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YEDITEPE UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

DEPARTMENT OF NUTRITION AND DIETETICS

INVESTIGATION OF THE ANTIBIOTIC (TETRACYCLINE) RESIDUES IN THE CHICK LIVER

Dietitian Sinem Demirci

MASTER’S THESIS

Supervisor

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TEZ ONAYI FORMU

Kurum : Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü

Program : Beslenme ve Diyetetik Yüksek Lisans Programı

Tez Başlığı : Tavuk Karaciğerinde Antibiyotik Kalıntılarının Belirlenmesi

Tez Sahibi : Sinem DEMİRCİ

Sınav Tarihi : 18.12.2017

Bu çalışma jurimiz tarafından kapsam ve kalite yönünden Yüksek Lisans Tezi olarak kabul edilmiştir.

	Unvanı, Adı-Soyadı (Kurumu)	İmza
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ONAY

Bu tez Yeditepe Üniversitesi Lisansüstü Eğitim-Öğretim ve Sınav Yönetmeliğinin ilgili maddeleri uyarınca yukarıdaki jüri tarafından uygun görülmüş ve Enstitü Yönetim Kurulu'nun 22/12/2017 tarih ve 2017/26-09 sayılı kararı ile onaylanmıştır.

Prof. Dr. Bayram YILMAZ
Sağlık Bilimleri Enstitüsü Müdürü

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PREFACE

In this thesis study, it is aimed to determine the presence and amount of tetracycline residues in chicken liver. Since the value of white meat is high, it is aimed to conduct detailed research in this regard.

First of all, I am thankful for my valuable thesis advisor Assist. Prof. Dr. İskender Karaltı who helped me while choosing my thesis topic and in the thesis phase and a valuable teacher who guides me on thesis Assist. Prof. Dr. Binnur Okan Bakır and I thank to all my teachers,

I am thankful for mother and father and my husband who gave me material and spiritual support throughout my life and the greatest assistant of my university life and my sisters Elif and Seda and my son Furkan and my daughter Meleknaz and I am giving this thesis as a present them because of their help in this achievement.

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Sinem DEMİRCİ

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LIST OF SYMBOLS AND ABBREVIATIONS

µ: Micro

ADI: Acceptible Daily Intake

ELİSA: Enzyme-Linked ImmunoSorbent Assay

FDA: Food Drug Administration

FSH: Follicle Stimulating Hormone

GnRH: Gonadotrophin Releasing Hormone

HCG: Human Chorionic Hormone

HPLC: High-Performance Liquid Chromatographic

IU: International Unit

MRL : Maximum Residue Limit

MRSA: Methicillin-resistant Staphylococcus aureus

NFLX: Norfloxacin

NGOs: Non-govermental Organizations

PBS: Phosphate buffered saline

ppb: per part billion

T.A.Ş: Turkish Incorporated Company

UV: Ultraviole

SUMMARY

DEMİRCİ, S. (2017). Investigation of Antibiotic (Tetracycline) Residues in Chicken Liver. Yeditepe University. Institute of Health Science, Master Thesis. İstanbul

This study was carried out on 100 chicken liver sold outdoor in order to investigate tetracycline residues in chicken liver. Chicken (Gallus gallus domesticus) is a bird species raised for meat and eggs in farms that have begun industrialization since World War II. It is a food that is consumed a lot because of reasons such as chicken has high quality protein, low cholesterol, cheaper than red meat in the world and our country. In production facilities, antibiotics are used for the purpose of protecting chickens from growing diseases and growing chickens that grow faster and faster with less loss. Tetracycline group antibiotics are widely used in chickens due to their broad spectrum and low toxic effects. Antibiotic residues arise because antibiotics are discontinued following the ten-day cutting ban after use or when high doses can not be eliminated from the tissues and organs of the chickens. This situation causes the antibiotic residues in the cut chicken to pass to humans and the environment. This study was conducted because of the high consumption of chickens in the world and especially in our country.

In the study, 100 poultry liver samples collected from the market were collected and antibiotic residue levels were examined. The qualitative test was used for the MeRA test, and tetracycline levels were quantitatively analyzed with elisa test. In all of the samples, antibiotic residue levels were found to be qualitatively positive. Specifically, when tetracycline residues are considered, it is found that 16% of the samples are above the maximum residue limits and 3% are at the limit values. In the remaining samples, tetracycline values were found to be within acceptable maximum residue limits. This suggests that antibiotics should be used in chickens and the cut date of chickens given antibiotics should be considered. In addition, when the legal requirements imposed in our country are complied with, such situations will also stop to exist.

Key Words: Chicken, chicken liver, antibiotic, residue, tetracycline, MeRA test, elisa test

ÖZET

DEMİRCİ, S. (2017). Tavuk Karaciğerinde Antibiyotik (Tetrasiklin) Kalıntılarının Araştırılması.

Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Master Tezi. İstanbul

Bu çalışma tavuk karaciğerinde tetrasiklin kalıntılarının araştırlması amacıyla açıkta satılan 100 tavuk karaciğeri üzerinde yapılmıştır. Tavuk (Gallus gallus domesticus), 2. Dünya Savaşı'ndan sonra endüstrileştirilmeye başlanmış çiftliklerde eti ve yumurtası için yetiştirilen bir kuş türüdür. Hem dünyada hem ülkemizde tavuk, kaliteli protein içermesi, kolesterolü düşük olması, kırmızı ete göre daha ucuz olması, gibi sebeplerden dolayı çokça tüketilen bir besindir. Üretim tesislerinde daha az kayıpla daha hızlı büyüyen ve etine dolgun tavuklar yetiştirmek için, hastalıklardan korumak için önlem amaçlı veya tavukları tedavi etmek için antibiyotik kullanılmaktadır. Tetrasiklin grubu antibiyotikler geniş spektrumlu olmaları ve toksik etkilerinin düşük olması nedeniyle tavuklarda sıklıkla kullanılmaktadır. Antibiyotik kalıntıları, antibiyotik kullanımı sonrası on gün kesim yasağına uyulmaksızın kesilmesinden veya yüksek dozlarda verilmesi sonucu tavukların doku ve organlarından elimine edilememesinden dolayı oluşur. Bu durum sonucu kesilen tavuktaki antibiyotik kalıntıları insanlara ve çevreye geçmektedir. Tavuğun dünyada ve özellikle ülkemizde tüketiminin yüksek olması nedeniyle bu çalışma yapılmıştır. Çalışmada piyasadan toplanan 100 adet tavuk karaciğeri örnekleri toplanmış ve antibiyotik kalıntı düzeylerine bakılmıştır. Kalitatif olarak tayinde MeRA test kullanılmış, elisa ile tetrasiklin düzeyleri kantitaif olarak analiz edilmiştir. Örneklerin tamamında antibiyotik kalıntı düzeyleri kalitatif olarak pozitif olarak saptanmışlardır. Spesifik olarak tetrasiklin kalıntısı bakıldığında örneklerin %16'sının maksimum kalıntı limitleri üzerinde olduğu ve %3'ünün sınır değerlerde olduğu bulunmuştur. Geri kalan örneklerde tetrasiklin değerleri kabul edilebilir maksimum kalıntı limitleri arasında bulunmuştur. Bu durum tavuklarda antibiyotik kullanımına ve antibiyotik verilen tavukların kesim tarihlerine dikkat edilmesi gerektiğini göstermektedir. Ayrıca ülkemizde uygulanan yasal gerekliliklere uyulduğunda bu tür durumlar da ortadan kalkacaktır.

Anahtar kelimeler: tavuk, tavuk karaciğeri, antibiyotik, kalıntı, tetrasiklin, MeRA test, elisa test

I. INTRODUCTION AND PURPOSE

Chicken is a bird species from the family of the Phasianidae and raised for meat and eggs in farms that have begun industrialization after World War II.

Poultry is the most open to growth technology and development sector because poultry has an important place in animal protein deficit and the production is faster and easier than cattle, and cost of the poultry is low.

These sectors are established with the aim of achieving maximum efficiency with minimum loss by consuming minimum energy. Chickens in these facilities consume a large amount of energy in confined areas and consume a large amount of fat to gain weight quickly (1).

In these facilities, the proximity of a large number of animals and the potential for rapid spread of the disease allows routine use of veterinary antibiotics to ensure the continuity of production there. Thus, the use of veterinary antibiotics has become an integral part of the growing animal food industry. The increased frequency of use of veterinary antibiotics, together with the erroneous use of these drugs and the interruption of follow-up post-treatment times, have had some negative effects on both the public and the public health. The remains of veterinary antibiotics are largely thrown away by animal feces. It is an important threat to our future because of the spreading of animal feces to the soil as fertilizer (2).

Antibiotic use in animals can cause direct and indirect effects on human health. Direct effects are effects that can be causally linked to antibiotic-resistant bacteria in food animals (1).

