiotics.
- Bacteriostatic antibiotics.

4. According to the chemical structure:

- β-lactams.
- Nitroimidazoles.
- Aminoglycosides.
- Phenicols.
- Lincosamides.
- Ionophores.
- Tetracyclines.
- Polypeptides.
- Quinolones.
- Quinoxalines.
- Macrolides.
- Phosphoglycolipids.
- Nitrofurans.
- Sulfonamides.

2.2 Common antibiotics used in poultry

2.2.1 Tetracyclines

The tetracyclines were discovered in the 1940s, they are a family of antibiotics that inhibit protein synthesis by preventing the attachment of aminoacyl-tRNA to the ribosomal acceptor (A) site (Chopra *et al.,* 2001). Tetracyclines consist of a common four-ring structure to which a variety of side chains are attached (Figure 2.1) (Prescott *et al.,* 2002). Chlortetracycline and oxytetracycline were the first members of the

tetracycline group to be described. Subsequently, a number of important semisynthetic tetracyclines were developed, e.g. doxycycline and minocycline (Michalova *et al.*, 2004).

Tetracyclines are the most commonly prescribed antibiotics; they have played an important role in veterinary medicine. Because of their broad spectrum activity and low cost, tetracyclines (TCs) including tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CTC) and doxycycline (DC) are widely used in animals for both prevention, treatment and as feed additives to promote growth (Mesgariabbasi *et al.*, 2011).

The widespread utilization of TCs leads to an increasing resistance factor, so accurate monitoring by public health agencies is required (Oka *et al.*, 2000). Three different tetracycline resistance mechanisms have been described; active efflux of the antibiotic, ribosomal protection and enzymatic inactivation of the drug. All these mechanisms are based on the acquisition of one or several tetracycline resistance determinants, which are widely distributed among bacterial genera. Additionally, mutations in the rRNA, multidrug transporter systems or permeability barriers may be involved in the resistance to several antibiotics including tetracyclines (Michalova *et al.*, 2004).

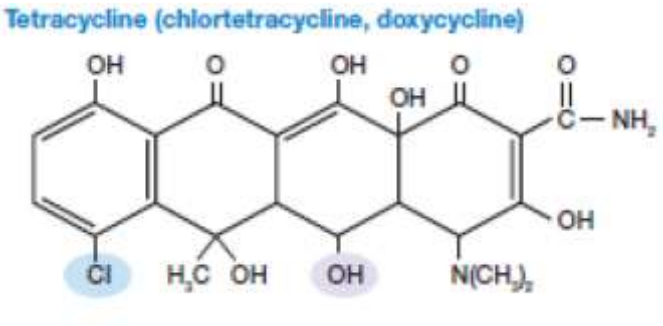

Figure 2.1: Tetracyclines: Three members of tetracycline family (Michalova *et al.*, 2004). Tetracycline lacks both of the groups that are shaded. Chlortetracycline differs from tetracycline in having a chlorine atom (blue); doxycycline consists of tetracycline with an extra hydroxyl (purple).

2.2.2 β-lactams

The β-lactam group is one of the most important families of antibiotics used in veterinary medicine and has been widely used for decades in animal husbandry. This group consists of penicillins and cephalosporins. The most common members of the penicillins used in veterinary practice are benzyl penicillin, amoxicillin, ampicillin and penicillin G. The

extensive use of penicillins may cause the presence of their residues in food products of animal origin and may have side effects to consumers. Moreover, penicillin residues in food products may be responsible of allergic reactions in humans and promote the occurrence of antibiotics resistant bacteria (Kowalski *et al.,* 2007). The cephalosporins are chemically related to the penicillins and both share the β-lactam ring structure. A number of cephalosporins, including cefalexin, cefuroxime, ceftiofur, cefquinome and cefotaxime are used in veterinary medicine in food animals (Woodward, 2009). Due to increased emergence of cephalosporin resistant bacteria (specially *E. coli* and *Salmonella*) (Forward *et al.,* 2004 and Dhanji *et al.,* 2010) the FDA prohibited the usage of cephalosporins in food producing animals including poultry (Schmidt and C.W., 2012).

2.2.3 Macrolides

Macrolides constitute a very important class of antibacterial compounds widely used in veterinary medicine to treat respiratory diseases. These antibiotics are molecules with a central lactone ring bearing 12 or 16 atoms to which several amino and/or neutral sugars are bound (Figure 2.2) (Stolker and Brinkman, 2005). The antibacterial action of macrolides is through the inhibition of protein synthesis by binding to the 50S ribosomal subunit of prokaryote organisms. Resistance to macrolides is usually plasmid-mediated, but modification of ribosomes may occur through chromosomal mutation, resistance can occur either by decreasing entry into bacteria, synthesis of bacterial enzymes that hydrolyze the drug or modification of the target (ribosome) (Riviere *et al.,* 2009).

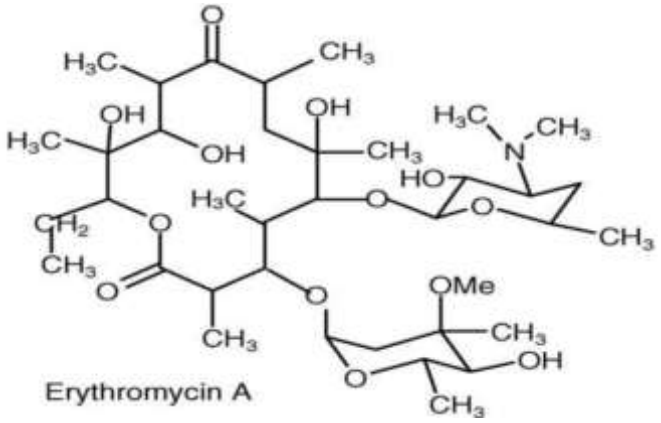

Figure 2.2: Typical structure of a macrolide member (Erythromycin A) (Riviere *et al.,* 2009).

2.2.4 Aminoglycosides

Aminoglycosides are a large class of antibiotics that are characterized by two or more amino sugars linked by glycosidic bonds to an aminocyclitol component, Aminoglycosides are broad-spectrum antibiotics and act primarily by impairing bacterial protein synthesis through binding to prokaryotic ribosomes (Mingeot-Leclercq *et al.,* 1999). In veterinary medicine and animal husbandry, aminoglycosides are widely used in the treatment of bacterial infections, and have been added to feeds for prophylaxis and for growth promotion. Those most commonly used are gentamicin, neomycin, streptomycin and dihydrostreptomycin (Stead, 2000).

2.3Antibiotics usage in veterinary medicine

Antibiotics are used largely for three purposes in animals: therapeutic use to treat sick animals, prophylactic use to prevent infection in animals and as growth promoters to improve feed utilization and production. In general, therapeutic treatment involves treatment of individual animals over a short period with doses of antibiotics exceeding the minimal inhibitory concentration of the known or suspected pathogen (Barton, 2000). Sometimes, with intensively farmed animals, therapeutic treatment is delivered through feed or drinking water. Prophylactic treatment involves moderate to high doses of antimicrobials, often given in feed or water for a defined period to a group of animals. Antibiotics used as growth promoters tend to be given in feed at sub-therapeutic levels over extended periods to entire herds and flocks (Wallace *et al.*, 1995).

2.4 Antibiotics resistance

Due to the excessive and inappropriate use of antibiotics, there has been a gradual emergence of populations of antibiotics–resistant bacteria, which pose a global public health problem. A resistant microbe is one which is not killed by an antimicrobial agent after a standard course of treatment (Levy *et al.,* 2004). Antibiotics used to combat infection forces bacteria to either adapt or die irrespective of the dosage or time span. The surviving bacteria carry the drug resistance gene, which can then be transferred either within the species/genus or to other unrelated species. Clinical resistance is a complex phenomenon and its manifestation is dependent on the type of bacterium, the site of infection, distribution of antibiotics in the body, concentration of the antibiotics at the site of infection and the immune status of the patient (Ahmed, 2012).

2.5 Emergence of resistant bacteria in chicken

In animals, antibiotics resistant enteropathogens (e.g., Salmonella, Campylobacter, Yersinia, and some strains of *Escherichia coli*) are of special concern to human health because these bacteria are most likely to be transferred through the food chain to humans, or resistance genes in commensal bacteria may be transferred to the zoonotic enteropathogens (McEwen *et al.,* 2002).

The most important antibiotics-resistant strains are the multiply antibiotics resistant Salmonella, macrolide or fluoroquinolone-resistant Campylobacter and multiply antibiotics-resistant *Escherichia coli*. In all cases, the hypothesis is that the food chain is the main mean of transmission (Phillips *et al.,* 2004). Direct physical contact, shared environments, and exposure through vectors and fomites are all routes for bacterial transmission between animal species. Poultry is considered a leading source for foodborne infections caused by Campylobacter and Salmonella. Food surveillance most commonly isolates Salmonella from fresh meat, commonly from poultry and less frequently from eggs, beef, fishery products, vegetables and milk (Rosengren *et al.,* 2010).

Elmanama and Abdelateef (2012) conducted a study to investigate the antibiotic resistance for enteric pathogens isolated from acute gastroenteritis patients in Gaza strip. The study showed that diarrhoea was more frequent among peoples living in houses rearing poultry and pigeons. They isolated Salmonella, *Campylobacter coli/jejuni*, *Aeromonas hydrophilia*, *Shigella boydii* and *Yersinia enterocolytica*. All isolates were resistant for more than one antimicrobials especially *Campylobacter coli/jejuni*.

Many researchers worldwide studied the prevalence and antibiotic resistance for bacteria isolated from chicken meat. In 2010, a study was carried out to investigate the prevalence and antimicrobial resistance profiles of Salmonella, Campylobacter and *Yersinia spp*. from retail chicken in Tehran, Iran. They revealed that a high proportion of chicken in markets were contaminated with Campylobacter and Salmonella. From 190 chicken samples, Campylobacter, Salmonella and Yersinia were isolated from 94(49.5%), 86(45%) and 41(21.5%) of samples, respectively. Concerning antibiotic resistance of isolated microbes, nalidixic acid resistance in Campylobacter and Salmonella isolates was greater than in Yersinia isolates. Resistance of Campylobacter to nalidixic acid (quinolone) was largely associated with ciprofloxacin (fluoroquinolone)

resistance and resistance to nalidixic acid and tetracycline was found in Salmonella. All Salmonella isolates were sensitive to ciprofloxacin. Tetracycline was the second most frequently observed type of antimicrobial resistance among the different genera tested (Dallal *et al.*, 2010).

Other researchers from Iran determined the prevalence and antibiotic resistance of *Campylobacter spp.* that were isolated during different stages of broiler processing. Samples were collected from four sites along the processing line including de-feathering stage, evisceration stage, twenty minutes after the chilling period started and 24 h after the chilling period completed. 186 of 336 carcasses (55.4%) were positive for *Campylobacter spp.* ten antimicrobial agents were used to asses isolates sensitivity. Of the 198 Campylobacter isolates tested, 178 (92.9%) were resistant to one or more antimicrobial agents. Resistance to tetracycline was the most common finding (78.3%), followed by resistance to ciprofloxacin (62.1%), nalidixic acid (58.6%), and enrofloxacin (44.4%) (Rahimi *et al.*, 2010).

A Korean study investigated the prevalence and antimicrobial resistance of Salmonella isolated from chicken meat produced by different integrated broiler operations. 210 samples from seven brands of conventional chicken meat were collected. There were differences in the number of bacteria isolated from different brands, but in general, 47 (22.4%) samples were positive for Salmonella. *S. enteritidis* was the dominant (57.4%) of the Salmonella-positive chickens. Twenty antibiotics agents were used to determine antibiotic resistance of isolates. Isolates were resistant to cephalothin 41(87%), nalidixic acid 41(85%), and streptomycin 33 (70%). All isolates of Salmonella were susceptible to amikacin, ciprofloxacin, imipenem, enrofloxacin, and trimethoprim (Kim *et al.*, 2012).

