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DEPARTMENT OF NUTRITION AND DIETEICS

Effects of Cooking Methods on Antibiotics Residues in Chicken Legs

MASTER THESIS

Selin İNAL

ADVISOR Assist. Prof. Dr. İskender KARALTI

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	Unvanı, Adı-Soyadı (Kurumu)	İmza
Jüri Başkanı:	Dr. Öğr. Üyesi Cenk SESAL	
Tez danışmanı:	Dr. Öğr. Üyesi İskender KARALTI	
Üye:	Dr. Öğr. Üyesi Hülya DEMİR	
Üye:		
Üye:		

ONAY

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Prof. Dr. Bayram YILMAZ
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DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

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ABBREVIATIONS

AG	Aminoglycoside
CAP	Chloramphenicol
ELISA	Enzyme-linked Immunosorbent Assay
EU	European Union
FAO	Food and Agriculture Organization
FDA	U.S. Food and Drug Administration
FIA	Fluoroimmunoassay
MRL	Maximum residue limits
OPD	o-phenylenediamine dihydrochloride
OTC	Oxytetracycline
PBPs	Penicillin Binding Proteins
PFA	Phytogenic Feed Additives
SPR	Surface-plasmon Resonance
TMB	Tetramethylbenzidine
TRF	Time-resolved Fluorescence
TRFIA	Time-resolved Fluoroimmunoassay
UV/VIS	Ultraviolet/Visible
WHO	World Health Organization

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ABSTRACT

İnal S. Effects of Cooking Methods on Antibiotics Residues in Chicken Legs. Yeditepe University, Institute of Health Science, Department of Nutrition and Dietetics, MSc thesis, İstanbul.

Antibiotics are medicine that use for treatment infectious diseases. Also antibiotics have an important role at poultry industry to accelerate growth. Using antibacterial drugs in animal may cause antibotic residue in meat, milk or eggs. Antibiotic residues on food threat human healths. Most of antibiotics such as chloramphenicol are legally banned to use on animal food cause of side effects. The purpose of this study is observed the cooking effect on chloramphenicol residues in food. In our study 10 different brands chicken samples were collected. Chicken samples were divided into 5 equal parts that each of 20 grams. Every samples were cooked in different methods; boiling, cooking in oven, sauteing and frying. Mera test and ELISA were used to determine residue levels both qualitative and quantitative. Mera test results showed that all raw samples are contaminated with antibiotic residues. After cooking methods 7 (14%) samples result changed through negative. Chloramphenicol residue was screened by ELISA. According to Elisa results there is no chloramphenicol residue in any sample (<0.03). As a result, illegal chloramphenicol was not detected, however antibiotic residues were observed in chickens. Thermal proccess may reduce residue concentration in foods. However cooking processes can not make sure a full elimination of antibiotic residue in foods and it is not making food totally safe.

Key words: Antibiotic, Cooking, Residue

ÖZET

İnal S. Pişirme Tekniklerinin Tavuk Butlarında Antibiyotik Kalıntı Düzeylerine Etkisi. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Beslenme ve Diyetetik Ana Bilim Dalı, Yüksek Lisans Tezi, İstanbul.

Antibiyotikler infeksiyon hastalıklarını tedavi etmek için kullanılan ilaçlardır. Aynı zamanda antibiyotikler kanatlı endüstrisinde büyütme faktörü olarak kullanılmaktadır. Çiftlik hayvanlarında antibiyotik kullanımı et, süt ve yumurtada kalıntıya neden olabilmektedir. Gıdalardaki antibiyotik kalıntısının riskleri vardır ve insan sağlığını tehdit etmektedir. Antibiyotik kullanımı yasalarla sınırlandırılmıştır. Klaromfenikol gibi bazı antibiyotiklerin hayvanlar üzerinde kullanımı tamamen yasaktır. Bu çalışmadaki amaç piyasada satılan tavuk butlarında klaromfenikol kalıntısını taramak ve farklı pişirme tekniklerinin kalıntı üzerine etkisini araştırmaktır. Çalışmamızda 10 farklı marka tavuk kullanılmıştır. Her tavuk 20 gramlık 5 eşit parçaya bölünmüştür. Kaynatma, fırınlama, soteleme ve kızartma yöntemleri ile pişirilmiştir. Kalıntı miktarlarını ölçmek için Mera test ve ELISA test kullanılmıştır. Mera test çalışma sonucunda tüm çiğ örneklerde antibiyotik kalıntısı gözlenmiştir. Pişirme tekniklerinden sonra 7 (14%) sonuç pozitiften negatife dönmüştür. ELISA test ile tavuklarda klaromfenikol kalıntısı taranmıştır. ELISA sonuçlarına göre hiçbir örnekte klaromfenikol kalıntısı gözlenmemiştir. Sonuç olarak kullanımı yasaklı olan klaromfenikole rastlanmamıştır, fakat tavuklarda antibiyotik kalıntısı gözlenmiştir. Pişirme teknikleri antibiyotik kalıntısını azaltmada yardımcı olabilir, fakat antibiyotik kalıntısını tamamen yok etmeyebilir.

Anahtar Kelimeler: Antibiyotik, Pişirme, Kalıntı

1. INTRODUCTION

1.1. Purpose and Contents

Proteins are one of the most important nutrients that should be consumed to ensure adequate and balanced diet. Animal origin foods include amino acids that can not release from body. For this reason a balanced diet should contain animal origin foods.

Population of World is increasing rapidly and food resources decrease. Poultry meat and egg are the main protein sources for all world. Considering that production costs are lower than beef meat or pork and the some of religious restrictions (for example; pork is forbidden), the industry of poultry is probably the most common food production industry at globally. The chicken meat marketing and egg marketing have grown rapidly in all World at last decades.

Antibiotics are medicine taht using for inhibit or kills the bacterias to protect or improve health. Also antibiotics have an important role at poultry industry to accelerate growth. When chickens take correct amount of antibiotics, residues has excreted by urine and so there can not be antibiotic residue on meat, egg or milk. Using illegal antibiotics, wrong route of applications, using excess amount antibiotics or lack of attention to residue (antibiotic) purification time from body may cause antibiotic residue on meat and other products. Antibiotic residues have been found in the blood and the other tissues of the animals when high concentrations of antibiotics were used cause of many reasons.

Limits of using antibiotics, dosages and way of application regulate by laws. At Turkey Health Ministry and Ministry of Food, Agricultre and Livestock control these laws. At another countries also there is too many organizations to control these laws such as World Health Organization(WHO), U.S. Food and Drug Administration (FDA), Food and Agriculture Organization (FAO), European Union (EU) Commisions.

According to laws some kind of antibiotics are legally banned to use on livestock. Chloramphenicols are cheap antibiotics that one of legally banned to use on livestock. Despite some antibiotics are banned to use, some researches show that antibiotics are using on livestock illegal or excess amount of limits. Using illegal antibiotics or excess amount of limits threat human health.

Antibiotic residues may have adverse effects on human health such as antibiotic residue, allergic/anaphylactic reactions and chronic toxic effects to exposure antibiotics long time. Adverse effects of antibiotic residues are one of the biggest concern on World. Researchers have been study to reduce the amount of antibiotic residues.