Indirect effects are those caused by antimicrobial resistance to food-borne antibiotics with resistance to various components of the ecosystem (eg, water and soil).

Considering the amount of chicken meat consumed in the world, chicken meat consumption in developed and developing countries is quite high (1).

In this study; poultry liver samples were studied because of the accumulation of antibiotic residues in the liver that were given in excess of the liver storage organ. Since chicken meat is widely consumed in our country, it is aimed to determine the presence of antibiotic residues and to determine the presence of tetracycline residues which are the most commonly used antibiotics in veterinary antibiotics.

II. LITERATURE REVIEW

II.1. History of Chicken Industry

Chicken is a domesticated bird species from the family of the Phasianidae, and is usually grown on farms for meat and eggs. Chicken production has been industrialized after World War II.

Chicken production facilities are making "profit" increasing selective production methods that will grow faster and give more white meat. These facilities were established on the principle of minimum energy expenditure of chickens in the form of breeding of chickens in tight places where they can not move in tight spaces (1).

It is also one of the important areas in the world for the closure of animal protein deficiencies.

The main reasons for the preference of poultry animals are that their production is easy and fast and their cost is low (3).

The first step towards the development of poultry in Turkey was taken in 1930 with the establishment of Central Poultry Research Institute in Ankara, but no significant progress was made until 1952.

In 1952, pure cultured breeds were imported and distributed to people and affiliates of the Racial Agriculture Ministry such as New Hampshire, Plymouth Rock, and Leghorn, who came as daily chicks from the U.S.

With this practice, the poultry was encouraged, but the desired high efficiency was not achieved because the conditions of care were not sufficient and the breeding was not carried out on any of these breed. Later in 1956, with the establishment of the Feed Industry Inc., rational feeding conditions started to occur. Interest in the private sector topic started with the import of hybrid parents in 1963.

The importation of grandparent parent and father lines was permitted in 1980 (1).

With the supply of genetic material through importation, branches of industry related to poultry, poultry industry, equipment industry, vaccine-drug production branches also started to develop. On the other hand, we focused on the development of domestic hybrid seeds, which was launched in 1968, in order to save our poultry from dependence on external sources.

As a result of these studies, white and brown egg-laying and master-parent lines were developed.

However, when the productivity levels of the produced lines are compared with the foreign genetic material, it is difficult to say that, in terms of some features, the reason for the weakness of competitiveness is achieved with the aim of aiming.

At present, Ankara Poultry Research Institute and Er-beyli Fig Research Institute produce and sell an average of 1.4 million layered hybrids and 1.2 million meat chicks. Efficiency levels are not as good as foreign hybrids, but they give positive results in some enterprises whose production conditions are not sufficient for foreign origin hybrids (1).

II.2. Chicken Meal Consumption in the World

15-20% of the calories come from the protein that should be taken daily during a balanced diet. These protein sources come from meat group, dairy group, legumes and eggs and small amounts of vegetables. The nutrient group for which we can supply protein, which contains all of the essential amino acids for the body, is red meat, chicken and fish (4). White meat cholesterol content is low, it can be preferred in diets compared to red meat. In a study conducted in 2016; When we look at the amount of poultry meat consumed in the world, it is found that it is consumed at least in Sudan and Ethiopia and most in Israel.

Japan, Turkey, Ukraine, Iran, European Union countries, Canada, Australia, USA, where the average poultry consumption in the world was calculated as 13.8 kilograms / capital and consumed by countries like India, Sudan, Pakistan, Indonesia, Egypt below the average of world poultry meat consumption, Countries such as Israel have consumed poultry meat above the world average (5). The poultry meat consumption graphic of the countries is shown in the figure 1.

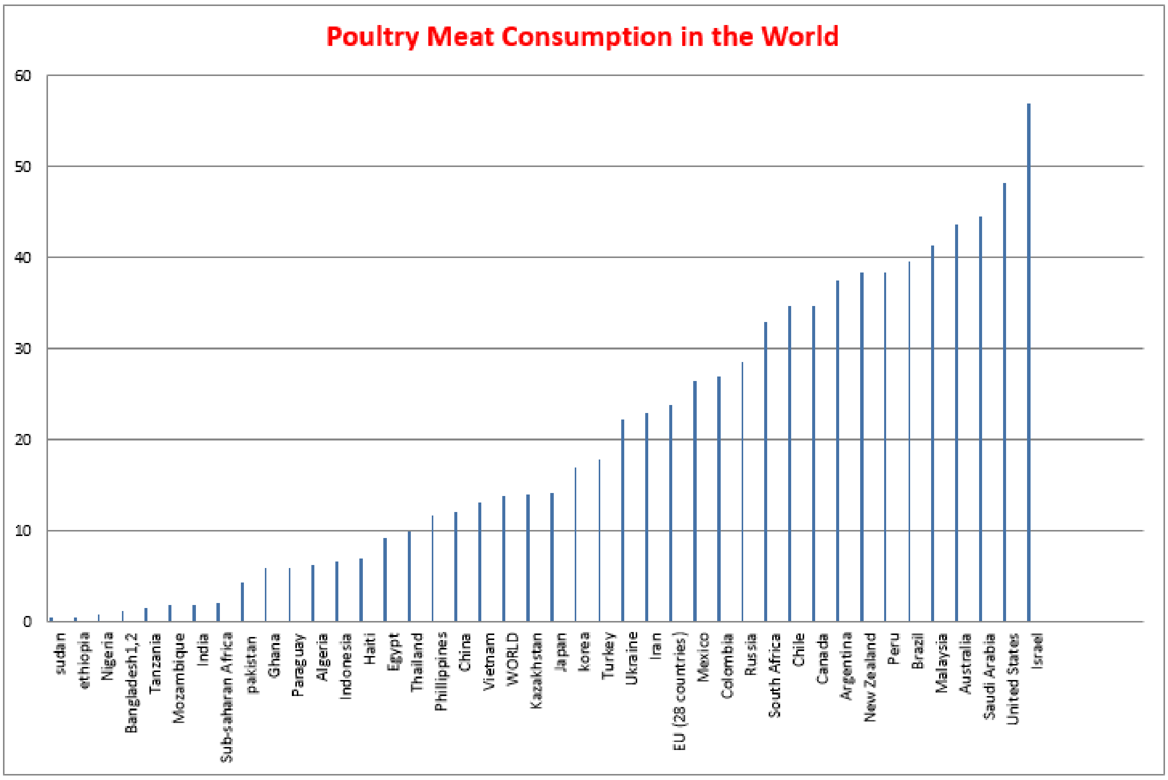


Figure 1: Poultry Meat Consumption kilograms/capital, 2016.

II.3 Use of Antibiotics in Animals

II.3.1. History

In the 1990s, the number of animal feeds in pigs, poultry and cattle increased significantly (6). In order to maintain economic vitality, large agricultural enterprises have begun to contract with individual farmers. This arrangement offered a guaranteed price for the market that produces controlled, stable animal food for farming and farming.

In these plants, the proximity of a large number of animals and the potential for rapid spread of the disease ensured the routine use of veterinary antibiotics to ensure the continuity of production there (2).

Thus, the use of veterinary medicines has become an integral part of the growing animal food industry (7).

Over 40,000 antibiotics have been discovered during this period, and about 80 have been used in veterinary-agriculture and fisheries (8).

Some of the important uses of veterinary medicines are:

- To treat and prevent infectious diseases (eg tetracycline, β-lactam antibiotics and steroid anti-inflammatories).
- To control the reproduction processes (eg: steroids, oxytocin, ergonovin, gonadotrophin releasing hormone (GnRH), human chorionic hormone (HCG) and prostaglandins, progesterone and follicle stimulating hormone (FSH)) and their production (eg bovine somatotropin, hormonal growth implants, ionophores, subtherapeutic antibiotics).
- To control parasites (eg, Dewormers, insect killers).

To control non-infectious diseases (eg nutritional supplements) (9).

Veterinary medicines are administered to animals as feed or water, by injection, by implant, orally in drops or as bolus.

Antibiotics approved for agriculture are widely used veterinary medicines for animal health and management (7).

II.3.2. Antibiotic and Types

“Antibiotic” term is normally used for the generic name given to various compounds with natural and semi-synthetic, antibacterial activity (2,10).

Antibiotics are chemical therapeutic agents used to kill or inhibit microorganisms (e.g. bacteria, viruses, fungi, or protozoa). Antibiotics that kill bacteria are called "bactericidal". Penicillins, cephalosporins, fluoroquinolones are bactericidal antibiotics that are used to treat existing bacterial killers. Antibiotics called antibiotics that prevent the growth of bacteria are called "bacteriostatic". Tetracyclines, sulphonamides, erythromycin are bacteriostatic antibiotics that inhibit the replication of bacteria (11).