A recent survey to estimate the prevalence of antibiotics resistance in, *Salmonella spp., E. coli, Enterococcus spp.* and *S. aureus* in meat in Saudi Arabia was published. A total number of 288 unprocessed meat samples of four different types (beef, camel, lamb and poultry) were analyzed. They were divided into domestic chilled (144) and imported frozen (144). All types of meat analyzed contained the four types of bacteria with *E. coli* being the most prevalent overall at 72.2%, Enterococcus prevalence was 26.2%, *S. aureus* prevalence was 24.6% and Salmonella prevalence was 10.7%. These bacteria were resistant to a number of antibiotics and some were multidrug resistant. They concluded that bacterial contamination of meat is a multi-country problem and

consideration should be made to improve methods of decontaminating food animals and work surfaces during meat processing (Greeson *et al.*, 2013).

2.6 Drug residues

2.6.1 Drug residues definition

The term "residues" is used to describe all active principles and their metabolites, which persist in meats or other food products from animals that have been treated with the drug in question. The term metabolite has not been defined, it is generally accepted that it applies to any by-product of biotransformation of the initial active principle (Burgat-sacaze *et al.*, 1981).

2.6.2 Effects of veterinary drug residues

A number of possible adverse health effects of veterinary drug residues have been suggested. These may include but not limited to the following (Doyle, 2006):

1. Allergic or toxic reactions to residues.

2. Chronic toxic effects occurring with prolonged exposure to low levels of antibiotics.

3. Development of antibiotic-resistant bacteria in treated animals. These bacteria might then cause difficult-to-treat human infections.

4. Disruption of normal human microbiota in the intestine. The bacteria that usually live in the intestine act as a barrier to prevent incoming pathogenic bacteria from getting established and causing disease. Antibiotics might reduce total numbers of these bacteria or selectively kill some important species.

2.6.3 Maximum residue limit

Maximum residue limit means the maximum concentration of residue resulting from the use of a veterinary medicinal product, which may be legally permitted or recognized as acceptable in or on a food, allocated to individual food commodities. It is based on the type and amount of residue considered to be without any toxicological hazard for human health as expressed by the Allowed Daily Intake (ADI), or on the basis of a temporary ADI that utilizes an additional safety factor (Myllyniemi, 2004).

The Codex Alimentarius Commission (CAC) is a commission jointly sponsored by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO). It is a collection of international food standards, guidelines, and codes of practice that protect the health of consumers and ensure fair practices in food trade. The Codex Alimentarius covers food safety matters (residues, hygiene, additives, contaminants, etc.) and quality matters (product descriptions, quality classes, labeling, and certification). Codex established the Codex Committee on Residues of Veterinary Drugs in Food in 1986. Codex has defined 590 MRLs for some fifty nine veterinary drugs. Most countries use Codex MRLs as a basis for establishing their national regulations for veterinary drug use, but still other organizations make their own MRLs to be used in their countries. (**Table 2.1**) shows the MRLs of some antimicrobial residues as stated by Codex Alimentarius Commission (Codex Alimentarius Commission, 2014).

Table 2.1: MRLs of some antimicrobials (Codex Alimentarius Commission, 2014).

Antibiotics	**Poultry muscle (µg/kg)**	**Poultry liver (µg/kg)**
Chlortetracycline/ Oxytetracycline/ Tetracycline	200	600
Neomycin	500	500
Spectinomycin	500	2000
Streptenomycin/ dihydrostreptomycin	600	600
Procaine benzylpenicillin	50	50
Flumequine	500	500
Danofloxacin	200	400
Tylosin	100	100
Erythromycin	100	100
Spiramycin	200	600
Colistin	150	150
Lincomycin	200	500

2.7 Withdrawal period (WP)

The withdrawal period is defined as the interval between the time of the last administration of a drug and the time when the animal can be safely slaughtered for food, milk or eggs can be safely consumed. The withdrawal period provides a high degree of assurance to both producers and consumers that concentration of residues in foods derived from treated animals will not exceed the MRLs (Almanama, 2011).

Each antibiotics has a WP which depends on drug type, drug concentration, route of administration, animal kind and the animal product (Riviere *et al.*, 2009) as demonstrated in (**Table 2.2**) (Hsu and W.H., 2008). All antibiotics are labeled with the appropriate WP, whether it is hours, days or weeks.

Table 2.2: Withdrawal periods of antimicrobials used in poultry production (Hsu and W.H., 2008)

Drug	**Administration Route**	**Animal**	**Withdrawal period (Days)**
Tylosin tartarate	D.W	Chicken	1
		Turkey	5
Erythromycin	D.W	Poultry	1
Gentamicin sulphate	SC.	Chicken	35
		Turkey	63
Neomycine sulphate	D.W	Poultry	0
Streptomycin	D.W	Chicken	4
Lincomycin	D.W	Chicken	0
Oxytetracycline HCl	D.W	Poultry	7-14
Chlortetracyclines	D.W	Poultry	1
Enrofloxacin	D.W	Poultry	8
Amoxicillin	D.W	Chicken	2
		Turkey	5
Sulfaquinoxaline	D.W	Poultry	10

Note: D.W. = Drinking water, SC= Subcutaneously.

2.8 Prohibition of some antibiotics

The extensive use of antibiotics as feed additives for long time may contribute to the development of resistant bacteria to drugs that are used to overcome infections. These microbes pose a potential risk for humans if they are transferred to people. Many European countries banned using antibiotics as food additives. Sweden prohibited in 1986 the use of additives belonging to the groups of antibiotics in feeding stuffs. Avoparcin was banned in Denmark (1995) and Germany (1996), spiramycin was prohibited in Finland (1998) because this product was used in human medicine, and virginiamycin was prohibited in Denmark (1998). Also zinc bacitracin was banned because its use in human medicine as treatment skin infections (Castanon, 2007).

Chloramphenicol, a broad-spectrum antibiotic, was previously widely used in veterinary and human medicine. Reports of aplastic anemia in humans arising from its use led to its ban in the USA and European Union (EU) in 1994. Thiamphenicol and florfenicol were permitted as substitutes (Stolker *et al.,* 2005). Nitrofurans, particularly furazolidone, furaltadone, nitrofurantoin and nitrofurazone for livestock production was completely prohibited in the EU in 1995 due to concerns about the carcinogenicity of the drug residues and their potential harmful effects on human health (Vass *et al.*, 2008).

Due to emergence of fluoroquinolone-resistant bacteria especially Campylobacter and Salmonella, the Food and Drug Administration (FDA) in 1977 banned the use of fluoroquinolones in treating poultry but the use of sarafloxacin and enrofloxacin in poultry was permitted, but an increase in fluoroquinolone-resistant Campylobacter spp. in poultry was linked to increased incidence of infection with resistant Campylobacter spp. in humans. Finally, FDA in 2005 prohibited the usage of enrofloxacin in poultry and sarafloxacin were withdrawn by the producer, thus usage of any members of fluoroquinolones in poultry species is illegal by FDA (Davis *et al.,* 2009).

2.9 Cooking effect on antibiotic residues

To determine the effect of cooking process on AMR, a study investigated the effect of cooking and cold storage on ampicillin, chloramphenicol, oxytetracycline, streptomycin and sulphadimidine residues in meat, the study showed that active AMR might be detected in animal tissue after roasting, grilling and prolonged cold storage. They concluded that it would be unwise to rely on cooking or cold storage to minimise or destroy such residues. The only way to ensure no residues would appear to be the strict

observance of the WP for each drug administered to domestic animals (O'brien *et al.*, 1981).

In another study, researchers investigated the effects of various ordinary cooking procedures (boiling, roasting and microwaving) on tetracyclines (TC) residues in chicken meat. The obtained data revealed that the reduction of TC residues in cooked samples was related to cooking procedures, cooking time and TC agents. The losses of TC residues increased with prolonged cooking time. Doxycycline was the most heat stable of TCs, less than 50% of the initial residues concentration was decreased in boiling and microwaving for 40 and 80 minutes respectively (Abou-Raya *et al.*, 2013).

In contrary, a different study concluded that oxytetracycline was the most heat labile. The time required to destroy more than 90% of the initial level of oxytetracycline (OTC) in breast meat was 15, 40 and 60 minutes for microwaving, boiling and roasting, respectively, OTC residues in breast meat were not detected after microwaving for 20 minutes. Generally, sufficient cooking temperature and time can have a significant effect on the losses of TC residues and provide an additional margin of safety for consumers (Al-Ghamdi *et al.*, 2000).

To determine the effect of different cooking processes (microwaving, roasting, boiling, grilling and frying) on enrofloxacin residues in chicken muscle, investigators used liquid chromatography mass spectrometry (LC-MS) method to evaluate stability of enrofloxacin in natural incurred chicken samples after cooking. They conducted the study on different parts of chicken (breast muscles, thigh muscles and liver). The study showed that enrofloxacin remained stable in boiling water for three hours. On the other hand, the amount of residue increased in the case of roasting and grilling. Also they noticed that when there was a reduction in residues percentage, the lost amount of analyte was found in water or exudates. These results rendered the investigators to inferred that cooking procedures did not affect the levels of quinolones (Lolo *et al.*, 2006).

In another study also evaluated the effects of different cooking processes on enrofloxacin residues in chicken muscle, liver and gizzard tissue from broiler chickens, results showed that enrofloxacin residues were reduced after different cooking processes. In cooked meat and gizzard, the most reduced levels of the residue were due to the boiling method. A high residue levels remained stable after microwave cooking/heating. They concluded

that cooking processes cannot destroy the total amounts of this drug but it can only decrease their amounts and most of the residues in boiling process are excreted from tissue to cooking fluid during the boiling process. Thus, exposure to residues can be reduced by discarding any juice that come from the edible tissues as they are cooked. Among the various agents affecting antibiotic residues after the cooking process, it was found that cooking time and temperature can play major roles (Javadi *et al.*, 2011).

2.10 Detection of drug residues

2.10.1 Screening methods

A screening method is defined as the first procedure that is applied to sample analyses. The purpose is to assure the presence or absence of veterinary drugs residues. This procedure should be as simple as possible. Still, it may be rather complex, due to, e.g. the properties of the drugs of interest or the desired limit of detection, and in certain cases, will provide (semi) quantitative next to the qualitative data (Aerts *et al.*, 1995).

2.10.2 Classification of screening methods by detection principle (Community Reference Laboratories (CRLs), 2014).

1. Biological methods: Detect cellular responses to analytes (e.g. inhibition of bacterial growth). These methods are not selective and can cover several chemical classes of active analytes (e.g. hormones, antibiotics). They do not allow the identification of individual analytes.

2. Biochemical methods: Detect molecular interactions (e.g. antigens, proteins) between analytes and antibodies or receptor proteins (e.g. ELISA), chemical labeling of either the analyte or antibody/receptor allows the interaction to be monitored and measured. These methods are either selective for a family of analytes having related molecular structures or are sometimes analyte specific.

3. Physicochemical methods: Distinguish the chemical structure and molecular characteristics of analytes by separation of molecules (e.g. TLC, GC, HPLC) and the detection of signals related to molecular characteristics (e.g. UV, DAD etc.). They are able to distinguish between similar molecular structures and allow the simultaneous analysis of several analytes.

Table 2.3: Advantages and disadvantages of some screening methods (Sirdar and M.M., 2010)

Test	Advantages	Disadvantages
ELISA	• Easy to use, • Availability for a good number of specific compounds, • Availability for families of compounds (e.g. sulfanomides, estilbenes), • Large number of samples (42) per kit for a single analyte, • Reduced time to obtain the results (2-2.5 h for most kits), • High sensitivity and specificity, • Possibility to use within the food processing facility.	• Increased cost, • Limited storage (few months) under refrigeration, • The need for waste disposal, • Interferences giving some false positives, • Only one kit per residue searched.
Biochip array biosensors	• Easy to use, • Results available in short time, • Multiples residues analyzed in one shot (as many as in an array), • Full automation: higher productivity, • High through-put technique: up to 120 samples per hour and array.	• High operative costs chips and equipment cost, • Analysis restricted to available chips.
HPLC	• Reduced time (few hours) to obtain results, • Sensitive, • Automation leading to higher productivity, • Specificity depending on a detector.	• Expertise required, • Needs sample preparation (Extraction, filtration, addition of internal standards, etc.), • Expensive.
Microbial methods	• Can be used for large surveillance programmers, • Basic laboratory equipment. • Broad spectrum, • Easy to use, • Economical.	• Difficult to standardize preparation procedures, • Some test could not insure MRLs compliance, • Sample preparation required to remove false positives due to protein bacterial inhibitors, • Low sensitivity.