Most of research on antibiotic residues study on raw meat products. However meat products do not consume raw from consumer. Before consuption, meat and meat products pass a heat treatment (cooking) or another process (freezing). The effect of cooking techniques on antibiotic residues is an issue that needs to be further investigated. The purpose of this study is searching effects of cooking on antibiotic residues on chicken legs.

2. GENERAL INFORMATIONS

2.1. Antibiotics

Antibiotics are medicines that used for protecting the humans and living-being health. It suppress or eliminates the growth of microorganisms such as bacteria, protozoa, fungi etc.. Approximately 250 chemical compounds are registered currently for use medication and veterinary (1).

The term of antibiotic is used in a general name for chemotherapeutic and antibiotic-like substances that use for treatment. Nowadays, it is possible that most of the antibiotics are obtained by synthetic or semi synthetic methods. Antibiotics have been used for the treatment of infectious diseases since the beginning 17th century. A scientific foundation of such treatments has occured at the beginning of this century when Paul Ehrlich put forth the concept of "selective toxic effect". Antimicrobial therapy started developing in 1935 when Domogk used sulfamides in treatment. Antibiotics started using for treatment when in 1940 Chain and Flarey obtained a substances from "*Penicillium notatum*" that kill microorganisms (2).

All bacteria have three proliferation stage, consisting of slow development, rapid development and rest periods. Antibiotics affect bacteria when slow and rapid development stages. This effect can be that kill bacterias (bactericidal effect) or stop develop and proliferation of bacteria (bacteriostatic effect). This effect occur by kill bacterias (bactericidal effect) or stop develop and proliferation of bacteria (bacteriostatic effect). For example; penicilines, aminoglikozides, sefalosproines, yankomisins, florokinos and basitrasin affectwith bactericisial effect, tetracylinds, macrolids and sulfonamids affect with bacteriostatic effect. Also antibiotics can be classified according to their effect spectrum as narrow and wide. Wide spectrum antibiotics are preferred more through veterinarians that working in field conditions acitvely (2).

2.1.1. Classifications of Antibiotics

Antibiotics are molecules that can be have different functions inside same molecule. For this reason, they can be neutral, cationic, anionic, or zwitterionic under different pH conditions. Antibiotics can be classified according to their chemical structure or mechanism of action. They are separated different subgroups. Tetracyclines, β-lactams, aminoglycosides, macrolides and fluoroquinolones are some of these subgroups (1).

2.1.1.1. Beta Lactams

Beta-lactam antibiotics is a huge group of different antibiotics that have different antibacterial domains, chemical structures and pharmacokinetics. Common characteristics of members of this group; their mechanisms of action, resistance ways towards themselves and all have a beta-lactam ring in the structure. Penicillins, monobactams, cephalosporins, carbapenems and combinations of beta lactam / beta lactamase inhibitors are antibiotics in this group (3).

All beta-lactam antibiotics affect bacteria by inhibit synthesis of peptidoglycan by blocking transpeptidase activity of penicillin binding proteins (PBPs) that responsible for cell wall synthesis. As a result, the bacterium that lacks cell wall synthesis has lysis and die.

Beta-lactams are identified as bactericidal antibiotic (3).

Research on antibiotics and their use are followed by the discovery of penicillin.

Penicillin

Penicillin is one of the most important antibiotics found. Penicillin has narrow impact but result of researches on penicillin main molecule, too many wide impact semisynthetic antibiotics have been found such as ampicillin, amoksisilin, azlocillin, carbenicillin (4)

Penicillin was discovered by A.Fleming at 1928. Researcher observed that bacterias do not proliferate around colonies of staphylococcus that contaminated by penicillium mould inside petri boxes and recorded this an antibiotic called penicillin. At 1956, penicillin were synthesized by Di Vigneaud and afetr one year a group from Batchclor, Doyle, Meyler and Robinson developed methods about penicillin main core synthesize (4).

Penicillins are generally spread in the extracellular fluid in the body after absorption. They pass biological membranes hardly since they are ionized and dissolve well in water. Benzylpenicillin that at circulation 65% is binds to albumin, 10% goes into the red blood cells. It is hard to break easily in the body and it is removed from from kidneys the more than %90 by a direct route (4).

2.1.1.2. Macrolides

Macrolides are antibiotics that primary and lipophilic. They have 14 macrocyclic members lactone ring dependent with glycosidic bonds. They are strong towards most kind of gram positive and negative bacteria. specially the bacteria which used for the cure of infectious diseases in edible animals (poultry, cattle etc) such as erythromycin, tylosine and lincomycin (1).

2.1.1.3. Aminoglycosides

Aminoglycosides consist of aminocyclitol ring and amino sugars bound with glycoside bonds to it. Different aminoglycoside (AG) molecules have alterations in the structure of and number of amino sugar groups. AG do not absorbed from the digestive tract. They do not pass to cerebrospinal fluid, intraocular fluid and bile except streptomisin. They excreted in urine without metabolised. Urine concentrations reach 80-100 times the serum (5).

2.1.1.4. Tetracyclines

Tetracylines are antibiotics that have four (tetra-), hydrocarbon rings (-cycl-), derivation (-ine). They are described as "a subgroup of polyketides with an octahydrotetracene-2-carboxamide skeleton". Oxytetracycline and chlortetracycline are subgroups of tetracylines. They are used in the cure of bacterial blood illnesses (1).

They pass to the placenta and milk, and accumulate in the teeth and bones. Tetracyclines are metabolized in the liver; reaches high densities in bile. They are mainly excreted in the urine and less in the faeces (5).

2.1.1.5. Fluoroquinolones

Fluoroquinolones are antibiotics with a fluorine atom bond to the central circle system, specifically at the 6-position. Another characteristic of fluoroquinolones is that they are growth promoters. Nucleic acids synthesis are inhibited by antibiotics such as enrofloxacin, ciprofloxacin and norfloxacin (1).

Fluoroquinolines constitute a class of active antibacterials that against important synthetic, gram positive and gram negative antibacterials. This group of antibiotics can penetrate into the cell easily, because of this they are frequently used in treatment for intracellular pathogens. Fluoroquinolones groups antibiotics such as ciprofloxacin is used in human treatment for infectious diseases (4).

2.1.1.6. Choloramphenicols

Chloramphenicols are cheap antibiotics that first introduced in 1949. They are the first choice in the cure of many infections such ad enteric fever. They are still one of the alternative therapies for the treatment of highly resistant bacterial infections (6).

Chloramphenicols enter into the cell by energy dependent. They are protein synthesis inhibitors, and bacteriostatic antibiotics. They affect pathogens such as *H.influenzae*, *Streptococcus pneumoniae* and *N.meningitidis*. The spectrum of actions are wide, they are effective on many microorganisms including bacteria, spirochetes, rickettsia, chlamydia and mycoplasmas. Chloramphenicols are one of the most important antibiotics for anaerobic bacterias. However, since 1989, the resistance of chloramphenicol is increasing rapidly in Korea, Vietnam, Peru, Mexico and Thailand.

Chloramphenicol can be used in the treatment of bacterial meningitis in infants and children in case of penicillin allergy. However, chloramphenicol has many side effects. The one of the most important side effect it is on the bone marrow called bone marrow depression. This effect occurs as a result of the direct pharmacological effect of the antibiotic and as a result of mitochondrial protein synthesis. Reticulocytopenia, anemia, leukopenia, thrombocytopenia may occur results of this. Gray baby syndrome may another side effect of chloramphenicol. Rarely, hypersensitivity reactions and anaphylaxis may occur. May cause bleeding if prolonged use (6).