Alexander Fleming's accidental discovery of penicillin in 1928 provided millions of other antibiotics to be produced and to be marketed to both humans and animals for treatment (2).

Antibiotics are the leading medical assets to fight against microbial infection, and are also one of the major causes of death worldwide. Therefore, it is crucial to screen antibiotic residues in animal-derived foods (12).

Veterinary antibiotics are widely used in many countries around the world to treat disease and protect the health of animals. These antibiotics are included in animal feed to increase growth rate and nutritional efficiency. Because antibiotics are absorbed in small amounts in the intestines of animals, the majority are eliminated unchanged from faeces and urine (2).

II.3.3. Classification of Antibiotics

Antimicrobial classes are classified as Category 1, 2 or 3 according to the health risk associated with the development of antimicrobial resistance in the population (13).

II.3.3.1. 1.Category: In terms of public health, the risk is now estimated to be low or limited by antimicrobial

Category 1 includes the most commonly used antibiotics among veterinary drugs (14).

Antibiotics in Category 1 are antibiotics that are considered as the first choice in many treatments. These are some penicillin species, tetracycline group, macrolide group and polymyxins. These antibiotics are shown in table 1 (13).

II.3.3.1.1 Penicillin

Penicillins (eg, Penicillin G and Penicillin V) are not in the very high risk group when used with tetracyclines in terms of people health (14).

Penicillins and cephalosporins are antibiotics of the β-lactam group and are among the veterinary drugs frequently used to treat and prevent bacterial infections (respiratory system infections, urinary system infections, cutaneous skin infections) in livestock and milk production establishments. Misuse of these veterinary antibiotics can cause microbial resistance in consumers and allergic reactions resulting in antibiotic or metabolite residues. Allergic reactions following food consumption, including antibiotic residues, have been reported in the literature (15).

II.3.3.1.2. Tetracyclines

Tetracyclines (TCs) are an important group of antibiotics used in farm animal breeding and poultry production. Tetracyclines are used to prevent and treat certain diseases in animals raised for human consumption, as well as to encourage the rapid growth of animals in fraud (16).

Tetracyclines are broad-spectrum antibiotics derived from naphthacencarboxamide, a structurally similar tetracyclic compound. The first tetracycline antibiotic was chlorotetracyclines isolated from Stremptomyces aurefaciens in 1948. Since its isolation, about 1000 derivatives have been obtained by chemical modification of tetracycline. However, most of these derivatives are uneconomic, unstable, and ineffective derivatives. Tetracyclines produce bacteriostatic effect by inhibiting protein synthesis in bacterial ribosomes (17).

Tetracyclines are effective against bacterials and rriquettes, chlamydiales, spirochetes, mycoplasma, leptospira and some protozoons from a large number and from various groups (18). Tetracyclines are used in the treatment of Brucella, but the prevalence of this pathogen is lower in the EU compared to other regions (14). Therefore, they are the broadest spectrum antibiotics. The tetracycline group is widely used for poultry breeding because of the wide spectrum of antibiotics (tetracycline, oxytetracycline and chortetracycline) and low toxic effects (18).

High doses and inappropriate use of tetracyclines can lead to the presence of residues in edible animal tissues. In this case, it can be toxic and dangerous for human health and may also cause potential allergic reactions. Moreover, the long-term existence of tetracycline residues may cause microorganisms to gain resistance to antibiotics (19). The tetracycline group can isolate 6 of the most important side effects of antibiotics.

- **Gastrointestinal Side Effects:**
- There may be a feeling of discomfort, epigastric pain, nausea, vomiting and anorexia. Tetracyclines can alter bowel flora and cause watery stools or diarrhea.
- **Allergy and Skin Reactions:** Hypersensitivity reactions in the tetracycline group can cause allergic reactions to the sun if exposed to the sun.
- **Hematologic Disorders:** Tetracyclines disrupt blood clotting mechanisms. They are rarely hemolytic anemia.
- **Liver and Kidney:** Rarely, liver and kidney also have negative effects. Especially in patients with hepatic insufficiency and given too much to pregnant women, degeneration can occur, such as lubrication of the liver (20).

II.3.3.1.3. Macrolides

Macrolides are widely used in the treatment of diseases that are common in food producing animals. This class has been categorized as an antimicrobial with critical prescription for veterinary medicine on the World Organization for Animal Health (OIE list) list.

Campylobacter spp. and Salmonella spp. specific antibiotic in the treatment of bacteria (21).

II.3.3.1.4. Polymyxine

Another commonly used antibiotic in veterinary antibiotics is Polymyxind (22). Despite the abundant use of colestin in a veterinary surgeon for over 50 years, informational colistin resistance transfer through current horizontal gene transfer or continuous clonal expansion has not been observed for target Gram-negative organisms (23). Antibiotic specific for Enterobacteriaceae bacteria (22).

Table 1: Antibiotics in the category 1

Antimicrobial class **Category 1** Antimicrobials have a low or limited risk of public health	**Hazard of zoonotic relevance (as detailed in Q2, Table 1)**	**Probability of resistance transfer (as detailed in Q2, Table 2)**	**Use in veterinary medicine (EMA/ESVAC, 2013) and information from Member States Marketing Authorisations**	**Concluding remarks**
Macrolides (contain ketolides)	Campylobacter spp. Salmonella spp.	High	Approved (including group medication)	Compliance with responsible use principles is necessary to reduce the risk Measures to reinforce responsible use principles are needed

Penicilines , Natural	None specific	High	Approved (including group medication)	Compliance with responsible use principles is necessary to reduce the risk for co-resistance
Penicillines : Narrow spectrum, β-lactamase-resistant penicillines	None specific	High	Approved (predominately intramammary formulations)	Compliance with responsible use principles is necessary to reduce the risk responsible use principles are needed due to risk for co-resistance
Polymyxins (e.g. colistin)	Enterobacteriaceae	Low	Approved (including group medication)	
Rifamycins	None specific	High	Approved (limited use predominantly in horses and intramammary formulations)	Compliance with responsible use principles is necessary to reduce the risk for co-resistance
Tetracyclines	Brucella spp.	High	Approved (including group medication)	Compliance with responsible use principles is necessary to reduce the risk for co-resistance

II.3.3.2. Category 2: Estimated Antibiotics with High Risk for Public Health

The antibiotics in this group are antimicrobials which can cause an effect that will threaten public health.

Antibiotics in Category 2; Cephalosporins 3rd- and 4th generation, fluoroquinolones, aminoglycosides, some penicillins. These antibiotics are shown in table 2 (13).

II.3.3.2.1. Fluoroquinolones

Fluoroquinolones (FQ) are an important synthetic antimicrobial group with good absorption and broad tissue distribution after oral administration, possessing a broad range of antibacterial activity.

Fluoroquinolones have found widespread application in the treatment and prevention of veterinary diseases in food producing animals and have even been used as growth-promoting agents. The wide range of applications and the possibility of abuse or abuse of fluoracinones reveal potential resistance to human resistance and the emergence and spread of bacterial strains and possible cancer induction (24).

II.3.3.2. 2. Norfloxacin

Norfloxacin (NFLX) is a synthetic antibacterial agent of the fluoroquinolone group. Norfloxacin residues remain stable during cooking and heating and are not affected (25).

Table 2: Antibiotics in the category 2

Category 2 Antimicrobials used in veterinary medicine where the risk for public health is currently estimated higher	**Hazard of zoonotic relevance**	**Probability of resistance transfer**	**Use in veterinary medicine**	**Concluding remark**
Cephalosporins, 3rd- and 4th generation	Enterobacteriaceae	High	Approved (restrictions apply)	Compliance with existing restrictions is needed

Fluoroquinolones and other quinolones	Campylobacter spp. Enterobacteriaceae	High	Approved (including group medication, restrictions apply)	Compliance with existing restrictions is needed (see Question 4)
Class of antimicrobials for which a risk profiling is required before a final decision on its category can be made:				
Aminoglycosides	Enterobacteriaceae Enterococcus spp.	High	Approved (including group medication)	Further risk profiling needed due to importance in vet med
Penicillins: Aminopenicillins including β-	Enterobacteriaceae Enterococcus spp.	High	Approved	Further risk profiling needed due to importance
Lactamase inhibitors combinations (e.g. coamoxiclav)				in vet med

II.3.3.3. Category 3: Use of Unapproved Antibiotics

In this category; Glycylcycles, lipopeptides, monobactams, oxazolidinones, penicillins (including carboxyphenicillins and ureidopenicillins including combinations of beta-lactamase inhibitors), riminophenazines, sulphones, antibiotics that have not been approved for use in the treatment of tuberculosis or other mycobacterial diseases, carbapenems and other penemes, ceftaroline and ceftobiprol, cyclic esters (eg phosphomycin) glycopeptides and are generally used for the treatment of enterococci derivatives, methicillin resistant Staphylococcus aureus (MRSA) infections in enterobacteria. These antibiotics are shown in table 3 (13).