Determination of antibiotic residues in food products such as meat, milk, and eggs by microbiological methods depends on the effect on a specific microorganism, the spectrum and the mode of action of the antibiotics which will be determined.

On the other side residue determination by chemical methods such as chromatography (by all its types) depends on the chemical properties (Mitchell *et al.*, 1998).

2.10.3 Confirmation methods

Various confirmation methods have been described for the detection of veterinary drugs in various matrices. Most techniques comprise a chromatographic separation and a detection method. Liquid chromatography (LC) is often combined with ultraviolet detection (UV), fluorescence detection and mass spectrometry, Gas chromatography (GC) can be combined with electron capture detection, infrared detection and mass spectrometry (Tothill, 2003). Confirmation methods can be both qualitative and quantitative. Quantitative methods are necessary to detect veterinary products that are permitted in some matrices in a maximum concentration; these methods need to confirm if the concentration of an analyte is below or above this limit. The quantification limit should be approximately 0.5 times the MRL. Qualitative methods are used for forbidden substances and violative use of veterinary medicinal products (Watson and D.H., 2004).

2.10.4 Microbiological assay

Microbiological assay screening methods for AMR exploit the primary property of these compounds, their selective toxicity towards specific bacteria. Growth inhibition assays for the detection of antibiotics mainly concern two types of formats: The tube test and the (multi) plate assay. Briefly, the first type comprises a growth medium inoculated with bacterial spores and a pH or redox indicator. In the absence of AMR, the test bacterium will start to grow, acidify the medium and cause a color change (Pikkemaat, 2009). A plate assay comprises a layer of inoculated growth medium. Samples can be applied on top of, or in a well in the agar layer. After over-night incubation, the presence of an antibiotic residue becomes visible as an inhibition zone around the sample. The size of the inhibition zone depends on the type of residue and its concentration, while the sensitivity of the test is affected by many factors, such as indicator organism, pH, type of growth medium, and thickness of the agar layer (Bovee *et al.*, 2009).

2.10.5 Microbiological assay methods

Microbiological assays can be classified depending on their mode of detection; growth inhibition and luminescence. If food samples do not contain AMR, or the concentrations are below the load of detection, the organisms grow producing acid compounds that change the indicator color, permitting visual or photometric detection. Nevertheless, if an antimicrobial is present in the sample no color change is observed (Pikkemaat and M.G., 2009).

Most of the microbiological inhibition tests with agar diffusion are based on inhibition-diameter measurement using a caliper. In these tests, samples are applied to plates of agar media inoculated with specific bacteria. Diffusion of an antibacterial substance is shown by the formation of inhibition zones (Okerman *et al.,* 1998).

2.11 Examples of microbiological assay methods

2.11.1 Four plate Test (FPT)

This test was developed as a mean of import control within the European Commission primarily to monitor residues of antimicrobials in fresh meat from Third World countries for use at national borders (Crosby and N.T., 1991). The test is comprised of four plates of agar medium inoculated with Bacillus subtilis (BGA) spores (at pH 6.0, 7.2 and 8.0) and Kocuria rhizophila ATCC 9341 (at pH 8.0). Meat samples are cut into small cylinders and applied to the plates (**Figure 2.3**). Trimethoprim is incorporated into the pH 7.2-medium to enhance the test's sensitivity toward sulfonamide residues. After incubation, diffusion of an antibacterial substance is shown by the formation of inhibition zones on any seeded plate (Heitzman, 1994).

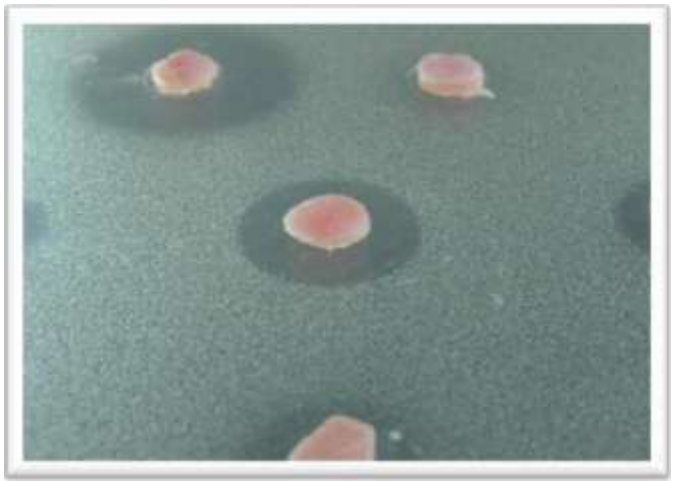

Figure 2.3: Muscle samples on a plate of FPT (Pikkemaat, 2009).

2.11.2 The Calf Antibiotic and Sulfonamide Test (CAST)

CAST is a microbial inhibition screening test for the detection of antibiotics and sulfonamides in veal calf carcasses. The test uses Bacillus megaterium ATCC 9885 as the indicator organism and Mueller Hinton agar as the growth medium. A sterile cotton swab is inserted into a kidney of a freshly slaughtered calf and the swab is allowed to soak in the kidney fluid for 30 min. Then the swab is removed from the kidney and placed on a plate seeded with specific concentration of B. megaterium. The plate is incubated at 45°C. After 16–20 h incubation, swabs are removed from the plate and the zone of inhibition (ZI) around each swab is measured vertically and horizontally and recorded (Dey *et al.,* 2005).

2.11.3 Screening Test for Antibiotic Residues (STAR)

The STAR protocol is intended for the qualitative detection of residues of substances with antibiotic activity in milk and muscle of pig, cattle, sheep, and poultry by using bacterial strains sensitive to antibiotics. This method is based on five different plates (Five-Plate Test) to detect specific families of antibiotics, the plate *B. cereus* ATCC 11778 for tetracyclines, the plate *E. coli* ATCC 11303 for quinolones, the plate *B. subtilis* B.G.A for aminoglycosides, the plate *K. rhizophila* ATCC 9341 for macrolides and the plate Bacillus stearothermophilus ATCC 10149 for sulfonamides and β-lactams. Slices of muscle samples of 2 mm in thickness and 8 mm in diameter are placed onto the plates. Then plates are incubated. If there is AMR, a zone of inhibition (ZI) around the meat sample will appear (Gaudin *et al.,* 2010).

2.11.4 Premi's test

The Premi® test is a commercial growth inhibitor test used for the detection of AMR in fresh meat, kidneys, fish and eggs in less than four hours. Premi test is an ampoule, containing a specific agar medium, imbedded spores of *B. stearothermophilus* var. calidolactis and a color indicator. The meat juice is placed in the ampule and after 20 min of pre-diffusion at room temperature; the meat juice is removed by washing step. Finally, the ampoule is incubated for approximately 3 h at 64°C. If no inhibitory substances are present, the germinated spores will multiply with the production of acid. This will be visible by a color change from purple to yellow. When anti-microbial compounds are present in sufficient amount (above the detection limit), the spores will be unable to germinate and therefore no color change will be observed (Gaudin *et al.,* 2008).

2.11.5 CHARM test

The CHARM test, a commercial test, is based on the irreversible binding reaction between functional groups of antibacterials and receptor sites on or within the cell of added microorganisms. For example, β-lactams bind to D-alanine carboxypeptidase on the cell wall, whereas other binding sites are found on ribosomes (Navrátilová, 2008). The Charm I test was developed exclusively for β-lactams in milk, further CHARM II test was developed to test a variety of antibiotics in both milk and other food of animal origin including honey. The test employs 14C-labeled or 3H-labeled antibacterials to compete for the binding sites. This competition for receptor sites prevents the radio labeled antibacterial from binding. Thus the more radio labeled compound bound the less analyte in the sample (Botsoglou *et al.,* 2001).

2.11.6 Residue Control Programs

Residue control programs are designed in accordance with country regulations. These programs generally control both domestic and imported products. Veterinary drugs for inclusion in these programs are selected on the basis of their risk profiles. Only the domestic residue sampling program includes steps for addressing the occurrence of violative residues in food-producing animals, on-farm. The import residue sampling program is primarily a verification program to determine that the domestic residue sampling program of an exporting country is operating effectively (Cunningham *et al.,* 2010).

Control programs have two principal components: monitoring and surveillance. Residue monitoring program randomly collect sample tissues from animals at slaughters then tissue samples are screened for residues of veterinary drugs, pesticides and environmental contaminants, and the residues are assessed for compliance with the applicable MRL or environmental standard. Surveillance programs collect sample tissues from animals suspected of violative residues depending on clinical signs or herd history. If monitoring reveals a potential residue problem, the action taken will vary in accordance with country rules (Paige *et al.,* 1999).

2.12 Previous studies

2.12.1 Effect of Antibiotics on growth performance

Singh *et al.* (1974) studied the effect of different levels of vitamin A supplement in diet of broiler chicken on growth performance. They observed that the actual requirement for weight gain in chicks was probably not more than 2000 IU/kg.

Mujeer *et al.* (1990) reported that Flavomycin 40 in broilers had 4.5 percent increase inbody weight over control group at 6 week of age.

Dhande *et al.* (1991) reported that Livol supplementation in diet @ 0.2 percent and 0.5percent showed better growth rate 1503 & 1594 gram respectively than control 1281gram at 8 week of age.

Kulkarni and Thakur (1992) reported that average weight gain was 1537 & 1518.33gram in control and treated group with biovet @ 2ml/10 litre of drinking water respectively at 6 week of age.

Baidya *et al.* (1993) conducted experiment on straight run broiler chickens fed with different growth promoters viz Auroface-100, 50, Biospur-50, G-Probiotic 50 or Bioboost forte 10g/100g and reported that average body weights as 1261.5, 1280.1, 1288.5, 1274.4, 1282.2, 1282.2 gram respectively at 6week of age.

Jamroz *et al.* (1995) studied the effects of feeding avilamycin @ 10mg/kg feed and Roxazyme -G 150 mg/kg feed or both on broiler performance. They observed that an improvement in live weight by 3 percent in feeding of both together resulted in the increase of body weight by 7.7 percent.

Use of antibiotics in poultry has facilitated efficient production, allowing the consumer to purchase good quality meat and eggs at a reasonable cost (Donoghue, 2003). This author also indicated that their use has enhanced the health and wellbeing of poultry by reducing the incidence of diseases.

Kannan *et al.* (2005) evaluated the effect of feeding antibiotic & probiotic on broiler performance. Body weight of broilers were significantly ($p<0.01$) influenced by all the dietary supplementation as compare to control.

Bozkurt (2008) reported that that birds fed with antibiotic growth promotor (AGP) mannan oligosaccharide (MOS) and dextran oligosaccharide (DOS) supplemented diets exhibited higher body weight gain ($P<0.05$).

Amer and Khan (2012) reported that better FCR was observed in birds those given drinking water with antibiotic and probiotic.

2.12.2 Detection of Antibiotic residue by various researchers

A Belgium study used a combination of three plates, seeded with strains of *Micrococcus luteus, B. cereus* and *E. coli* to detect residues of β-lactams, tetracyclines and fluoroquinolones in poultry meat. Confirmation and quantification of positive samples were performed using a validated HPLC method with fluorescence detection. 18 out of the 228(7.9%) broilers contained inhibiting substances. Seventeen samples inhibited *B. cereus*. Doxycycline was detected in the 16 samples that were investigated with HPLC with fluorescence detection. One sample inhibited *M. luteus* and it was confirmed to be amoxicillin. No fluoroquinolones were detected (Okerman *et al.,* 2001).

In a study conducted to investigate AMR in chicken, three microbial screening tests were used; fast antimicrobial screening test (FAST), *B. stearothermophilus* disc assay (BSDA) and a commercial test kit (TAT). Four hundreds chicken meat samples were screened; the prevalence of AMR in chicken meat was from 11.1% to 21.7%. Test performances were evaluated on sensitivity, specificity, positive predictive value and negative predictive value, the researcher concluded that BSDA is the screening test of choice, in addition to simplicity, short incubation period as well as the low cost (Tin and T.M., 2003).