2.2. Usage Antibiotics in Food-Producing Aminals

Antibiotics are one of the most necessary and effective veterinary medicines of intensive animal food production in our country and the World. Almost 80% of the edible animals are currently being treated with medication in a part of their lives or in many times (7).

In 2004 at member states of european union; 4.6 tones hormones, 194 tones antiparasitic, 221 tones metabolism regulators and 5.393 tones antibiotics, totally 6.501 tones veterinary medicines have used. According to records in 2006 at Turkey; in veterinary medicines in 77% of the total consumption in terms of main groups; bacterial (33%) , parasiticis (28%) and use to increase in animal yield (%16). These numbers show that the extent of medicine use in livestock.

A study at India show that usage of antibiotics will increase 82% in next 20 years (8). Another study found that the most consuption of antimicrobial medicine on livestock was at China in 2010, and in next 20 years it will increase 30% more. Another country is India that causes to a large share of the total growth in antimicrobial consumption in edible animals. India is a country that already face up with very high (and increasing) prevalence of antibiotic resistance. Also anitibiotics use over in India as a human medicine (9).

First antibiotics have been started using with other veterinary medicines to prevent and control disease at 1950 as an animal food additives. Antibiotics are added in animal's foods and drinking water to prevent side effects of stress that environment or vaccination causes and increase growth. Until today more than 40.000 antibiotics were discovered, almost 80 of them are using on animals as veterinary medicines or fishery industry. Beta lactams, tetracyclines, Chloramphenicols and macrolides are the most usage one at veterinary. Also most of antibiotics dose (such as 30-80%) used in edible animals are removed out because of partial antibiotic metabolism (7,8).

There is three hypotheses about how antibiotics affect growth and increase animal yield;

1) By inhibiting to produce toxic metabolites which inhibit nutrition absorption

2) By preventing to development pathogenic microorganism at gastrointestinal system

3) By decreasing or preventing subclinical infections in food-processing animals (7).

Also, antibiotic contaminated feeds will reach the soil residues directly when animals do not consume. Manure is te other antibiotic source for the environment. Manure that occur from waste of solid created from animals is used to fertilize the soil. Nevertheless, most of the antibiotics filter in aquatic ecosystem such as lakes during run off and end up. (8).

There is not definitely possible to check whether the producers of our country comply with the legal requirements for hormones, drugs and antibiotics used as high growth and growth factors (7).

2.2.1. Usage Antibiotics in Poultry

Population of World is increasing rapidly and food resources decrease. Poultry meat and egg are the main protein sources for all world. Considering that production costs are lower than beef meat or pork and the some of religious restrictions (for example; pork is forbidden), the industry of poultry is probably the most common food production industry at globally. Last decades in all World, the global market of chicken meat and egg has grown rapidly. Commercial poultry production is a very intensive animal agricultural system. For example; it can contain as many as 100,000 broilers in one poultry house. This means that control/prevention of disease at all levels should be a main focus for the poultry veterinarian (10).

Antibiotics have an important role at poultry industry to accelerate growth. In 1940s, antibiotics were found to have an anabolic effect by accelerating the live weight increase of antibiotics added to the chick rations. The antibiotics that most commonly used in poultry are the polypeptides, aminoglycosides, betalactams and aminocyclitols, macrolides and lincosamides, florfenicol, quinolones, sulphonamides, tetracyclines and fluoroquinolones and ionophores (11, 12).

In 1969, reserachs on using antibiotics that accelerating chicken growth started by Swedish Committee. Result of this, restrictions have been initiated in the use of antibiotic growth factors without veterinary prescription. Beginning of 1970, European Union also initiated prohibition in the use of antibiotics (12).

When chickens take antibiotics, bacterias that inside and around animals also expose antibiotics and most of them die. But sometimes antibiotics do not affect bacterias, they live and proliferate. This antibiotic resistant bacterias do not affect humans directly at farms. Antibiotic resistant bacterias can move inside kitchen through raw chicken. 60% of bacteria that found at chicken have antibiotic resistance (4).

In recent years, researchers have searched for natural and accelerating substances that may be an alternative to antibiotics. For this purpose, the use of probiotics, organic acids and enzymes as an alternative has been updated (13).

2.3. The Reasons of Antibiotic Residues

Antibiotic residues can be observed in the blood and the other tissues of the animals when overdose antibiotics were used. There can be many reasons for this. Lack of attention to residue (antibiotic) purification time from body, pharmaceutical shape,

route of application, kind of drugs, using unlabeled drugs or using human medicines are some of these reasons.

2.3.1. Lack of Attention to Residue (Antibiotic) Purification Time from Body

Pre-cutting holding time, egg and milk usage time and fishes hunting time can affect antibiotic residue purification time from animal body.

Pre-cutting holding time means the time that toxic or unwanted drug treated animals should not be cut until decrease residues in tissues and organs for consumers. At the end of this period the animals chemical residues and antibiotics found in their edible parts are considered to be at a level that will not adversely affect human health (14). Pre-cutting holding time s determined by experimental studies in healthy animals (Table 1) (15).

Pre-cutting holding time depends on too many factors, generally it changes between a few days and 1-2 weeks. If there is no record about drugs, pre-cutting holding time is considered 28 days by temporarily (14).

Table 1: Pre-cutting Holding Times for Some Antibiotics (15).

Type of Drug	**Type of Animal**	**Application Way**	**Pre-cutting Holding Times (day)**
Diminazen	Ruminants	Injection	28
Sulfaquinoxaline	Poultry	Oral	10
Sulfadimidin	Ruminants	Injection	10
	Ruminants	Oral	12
	Poultry	Oral	14
A*mprolyum	Poultry	Oral	3
Imidocarb	Ruminants	Injection	28
Lasalosid	Poultry	Oral	5
Buparvaquon	Ruminants	Injection	42

Tetracylins	Ruminants	Injection	15

2.3.2. Pharmaceutical Shape

Drug formulation is another important reason for residues. Specially ready injections drugs, tablets and intramuscular preparations are more important than others. Time-release formulations such as penisilin, amoksilisin are also important (14).

2.3.3. Route of Application

Route of application follows the sequence indicated in their pharmaceutical shapes. Injections drugs, tablets and intramuscular preparations are more important than others for making residue. There is a risk of prolonged residual residues at the site of application, specially when long-acting drugs are used, even when the level of the drug in plasma, tissue, and organs falls below the permitted level (14).

2.3.4. Kind of Drug

The main drugs that reason of residue are such as streptomycin, penicillin, oxytetracycline, gentamicin, sulfonamides. Some antibiotics such as stroptomycin, gentamicin cause residue due to their special interest in kidney (14).

2.3.5. Using Unlabeled Drugs

Using unlabeled drugs or formulations on edible animals or using labeled drugs or formulations by forbidden way (such as overdose, different application route..) cause residue. In rules, labeled drugs can not used in different way from label on edible animals, but sometimes veterinary can have to use unlabeled drugs. Main reasons for this are there is no labelled drugs for treatment some diseases or recommended dosage of labeled drug is not enough for animals. Because of these reasons, overdose drugs (unlabeled) can have to use for treatment (14).