Table 3: 3. Antibiotics in the category

Antimicrobial class Category 3 Antimicrobials currently not approved for use in veterinary medicine	**Hazard of zoonotic relevance**	**Probability of resistance transfer**	**Use in veterinary medicine**	**Concluding remark**
Glycylcyclines	Enterobacteriaceae MRSA	Low	Not approved	See response to Question 1
Lipopeptides	Enterococcus spp. MRSA	Low	Not approved	No specific concern identified yet
Monobactams	Enterobacteriaceae	High	Not approved	Use in veterinary medicine should be kept at an absolute minimum due to high risk for spread of resistance
Oxazolidinones	Enterococcus spp. MRSA	High	Not approved	Use in veterinary medicine should be kept at an absolute minimum due to high risk for spread of resistance
Penicillins: carboxypenicillins and ureidopenicillins including β-lactamase inhibitors combinations	Enterobacteriaceae Enterococcus spp.	High	Not approved	
Riminofenazines	None specific	Low	Not approved	No specific concern identified yet
Sulfones	None specific	Low	Not approved	No specific concern identified yet
Drugs used solely to treat tuberculosis or	None specific	High	Not approved	No specific concern identified yet

other mycobacterial diseases				
Carbapenems and other penems	Enterobacteriaceae	High	Not approved	Use in veterinary medicine should be kept at an absolute minimum due to high risk for spread of resistance. As coresistance is an important issue, it is of high priority to decrease the total antimicrobial use in animal production in the EU
Ceftaroline and ceftobiprole	MRSA (Methicillinresistant Staphylococcus aureus)	Low	Not approved	No specific concern identified yet
Cyclic esters (e.g. fosfomycin)	Enterobacteriaceae	High	Not approved	Use in veterinary medicine should be kept at an absolute minimum due to high risk for spread of resistance
Glycopeptides	Enterococcus spp. MRSA	High	Not approved	Use in veterinary medicine should be kept at an absolute minimum due to high risk for spread of resistance

II.3.4. Use of Veterinary Antibiotics

The issue of the use of antibiotics is a matter of concern to business owners, farmers and integration owners with the ministry, veterinary drug and feed additive manufacturers and importers, drug stores, pharmacies, veterinarians, professional organizations, universities and related sectoral non-govermental organizations (NGOs) all over the world and in our country. Easy control mechanisms to be carried out by the Ministry of Food, Agriculture and Livestock, strict controls for better operation of the existing mechanism and serious sanctions are important to reduce residual food consumption of consumers (8).

II.3.4.1. Use of Veterinary Antibiotics by Countries

In our country, there are no healthy persons related to the usage amount of veterinary medicines. This is especially possible with the application of data matrix and traceability schemes in medicines.

However, the number of licensed medicines will give an idea on this point. As of February 2013, the number of licensed products in our country is expressed as 2 thousand 161. Of these, 526 are native and 635 are imported products. One thousand 17 active substances are used to prepare these products. The 949 antibiotics from the mentioned medicines are drugs. When evaluated according to animal species, it is the antibiotic of 371 of 171 drugs in the cattle, 155 of the 670 drugs in the horses, 174 of the 473 drugs in the cat and dogs, 136 of the 437 drugs in the sheep and goats, 68 of the 147 drugs in the wings and 44 of the drugs in the cattle. .

In the United States, total annual production and use pharmaceutical information, including antibiotics, is generally not available. For this reason, estimates of annual production and use of antibiotics for human health and agriculture should be discussed (2).

In the UK some class antibiotics are included in animal nutrition to increase growth rates. Tetracyclines are the most commonly used antibacterial compounds and are followed by sulfonamides, b-lactams, macrolides, aminoglycosides, fluoroquinolones, and others. Sulfonamides are the second most widely used veterinary antibiotics in the United Kingdom, accounting for approximately 21% of total sales (27). Tylosin and virginiamycin are prohibited in the European Union (28).

The use of antibiotics as growth promoters in the European Union is subject to Directive 70/524 / EEC including additives in feedstuffs and also does not adversely affect human, animal health or animal health at the level permitted in animal feed (29).

Prior to 2000, many antimicrobials in Australia, including arsenic, glycopeptides, macrolides, ionophores, polypeptides, quinoxalines, streptogramins and others, have been registered as growth promoters and sold to livestock owners, feed mills and feed mixes. However, after the joint expert technical advisory committee's report on antibiotic resistance, the Australian government adopted recommendations to oversee the use of antibiotics used for growth on animals. In June 2000, the glycopeptides were voluntarily withdrawn from the market. Due to the Australian regulatory system, the use of veterinary antibiotics fluoroclonon or amfenicol antibiotic classes, colistin or gentamicin (aminoglycoside) in animals producing food is not legal, although there is no data available on the amount of antibiotics used for growth in Australia, as in Canada (27).

In general, animals account for about 57% of the approximately 93,000 kg of antibiotic use in New Zealand. Approximately 34% of these antibiotics are ionophores with a mode of action that is quite different from other groups. When it does not contain ionophores, total antibiotic use in animals accounts for about 47% of the remaining 75000 kg. New Zealand uses antibiotics used in animals for feeding (27).

The data obtained in the studies conducted indicate that food-producing animals in South Africa use antibiotics forbidden to use in other countries. pharmacy and poisons board of the ministry of health; It has been shown that about 14,600 kg of active antimicrobial agent is used in animal food production in Kenya. In other African countries such as the United States of Rep. Of Tanzania and Uganda, access to antimicrobials is very easy due to the government's low control levels (27).

Sales and / or use of veterinary antibiotics in other countries are now missing in the public domain (27).

Canadian food animal production is a large, diverse, and dynamic industry, as is the use of growth-promoting antimicrobial substances in the United States. However, there is no comprehensive estimate of antimicrobial consumption in animal production for Canada (27).

In Japan, food-producing animals are prohibited from using antibiotics for rapid growth. Currently no antibiotics are registered for such use. However, after the approval of the Ministry; antibiotics are allowed to be used as a component of feed additives (27).

The use of antibiotics in animal feed in China has been regulated since 1989. Currently registered antibiotics for use in China include antibiotics such as moensin salinomycin, destomycin, simplexin, colistin. Apart from this, tetracyclines are already used (27).

In Russia, the use of antibiotics on the feed is mainly restricted to non-medicinal drugs such as basilix, grisine, flavomycin and virginiamics registered (27).

According to a report by the World Health Organization 2001, there are no data on veterinary antibiotic species in many developing countries, such as India, Thailand and Indonesia, due to lack of control of antimicrobial use in food animals (27).

II.3.5. The Influence to Enviroment of Antibiotic Usage in Food Animal

Veterinary antibiotics are widely used in many countries around the world to treat disease and protect the health of animals. These antibiotics are included in animal feed to increase growth rate and nutritional efficiency. Because antibiotics are absorbed in small amounts in the intestines of animals, the majority are discarded unchanged from stools and urine. Given the widespread application of animal feces to soil as fertilizer in many countries, there is a growing concern about the potential environmental impact of antibiotic residues (2).

The use of antibiotics in poultry production has changed significantly over the last decade, especially due to concerns about the potential negative human health consequences of these uses.

Managing the possible effects of antibiotic use in poultry requires a simple estimate of the risks attributable to the use of antibiotics in poultry.

There is a need for risk models and empirical studies to evaluate the interventions that reduce the negative consequences associated with specific antibiotic use (30).

II.3.6. Antibiotic Residue in Food Animals and Effects on Human Health

The main theme of consumer health is food safety. Food safety is important not only in terms of public health but also in ensuring the continuation of food safety at high levels. Unconscious and uncontrolled use of antibiotics in the consumption of food residue in the risk of blood urine, waste water to the surrounding environment, as well as the development of drug resistant strains of bacteria in the consumer concern (8).

In food-grade tissues and organs, drugs or chemical residues that are important for undesirable or toxic effects should not be cut off from animals that are drugged until a safe level or concentration is reached for the consumer. Those who consume from untimely stopping animals during this period pass these drug residues (31).

Antibiotic use in animals can cause direct and indirect effects on human health:

- Direct effects are effects that can be causally linked to antibiotic-resistant bacteria of food animals.
- Indirect effects are the consequences of antimicrobial use of resistant organisms spread to various components of the ecosystem (eg, water and soil) as a result of antibiotic use in food animals (32).

Antibiotic resistance is a problem that affects public health critically. While once believed to be the province of hospitals and other health-care facilities, a host of community factors are now known to promote antibiotic resistance, and community-associated resistant strains have now been implicated as the cause of many hospital-acquired infections (33,34).

Evidence that the use of antibiotics in food animals may cause antibiotic-resistant infections in humans has been identified about 10 years ago (32).

As a result of the inherent nature of exposure to antibiotic compounds, antibiotic resistance arises as a result of natural selection (35).