In a study done in Pakistan using *B. subtilis* as a test organism, screening of AMR in a total of 100 broiler tissue samples (33 livers, 33 kidney and 33 muscles) revealed that 13(39.4%) livers, 9(27.3%) kidneys and 7(20.6%) muscles contained antimicrobial residus (Shahid *et al.,* 2007).

A Bulgarian study carried out to investigate the presence of antimicrobial drugs residues in chicken (breast muscles, liver and kidneys). Samples from meat (breast muscles), liver and kidneys were taken as follows: 115, 192, 155 for meat, liver and kidneys respectively, samples were screened using FPT method, 2 samples (1.7%) from meat

were identified as antimicrobials-residue-positive while 17(8.8%) from liver and 33(21.%) from kidney (Pavlov *et al.,* 2008).

Shareef and colleagues used thin layer chromatography (TLC) to screen the presence of oxytetracycline, sulfadiazine, neomycin, and gentamycin in stored poultry products in Mosul, Iraq. 25 samples from each (livers, thigh muscle, and breast muscle) were screened. Total of 75 samples of stored poultry products were tested. 39 (52%) of the samples were positive. In more details, 56% of samples were positive for each liver and breast muscle while 44% of samples were positive in thigh muscle. In that study neither gentamicin nor neomycin were detected. On the other hand, oxytetracycline and sulfadiazine were detected in equal number of positive results, 18 for each type (Shareef *et al.,* 2009).

A study done in the Dominican Republic, Santiago province to determine whether retail broiler meat contained quinolone residues, a total of 135 chicken breast samples were screened using colorimetric assay based on the inhibition of an *E. coli* strain which is sensitive to quinolones. 9(6.6%) of samples were containing quinolones above MRL (Silfrany *et al.,* 2013).

An Egyptian study carried out to assess the safety of broiler fillet through residues monitoring of antimicrobials especially (Oxytetracycline & Enrofloxacin). In that study, two methods were used for the determination of AMR in broiler fillet, a screening method by microbiological inhibition assay using *B. subtilis* (ATCC-6633) as indicator organism and a confirmation method using HPLC analysis. From one hundred random broiler fillet samples (50 fresh and 50 frozen), the screening test found that 21% of total examined samples contained AMR. HPLC method for confirmation and quantification proved that six samples were containing oxytetracycline and three samples were containing enrofloxacin, all samples except one had violative values of AMR comparing to MRLs determined by European Union Commission (Hussein *et al.,* 2013).

A study was done in Nigeria to determine the prevalence of AMR in commercial broiler chickens using Premi® test Kit. From 70 sampled commercial birds from three major poultry markets in the study area, 42 (60%) of birds contained antimicrobial residues. It detected also residues in 90 out of the 280 different organ matrices made up of 70 samples of each organ, kidney was the highest at 48.6%, gizzard (30.1%), liver (25.8%), and muscle (24.3%) (Ezenduka *et al.,* 2014).

CHAPTER - 3

MATERIALS AND METHODS

CHAPTER - 3

MATERIALS AND METHODS

This chapter describes materials and methods that used to achieve the objectives of the study. This is a cross-sectional analytical study that aimed for detection of three antibiotics residue which are largely used in Sonali chicken.

3.1 Location of the study

The experiment was conducted at the Dairy and Poultry Science farm of Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during the period from mid February to mid April, 2018. The commercial Sonali chicken was used in this experiment for a period of 9 weeks to find out the dietary effects of antibiotics and detection of antibiotics residue in the meat.

3.2 Experimental birds

One hundred twenty vigorous day-old Sonali chicks were procured from Rafid Hatchery Limited, Joypurhat.

3.3 Layout of the experiment

The experiment was conducted in Complete Randomized Design (CRD)**.** The chicks were randomly distributed to four dietary treatment groups (T_0, T_1, T_2 and T_3) having three replications in each treatment. The chicks were reared in separated pens according to treatments and replications, each dietary treatment group contains of 10 birds. The layout of the experiment is shown in the following table:

Table 3.1: Layout of the experiment.

Dietary Treatment	No. of chicks in each replication			Total number of chicks in each treatment
	R_1	R_2	R_3	
T_0	10	10	10	30
T_1	10	10	10	30
T_2	10	10	10	30
T_3	10	10	10	30
Total				120

Where,
T_0: Control group, **T_1**: Ciprofloxacin @ 1ml/L drinking water, **T_2**: Oxytetracycline @ 1gm/L drinking water and **T_3**: Amoxicillin @ 1gm/L drinking water.

3.4 Preparation of the experimental house

HSTU poultry farm was used for rearing experimental birds to evaluate the efficacy of different antibiotics for growth as well as detecting those antibiotics residue remaining in

of Sonali chicken. Experimental shed was constructed with compartment of housing for ten birds. Each compartment was 54×42 inches for length and breadth respectively. The shed was constructed by iron net and wooden materials. At first, the experimental house was properly washed and cleaned by using tap water. Ceiling, walls, and floor were thoroughly cleaned and subsequently disinfected with bleaching powder, then the room was kept closing for two weeks. After that, the house was again disinfected with virocid solution 1ml/3liter water. At the same time, all feeders, waterers and other necessary equipment were also properly cleaned, washed and disinfected with bleaching powder. After proper drying, the house was used for this study.

3.5 Collection of Antibiotics

The commonly used antibiotics such as ciprofloxacin, oxytetracycline, amoxicillinwere bought from a veterinary pharmacy named "Poshu Shasthow" in the local market of Dinajpur.

3.6 Experimental diet

The experimental diet was divided into two phages (Sonali-starter, Sonali-grower). Sonali starter was provided 0 to 20 days and Sonali grower was provided from 21 days to end day of experiment. Only a bag of Sonali-starter of experimental diet was purchased from local market in Dinajpur, namely company (Nourish poultry and hatchery limited). Then, the rest of the feed named Sonali-grower was made by own to keep free from external antibiotics. All the ingredients were taken at proper rate according to their standard composition. All the treatments were provided through drinking water during experimental period.

Table 3.2: Nutrient Composition of Sonali Starter.

Chemical composition	Starter (Upto 20 days)
Moisture (%)	11-12
Crude protein (%)	21
Crude fiber (%)	5
Crude fat (%)	-
Ether extract (%)	4
Calcium (%)	1
Available phosphorus (%)	0.5
ME (Kcal/Kg)	2850

Table 3.3: Ingredients amount of formulated ration of Sonali Grower with their chemical Composition.

Ingredients (Kg)	**Treatments**			
	T_0	T_1	T_2	T_3
Maize	56.5	56.5	56.5	56.5
Soybean	25	25	25	25
Rice Polish	5	5	5	5
Wheat Bran	5	5	5	5
Meat and Bone Meal	2	2	2	2
CGM (Corn Gluten Meal)	1.5	1.5	1.5	1.5
Propec	1	1	1	1
Soybean Oil	1.5	1.5	1.5	1.5
DCP	0.5	0.5	0.5	0.5
Oyster Shell	0.9	0.9	0.9	0.9
Limestone	0.75	0.75	0.75	0.75
Salt	0.35	0.35	0.35	0.35
Total	**100**	**100**	**100**	**100**
Chemical composition of Sonali Grower				
ME (Kcal/Kg)	2924.302	2924.302	2924.302	2924.302
Crude Protein (%)	20.48	20.48	20.48	20.48
Crude Fiber (%)	3.13	3.13	3.13	3.13
Ether Extract (%)	4.63	4.63	4.63	4.63
Calcium (%)	0.67	0.67	0.67	0.67
Phosphorus (%)	0.7343	0.7343	0.7343	0.7343
Lysine (%)	1.0127	1.0127	1.0127	1.0127
Methionine (%)	0.31745	0.31745	0.31745	0.31745

***** Added vitamin-mineral premix @ 250gm, Lysine @ 50gm, Methionine @ 50gm, Toxin Binder @ 150gm, Anti-Salmonella @ 150gm, Enzyme @ 50gm, Emulex @ 50gm and Maduramysin @ 50gm per 100 kg feed.

3.7 Routine Management

The birds were reared to similar care and management in all treatment groups throughout the experimental period. The following management practices were followed whole experimental period.

3.8 Litter Management

Fresh and dried rice husk was used as litter at a depth of 2-3 inch. After 5 weeks, old litter was totally removed and new litter was provided as same depth. The litter was stirred one time per day from four weeks upto the last day of experimental period.

3.9 Floor Space

Each pen was 4.5×3.5 sq. ft. allocated for feeding, watering, and housing for 10 experimental birds.

3.10 Brooding Management

Brooding is the first management of day old chick. In brooding period, electric brooder was used to provide suitable heat for maintaining their body temperature. The brooder was hanged just above the bird level at the center of chick guard. Before entrance of day old chicks, fresh dried litter was provided at depth 3 inches then covered by newspaper. Pre-heating the brooding space and temperature adjust at 33 ± 2^0C. After entrance, day old chicks were provided with vitamin C and glucose, one hour later feed was provided. At first day, temperature was maintained 33 ± 2^0C then gradually decreased 1^0C per day. Temperature and humidity were recorded by using clinical thermometer and hygrometer.

Figure 3.1: Preparation of brooding house.

Figure 3.2: Brooding management.

Figure 3.3: After separation of birds into different treatments with shed.

3.11 Lighting Management

The birds were exposed to 23 hours of lighting and 1-hour dark period throughout the experimental period.

3.12 Feeding and drinking

Provide adlibitum feed and water through the experimental period.

Table 3.4Vaccination

Name of Vaccine	Name of diseases	Age (Days)	Route of administration
IB+ND	Infectious Bronchitis & New Castle	5th	One drop in one eye
IBD	Gumboro	10th	One drop in one eye
IBD	Gumboro	17th	Through drinking water
ND	New Castle	22nd	Through drinking water
ND	New Castle	42nd	Through drinking water

Vaccine, prepared by Intervet International, Netherland, was applied as per recommendation of the manufacturer.

3.13 Sanitation

Drinkers were washed daily in the morning and feeders were cleaned weekly before being used. Strict sanitary measures were followed during the experimental period.

3.14 Temperature and relative Humidity measure

Temperature (^{0}C) was recorded by clinical thermometer and relative humidity (%) was recorded by digital hygrometer three time daily.

3.15 Debeaking

Debeaking of the birds was done successfully by electric debeaker at the age of 42-45 days to reduce cannibalism and other external injuries.

Figure 3.4: Debeaking of birds.

3.16 Slaughtering of the Birds

Prior to slaughtering the birds were fasted for 10 hours, but water was provided adlibitum. Two birds were randomly selected in each replication for slaughtering. The live weight of birds was taken individually before slaughtering. At the time of slaughtering, the birds were secured by holding both shanks with one hand and both wings with other hand by the help of an assistant to prevent struggling. Slaughtering was done by Halal method with sharp knife. Complete bleeding was accomplished by raising the bird approximately 45^{0}C so that the caudal part will be higher than the head. After complete bleeding was done then removal of shank, head and skin. Finally evisceration was done manually to separate liver, spleen, heart, gizzard, and meat yield.

Figure 3.5: Live weight

Figure 3.6: Carcass weight

Figure 3.7: Thigh meat weight

Figure 3.8: Breast meat weight

3.17 Methods of the study

3.17.1 Procurement of meat samples

Chicken meat and liver samples of same poultry birds were procured from freshly slaughtered birds. The samples were collected in a sterile polythene bags, labeled, packed and brought in insulated ice container to the laboratory for analysis. All the samples were stored at -18^0C to -20^0C till further analysis and thawed at room temperature immediately before analysis.

After that, all the collected samples were sent to Poultry Research & Training Centre (PRTC) situated in Chittagong Veterinary & Animal Sciences University, Khulshi, for the detection of antibiotics residue.

The detection methodology was followed by Ultra High Performance Liquid Chromatography (UHPLC) which is the best method for antibiotics residue detection.