2.3.6. Using Human Medicines

Some of the licensed medicines for human medicine are used compulsorily because they do not have licensed drugs in veterinary medicine. Since these drugs do not contain any regulation related to the residues, they have the danger of contamination in food when they are used in edible animals. These drugs should not be used unless they

are necessary; if used, the veterinary should monitor the animals because of residual danger (14).

2.4. Legal Regulations on Antibiotic Residues

Laws limit the amount of antibiotic residue taken by consumers. Another purpose of laws is that to reduce antibiotic use for retarding the evolution and extended of antibiotic-resistant bacteria in humans and animals (16).

Organizations and laws should work together such as; World Health Organizatyion (WHO), U.S. Food and Drug Administration (FDA), European Union (EU) Commisions, Food and Agriculture Organization (FAO), in Turkey; Misintry of Health and Ministry of Food, Agriculture and Livestock.

2.4.1. Legal Regulations at Turkey

The maximum residue limits (MRL) are specified in the communiqué number 2011/20 of the Turkish Food Codex. Some MRL of antibiotics shown at Table 2. The violation of the residue levels and the legal infrastructure are regulated by Law number 3285. With the communiqué number 28145 published in the official newspaper regulated procedures and principles for monitoring of certain substances and their residues in live animal and animal primary products to protect consumer's health (17, 18). Prohibited substances at Turkey shown that Table 3.

According to this communiqué prohibited substances; Group A; Anabolic and prohibited substances

1. Stilbenes, stilbened derivatives
2. Antithyroid substances
3. Steroides
4. Resormic acid lactones include zeranol
5. Beta-agonists
6. Dapsone, dimethridazole, furazolidone, chloramphenicol, chloroform, chlorpromazine, colchicine, metronidazole, nitrofurans, ronidazole

Table 2: Maximum Residue Limits of Antibiotics (17)

Antibiotic	**Type of Animal**	**Maximum Residue Limits**	
Benzilpenisilin (Beta Lactam)	All edible animals	50 µg/kg	Muslce
		50 µg/kg	Liver
		50 µg/kg	Kidney
		4 µg/kg	Egg
Streptomycin (Aminoglycosides)	Ruminant	200 µg/kg	Milk
	All edible animals	500 µg/kg	Muslce
		500 µg/kg	Liver
		1000 µg/kg	Kidney
Tetracylines	All edible animals	100 µg/kg	Muslce
		300 µg/kg	Liver
		600 µg/kg	Kidney
		100 µg/kg	Milk
		200 µg/kg	Egg
Fluoroquinoloes	Cattle, sheep, goat	200 µg/kg	Muslce
		3000 µg/kg	Liver
		300 µg/kg	Kidney
	Poultry	100 µg/kg	Muslce
		2500 µg/kg	Liver
		750 µg/kg	Kidney
	Fish	1000 µg/kg	

Group B; Veterinary medicines and contaminants

1. Antibacterial agents, including sulfonamides and quinolones
2. Other veterinary medicines
    - Anthelmintics

- Anticoccidial agents, including nitroimidazoles
- Carbamates and pyrethroids
- Sedatives
- Non-steroidal painkillers
- Other pharmacologically active substances

3. Other substances and environmental contaminants
    - Organic chlorinated substances
    - Organic phosphorescent substances
    - Chemical elements
    - Mycotoxins
    - Paints
    - Others

Table 3: Prohibitied Antibiotics at Turkey

Antibiotic	**Maximum Residue Limits**
Aristolochia spp. and preparations thereof	MRL can not acceptable
Chlorpromazine	MRL can not acceptable
Chloramphenicol	MRL can not acceptable
Chloroform	MRL can not acceptable
Colchicine	MRL can not acceptable
Dimetridazole	MRL can not acceptable
Dapsone	MRL can not acceptable
Dimetridazole	MRL can not acceptable
Metronidazole	MRL can not acceptable
Nitrofurans	MRL can not acceptable
Ronidazole	MRL can not acceptable

2.4.2. Legal Regulations at World

After 1940s there has been seen using antibiotics as growth promoters has side effects. After this using antibiotic as growth promoters banned in many countries. In 1969,

Sweden commison made a research for using antibiotics as growth promoters. After result of research using antibiotics were restricted. Antibiotics that were banned according to years were shown at Table 4 (12, 19).

After some antibiotics such as avilamycin, erythromycin, vancomycin, and virginiamycin ban to use as growth promoters in Denmark , levels of antibiotic resistance in humans have reduced. For example, in 1998 using virginiamycin as a growth promoter were banned, resistance of virgininiamycin reduced 33% in 2000 (16).

Table 4: Antibiotics That Were Banned According to Years (12)

YEAR	**COUNTRY**	**DECISION**
1969	Sweden	Using antibiotics as growth promoters were banned.
1970	EU	Using antibiotics as growth promoters were restricted.
1970	England	Penicilin and tetracylin were banned.
1971	EU	Tetracylin were banned.
1971	Sweden	Some of antibiotics as growth promoters and tetracylin were banned.
1986	Sweden	All antibiotics as growth promoters were banned.
1997	EU	Avoparcin were banned.
1998	Netherland	Olaquindox were banned.
1998	Denmark	Virginamycin and antibiotics as growth promoters were banned.
1998	Switzerland	All antibiotics as growth promoters were banned.
1999	EU	Tylosin phosphate, zinc bacitracin, spiramysin, virginiamycin were banned.
1999	England	Tylosin phosphate, zinc bacitracin, spiramysin, virginiamycin were banned.
2006	EU	All antibiotics as growth promoters were banned.
2006	Turkey	All antibiotics as growth promoters were banned.

Based on the European Parliament council regulation 2377/90 in 2009 (EEC), Commission Regulation 37/2010 (EU) summarizes the maximum antibiotic levels in foodstuffs of animal origin. This regulation also contains a list of some antimicrobials that are prohibited for use in food products due to safety (20). These antibiotics are same antibiotics that banned at Turkey and showed at Table 3.

In addition to laws, FAO and WHO developed the Codex Alimentarius, makes many advices to 'provide safety and quality in international food commerce'. At July 2015 "The Maximum Residue Limits (MRL) for Veterinary Drugs in Foods" were updated. MRLs for veterinary drugs, including antibiotics were recomended (16).

2.5. Methods Of Antibiotic Residues Detections

Antibiotic screening methods can be classified in two main groups according to the specific reaction taking place. First group is celled microbiologicalassays and second group is called immunoassays. In addition to that, biosensors can be added as new group for screening antibiotic residues (21).

Regarding the screening methods in the literature, those applied most were diameter of inhibition zones, ultraviolet/visible (UV/vis), fluorescence, time-resolved fluorescence (TRF), luminescence and surface-plasmon resonance (SPR).

2.5.1. Microbiological Inhibition Screening Methods

Microbiological assays can be qualitative or semi-quantitative methods. They are depending upon a certain reaction between a sensitive organism (usually bacteria) and the antibiotic available in the sample. Their stability, cost efficiency and simpleness are some advantage of these assays. When compared to the LC-MS systems, another important advantage of microbiological assays, is that antibiotics and metabolites with antibacterial activity can be detected by microbiological tests. However LC-MS systems are generally used on compounds already chosen as targets, in this way another antibiotics available may pass without detection. But also, the long times for incubation needed in some conditions and shortage of specificity are the most significant disadvantage of the microbiological assays.

there are many different trade microbiological assays that produced in different names by many companies (such as; BR test, Delvotest, Copan test, Eclipse test, Lumac, and Arla) in recent years. These assays analyse several antibiotics at thresholds usually so close to the MRL (21).