Advantageous mutations in the presence of antibiotics can also be transferred by plasmid exchange in the bacterial colon and cause increased resistance properties (32,36).

From the public health standpoint, the presence of antimicrobial residues poses various problems with potential risks for consumers (12).After the emergence of each new class of antibiotics, the emergence of drug resistance has been observed (32).

International, national and local antibiotic management centers have been developed to encourage antibiotic efficacy against serious and life-threatening infections, to limit unnecessary exposure to antibiotics (37,38).

In practice, however, practitioners are under the obligation to ethically use antibiotics for patients who can benefit from antibiotic use, protecting the efficacy of antibiotics. In veterinary medicine, there are also important debates about the use of antibiotics in animals raised for human consumption. The potential human health hazard posed by the use of inappropriate antibiotics in food animals is important because pathogenic resistant organisms that breed in these animals are ready to enter the food supply and spread widely in food products (39,40).

Commensal bacteria found in animal husbandry are often found in fresh meat products and may serve as reservoirs for resistant genes that can potentially infect pathogenic organisms in humans (41,42).

II.3.7. Resistance Occurrence as a Result of Antibiotic Use in Food Animal

Given that some antibiotics will continue to be used in the poultry industry, methods are needed to estimate the causal relationship between these antibiotic uses and actual animal and human health effects (43,44).

The use of most antibiotics in animal husbandry requires a veterinary prescription, but individual treatment decisions are often made and implemented by employees of a farmer in accordance with instructions given by a veterinarian (43,44).

Although the use of antibiotics in food animals is widely accepted, there is no reliable data on the amount and manner of use (eg, dose and frequency) (27).

The Food & Drug Administration (FDA) has developed guidelines to determine the risk of remaining antibiotics in food products. This guide shows the antibiotic residues on the normal human intestinal flora and the resistance of these strains. It also provides rules for the calculation of Acceptable Daily Intake Quantity (ADI) for antibiotic residues, which pose a significant risk to human health (32,45).

Given the importance of antibiotic resistance as a public health problem, many governments and professional societies have observed current scientific findings and have developed recommendations to limit the use of any antibiotic, including food animals (32). These recommendations are shown in table 4.

Table 4: Recommendations on the use of antibiotics

Title (organization, year)	Recommendations
Antimicrobil Resistance from Food Animals (WHO,2008)	• Promote/assist national implementation of Who and OIE guidelines and identify barriers to implementations • Encourage national participation in development of international antimicrobial use guidelines. • Promote national surveillance of antimicrobial use and AMR, and creation of national AMR containment task forces • Establish international information sharing/monitoring networks for antimicrobial use and emergingAmr usingexisting training platforms • Conduct national surveillance of Amr in animals, humans, and food; use integrated data to identify AMRemergency arising from non-human use;implement timely measures to contain AMR • Consider potential development of AMR and cross-resistance during national prelicensing safety evaluation of veterinary drugs; conduct post-licensing surveillance of emerging AMR. • Provide incentives for development of new antimicrobials; fund educational and research. • Control national sale and distribution of antimicrobials; prevent ilicit manufacture, importation, and sale of veterinary drugs. • Encourage good hygienic practices and farm management to ensure animal health without the use of antimicrobials. • Require prescriptions for animal antimicrobial use;develop national veterinary guidelines for appropriate antimicrobial prescribing.
Foodborne Antimicrobial Resistance as a Biological Hazard: Scientific Opinion of the Panel on Biological Hazards (European Food Safety Authority, 2008)	• Target Known routes of transmission for spesific resistance traits. • Prioritize interventions to reduce emerging resistance to fluoroquinolons and to third and fourth generations cephalosporins. • Implement uniform adoption of risk assessment methodologies at the national and international level; encourage global adoption of EU standarts for measuring the development of AMR • Investigate role of food, water and environment in the spread of epidemic plasmids that encode resistance. • Consider the role of commensals and bacteria intentionally added to aid food processing in transmission of resistance. • Develop new approaches for recognizing and controlling transmission of resistance genes based on epidemiologic

	and source attribution research.
Putting Meat on the Table: Industrial Farm Animal Production in America (Pew Commission on Ondustrial Farm Animal Production, 2008)	• Phase out nontherapeutic antimicrobial use in food animals; immediately ban nontherapeutic use of newly approved antimicrobials; clarify antimicrobial definitions to provide clear estimates of use and facilitate clear policy-making. • Consolidate food safety roles of the USDA, FDA, and EPA into a single Food Safety Administration • Develop risk-based food safety system; strengthen FDA Guidance 152 and retroactively investigate previously approved antimicrobials with new criteria. • Expand USDA's extension service to include educational programs on best practices for disease mitigation in animal husbandry; expands interdisciplinary education to strenghthen aprtnerships among physicians, veterinarians, and public health professionals. • Restrict public access to agricultural sources of antimicrobials; enforce restricted access to prescription drugs and veterinary oversight an authorization of all antimicrobial use in food animals. • Implement national disease-monitoring database to allow 48-hour trace-back to individual animals. • Require reporting of gross annual antimicrobial sales; incorporate into NAIS; link to NARMS. • Increase monitoring of effects of antimicrobial exposure among farm workers and people living close to farms.
Antimicrobial Resistance: Implications for The Food Syste(Institute of Food Technologists, 2006)	• Conduct risk assessment to determine effects of specific antimicrobial-organism exposures on the development of resistance under various conditions and environments; determine relationsship between use of specific antibiotics in food animal husbandry and resistance selection rates among major foodborne bacteria at slaughter; compare resistance rates between antibiotic-use farms and antibiotic-free farms. • Advance understanding of the mechanism of resistance; improve ability to predict the potential for cross-resistance. • Increase research and development of anitibotic alternatives; examine whether microbial interventions are equally effective for antimicrobial-susceptible and resistant microorganisms. • Expand development of prudent use guidelines to include all antibiotic uses and modify as new evidence becomes available; use surveillance and food attribution models to measure the effectiveness of interventions.

II.3.8. Antibiotic Regulations

Risk models and empirical studies are needed to evaluate the interventions that reduce the negative consequences associated with specific antibiotic use (30).

In Turkey; The residual regulation of our Food, Agriculture and Animal Husbandry contains the principles that the European Union countries apply. The provisions of this regulation shall be executed by the Minister of Food, Agriculture and Livestock.

The aim of the Turkish Food Codex, Regulation on the classification of pharmacological active ingredients and maximum residue limits to be found in animal husbandry; the classification of the pharmacologically active substances that can be scientifically and technically detectable and the maximum residue limits of veterinary drugs which may be present in animal foods in order to ensure food safety.

This Regulation covers the classification of the pharmacological active substances belonging to veterinary drugs which can be found in animal foods and the maximum residue limits (46).

Article 12 of this regulation states that "(1) Veterinary drug residues that may be present in the products covered by this Communiqué are the provisions contained in the classification of pharmacological active substances and regulation on maximum residue limits to be found in the Turkish Food Codex Animal Diseases published in the Official Gazette dated 4/5/2012 and numbered 28282 it will be appropriate. "(46).

In the European Union (EU); (MRLs) for veterinary drug residues in animal tissues entering the human food chain to protect human health. Commission Regulation (EC) no. 2377/90 (EEC) 37/2010, repealing the Council Regulation and its amendments, regulates drugs permitted for therapeutic veterinary use in animals intended for food production. Due to ease of use, all pharmacologically active substances are listed alphabetically in a single EC. Regulation No. 2377/90 has created two separate tables, one for the competent substances, including penicillins, listed in Annexes 1, 2 and 3, and one for the prohibited substances listed in Annex 4 (47).

The targets in the new legislation are:

- Increase the availability of veterinary medicinal products for animals producing food to avoid animal health and well-being and the use of illegal substances.
- Providing explicit references to control of residues of pharmacologically active substances in foods to protect consumer health and improve the functioning of the Single Market.

- To clarify Community procedures that establish maximum residue limits (MRLs) by making them consistent with international standards.
- simplify the existing legislation by increasing the legibility of the provisions on MRLs designated for end-users (animal health professionals, competent authorities in Member States and third countries).

The Commission's proposal was adopted by the European Parliament and Council in accordance with the joint decision procedure and was published in the Official Gazette on 16 June 2009. The new Regulation (470/2009 / EC Regulation) will enter into force on the twentieth day following its publication in the Official Gazette (47).

II.4. Place and Importance of Chicken in Nutrition

Nutrition is the use of nutrients for growth, survival and health. Nutrition comes at the head of human needs. The human body consists of water, protein, lipids, minerals, and other items in our food. The adult human body has an average of 59% water, 18% protein, 18% lipid, 4.3% minerals and 0.7% carbohydrates, vitamins, nucleic acids, hormones (3).

To date, research on nutrition has shown that over 50 per cent of the species need nutrients to survive, grow and develop. We can gather the nutritional items people need in 5 groups.