3.17.2 Chemical examination: The collected samples divided into three groups. Ciprofloxacin group, Oxytetracycline group and Amoxicillin group each group contained chicken breast meat& thigh meat and chicken liver (Meat=9, Liver=9).

Ciprofloxacin group:

Extraction: (Verdon *et al.,* 2004) 2 g of sample was weighed in a 50 ml polypropylene centrifuge tube, homogenized for 2 min and then 8 ml of trichloro acetic acid 5% (TCA) was added and vortex for 1 min., rotary agitated for 10 minutes, then centrifuged for 5 minutes at 14000 rpm, filtered through a 0.45 µm nylon filter.

Chromatographic condition: Mobile phase was 0.01 M phosphoric acid / acetonitrile (80:20 v/v),

Column: (c 18 – 250 x 4.6 mm –5 µm),

Flow rate: 0.3 ml/min,

Fluorescence detection wave length: excitation 280 nm – emission 450 nm,

Detection time: 10-12 min,

Injected volume: 25µl.

Oxytetracycline group:

Extraction: (Şenyuva *et al.,* 2000) 2 g of sample was homogenized in a blender for 2 min and then 0.1 g citric acid, 1 ml nitric acid (30%), 4 ml methanol and 1 ml deionized water were added respectively. The suspension with solid particles was put in a vortex for good mixing, kept in an ultrasonic bath for 15 min and then centrifuged for 10 min at 4000 rpm, Filtered through a 0.45 µm nylon filter.

Chromatographic condition: Mobile phase was distal water / acetonitrile (85:15 v/v),

Column: (C18 – 150 x 4.6 mm –5 µm),

Temp. of column 25ºc,

Flow rate 1.5 ml/min,

Fluorescence detection wave length 360 nm,

Detection time (4-6 min),

Injected volume: 25µl.

Amoxicillin group:

A total of 30 mg of amoxicillin trihydrate CRS was dissolved in mobile phase-A and diluted to 50 ml with mobile phase-A. Extracted antibiotic solutions for TLC were filtered through 0.2 MFS syringe filters (0.2 m, Advantech MFD, Inc., Japan). The chromatographic procedure was carried out by the following ways:

1. A stainless steel column C18 (2µm) P/N 8915002, 2 mm ID×100 mmL No. 22G2C-001 was used for chromatography.

2. Mobile phase was run at a flow rate of 0.2 ml/min.

Dilute sodium hydrogen was added to 250 ml of 0.2 M potassium di-hydrogen phosphate R up to pH 5.0 and diluted to 1000 ml with water R. A spectrophotometer detector was set at 254 nm to measure the wavelength. Injection volume was 20µl.

Regression equation: Y=ax

Here, Y=Component area or height,

a=Slope of the calibration line,

x=Uncorrected amount,

b=Intercept.

Figure 3.9: Poultry Research & Training Centre (PRTC), CVASU.

3.17.3 Calculation:

1. Total weight gain in (kg): This was computed as a group by subtracting the initial weight from the final weight.

Total gain in weight = Final weight – Initial weight

2. Dressing percentage: The dressing percentage of Sonali chicken was calculated as follows:

Dressing (%) = (Dressed Weight ÷ Body Weight) × 100

3. Total feed consumption (kg): The amount of feeds consumed by the birds from the start until the end of the experiment (63 days). This was computed by adding the total feeds offered after the total left- over have been subtracted.

Total feed consumption = Total feed offered – Total left over.

4. Feed efficiency: This was obtained per treatment by dividing the total feed consumed by the total gain in weight. Feed efficiency is computed for the whole duration of the experiment (63 days).

Feed efficiency = Total feed consumed / Total gain in weight

5. Total cost of the total feed consumed (PhP): This was obtained by multiplying the cost of feed per kilogram to the total feed consumed.

Cost of the total feed consumed = Cost of feed per kilogram × Total feed consumed

6. Feed cost per kg gain of Sonali chicken (PhP): The feed cost per kilogram of gain in weight and this was computed as the price of feeds per kilogram multiplied by the total gain in weight.

Feed cost per kilogram gain (PhP) = Price of feeds per kg × Total gain in weight

7. Mortality rate (%) = No. of dead chickens / Total no. of birds as a group × 100

3.18 Data collection and record keeping:

The following records were kept during the experimental period:

Initial DOCs weight and after brooding weight of chicks. Weekly Body weight gain and feed intake were recorded replication wise in each treatment group at last day of week. Mortality was recorded daily if death occurred. The different meat yield parameters like, carcass, thigh, breast meat, heart, liver weight for individual birds were recorded after slaughtering. Temperature and relative humidity were recorded three times daily.

3.19 Statistical analysis

The data of feed consumption, growth performance, carcass characteristics were recorded and analyzed by SPSS version-20 software by using one way ANOVA accordance with the principles of Complete Randomized Design (CRD). All values were expressed as Mean±SEM and significance was determined when $P<0.05$. Mean was compared among the treatment groups by using Duncan test.

CHAPTER - 4

RESULTS AND DISCUSSION

CHAPTER - 4
RESULTS AND DISCUSSION

This experiment was conducted to evaluate the efficacy of various antibiotics on production performance in terms of weekly body weight gain, final live weight gain, feed intake, feed efficiency, dressing percentage and detection of antibiotic residue in the meat of Sonali Chicken at different dietary treatments. This experiment was held under the Department of Dairy and Poultry Science, Faculty of Veterinary and Animal Science HSTU, Dinajpur.

Day-old chicks were randomly divided into 4 groups (T_0, T_1, T_2 and T_3) after 7 days for assessment the efficacy of different antibiotics as antimicrobial action, growth promoter on Sonali birds. Finally, the residue of antibiotics in the raw meat was examined.

4.1 Weekly Body weight gain

At the start of the experiment, the average body weight of the birds in different groups was not significantly differed. In **Table 4.1** showed that after 7 days of brooding, initial body weight of chicks in different dietary treatments were similar. The live weight of birds at 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th} , 7^{th}, 8^{th} and 9^{th} weeks did not significantly ($P>0.05$) vary among the treatment groups. The efficacy of supplementation of different antibiotics such as ciprofloxacin, oxytetracycline and amoxicillin @ 1 ml/L, 1 gm/L and 1 gm/L respectively in drinking water upto 9 weeks increase live weight gain day by day compared to the control T_0 group. In 9^{th} weeks, the highest value (720.33±17.29g) was found in T_2 group that received Oxytetracycline @ 1gm/L water and the lowest value (682±27.30g) was found in T_0 group & that was control group. Within the different antibiotics groups named ciprofloxacin, oxytetracycline and amoxicillin respective treatment @ 1ml/L, @ 1gm/L and @ 1gm/L in drinking water, live weight was found (686.63±29.77g), (720.33±17.29g) and (703.77±6.48g). The result of this study clearly showed that oxytetracycline antibiotic @ 1gm/L of drinking water increase live weight up to 9 weeks of age. These results agree with Zulkifli *et al.*, 2000 who reported that *Lactobacillus* cultures and oxytetracyclines (OTC) have similar effect on improving the body weight and weight gain of broiler chicken. Control group (T_0) showed as similar result compared with T_1 group. The highest weight gain found in T_3 (Oxytetracycline) group.

4.2 Body weight gain

Initial body weight of Sonali chicks fed on different dietary treatments was similar (P>0.05). Final live weight gain was not significantly (P>0.05) varied among the different treatment (T_0, T_1, T_2 and T_3) groups. The highest body weight gain was gained that received oxytetracycline @ 1gm/L drinking water. However, treatment group T_2 gained the highest body weight compared to control group T_0, treatment group T_1 and T_3. The result of this study indicated that T_2 group is better for weekly live weight gain as compared to other groups T_0, T_1 and T_3. The highest body weight (688.57±17.29g) found in T_2 group and the lowest body weight (647.48±27.30g) found in T_0 group. Antibiotics can be used successfully at sub-therapeutic doses in poultry production to promote growth (Barceló, 2007, Chattopadhyay, 2014, Harms et al., 1986, Khodambashi Emami, 2012, Rosen, 1996) and protect the health of birds by modifying the immune status of broiler chickens (Lee *et al.*, 2012).

Table 4.1: Dietary effect of supplementation of different antibiotics on weekly body weight, and body weight gain of Sonali chicken.

Parameters	**Treatments**				**Level of significance**
	T_0	**T_1**	**T_2**	**T_3**	
	Control Group	**Ciprofloxacin 1ml/L**	**Oxytetracycline 1gm/L**	**Amoxicillin 1gm/L**	
Initial weight	34.52±0.00	33±0.00	31.76±0.00	38.6±0.00	NS
1st week	102.8±1.67	99.03±2.53	99.67±3.24	102±1.6	NS
2nd week	142.5±3.58	139±2.47	138.07±2.37	141.4±2.31	NS
3rd week	202.53±2.3	190.17±5.99	195.23±4.72	195.4±5.99	NS
4th week	290.7±5.78	274.6±6.42	281.87±9.37	283.73±9.81	NS
5th week	362.97±4.52	351±19.16	358.13±12.37	341.47±1.03	NS
6th week	422.3±9.9	389.93±4.27	406.73±0.57	397.5±19.56	NS
7th week	500±10.33	447.6±26.29	477.2±8.73	481.97±5.20	NS
8th week	564.33±20.54	564.07±28.02	592±15.91	566.27±9.97	NS
Final Body wt.	**682±27.30**	**686.63±29.77**	**720.33±17.29**	**703.77±6.48**	**NS**
Body wt. gain	647.48±27.30	653.63±29.77	688.57±17.29	665.17±6.48	NS

The mean values with different superscript (a to c) within the same row differs significantly, at least ($P<0.05$). All values indicate mean ± Standard error of mean

NS=Non significant, * statistically significant ($P<0.05$)

4.3 Feed intake

The cumulative feed intake of Sonali chicken in different dietary treatment during experimental periods was almost statistically similar and the differences were insignificant (P>0.05). However, the lowest feed intake (1727.43±25.38 g) was found in T_3 group and the highest feed intake (1837.93±52.93 g) was found in T_0 group. In **Table 4.2,** we found that control group and T_1 group were near similar with each other. Abdulrahim et al. (1999) reported that the feed conversion ratio was best in broilers supplemented with *L. acidophilus* in combination with bacitracin and poorest in broilers fed bacitracin alone, while broilers fed *L. acidophilus* alone had feed conversion ratio similar to that of the control. In contrast, Mohan *et al.* (1996), Yeo and Kim (1997) and Cavazzoni *et al.* (1998) did not find any improvement in the feed to gain ratio or feed intake of broilers fed either probiotics or different antibiotics.

4.4 Feed efficiency

At the experimental period, feed efficiency of different treatment groups statistically significant (P<0.05). The birds of T_3 groups took containing amoxicillin 1 gm/L through drinking water converted feed to meat most efficiently. The feed efficiency of T_3 treatment group was statistically significant (P<0.05) with T_0, T_1 and T_2.

Table 4.2: Dietary effect of supplementation of different antibiotics on feed intake, feed efficiency and mortality of Sonali chicken.

Parameters	**Treatments**				**Level of significance**
	T_0	**T_1**	**T_2**	**T_3**	
	Control Group	**Ciprofloxacin 1ml/L**	**Oxytetracycline 1gm/L**	**Amoxicillin 1gm/L**	
Feed intake (gm)	1837.93±52.93	1819.63±86.66	1756.93±19.6	1727.43±25.38	0.078(NS)
Feed efficiency	2.67^{b}±0.03	2.7^{b}±0.04	2.55^{ab}±0.09	2.45^{a}±0.01	0.011*
Mortality (%)	1	0	0	0	NS

The mean values with different superscript (a to b) within the same row differs significantly, at least (p<0.05). All values indicate mean ± Standard error of mean

NS=Non Significant, * statistically significant (P<0.05).

4.5 Dressing percentage

After slaughtering, defeathering and eviscerating, remove all edible and non edible by-products, dressing percentage of different treatment groups showed in **Table 4.3**. The table 4.3 indicated that, there were no significant differences among the treatment groups. The highest dressing percentage was observed in T_2 group (52.36±1.77%) than other treatment groups T_1 (51.86±0.68%), T_3 (51.61±0.23%) and control group T_0 (51.11±0.59%) respectively.