MeRa test is also an example of microbiological tests. MeRa test used *Geobacillus stearothermophilus* spores to detect of antimicrobial agent residues.

2.5.2. Immunoassay

The other semi-quantitative methods are immunoassays. Advantage of these methods are their high specificity, high sensitiveness, simpleness and cost efficiency. They are specially useful in common work because of these advantages. Their attribute is the certain reaction between antigen and antibody. Classification of immunoassays depends on the analyse label; fluoroimmunoassay (FIA), enzyme-linked immunosorbent assay (ELISA), and time-resolved fluoroimmunoassay (TRFIA) (21).

ELISA procedure are based on a catalyst enzyme and detection of UV/vis. It is incubated by the enzyme-secondary antibody conjugate, after this, o-phenylenediamine dihydrochloride (OPD) or tetramethylbenzidine (TMB) substrate solutions are thrown in. The colour change is stopped by addition of interruption solutions, and absorbances are calculated at 492 nm or 450 nm, in order of (22).

ELISA problems have been handled by utilize. For example; highly background absorption, several times incubation and wash steps, and degradation of enzyme.

2.5.3. Biosensors

Biosensors represent an interesting approach between the new generation of detection methods because of their ability that can manage exactly automatic. The established biosensor define them; contain a biological recognition element (enzymes, proteins, nucleic acids, cells, tissues...) attached to a signal transduction element.

Biosensors are so specific as the biorecognition element used and quick, constant control and in place applications are allowed by them. These are some advantages of biosensors. Even thought these advantages, there is two main limitation of biosensor design. The first disadvantage is the uncertainty of the biological sensing component. They can lose their activity in hours or days depending on the internal of the molecule and environmental stresses, such as pH or temperature. Size of the physico-chemical transducers used in biosensors is the second disadvantage for biosensors. At present, the smallest signal transducers declared are in the mm – lm range (21).

2.6. Health Effects of Antibiotic Residue

Antibiotic residues may have adverse effects on human healths.

Allergic/anaphylactic reactions, chronic toxic effects due to prolonged exposure to low levels of antibiotics and antibiotic resistance are possible adverse affect of using antibiotics in livestock.

2.6.1. Allergic/Anaphylactic Reactions

Allergic reactions are one of the most adverse effect of antibiotic resiudes. Many of antibiotics and drugs can cause out allergic reactions on human. Penicillin, aminoglycosides and tetracyclines are the most knowing antibiotics related to allergic reactions. Unfortunately, there is no information yet about long term effect of antibiotics on human health (23).

Less toxic antibiotics are known that beta lactams. In addition to this, it is concluded that they were responsible for the most of reported allergic reactions by the reason of antimicrobials in humans. Distinctive reactions like allergy, skin rashes and phototoxic dermatitis have been reported result of the use of tetracyclines. Streptomycin has critical adverse effects. One of them is the effect on vestibular mechanisms in the inner ear and it causes balance lost. Other adverse effects of this drug are hypersensitivity, skin rashes and induced fever (23, 24).

2.6.2. Toxicity

Toxicity is the one of other adverse effect for antibiotic resiude. Being exposed to oxytetracycline (OTC) causes blood changes such as leucocytosis, atypical lymphocytes, lung congestion, toxic granulation of granulocytes and thrombocytopenia purpura. Also liver injury and delayed blood coagulation can be occur. OTC can afect badly calcium rich organs such as bones and teeth and also occasionally erosion of nasal cavities. It may be reason of brown discolouration of the teeth on under 7 years children. Taking OTC treatment during pregnancy can cause discolouration of the teeth on infants (25).

Beta-lactams group of antibiotics are generally clear fastly from the blood via the kidneys and excreted in to the urine (residues in muscles are about 100 times less than kidney and liver). According to researches; 10 IU penicillin can cause an allergic reaction in a sensitive individual. In very sensitive individual 0.01 IU/ml of milk can cause allergic reactions (25).

A common finding with fluoroquinolones like norfloxacin, ofloxacin, ciprofloxacin, pefloxacin, and levofloxacin has been observed that the induction of embryotoxic effects such as reduced fetal weight, embryonic injury, declaration of

ossification. There is no teratological effects have been reported for these compounds. And so the researchers describe that embryonic effects are usually considered secondary to maternal toxic effects after using high-dose antibiotics. Teratogenic effects in rats have been observed only for sparfloxacin between clinically used fluoroquinolones that dose-related rise in incidence fetuses with ventricular septal defect, but clearly only at maternal toxic doses.

Although being generally considered devoid of teratogenic potential in preclinical studies, use of quinolones in clinical is generally not accepted during pregnancy (26).

2.6.3. Antibiotic Resistance

The one of the most important adverse effect of antibiotic residues is the antibiotic resistant. It is a a big global health concern nowadays. Antibiotic resistance has described as one of the world's most pressing health problems by The U.S. Centers for Disease Control and Prevention because of increasing the number of bacteria resistance to antibiotics in the last decade. Also many bacterial infections are becoming resistance to the most commonly prescribed treatment. Antibiotic resistance has identified as one of the three greatest threats to human health by WHO (27).

Antibiotic resistant bacterial infections cause dead of more people. It is estimated that antibiotic resistant microorganisms will kill people more than cancer by the year 2050 (28). Bacteria resistance increase rapidly. For instance in 1990s less than 10% Staphylococci bacteria were resistance to penicillin but in 2000 over 90% of the *Staphylococci* were resistance to penicillin (27).

Transfer of resistant bacteria to humans by livestocks was noted more than 35 years ago after high limits of antibiotic resistance were found in the intestinal flora of both farm animals and farmers. Subsequent researches have shown that resistant bacteria in farm animals reach consumers through meat products. This occurs by a few steps. First step, antibiotic use in food-producing animals to kills or suppresses susceptible bacteria, allowing antibiotic resistant bacteria to develop. Second step, resistant bacteria are transferred to humans through the food. Last step, infections in human can be occur because of these bacterias and may lead to adverse health consequences (29).

2.7. Alternatives to the Use of Antibiotics

Side effect of using antibiotics, legal regulations of antibiotics and worries about health causes to search alternatives to antibiotics. The purpose of these researches is to

decrease mortality rate, protect to environment and consumer health at same time increase of animal yield. There are many study focused on to find natural compounds that has similar effects of growth promoter without harmful effects.

There are actually too many non-therapeutic alternatives that can use instead of antibiotics. Probiotics, prebiotics, enzymes, organic acids, immunostimulants, bacteriocins, bacteriophages, phytogenic feed additives, phytocides, nanoparticles and essential oils are the most popular ones among them (30).

Phytogenic feed additives (PFA) derived from plants, herbs and spices are used to improve animal performance. Their results are very successful. Results showed that PFA has positive effects on growth and immune system. Also PFA decreased stress response. Recent researchers demonstrated that PFA may good alternatives instead of antibiotics. However, there is still a need to clarify the phytochemical composition to use safely (30,31).