1-Proteins

2-Fats

3- Carbohydrates

4-Minerals

5-Vitamins

When one or more of these nutritional items are not provided, the body workout is accompanied by consequent growth, developmental deficits, and health problems. The aim of this nutrition is that the individual can provide all the nutritional items that are needed according to his / her age, gender and physiological condition. This is called adequate and balanced nutrition (3).

The word "protein" in Latin is "the elemental nitrogenous element for living beings". Proteins are also essential for growth, development, renewal of damaged cells as they are the main ingredients of the cell besides energy. Proteins also constitute the structure of enzymes and some hormones involved in the use of nutrients in the body (48).

All animal and vegetable foods have protein. However, the amount of protein in each meal and the proportions of essential amino acids vary. Essential amino acid associations of proteins found in animal nutrients generally fit the needs of the body and in the proteins of plant nutrients, one or two of the essential amino acids are less than necessary.

Amino acids are composed of carbon, hydrogen, oxygen, and nitrogen. Some may contain minerals such as iron, copper, zinc, iodine, sulfur, phosphate. Amino acids are separated into 2 groups as essential and non-essential amino acids. Essential and nonessential amino acids are shown in the table 5 (48).

Table 5: Classification of aminoacids

Classification of aminoacids	
<u>Essential Aminoacids</u>	<u>Non-Essential Aminoacids</u>
• Histidine • İsoleucine • Leucine • Lyzine • Methionine • Phenylalanine • Tiron • Tryptophan • Valine	• Alanine • Arginine • Asparagine • Aspartic acid • Cysteine • Sistin • Glutamic acid • Glutamine • Glycine • Hydroxyproline • Proline • Cerine • Tyrosine

The digestibility of proteins obtained from animal, egg, meat, milk and similar animal foods is 91-100%, 79-90% of cereal proteins and 69-90% of legumes proteins.

The majority of the protein taken from meat, fish, milk and its derivatives can be converted into body protein. These are good quality protein sources (49). Essential amino acids include isoleucine, leucine, lysine, methionine, phenylalanine, threonine, histidine, tryptophan and valine amino acid (50).

Changes in socio-economic structures of societies are also reflected in consumption habits. In other words, a community's nutrition culture; geography, climate, agriculture, animal husbandry, industrialization, and the spread of mass media are many factors (4).

Poultry resembles red flesh in terms of nutritional value. They are only less fat and consequently, energy values are lower than sheep and beef. Saturated fat and cholesterol are also less. Especially the white flesh of chicken is very little fat. For this reason, chicken meat is preferred over red meat in recent years. In general, the flesh of poultry contains more protein than the fowl meat (sheep & cattle). However, their iron content is less. They are also considered to be rich in riboflavin, niacin, B6 and B12 vitamins (49). Macronutrients provided by 100 grams of various meat are shown in table 6 and micronutrients provided by 100 grams of various meat are shown in table 7 (49).

Table 6: Macronutrients Provided by 100 Grams of Various Meats (49)

Meat Type	Amount (100gr)	Energy (kkal)	Kilojul	Carbohydrate (g)	Protein (g)	Fat (g)
Beef (medıım-fat)	1 portion boneless	240	1004	0	18,7	18.2
Sheep(medium-fat)	1 portion boneless	267	1117	0	17,0	21,0
Chicken	1 portion boneless	149	623	0	19,0	8,0
Rabbit	1 portion boneless	137	573	0	21,0	5,8
Goat	1 portion boneless	157	657	-	18,4	9,2
Pig	1 portion boneless	377	1577	0	13,00	36,0
Brain	3 matchbox size	125	523	1,2	10,3	8,6
Heart	3 matchbox size	116	458	2,0	16,5	4,5
Kidney	1-2 unit	131	548	0,8	16,0	7,0
Liver	3 matchbox size	136	569	4,5	20,0	4,0
Lungs	3 matchbox size	81	339	0	14,6	2,4
Tongue	4-5 slices	194	812	0,5	16,2	14,0
Sausage	2-3 unit	309	1293	1,8	12,5	27,6
Salami	4-5 slices	304	1272	1,1	12,1	27,5
Fish (medium fat)	1 porstion	149	624	0,0	19,0	8,0
Egg	2 unit	159	665	0,7	12,8	11,5

Table 7: Micronutrients Provided by 100 Grams of Various Meats (49)

(1) *Values show activity of vitamin A.

Meat Type	Amount (100gr)	Calsium (mg)	Iron (mg)	Vit.A IU(1)	Vit. B_1 (mg)	Vit. B_2 (mg)	Vit.C (mg)
Beef(medium-fat)	1 portion boneless	8	2,6	0	0,06	0,16	0
Sheep(medium-fat)	1 portion boneless	7	2,2	0	0,10	0,20	0
Chicken	1 portion boneless	15	1,5	-	8	0,16	0
Rabbit	1 portion boneless	17	1,6	0	0,05	0,15	0
Goat	1 portion boneless	1	2,2	0	0,17	0,32	0
Pig	1 portion boneless	6	1,6	0	0,60	0,20	0
Brain	3 matchbox size	12	3,2	500	0,25	0,24	14
Heart	3 matchbox size	10	4,5	40	0,30	0,90	4
Kidney	1-2 unit	13	6,0	1000	0.35	2,50	12
Liver	3 matchbox size	10	8,0	25000	0,30	3,00	20
Lungs	3 matchbox size	16	6,6	165	0,09	0,40	2
Tongue	4-5 slices	12	2,0	0	0,10	0,30	0
Sausage	2-3 unit	7	1,9	-	0,16	0,20	-
Salami	4-5 slices	7	1,8	-	0,16	0,22	-
Fish (medium-fat)	1 portion	50	1,1	100	0,10	0,20	0
Egg	2 unit	54	2,7	100	0,14	0,37	0

III. MATERIAL AND METHODS

III.1. Material

100 unbranded chicken liver were collected from various butcheries in various districts of Istanbul. These chicken liver samples were numbered 1-100 and placed in separate storage pouches and stored at -18 ° C in the freezer.

Two grams of these samples were cut into 10 ml test tubes numbered 1-100. The samples we put in the test tubes were prepared for the MeRA test. ELISA samples were also prepared for ELISA test.

III.2. Method

Firstly, the antibiotic residues in the samples were qualitatively determined by MeRA Test. Tetracycline levels were quantitatively determined by ELISA method in all the samples in which we detected antibiotics.

III.2.1. Antibiotic Assay with MeRA Test Kit

It is a qualitative microbiological test containing Geobacillus stearothermophilus spores for the detection of antimicrobial substance residues in meat products. There are two advantages to choosing this test. The first is the ease of making comments with color change, and the second is the ease of getting results within 4 hours (60).

Some antimicrobial agent groups, such as beta-lactams and tetracyclines, are thermostable; molecules belonging to these chemical classes are inactivated shortly at the growth temperature of thermophilic bacteria. The MeRA test includes a rapid pre-incubation step which allows growth and multiplication of G. stearothermophilus. Following this step, the interaction between the vegetative form of G. stearothermophilus and the heat-sensitive antibiotics, if present in the sample, is carried out at room temperature (60).

Finally, the test tubes are subjected to a final incubation. This incubation step of the MeRA test is a critical step in achieving extremely low detection limits (60).

III.2.1. 1. The Making of the MeRA Test Experiment

- 2 gr chicken liver sample and 6 ml distilled water were transferred to a 10 ml test tube. (meat: water ratio will be 1: 3)
- The capped test tube was mixed with the vortex.
- The homogenized sample was centrifuged at 4000 rpm for 15 min.

- One sports disk was added to the food solution.
- Pre-incubation of sports disk with solution at 64 ° C for 20 min was performed.
- The incubated solution was brought to room temperature after incubation. 1 ml of homogenized supernatant (test sample) was transferred into the incubated solution. The solution was allowed to stand at room temperature for 20 min so that the antimicrobial agent in the test sample (if present).
- Test sample was incubated at 64 ° C on a water bath or thermoblock for 3 to 3.5 hours.
- Observed the color change in the tube.

  - If there is no color change (Blue-green color): The antimicrobial agent concentration in the meat sample is above the detection limits.

  - If there is a color change (Yellow color): There is no antimicrobial agent in the meat sample or its concentration is below the detection limits (Figure 2).