4.6 Breast meat weight

Breast meat weight obtained in **Table 4.3** was statistically insignificant (P>0.05) among the different treatment groups. Breast weight was the highest found in T_2 group (105.33±6.17g) compared to treatment groups T_1 (104.5±2.31g), T_3 (104.2±2.6g) and control group T_0 (104.1±0.58g).

4.7 Thigh meat weight

Data obtained from **Table 4.3**, thigh meat weight of Sonali chicken was statistically insignificant (P>0.05) among the different treatment groups. Highest weight was found in T_2 group (124.3±9.87g) whereas the lowest was found in T_1 group (118.67±2.91g).

4.8 Liver weight

Liver weight of Sonali chicken in different dietary treatment groups was statistically insignificant (P>0.05). From **Table 4.3**, it was seen that liver weight maximum in T_2 treatment group and minimum in T_1 treatment group.

Table 4.3: Dietary effect of supplementation of different antibiotics on meat yield parameters of Sonali chicken.

Parameters	Treatments				Level of significance
	T_0	T_1	T_2	T_3	
	Control Group	Ciprofloxacin 1ml/L	Oxytetracycline 1gm/L	Amoxicillin 1gm/L	
Final live wt. (g)	654.15±27.47	680.2±18.77	708.33±21.18	706.4±8.33	NS
Dressing (%)	51.11±0.59	51.86±0.68	52.36±1.77	51.61±0.23	NS
Breast meat wt. (g)	104.1 ±0.58	104. 5 ±2.31	105.33±6.17	104.2±2.6	NS
Thigh meat wt. (g)	120.2±2.31	118.67±2.91	124.3±9.87	121.52±1.2	NS
Liver (g)	25.33±1.33	25. 05 ±1.76	26.33±0.67	25.95±2.3	NS

The mean values with different superscript (a to b) within the same row differs significantly, at least ($p<0.05$). All values indicate mean ± Standard error of mean

NS=Non significant, *Statistically significant ($P<0.05$)

4.9 Antibiotic residue detection

The test for detection of antibiotic residue was performed by Poultry Research and Training Centre (PRTC) Laboratory situated in CVASU, Khulshi, Chattagram. Ultra High Performance Liquid Chromatography (UHPLC) test was applied to detect the antibiotic residue in the meat and liver of Sonali chicken.

Table 4.4. Different antibiotic residues found in liver and muscle samples.

Name	Antibiotics Residue (mg/kg)		Mean±SE	
	Liver	Meat	Liver	Meat
Control	Nil	Nil	Nil	Nil
Ciprofloxacin	Nil	Nil	Nil	Nil
	Nil	Nil		
	Nil	Nil		
Oxytetracycline	2.30	0.38	2.56±0.6	0.41±0.09
	1.67	0.26		
	3.70	0.58		
Amoxicillin	Nil	1.095	Nil	0.92±0.09
	Nil	0.784		
	Nil	0.883		

The results in the above **Table 4.4** expressed the oxytetracycline residuals level which detected in chicken meat as 0.38 mg/kg, 0.26 mg/kg and 0.58 mg/kg with a mean value 0.41 ± 0.09 mg/kg while in chicken liver the results were 2.30 mg/kg, 1.67 mg/kg and 3.70 mg/kg with a mean value 2.56± 0.6 mg/kg. Ciprofloxacin found as nil in both liver & meat samples. Amoxicillin was absent in liver but present a certain amount in the raw meat as 1.095 mg/kg, 0.784 mg/kg, 0.883 mg/kg with a mean value 0.92±0.09 mg/kg.

From the taken samples in this study, the results revealed that the highest antibiotic residue in meat found in amoxicillin supplemented group followed by oxytetracycline supplemented group. In liver, the highest antibiotic residue found in oxytetracycline supplemented group.

Among the poultry tissues, liver contained the highest level of antibiotic residues in comparison to other samples. The order of sequences from the present study was the highest in liver followed by meat respectively.

Table 4.5. Residue Percentage (Ciprofloxacin, Oxytetracycline and Amoxicillin).

Ciprofloxacin				**Oxytetracycline**				**Amoxicillin**			
Chicken Meat		Chicken Liver		Chicken Meat		Chicken Liver		Chicken Meat		Chicken Liver	
No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
3	0	3	0	3	100	3	100	3	100	3	0

The results in **Table 4.5** represented that, residue of oxytetracycline was found in both meta & liver sample. But residue of ciprofloxacin was absent and residue of amoxicillin was found only in meat. These results agreed with Petrovi *et al.,* 2006 and Sattar *et al.,* 2014 that recorded the lowest residue level of ciprofloxacin in chicken muscles than chicken organ especially liver. But lower results were recorded by Omotoso and Omojola, 2015.

From the above discussion, it could be said that body weight gain, feed efficiency, presence of antibiotic residue, dressing percentage were better enhanced by the effect of different antibiotics with normal and increased level of dosage.

CHAPTER - 5

SUMMARY AND CONCLUSION

CHAPTER - 5

SUMMARY AND CONCLUSION

The experiment was conducted to evaluate the efficacy of different antibiotics on production performance, dressing yield and detection of antibiotic residue in the meat of Sonali chicken at Dairy & Poultry Science farm situated in Hajee Mohammad Danesh Science and Technology University, Dinajpur from mid February to May 2018. The test, Ultra High Performance Liquid Chromatography (UHPLC) required for detection of antibiotic residue was performed by Poultry Research & Training Centre (PRTC) laboratory, CVASU, Khulshi, Chattagram. A total of 120 day old Sonali chicks were randomly distributed into four dietary treatments with three replications each contains 10 birds. Treatments are namely, T_0 (control group), T_1 (Ciprofloxacin @ 1ml/L), T_2 (Oxytetracycline @ 1gm/L), T_3 (Amoxicillin @ 1gm/L) through drinking water. At the terminal stage of experiment the cumulative body weight gain of different treatment groups were T_0 (654.15±27.47 g), T_1 (680.2±18.77 g), T_2 (708.33±21.18 g) and T_3 (706.4±8.33 g) respectively. Birds that received oxytetracycline (1gm/L) through drinking water was gained the highest (708.33±21.18 g) body weight and the lowest (680.2±18.77 g) in control group (T_0). Within different antibiotics group, live weight was increased along with standard dose. The feed intake among different treatments were statistically similar ($P>0.05$). The cumulative maximum feed intake was observed in T_0 group (1837.93±52.93g) and minimum in T_3 group (1727.43±25.38 g). Feed efficiency of different treatments were statistically significant ($P<0.05$). Respective feed efficiency was found T_0 (2.67±0.03), T_1 (2.7±0.04), T_2 (2.55 ±0.09) and T_3 (2.45±0.01). Group T_3 converted feed to meat most efficiently then T_2, T_0 and T_1 treatment respectively.

Obtained data on meat yield parameters and dressing percentage there were no significant ($P>0.05$) difference among treatment groups. The breast meat weight was the highest in treatment T_2 group compare to control group and other treatments group. Among the treatments the highest (52.36±1.77%) dressing percentage was observed in T_2 group and the lowest (51.11±0.59%) in T_0 group.

Detection of antibiotic residue was statistically significant ($P<0.05$) among the treatments group. The highest percentage was observed in group T_2 and the lowest was observed in group T_1.

Based on the result of present study, it may be concluded that antibiotic is directly acted as antimicrobial agent, growth promoter and its effects on body weight gain and feed efficiency on Sonali chicken. Antibiotic presents as residual part in the meat and liver of Sonali chicken.

Antibiotics that are clinically used in the industrialized world also found in Bangladesh. Government policy in general is liberal with antibiotic import, admittedly because the need is great due to a high prevalence of diseases and heavy germ load in the environment. Indiscriminate use of antibiotics in poultry production now-a-days in Bangladesh causes potential antibiotic residues in poultry products. Besides, the live birds sellers play a vital role in this aspect as the birds stay in the market for a couple of days when injudicious use of antibiotics might bring harmful consequences though it reduces the birds' morbidity and mortality and ensures profitability.

REFERENCES

REFERENCES

Abbasi, M. M; Babaei, H. and Ansarin, M. 2011. Simultaneous determination of tetracyclines residues in bovine milk samples by solid phase extraction and HPLC-FL method. Advanced pharmaceutical bulletin, 1(1): 34-39.

Abdulrahim, S. M; Haddadin, M. S. Y., Odetallah, N. H. M. and Robinson R. K. 1999.Effect of Lactobacillus acidophilus and zinc bacitracin as dietary additives for broiler chickens. British Poultry Science. 40: 91-94.

Abou-Raya, S. H; Shalaby, A. R; Salama, N. A; Emam, W. H. and Mehaya, F. M. 2013. Effect of Ordinary Cooking Procedures on Tetracycline Residues in Chicken Meat.Journal of Food & Drug Analysis. 21(1): 80-86.

Aerts, M.M.L; Hogenboom, A.C. and Brinkman, U.A.T. 1995. Analytical strategies for the screening of veterinary drugs and their residues in edible products. Journal of Chromatography B: Biomedical Sciences and Applications. 667(1): 1-40.

Ahmed, M. 2012. Antibiotic Resistance: An Emerging Global Headache, in Antibiotic Resistant Bacteria - A Continuous Challenge in the New Millennium, M. Pana, Editor. In Tech. p. 3-14.

Al-Ghamdi, M. S; Al-Mustafa, Z. H; El-Morsy, F; Al-Faky, A; Haider, I. and Essa, H. 2000.Residues of tetracycline compounds in poultry products in the eastern province of Saudi Arabia. Public health. 114(4): 300-304.

Almanama, M. A. 2011. Prevalence of Mycoplasma gallisepticum in the Ten licensed Hatcheries in Gaza strip, Palestine, Biological Sciences, Islamic University Gaza, Master Thesis, Unpublished data.

Amer, M. Y. and Khan, S. H. 2012. A comparison between the effects of a probiotic and an antibiotic on the performance of Desi chickens. Veterinary World. 5(3): 160-165.

Apu, A.S. and Saleque, M.A. 2012. Impact of innovative approaches for increased family poultry production and scaling up the livelihoods in Bangladesh. Paper presented at the XXIV World's Poultry Congress, 5–9 August 2012, Salvador, Brazil.

Baidya N; Mandal, L. and Banerjee, G.C. 1993. Efficacy of feeding antibiotic and probiotics in broiler. Journal of Veterinary and Animal Sciences. 24: 120-124(Cited from CAB Abst. 1996-1998/07).

Barceló, D. 2007. Pharmaceutical-residue analysis. Trends Anal Chem, 26: 454-455.

Barton, M.D. 2000. Antibiotic use in animal feed and its impact on human health. Nutrition Research Reviews. 13(02): 279-299.

Bhuiyan, A.K.F.H. 2011. Implementation of National Livestock Development Policy (2007) and National Poultry Development Policy (2008): Impact on smallholder livestock rearers. Keynote paper presented at the workshop organized by the South Asia Pro Poor Livestock Policy Programme (SAPPLPP) and BRAC held at the BRAC Centre Inn, Dhaka.

Botsoglou, N.A. and Fletouris, D.J. 2001. Drug residues in foods: pharmacology, food safety, and analysis. Food Science and Technology. New York: Marcel Dekker.

Bovee, T.F. and Pikkemaat, M.G. 2009. Bioactivity-based screening of antibiotics and hormones. Journal of Chromatography A. 1216(46): 8035-8050.

Bozkurt, M; Küçükyilma, K; Çatli, A. U. and Çinar, M. 2008. Growth Performance and Slaughter Characteristics of Broiler Chickens Fed with Antibiotic, Mannan Oligosaccharide and Dextran Oligosaccharide Supplemented Diets. International Journal of Poultry Science. 7(10): 969-977.

Burgat-sacaze, V; Delatour, P. and Rico, A. 1981. Bound residues of veterinary drugs: bioavailability and toxicological implications. Annales de Recherches Vétérinaires. 12(3): 277-289.

Castanon, J. 2007. History of the use of antibiotic as growth promoters in European poultry feeds. Poultry Science. 86(11): 2466-2471.