World Helath Organization define probiotics as live microorganism that improve helath of host when administered in adequate amounts. Probiotic supplementation support growth, feed efficiency and intestine health. Also positive efect on poultry meat quality observed that after probiotic. This effect occur by affecting protein and fat content of meat.

Essential acids are the volatile matter of plant and hydrophobic liquid of odoriferous substances. They can be synthetic or vegetable origin (natural). A study showed taht using essential acids may increase growth and improve meat quality. However, not all essesntial acids can be used alternative of antibiotics. A few of them have properties of antimicrobial (30).

Prebiotics are non-digestable compounds that improve host health thanks to fermentable proporties. Prebiotics improve intestinal health and have effects on poultry productivity positevly. Therefore they can be used instead of antibiotics as an alternative.

Organic acids are another alternative to antibiotics. A research showed that adding butyric acid (a kind of organic acid) as a dietary supplement chickens growth performance increased.

Other experiments aimed that identify compounds that have positive effects on chicken health and productivity have focused on the use of natural products such as resinous mixtures produced by honey bees, milk and propolis. Positive effect of propolis were observed in broilers when propolis were added their feed (30).

2.8. Effect of Processing on Antibiotic Residues

Analyses for antibiotic residues are generally measured on raw products. However, products of animal origin are generally undergoes proccessing before consumption (thermal or food additive treatments). Because most of products are cooked before consumption, more informations are required about effects of processing on antibiotic residues.

Studies that carried out on the occurrence of the antibiotic residues in cooked foods and the stability of these compounds following heat treatment are so less. A few studies has been studied on cooking of foods containing residues. Incurred residues are generally distributed unequally in tissues. Some researchers have studied on degradation in liquid media such as milk. These allow better control of the time and temperature of heating, but show less actual cooking conditions. The residues of antibiotics have varying degrees of stability during cooking and, because of this, the cooking influences the level of risk posed by such residues (32,33).

2.8.1. Basic Cooking Methods

There are many methods to cook animal origin products. Generally these methods include different heat transfer type. Conventional cooking methods use conduction, convection and radiation as means for heat transfer. In non-traditional cooking methods; different energy sources are used such as microwaves. Cooking in oven, boiling, sauteing on pan, frying in deep oil, grill and cooking at microwave are the most common methods that using (34).

2.8.1.1. Cooked in Oven

Heat transfer in oven takes place by the combination of conduction, convection and radiation. Meat are put into preheated oven. Baking in the oven can be in two different temperatures; a constant cooking temperature of 150-160 ℃ or high temperature frying of up to 250 ℃ followed by a lower temperature of 150 ℃ until the required core temperature is achieved.

2.8.1.2. Grill

The grill is a dry cooking method using direct radiant heat. The heat source may be an oven grill, electric grill or outdoor grill. Meat can be over or under heat source. The meat must be turned during cooking because the heat is spread in one direction.

2.8.1.3. Sauteing

It is a method cooked by direct heat conduction. Heat is transmitted to the meat by contacting the pan with the meat. The meat is placed in a preheated pan, cooked with or without oil. The meat always must be turned because of high temperature.

2.8.1.4. Boiling

It is a method in that meat is cooked by immersion in boiling water at 100 ℃. Heat transfer occurs with water. The maximum heat can be 100℃ .

2.8.1.5. Microwave

The microwave is an ideal method for reheating and defrosting pre-cooked dishes. The principle of microwave cooking; is the conversion of electromagnetic energy into thermal energy in meat. While cooking the meat is placed in a container suitable for microwave cooking, covered with a film or lid and cooked in a microwave oven. The cooking time depends on the cooking rate (power output in watts). The total cooking time is 1 / 3th or half of the traditional baking time (34).

3. MATERIALS AND METHODS

3.1. Material

The legs of the chickens that 10 different brands sold in the province of Istanbul were collected at same day. The chickens were brought to the laboratory in the cold chain and stored at 2-4 ° C until the research day. All chickens were kept original package until research day to prevent contamination.

3.2. Methods

Chicken samples were divided into 5 equal parts that each of 20 grams. First samples were kept raw. Other 4 parts for every groups were cooked by different cooking techniques.

Second groups; 20 gram samples were immersed in water that preheated before 100 ° C and cooked for 10 minutes.

Third groups; 20 gram samples were placed on metal baking tray with oven baking paper and cooked in electric oven in 200 ° C for 12 minutes.

Fourth group; 20 gram samples were sauteed for 10 minutes with 1 teaspoon of oil in a preheated non-stick pan.

Fifth group; 20 gram samples were immersed prehetated before deep oil between 150-180 ° C and cooked for 10 minutes.

All samples were blendered and put inside plastic bags (Picture 1).

Picture 1: Samples in plastic bags

Firstly, the antibiotic residues in the samples were determined qualitatively with MeRA test. Then, Chloramphenicol levels in the samples were determined quantitatively by elisa method. Chloramphenicol is a group of antibiotics that is forbidden to be used in edible animals and humans.

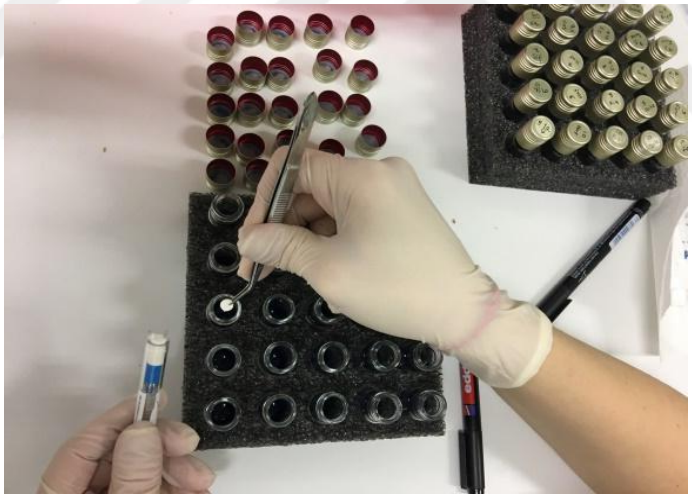
3.2.1. Application of Mera Test

The MeRA Test includes a quick pre-incubation in which G. stearothermophilus is allowed to germinate and proliferate, followed by a phase at room temperature suitable to allow the contact between the vegetative form of Geobacillus and the thermo-sensitive antibiotics, if present in the sample. Finally, the tube is reintroduced into the incubator for the last incubation. The incubation sequence of MeRA Test is the critical characteristic that allows the method to reach extremely low detection limits(35).

Mera Test Steps

1. 2 g of minced chicken meat and mL 6 of distilled water were added in a 10 mL tube. (chicken:water 1:3 ratio)
2. Samples were homogenized for a few seconds.
3. The homogenized meat were centrifuged by tabletop centrifuge set at 4000 rpm for 15 minutes. (picture 2)
4. 1 disc of spores were added into the medium. (picture 3)
5. Solution with spores were preincubated for 20 minutes at 64 °C at in the water bath.
6. After incubation, solution were waited at room temperature. 1 mL of the matrix sample was introduced and let the antimicrobial agent, if present, act at room temperature for 20 minutes.
7. Samples were reintroduced in the waterbath or in the Termoblock at 64 °C for the second incubation for 3h - 3h 30 minutes. (picture 4)
8. The color change of the medium in the vials were watched. No color change (gree-blue color): meat sample containing antimicrobial agent residues in concentration above the detection limits. Color change (yellow color): meat sample containing no antimicrobial agent residues, or residues in concentration under the detection limits.