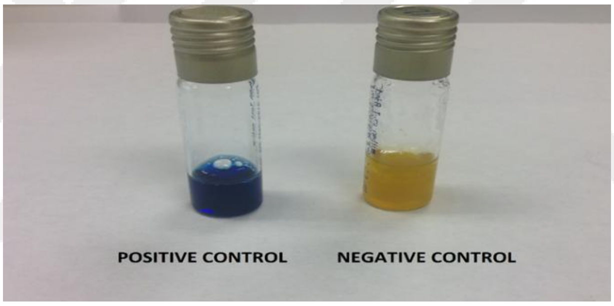


Figure 2: Positive Control and Negative Control

Table 8: MeRA test maximum residue limits

Antimicrobial agent	Maximum Residue Limits For meat (µg/kg)	Determination Limits2 (µg/kg) MeRA Test sensitivity
BETA-LACTAMS		
Ampicillin	50	25-50
Oxacillin	300	150-300
Cloxacillin	300	150-300
Dicloxacillin	300	150-300
Amoxicillin	50	25-50
Benzylpenicillin	50	25-50
Penethamate	50	25-50
Cefalexin	200	100-200
Ceftiofur	1000	< 500
Cefquinome	50	25-50
TETRACYCLINES		
Tetracycline	100	100-200
Clorotetracycline	100	100-200
Oxytetracycline	100	100-200
Doxycycline	100	100-200
MACROLIDES		
Erithromycin	200	200-400
Tylosin	100	100-200
Tilmicosin	50	100-200
Spiramycin	200	200-400
LINCOSAMIDES		
Lincomycin	100	100-200
Pirlimycin	100	50-100

AMINOGLYCOSIDES		
Gentamicin	50	100-200
Neomycin	500	500-1000
Streptomycin	500	500-1000
Dihydrostreptomycin	500	500-1000
SULPHAMIDES		
Sulfadiazine	100	50-100
SULFANILAMIDES		
Sulfadimidine	100	50-100
BENZIL PIRIMIDINE		
Trimethoprim	50	50-100
QUINOLONES		
Flumequine	200	200-400
Enrofloxacin	100	50-100

**1. It is referenced from beef cattle. Read the 37/2010 Editing for all data.

**2. The MeRA test is generally based on the sensitivity to antimicrobial agents used in veterinary medicine and the current maximum limit values in Europe (37/2010 EC Regulation)

The maximum residue is the amount (maximum) allowed for the presence of metabolites of a veterinary drug or veterinary drug in a food. Exceeding this limit poses a great risk to human health. It is important in the ADI that the maximum residue limit is specified. As the amount to be given daily in animal foods increases, the residual risk will increase (60).

III.2.2. Elisa Test

At the same time, antiserum levels of tetracycline were measured by elisa method in chicken liver samples. For this study, Elisa kit of Ridascreen Tetracycline (r-BioPharm, Germany) was used. This kit is a quantitative competitive ELISA test used in the determination of tetracycline residues in food samples such as milk, dairy cheese, butter, yoghurt, kefir, cream, honey, meat, sausage, fish, eggs (61).

III.2.2.1. Tetracycline Elisa Test Method:

Preparation of Samples:

- One gram of chicken liver livers were placed in the test tubes and the number of the chicken liver sampled on the tubes was recorded.
- After 100 samples were prepared in this way, the tubes were closed by adding 9 ml of PBS.
- The samples were vortexed and then centrifuged at 4,000 g for 10 min at room temperature (20-25 ° C).
- 1 ml supernatants from centrifuged specimens were seeded with new tubes.
- 2 ml of ether was added to the prepared 1 ml supernatants.
- The prepared tubes were centrifuged again at 4,000 g for 10 min and the prepared meat broth was separated.
- 1 ml samples from the bottom of the tubes were transferred to new tubes for elisa test application.

Preparation of Standards: Standards were diluted because they were concentrated. Each standard (50 µl) was diluted with 450 µl of sample buffer 1. The standards are prepared on the working day as it should be fresh.

The Applying of Elisa Test: The solutions and plates in the kit were brought to room temperature before the operation and the following steps were followed step by step and the operation was completed. (Figure 3)

Figure 3: Elisa test kit

1. Sample and standard number of test beads were placed in the plate.
2. Pipet 50 µl of the standard and samples into wells.
3. 50 µl of anti-tetracycline antibody was pipetted into each incubator. It was then incubated for 1 hour at room temperature.
4. In an automatic elisa washer, 250 l wash buffer was washed in each wash 3 times.
5. Add 100 µl of conjugate to each buffer with the help of a multi-channel pipette, shake, and incubate at room temperature for 15 min (Figure 6).
6. In an automatic elisa washer, 250 l wash buffer was washed 3 times in each wash.
7. Add 100 µl of sucrose / chromogen to each of the plates, shake, and incubate at room temperature for 15 min.
8. 100 µl stop solution was added and the Elisa was read aliquot using a 450 nm filter.

A standard curve graph was drawn using the Rida Soft Win program (Figure 4). Values of tetracycline values in ppb in terms of absorbance sample / zero standard absorbance x100 were calculated (20).

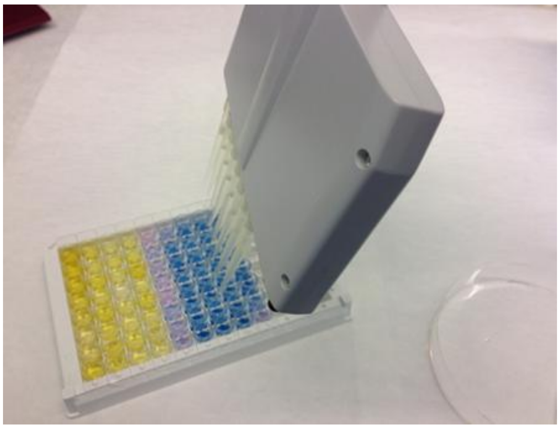
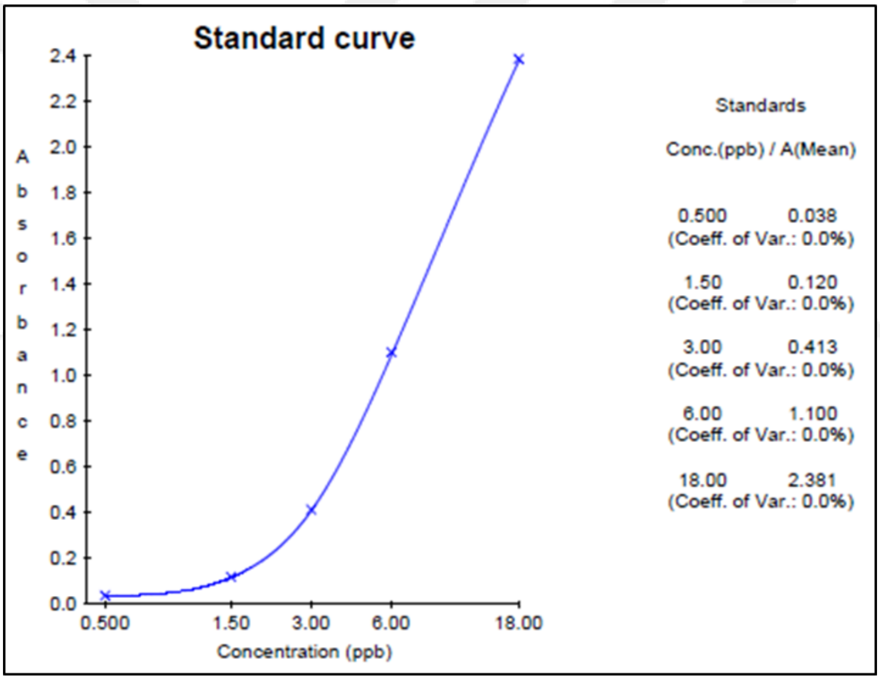


Figure 4: Study of Elisa Test

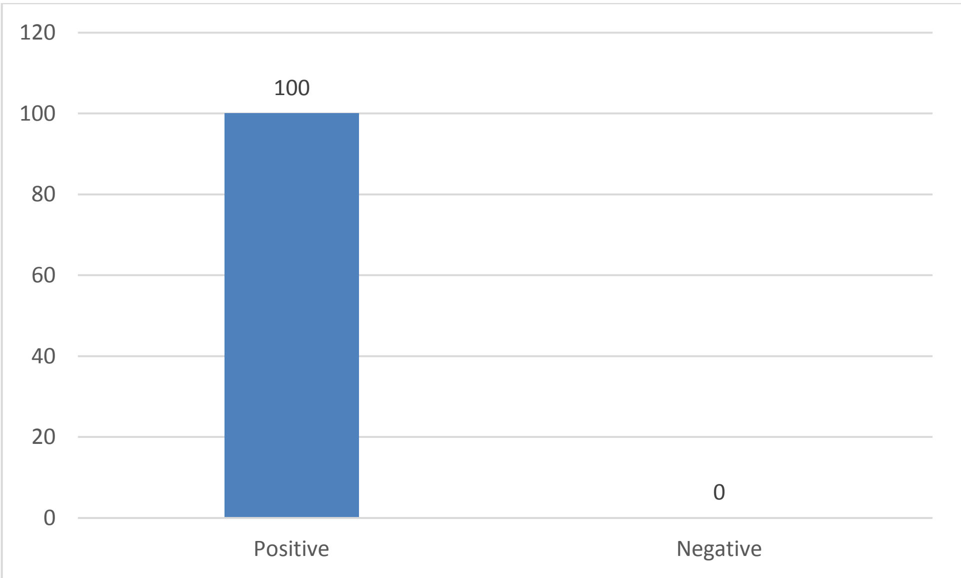


Graphic 1: Standard Curve Graphic of Tetracycline Elisa Test

IV. RESULTS

IV.1. Results of MeRA Test

It was aimed to qualitatively determine the results with this test. The concentration of antimicrobial agent in 100 samples of 100 chickens weighing pasture according to pasture test results is above the detection limits. Since pasture does not have specific maximum limits for chicken liver in the test, we evaluated it according to the maximum limits of meat. The color change in the tube at the 1st, 6th, 15th, 16th, 22nd, 79th, 84th, 85th and 96th samples of the chicken liver samples we enumerated in the preparation phase of the experiment is in the light green-dark green range; all of the other tubes were observed in dark blue color. However, the colors in the light green-dark green range do not show that the antimicrobial agent concentration is negative (Graphic 2-Figure 5).