Cavazzoni, V; Adami, A. and Castrovilli, C. 1998. Performance of broiler chickens supplemented with Bacillus coagulans as probiotic. British Poultry Science. 39: 526-529.

Chattopadhyay, M. K. 2014. Use of antibiotics as feed additives: a burning question. Front Microbiol, 5: 334.

Chopra, I. and Roberts, M. 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiology and Molecular Biology Reviews. 65(2): 232-260.

Codex Alimentarius Commission 2014. Maximum residue limits (MRLS) and risk management recommendations (RMRs) for residues of veterinary drugs in foods. Updated as at the 37th Session of the Codex Alimentarius Commission, 2014.CAC/MRL 2.

Community Reference Laboratories (CRLs) 2014. Guidelines for the validation of screening methods for residues of veterinary medicines (initial validation and transfer). Last accessed [10/12/2010], http://ec.europa.eu/food/food/chemicalsafety/residues/Guideline_Validation_Screening_en.pdf.

Crosby, N.T. 1991. Determination of veterinary residues in food. 1st ed., New York: Ellis Horwood.

Cunningham, F.M; Elliott, J. and Lees, P. 2010. Comparative and veterinary pharmacology.1st ed. Handbook of experimental pharmacology. Vol. 199. Heidelberg; New York: Springer.

Dallal, M. M. S; Doyle, M. P; Rezadehbashi, M; Dabiri, H; Sanaei, M; Modarresi, S. and Sharifi-Yazdi, M. K. 2010. Prevalence and antimicrobial resistance profiles of Salmonella serotypes, Campylobacter and Yersinia spp. isolated from retail chicken and beef, Tehran, Iran. Food Control. 21(4): 388-392.

Davis, J. L; Smith, G. W; Baynes, R. E; Tell, L. A; Webb, A. I. and Riviere, J. E. 2009. Update on drugs prohibited from extra label use in food animals. Journal of the American Veterinary Medical Association. 235(5): 528-534.

Dey, B. P., Reamer, R. P., Thaker, N. H. and Thaler, A. M. 2005. Calf antibiotic and sulfonamide test (CAST) for screening antibiotic and sulfonamide residues in calf carcasses. Journal of AOAC international. 88(2): 440-446.

Dhande, V. U.; Kukde, R. J. and Thakur, B. S. 1991. Effect of Livol on performance of broilers. Poultry Guide. 28: 35-36.

Dhanji, H; Murphy, N. M; Doumith, M; Durmus, S; Surman Lee, S; Hope, R. and Livermore, D. M. 2010. Cephalosporin resistance mechanisms in Escherichia coli isolated from raw chicken imported into the UK. Journal of Antimicrobial Chemotherapy. 65(12): 2534-2537.

Dipeolu, M. A. and Alonge, D. O. 2002. Residues of streptomycin antibiotic in meat sold for human consumption in some states of SW Nigeria. Archivos de zootecnia, 51(196): 477-480.

Dolberg, F. 2008. Poultry sector country review: Bangladesh, pp. 34–35. Rome, FAO Animal Production and Health Division, Emergency Centre for Transboundary Animal Diseases, Socioeconomics, Production and Biodiversity Unit.

Donoghue, D. J. 2003. Antibiotic Residue in Poultry Tissues and Eggs: Human Health concerns? Poultry Science 82: 618-621.

Doyle, M. 2006. Veterinary drug residues in processed meats—potential health risk, a review of the scientific literature. Food Research Institute (FRI Briefings), University of Wisconsin–Madison, March 2006. Last accessed [15/3/2014], https://fri.wisc.edu/files/Briefs_File/FRIBrief_VetDrgRes.pdf.

Elmanama, A.A. and Abdelateef, N. 2012. Antimicrobial Resistance of Enteric Pathogens Isolated from Acute Gastroenteritis Patients in Gaza strip, Palestine. The International Arabic Journal of Antimicrobial Agents. 2(4): 1-4.

Emami, K. N; Samie, A; Rahmani, H.R. and Ruiz-Feria, C.A. 2012. The effect of peppermint essential Oil and fructo-oligosaccharides, as alternatives to virginiamycin, on growth performance, digestibility, gut morphology and immune response of male broilers. Animal Feed Science and Technology. 175: 57-64.

Ezenduka, E.V; Ike, O.S. and Anaelom, N.J. 2014. Rapid detection of antimicrobial residues in poultry: A consequence of non-prudent use of antimicrobials. Health. 6(2): 149-152.

Forward, K. R; Matheson, K. M; Hiltz, M; Musgrave, H. and Poppe, C. 2004. Recovery of cephalosporin resistant Escherichia coli and Salmonella from pork, beef and

chicken marketed in Nova Scotia. Canadian Journal of Infectious Diseases and Medical Microbiology. 15(4): 226-230.

Gaudin, V; Hedou, C; Rault, A. and Verdon, E. 2010. Validation of a Five Plate Test, the STAR protocol, for the screening of antibiotic residues in muscle from different animal species according to European Decision 2002/657/EC. Food Additives and Contaminants. 27(7): 935-952.

Gaudin, V; Juhel-Gaugain, M; Morétain, J. P. and Sanders, P. 2008. AFNOR validation of Premi® Test, a microbiological-based screening tube-test for the detection of antimicrobial residues in animal muscle tissue. Food Additives and Contaminants. 25(12): 1451-1464.

Greeson, K; Suliman, G. M; Sami, A; Alowaimer, A. and Koohmaraie, M. 2013. Frequency of antibiotic resistant Salmonella, Escherichia coli, Enterococcus, and Staphylococcus aureus in meat in Saudi Arabia. African Journal of Microbiology Research. 7(4): 309-316.

Guardabassi, L; Jensen, L.B. and Kruse, H. 2008. Guide to antimicrobial use in animals. 1st ed., Oxford, UK: Blackwell.

Harms, R.H; Ruiz, N. and Miles, R.D. 1986. Influence of virginiamycin on broilers fed four levels of energy. Poultry Science. 65: 1984-1986.

Heitzman, R. J. 1994. Veterinary drug residues: residues in food producing animals and their products: reference materials and methods: final report. 2nd ed., Oxford: Blackwell Scientific.

Hsu, W. H. 2008. Handbook of veterinary pharmacology. 1st ed., Ames, Iowa, USA: Wiley-Blackwell.

Huque, Q. M. E. 2011.Commercial poultry production in Bangladesh. Souvenir of 7th International Poultry Show and Seminar, 25–27, Dhaka, Bangladesh.

Hussein, M. A. and Khalil, S. 2013. Screening of some antibiotics and anabolic steroids residues in broiler fillet marketed in El-Sharkia governorate. Life Science Journal. 10(1): 2111-2118.

Jamroz, D; Skorupinska, J; Orda, J; Wiliezkiewiez, A. and Kirchgessner, M. 1995. Effect of Avilamycin (Maxus) and Roxazyme Supplementation on broiler performance. Archiv-fur-Geflugelkunde. 59: 228-233.

Javadi, A; Mirzaie, H. and Khatibi, S.A. 2011. Effect of roasting, boiling and microwaving cooking methods on Enrofloxacin residues in edible tissues of broiler. African Journal of Pharmacy and Pharmacology. 5(2): 214-218.

Kannan, D; Vishwanathan, K. and Mohan B. 2005. Production performance of broilers by feeding antibiotics and Probiotics diet, Abstract, IPSA. Co. No. 2005.

Karraouan, B; Bouchrif, B; Ziyate, N; Talmi, A; Yahia, Kh. I. S; Cohen, N. and Fassouane, A. 2009. Evaluation of multi-plate microbial assay for the screening of antibacterial residues in poultry muscle. European Journal of Scientific Research 35(2): 311-317.

Kaya, S. E. and Filazi, A. 2010. Determination of antibiotic residues in milk samples. Kafkas Universitesi Veteriner Fakultesi Dergisi. 16: 31-35.

Kim, M. S; Lim, T. H; Jang, J. H; Lee, D. H; Kim, B. Y; Kwon, J. H. and Yang, S. Y. 2012. Prevalence and antimicrobial resistance of Salmonella species isolated from chicken meats produced by different integrated broiler operations in Korea. Poultry science, 91(9): 2370-2375.

Kirbis, A. 2007. Microbiological screening method for detection of aminoglycosides, β-lactames, macrolides, tetracyclines and quinolones in meat samples. Slov Vet Res 44(1/2): 11-18.

Kowalski, P. and Konieczna, L. 2007. Determination of penicillin antibiotics in poultry muscle by capillary electrophoresis. Bulletin-Veterinary Institute In Pulawy,. 51(4): 595-598.

Kulkarni, V. V. and Thakur, Y. P. 1992. Effect of 'Biovet' on the growth performance and carcass components of Broilers. Poult. Guide, 29: 55-57.

Lee, K. W; Ho Hong, Y; Lee, S. H; Jang, S. I; Park, M. S. and Bautista, D. A. 2012. Effects of anticoccidial and antibiotic growth promoter programs on broiler performance and immune status. Research in Veterinary Science. 93: 721-728.

Lemus, J. A; Blanco, G; Grande, J; Arroyo, B; Garcia-Montijano, M. and Martinez, F. 2008. Antibiotics threaten wildlife: circulating quinolone residues and disease in avian scavengers. PLOS ONE. 3(1): 1-6.

Levy, S. B. and Marshall, B. 2004. Antibacterial resistance worldwide: causes, challenges and responses. Nature Medicine. 10: S122-S129.

Lolo, M; Pedreira, S; Miranda, J. M; Vazquez, B. I; Franco, C. M; Cepeda, A. and Fente, C. 2006. Effect of cooking on enrofloxacin residues in chicken tissue. Food additives and contaminants, 23(10): 988-993.

Mahgoub, O; Kadim, I. T; Mothershaw, A; Al Zadjali, S. A. and Annamalai, K. 2006. Use of enzyme linked immunosorbent assay (ELISA) for detection of antbiotic and anabolic residues in goat and sheep meat. World Journal of Agricultural Sciences. 2(3): 298-302.

McEwen, S. A. and Fedorka-Cray, P. J. 2002. Antimicrobial use and resistance in animals. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 34(3): 93-106.

Meredith, W. E; Weiser, H. H. and Winter, A. R. 1965. Chlortetracycline and Oxytetracycline residues in poultry tissues and eggs. Applied Microbiology 13(1): 86-88.

Michalova, E; Novotna, P. and Schlegelova, J. 2004. Tetracyclines in veterinary medicine and bacterial resistance to them. A review. Veterinarni Medicina (Czech). 49(3): 79-100.

Mingeot-Leclercq, M.-P; Glupczynski, Y. and Tulkens, P. M. 1999. Aminoglycosides: Activity and Resistance. Antimicrobial Agents and Chemotherapy. 43(4): 727-737.

Mitchell, J. M; Griffiths, M. W; McEwen, S. A; McNab, W. B. and Yee, A. J. 1998. Antimicrobial drug residues in milk and meat: causes, concerns, prevalence, regulations, tests, and test performance. Journal of food protection, 61(6): 742-756.

Mohan, B; Kadirvel, R; Bhaskaran M. and Natarajan, A. 1996. Effect of probiotic supplementation on growth, nitrogen utilisation and serum cholesterol in broilers. British Poultry Science. 37: 395-401.

Mujeer, K. A; Ramakrishna, J; Thyagarajan, D; Thangavel, A. and Sundararasu, V. 1988. Efficacy of Brotone on broilers. Indian Poultry Revolution. 19: 41-43.

Myllyniemi, A. L. 2004. Development of microbiological methods for the detection and identification of antimicrobial residues in meat. National Veterinary and Food Research Institute. Helsinki, Finland.

Myllyniemi, A. L. 2004. Development of microbiological methods for the detection and identification of antimicrobial residues in meat, Faculty of Veterinary Medicine, University of Helsinki, Master Thesis, Unpublished data.

Navrátilová, P. 2008. Screening methods used for the detection of veterinary drug residues in raw cow milk – a review. Czech Journal of Food Science. 26(6): 393-401.

O'brien, J; Campbell, N. and Conaghan, T. 1981. Effect of cooking and cold storage on biologically active antibiotic residues in meat. Journal of Hygiene. 87(03): 511-523.