Picture 2: centrifuged by tabletop centrifuge set at 4000 rpm



Picture 3: 1 disc of spores were added into the medium

Picture 4: Samples that inside water bath

3.2.2. Application of Elisa

Enzyme-linked immunosorbent assay (ELISA) is a direct competitive assay which operates on the basis of competition between the drug / metabolite in the sample (36).

Preparation of Samples

Meat samples were homogenized in a bowl. 4 gr meat sample are put into 50 ml falcon tubes and 20 of 50 mm succinic acid were added. Tubes are shaken in shaker incubator for 15 minutes in room temperature. Then, it were centrifuged at 4000 g for 15 minutes. After centrifuge, supernatant were diluted 1/10 (100 µl supernatant, 900 µl PBS- Phosphate buffered saline). 50 µL of the upper layer were taken and proceed to the assay protocol.

Elisa Test Steps

1. 50 µL samples and calibrators were pipetted into the wells.
2. 50 µL conjugate solution were added and plates were shaked well. Plates sealed and were incubated 60 minutes.
3. Plates were washed 3 times with diluted wash buffer.
4. 100 µl conjugate were added by multi-channel pipette and shaken and incubated in room temperature for 15 minutes.

5. Plates were washed 3 times for each wash to come 250 µl wash buffer in automatic ELISA washer.
6. 100 µl substrate/chromogen were added and incubated in room temperature for 15 minutes.
7. 100 µL stop solution were added into the wells.
8. The strips were read with ELISA reader using 450nm filter.
9. Coloramphenicol ppb values were calculated using the sample / zero standard absorbance x100 formula.

4. RESULTS

4.1. Study Results

In our study Mera results showed that 43 of 50 samples are contaminated with antibiotic residues. After different cooking methods, significant reduction of antibiotic residue are observed. According to Elisa results there is no chloramphenicol residue in any sample (<0.03).

4.1.1. Mera Test Results

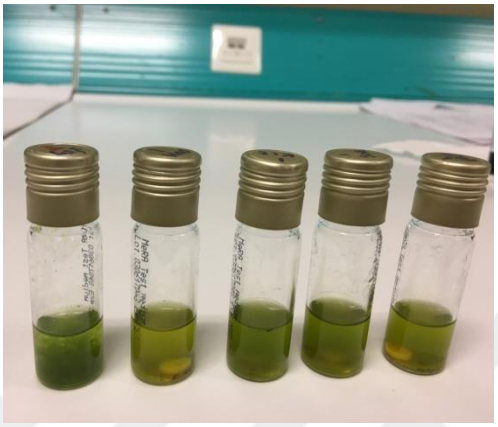
The purpose of Mera test was determined the results qualitatively. The color change of the medium in the vials showed the results. If meat samples contain antimicrobial agent residues in concentration more than the detection limits, colour did not change (green – blue colour). If meat sample do not contain antimicrobial agent residues, or residues in concentration lower than the limits, colour changed (yellow colour) (picture 5).

Picture 5: Positive and Negative Results

All raw samples' results are positive. After different cooking methods, some positive results changed through negative. First raw sample results change to negative

after boiled, cooked in oven, sauted and fired. This difference were showed that at picture 6. However, second sample results stays positive after boiled, while changing after three different cooking methods. There is no negative results on other 40 samples.

(table 5).



Picture 6: Results of Sample One

Table 5: Mera Test Results

Mera Test Results					
Sample	Raw	Boiled	Cooked in oven	Sauted	Fried
1	Positive	Negative	Negative	Negative	Negative
2	Positive	Positive	Negative	Negative	Negative
3	Positive	Positive	Positive	Positive	Positive
4	Positive	Positive	Positive	Positive	Positive
5	Positive	Positive	Positive	Positive	Positive
6	Positive	Positive	Positive	Positive	Positive
7	Positive	Positive	Positive	Positive	Positive
8	Positive	Positive	Positive	Positive	Positive
9	Positive	Positive	Positive	Positive	Positive
10	Positive	Positive	Positive	Positive	Positive

4.1.2. Elisa Test Results

Chloramphenicol residue was screened by ELISA. According to Elisa results results there is no chloramphenicol residue in any sample (<0.03) (Table 6).

Table 6: Elisa Test Results

	Elisa Test Results				
Sample	**Raw**	**Boiled**	**Cooked in oven**	**Sauted**	**Fried**
1	<0.03	<0.03	<0.03	<0.03	<0.03
2	<0.03	<0.03	<0.03	<0.03	<0.03
3	<0.03	<0.03	<0.03	<0.03	<0.03
4	<0.03	<0.03	<0.03	<0.03	<0.03
5	<0.03	<0.03	<0.03	<0.03	<0.03
6	<0.03	<0.03	<0.03	<0.03	<0.03
7	<0.03	<0.03	<0.03	<0.03	<0.03
8	<0.03	<0.03	<0.03	<0.03	<0.03
9	<0.03	<0.03	<0.03	<0.03	<0.03
10	<0.03	<0.03	<0.03	<0.03	<0.03

5. DISCUSSION AND CONCLUSION

5.1. Discussion

Our study result show that 10 different brand samples that are selling at İstanbul province have antibiotic residue. After different cooking methods, some positive Mera test results changed through negative. 1 sample after boiled, 2 samples after cooked in oven, 2 samples after sauted and 2 samples after fried result changed from positive to negative.

According to Elisa results there is no chloramphenicol residue in any sample (<0.03).

The uses of chloramphenicol in food animals has been legally banned, therefore there are not many studies about it in our country. In the study conducted by Yüksek in 2000, it was aimed that determine residues in various tissues (muscle, liver, kidney and spleen) of the animals OTC, Chloramphenicol (CAP) and Zinc Bacitracin. It was observed that the residues are located respectively the most in kidney, liver, muscle, and spleen. At the end of the analysis, OTC residues were found only in the kidney tissues. However no residue of CAP was seen in the tissues of the chicken (37). The results are consistent with our study.

In other study conducted on 90 broilers at Iran in 2014 observed that 28 (31%) and 82 (%91) samples contained CAP and enrofloxacin residue, respectively. As the uses of CAP in food animals has been legally banned the zero MRL should be anticipated. This study showed that illegal uses of CAP is still continue despite the international prohibition (38).

Tajik et al. investigated the chloramphenicol (CAP) residue occurance in broiler chickens. Samples (160) were scanned by Four Plate Test (FTP), ELISA and HPLC, respectively. The FTP test showed that 28 (17.5%) samples contamineted by CAP. ELISA and HPLC confirmed the result. However HPLC findings was lower than ELISA. This study also showed that illegal uses of CAP is contunie despite prohibitons
(39).

Antibiotics are among the most commonly used compounds in livestock production. Also antibiotic residues may have adverse effects on human healths.

Generally studies search on raw samples. Products of animal origin are generally undergoes proccessing before consumption (thermal or food additive treatments). Because most of products are cooked before consumption, more informations are required about effects of processing on antibiotic residues.

Javadi observed that reduction in the concentration of Doxycline residue after different cooking techniques. Researcher used boiled, microwave and roasting techniques for cooking in this study. The most reduction of residue were observed in microwave process. According to this study, conclusion is cooking processes do not guarantee a full elimination of residue however it can only decrease its amounts (40).

In a study conducted by Al-Ghamdi et al., 247 chicken muscle, 719 chicken liver and 630 egg samples were collected and analysed for antibiotic residues. The results showed that 110 raw liver samples and 117 raw muscle samples contaminated by antibiotic residue. After cooking the overall concentration of Tetracyline, chlortetracyline and doxcyline decreased. Unlike this result after cooking raw liver, concentration of Oxatetracylin increased. However this difference does not reach statical significance ($p>0.05$). After cooking raw muscle samples, all kind of antibiotics residue level decreased (41).

Abou Raya et al. aimed that to observe changes antibiotic residues in chicken meat after different cooking proccess, and determine the cooking time required to make cooked sample safer for consumption. Study results showed that cooking causes a reduction of antibiotic residues in the samples, and microwave cooking is more effective than roasting and boiling. Regarding to cooking time, it was observed that regardless of the cooking procedures, prolonged cooking time decreased residues significantly ($p < 0.01$) (42).

In the other study conducted by Vivienne et al. observed that the effect of different cooking methods on oxytetracycline OTC residues in broiler. Results showed that oxytetracycline concentration reduced significantly in muscle by roasting and boiling 53.6% and 69.6%, respectively, at pH 6.0. The concentration reduced by microwaving 49.1% but was not statistically significant. The same pattern were observed that at pH 7.2 with reduction of 34.3%, 53.2%, and 67.7% for microwaved, roasted, and boiled. Also for the liver tissues, there was a significant reduction in the concentration for both pH 6.0 and 7.2 for all samples. The best reduction effect for muscle samples was boiling method, however the best reduction was observed by roasting in liver samples (43).

Nguyen et al. investigated that the effect of different cooking techniques to reduce tetracyclines in pork. Pig muscles which contain tetracyclines residue were used as samples. Results showed that residues decreased by 45.35 to 67.05% after boiling, 38.17 to 65.74% after deep-frying and 38.17 to 48.47% after microwaving. According to results, microwaving (1 min) is the faster than other methods (9 min) (44).

In another study, Furusawa and Hanabusa observed that three different cooking methods affect on chicken muscle. They used boiling, roasting and microwaving and examined the change of four different kind of sulfonamides. Results showed that residues reduce in the muscle by boiling 45-61% in 12 min and microwaving 35–41% in 1 min. In roasting, sulfonamides residues except sulfadiazine reduced 38-40% in 12 mins. No significant reduction of sulfadiazine was observed (45).

Roca et al. observed that quinolones are very stable during thermal procedures. The maximum reduction of ciprofloxacin and norfloxacin concentration were 12.71% and 12.01%, respectively, at 120 °C and 20 min. Therefore, quinolon residues threaten human health significantly, because these antibiotic residues can stay in milk after heat processing (46).

In another study conducted by Gratacos-Cubarsi et al. searched that effect of microwave and boiling effect on chicken meat contaminated with tetracycline. Study results showed that between 56 – 82% reduction of initial residue concentration in microwave and boiling respectively (47).

In the study conducted on milk investigated the effect of heating on antimicrobial activity of macrolides residues. Results showed that different effects of heat treatment. After sterilization at 120 ºC for 20 min, 93% of erythromycin, 64% of spiramycin, 51% of tylosin and 5% of lincomycin was observed inactivate. While treatment at 140 ºC for 10 seconds results are generally lower than sterilization 30%, 35%, 12% and 5%. The lowest reduction of antimicrobial activity (21% of erythromycin and 13 % of spiramycin) observed at pasteurazation at 60 ºC for 30 min
(48).

5.2. Conclusion

Veterinary medicines specially antibiotics, are between the most necessary and effective components of intensive animal food production in our country and the world. Approximately 80% of the animals that used in food production are currently being cured

with medication in a part of their lives or in many times (7). Also antibiotics have an important role at poultry industry to accelerate growth.

Beta lactams, tetracyclines, chloramphenicols and macrolides are the most usage one at veterinary. Also most of antibiotics dose (30-80%) given to food animals are excreted due to partial antibiotic metabolism (7,8).

Using antibacterial drugs in animal may cause deposition of residue in meat, milk and eggs. There can be many reasons for this. Using unlabeled drugs, wrong applications or kind of drugs are some of reason for residue occurence. According to a study conducted on 33 broilers, results confirm that 29 (69,7%) broilers has antibiotic residue over MRL (49).

Antibiotic residues may have adverse effects on human healths. Allergic/anaphylactic reactions, chronic toxic effects due to prolonged exposure to low levels of antibiotics and antibiotic resistance are possible adverse affect of using antibiotics in livestock. Laws limit the amount of antibiotic residue taken by consumers. Another purpose of laws is that to reduce antibiotic use for retarding the evolution and extended of antibiotic-resistant bacteria in humans and animals. In Turkey; Misintry of Health and Ministry of Food, Agriculture and Livestock are working to reduce antibiotic use. The MRLs are specified in Turkish Food Codex. According to codex the uses of chloramphenicol in food animals has been banned (17).

There are not many studies about CAP in our country, because it is legally banned. However there is a study work on CAP at Turkey, and results showed there is no CAP residue (37). In contrast other two studies at others countries showed that CAP residues, althoughit is legally banned (38,39). Our results are consistent with first study at Turkey. There is no CAP residue on samples.

Antibiotic residues are generally analysed on raw products. However, products of animal origin are not consume raw, generally undergoes proccessing before consumption (thermal or food additive treatments). The Codex Alimentarius Commission of the FAO and the WHO have stated that the scientific literatures about effect of processing on residues in food are inadequate to consider clear determination, therefore there are need more studies (50).

A study conducted by Pavlov searched that freezing process and storage time effect on residue levels. Results showed that significant reduction on residue levels in poultry meat during cold storage (51). Another studies also showed that cooking may

decrease residue levels on animal products (40, 41, 42, 43, 44). Our results are consistent with these studies results. After using of cooking methods, significant reduction of antibiotic residue was observed.

Study results showed that antibiotics are using on edible animals and antibiotic residues were observed in chickens. However there is no CAP residue which is banned. Also results showed that thermal proccess may reduce residue concentration in foods. However cooking processes can not make sure a full elimination of antibiotic residue in foods and it is not making food totally safe.

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7. CURRICULUM VITAE

Personal Informations

Name	Selin	**Surname**	İnal
Place of Birth	Tarsus	**Date of Birth**	29.06.1993
Nationality	Turkish	**TR ID Number**	12236446132
E-mail	selin.inal@hotmail.com	**Phone Number**	+905342098154

Education

Degree	Department	Graduated From	Graduated year
Master	Nutrition and Dietetic	Yeditepe University	
Bachelor's Degree	Nutrition and Dietetic	Yeditepe University	2016
Undergraduate degree	Helathcare Management	Anadolu University	2016
High School		İstiklal Makzume Anadolu Lisesi	2011

Languages	**Grades**
English	Very good

Work Experiences

Position	**Instutute**	**Duration**
Dietician	Nuhil Gıda A.Ş.	2017-2018

Computer Skills

Program	**Level**
Microsoft Office	Very good
SPSS	Good