Oka, H; Ito, Y. and Matsumoto, H. 2000. Chromatographic analysis of tetracycline antibiotics in foods. Journal of Chromatography A. 882(1–2): 109-133.

Okerman, L; Croubels, S; De Baere, S; Hoof, J. V; De Backer, P. and De Brabander, H. 2001. Inhibition tests for detection and presumptive identification of tetracyclines, beta-lactam antibiotics and quinolones in poultry meat. Food Additives & Contaminants. 18(5): 385-393.

Okerman, L; De Wasch, K. and Van Hoof, J. 1998. Detection of antibiotics in muscle tissue with microbiological inhibition tests: effects of the matrix. Analyst. 123(11): 2361-2365.

Omotoso, A. B. and Omojola, B. 2015. Fluoroquinolone residues in raw meat from open markets in Ibadan, Southwest, Nigeria. International Journal of Health, Animal Science and Food Safety. 2: 32.

Paige, J. C; Chaudry, M. H. and Pell, F. M. 1999. Federal surveillance of veterinary drugs and chemical residues (with recent data).The Veterinary clinics of North America. Food animal practice. 15(1): 45-61.

Pavlov, A; Lashev, L; Vachin, I. and Rusev, V. 2008. Residues of antimicrobial drugs in chicken meat and offals. Trakia J. Sci. 6(1): 23-25.

Petrovi, J; Balti, M; Upi, V; Stefanovi, S. and Stojanovi, D. 2006. Residues of enrofloxacin and its main metabolite ciprofloxacin in broiler chickens. Acta Veterinaria (Beograd). 56(5-6): 497-506.

Phillips, I; Casewell, M; Cox, T; De Groot, B; Friis, C; Jones, R. and Waddell, J. 2004. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. Journal of Antimicrobial Chemotherapy. 53(1): 28-52.

Pikkemaat, M. G. 2009. Microbial screening methods for detection of antibiotic residues in slaughter animals. Analytical and Bioanalytical Chemistry. 395(4): 893-905.

Prescott, L. M; Harley, J. P. and Klein, D. A. 2002. Microbiology. 5th editions, Boston, UK: McGraw-Hill.

Rahimi, E; Momtaz, H; Ameri, M; Ghasemian-Safaei, H. and Ali-Kasemi, M. 2010. Prevalence and antimicrobial resistance of Campylobacter species isolated from chicken carcasses during processing in Iran. Poultry Science. 89(5): 1015-1020.

Reyes-Herrera, I; Schneider, M. J; Cole, K; Farnell, M. B; Blore, P. J. and Donoghue, D. J. 2005. Concentration of antibiotic residues vary between different edible muscle tissues in poultry. Journal of Food Protection 68(10): 2217-2219.

Riviere, J.E. and Papich, M.G. 2009. Veterinary pharmacology and therapeutics. 9th editions. Ames, Iowa, USA: Wiley-Blackwell.

Rosen, G. D. 1996. Pronutrient antibiotic replacement standards discussed. Feedstuffs, 75: 11-13.

Rosengren, L; Gow, S. and Weese, J. 2010. Antimicrobial use and resistance in pigs and chickens: A review of the science, policy, and control practices from farm to slaughter - executive summary. Canadian Journal of Infectious Diseases & Medical Microbiology. 21(3): 123-127.

Saleque, M. A. 2009. Poultry industry in Bangladesh: Current status and its challenges and opportunities in the emerging market environment published. In Poultry Business Directory, pp. 13–24. Dhaka.

Saleque, M. A. and Saha, A. A. 2013. Production and economic performance of small scale Sonali bird farming for meat production in Bangladesh. In Proceedings of the Seminar, 8th International Poultry Show and Seminar pp. 20–24. Dhaka, World's Poultry Science Association, Bangladesh Branch.

Sattar, S; Hassan, M. M; Islam, S. K. M. A; Alam, M; Faruk, M. S. A; Chowdhury, S. and Saifuddin, A. K. M. 2014. Antibiotic residues in broiler and layer meat in Chittagong district of Bangladesh. Veterinary World. 7(9): 738-743.

Schmidt, C.W. 2012. FDA Proposes to Ban Cephalosporins from Livestock Feed. Environmental Health Perspectives. 120(3): a106-a106.

Şenyuva, H; Özden, T. and Sarica, D. Y. 2000. High Performance Liquid Chroma tographic Determination of Oxytetracycline Residue in Cured Meat Products. Turkish Journal of Chemistry. 24: 395-400.

Shahid, M. A; Siddique, M; Rehman, U. S; Hameed, S. and Hussain, A. 2007. Evaluation of a microbiological growth inhibition assay as a screening test for the presence of antibiotic residues in poultry meat. American Journal of Food Technology. 2(5): 457-461.

Shamsuddoha, M. and Sohel, M. H. 2003. Problems and Prospects of Poultry Industry in Bangladesh: A Study on Some Selected Areas. The Chittagong University Journal of Business Administration. 19: 200.

Shareef, A. M; Jamel, Z. T. and Yonis, K. M. 2009. Detection of antibiotic residues in stored poultry products. Iraqi Journal of Veterinary Sciences. 23(1): 4548.

Silfrany, R. O; Caba, R. E; de Los Santos, F. S. and Hanning, I. 2013. Detection of quinolones in poultry meat obtained from retail centers in Santiago Province, the Dominican Republic. Journal of food protection. 76(2): 352-354.

Singh, S. K; Sadagopan, V. R. and Panda, B. 1974. Studies on the effect of different levels of Vit-A supplementation in the diet of broiler chicken. Indian Veterinary Journal. 51: 194-198.

Sirdar, M. M. 2010. Antibiotic residues in commercial layer hens in Khartoum state, Sudan 2007-2008. Veterinary Science, University of Pretoria, Master Thesis, Unpublished data.

Stead, D. A. 2000. Current methodologies for the analysis of aminoglycosides. Journal of Chromatography B: Biomedical Sciences and Applications. 747(1–2): 69-93.

Stolker, A. and Brinkman, U. T. 2005. Analytical strategies for residue analysis of veterinary drugs and growth-promoting agents in food-producing animals—a review. Journal of Chromatography A. 1067(1): 15-53.

Tin, T. M. 2003. Detection of Antibiotic Residues and Isolation of Antibiotic Resistant Escherichia Coli from Chicken Meat and Chickens in Malaysia, Veterinary Medicine, Universiti Putra Malaysia, Ph.D Thesis, Unpublished data.

Tothill, I. E. 2003. Rapid and on-line instrumentation for food quality assurance. 1st ed., Boca Raton, Cambridge, England: CRC Press; Woodhead.

Vass, M; Hruska, K. and Franek, M. 2008. Nitrofuran antibiotics: a review on the application, prohibition and residual analysis. Veterinarni Medicina. 53(9): 469-500.

Verdon, E; Couëdor, P. and Sanders, P. 2004. Validation of a Multi-Quinolone, Multi-Matrix, Multi-Species Method for the determination of Quinolone residues by HPLC with fluorescence detection. AFSSA; French agency for food safety Euroresidue V, Noodwijkerhout, The Netherlands, May 10-12.

Wallace, R. J. and Chesson, A. 1995. Biotechnology in animal feeds and animal feeding. 1st edition. Weinheim; Cambridge: VCH.

Wang, J; MacNeil, J. D. and Kay, J. F. 2012. Chemical analysis of antibiotic residues in food. Hoboken, N.J.: Wiley & Sons.

Watson, D. H. 2004. Pesticide, veterinary and other residues in food. 1st edition. Boca Raton, Cambridge, England: CRC Press; Woodhead.

Woodward, K. N. 2009. Veterinary pharmacovigilance: adverse reactions to veterinary medicinal products. 1st ed., Oxford, UK: Wiley-Blackwell.

Yeo, J. and Kim, K. 1997. Effect of feeding diets containing an antibiotic, a probiotic or yucca extract on growth and intestinal urease activity in broiler chicks. Poultry Science. 76: 381-385.

Zulkifli, I; Abdullah, N; Azrin M. N. and Ho, Y. W. 2000. Growth performance and immune response of two commercial broiler strains fed diets containing Lactobacillus cultures and oxytetracycline under heat stress conditions. British Poultry Science. 41: 593-597.

APPENDICES

APPENDICES

Appendix I: Daily temperature (^{0}C) was recorded by clinical thermometer at 7.00 AM, 2.00 PM and 7.00 PM

Sl. No	Date	7.00 AM	2.00 PM	7.00 PM
1	18-02-18	27	30	25
2	19-02-18	25	30	28
3	20-02-18	29	31	30
4	21-02-18	31	32	32
5	22-02-18	29	30	27
6	23-02-18	28	31	29
7	24-02-18	29	33	29
8	25-02-18	30	32	29
9	26-02-18	29	32	29
10	27-02-18	28	33	30
11	28-02-18	29	33	31
12	01-03-18	30	34	30
13	02-03-18	32	33	30
14	03-03-18	32	33	29
15	04-03-18	29	31	29
16	05-03-18	30	32	31
17	06-03-18	25	23	24
18	07-03-18	26	22	23
19	08-03-18	22	25	24
20	09-03-18	27	29	28
21	10-03-18	31	32	30
22	11-03-18	28	32	29
23	12-03-18	30	32	29
24	13-03-18	31	33	30
25	14-03-18	29	32	31
26	15-03-18	29	33	30
27	16-03-18	30	34	30

Sl. No	Date	7.00 AM	2.00 PM	7.00 PM
28	17-03-18	29	33	30
29	18-03-18	28	32	29
30	19-03-18	29	31	30
31	20-03-18	30	33	29
32	21-03-18	27	32	29
33	22-03-18	30	33	31
34	23-03-18	28	30	29
35	24-03-18	29	31	28
36	25-03-18	30	32	30
37	26-03-18	27	31	28
38	27-03-18	28	30	29
39	28-03-18	29	31	30
40	29-03-18	30	33	31
41	30-03-18	26	29	28
42	31-03-18	27	30	28
43	01-04-18	29	31	29
44	02-04-18	30	32	30
45	03-04-18	29	31	28
46	04-04-18	28	32	29
47	05-04-18	27	30	27
48	06-04-18	29	31	30
49	07-04-18	28	32	29
50	08-04-18	30	33	30
51	09-04-18	28	30	29
52	10-04-18	27	31	28
53	11-04-18	30	33	29
54	12-04-18	29	33	30
55	13-04-18	28	31	29
56	14-04-18	27	30	28

Appendix II: Average body weight gain per birds (gm)/week:

Week	Replication	T_0 Control	T_1 Ciprofloxacin 1ml/L	T_2 Oxytetracycline 1gm/L	T_3 Amoxicillin 1gm/L
1st	R_1	105.2	98.1	97.7	100.5
	R_2	103.6	103.8	95.3	100.3
	R_3	99.6	95.2	106	105.2
2nd	R_1	146.6	137.6	135.8	145.4
	R_2	145.6	143.8	135.6	137.4
	R_3	135.4	135.6	142.8	141.4
3rd	R_1	205	196.8	199.9	206.2
	R_2	204.6	178.2	185.8	185.5
	R_3	197.9	195.5	200	194.5
4th	R_1	293.8	281.8	287.4	301.4
	R_2	298.8	261.8	263.6	267.5
	R_3	279.5	280.2	294.6	282.3
5th	R_1	360.2	385.5	360.1	342.4
	R_2	371.8	319.3	335.8	342.6
	R_3	356.9	348.2	378.5	339.4
6th	R_1	407.7	389.8	407.2	380.7
	R_2	441.2	382.6	405.6	436.5
	R_3	418.1	397.4	407.4	375.3
7th	R_1	480	445.2	494.5	471.6
	R_2	514.5	403.3	466.5	486.4
	R_3	505.5	494.3	470.6	487.9
8th	R_1	550.2	545.2	604.6	550
	R_2	604.8	527.8	560.4	584.4
	R_3	538	619.2	611	564.4
9th	R_1	668.4	656.8	720.7	690.8
	R_2	734.6	667.3	690.2	710.5
	R_3	643	735.8	750.1	700.8