Graphic 2: Positive-Negative Distribution of Pasture Test Results

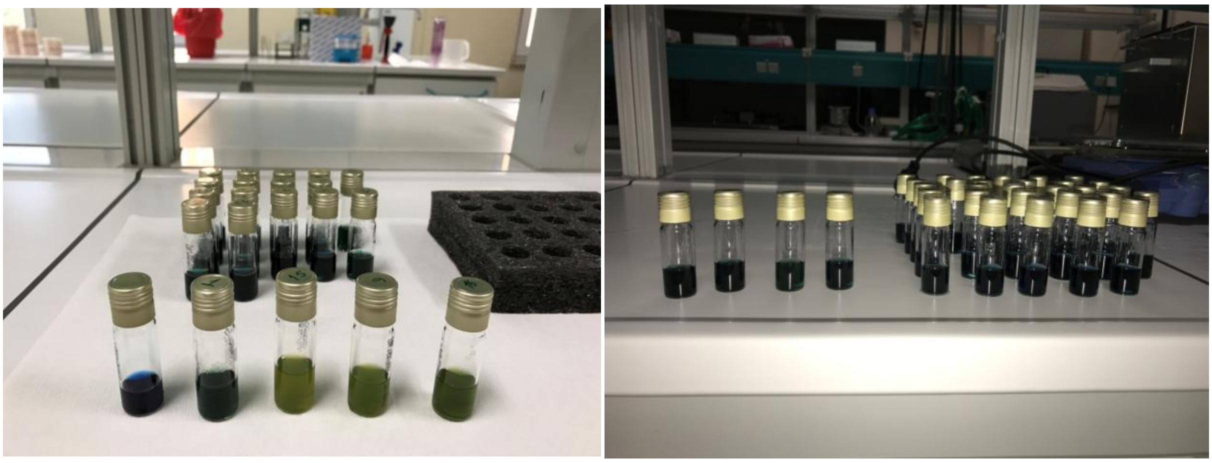
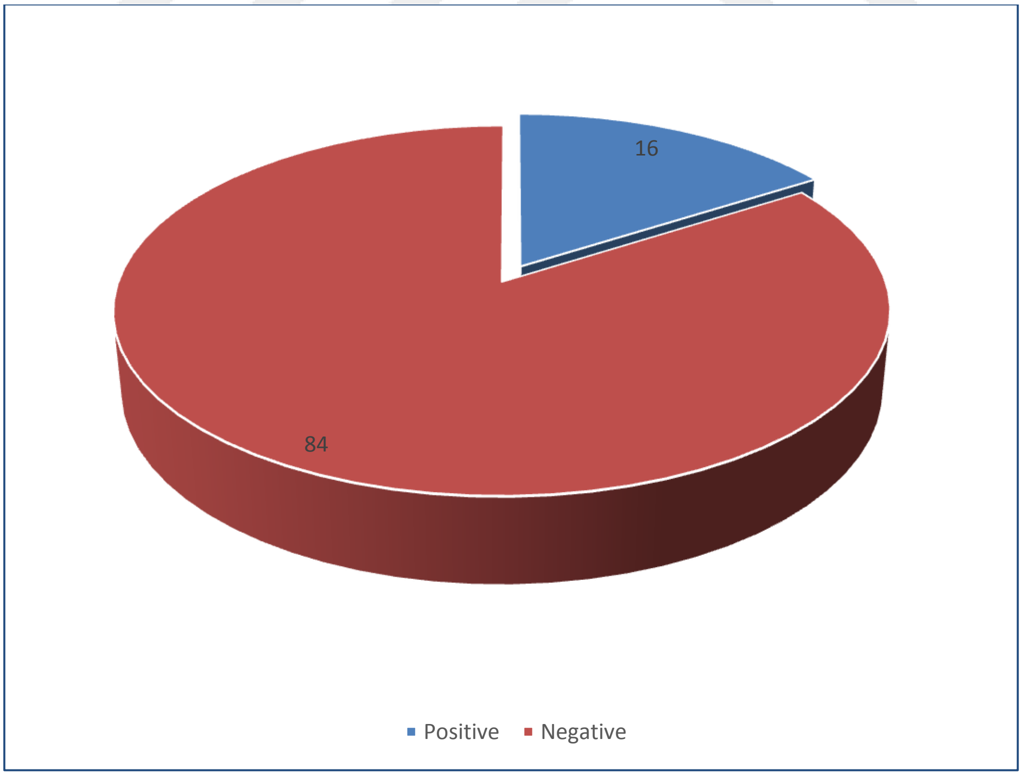


Figure V: MeRA test results

IV.2. Elisa Test Results

As a result of the elisa study with tetracycline (r-bioPharm), positivity was detected in 16 out of the samples of chicken liver sold out. 3 of them were found to be close to the border (97.34). In total 84 tetracyclin levels were found among acceptable limits (Graphic 3,Table 9).



Graphic 3: Positive-Negative Distribution of Elisa Test Results

Table 9: Tetracycline Elisa Test Results

Sample No	Results	Sample No	Results	Sample No	Results	Sample No	Results
1	30,74	**26**	12,88	**51**	56	**76**	113,99
2	47,39	**27**	56	**52**	8,2	**77**	130,64
3	39,68	**28**	12,88	**53**	113,99	**78**	80,69
4	15,03	**29**	8,2	**54**	8,2	**79**	97,34
5	8,26	**30**	12,88	**55**	56	**80**	113,99
6	38,38	**31**	56	**56**	147,29	**81**	130,64
7	42,1	**32**	12,88	**57**	8,2	**82**	21,08
8	8,2	**33**	12,88	**58**	8,2	**83**	30,74
9	7,62	**34**	12,88	**59**	8,05	**84**	113,99
10	12,93	**35**	8,05	**60**	56	**85**	130,64
11	8,05	**36**	21,08	**61**	21,08	**86**	80,69
12	36,04	**37**	21,08	**62**	56	**87**	97,34
13	5,13	**38**	8,2	**63**	21,08	**88**	113,99
14	7,51	**39**	21,08	**64**	21,08	**89**	130,64
15	12,88	**40**	56	**65**	8,05	**90**	21,08
16	21,08	**41**	8,05	**66**	21,08	**91**	30,74
17	11,53	**42**	21,08	**67**	30,74	**92**	113,99
18	12,05	**43**	36,04	**68**	47,39	**93**	130,64
19	30,74	**44**	56	**69**	64,04	**94**	80,69
20	8,2	**45**	36,04	**70**	80,69	**95**	56
21	56	**46**	8,2	**71**	97,34	**96**	113,99
22	12,88	**47**	21,08	**72**	113,99	**97**	130,64
23	12,88	**48**	36,04	**73**	56	**98**	21,08
24	8,2	**49**	8,05	**74**	147,29	**99**	30,74
25	12,88	**50**	8,05	**75**	30,74	**100**	47,39

*Results are given as ppb.

V. DISCUSSION AND CONCLUSION

Medicines and other chemical substances are used in animals to prevent diseases and accelerate their development. However, it is considered to be a residue if the food value exceeds the permissible limits in food items such as meat, milk, eggs, honey (51,52). In today's plants where food-grade animals are produced, the aim is to minimize the energy, to maximize efficiency, to keep animals out of sickness, and to use veterinary antibiotics for faster growth and cutting. These antibiotics are included in animal feed to increase growth rate and nutritional efficiency (2). In livestock applications, the use of veterinary medicines is one of the most importance for the efficient and safe production of meat, fish, milk, eggs and honey. The use of these drugs is subject to strict licensing and approval procedures with a system similar to that of medicinal products (53,54). However, excessive doses of antibiotics in animals can cause unnecessary antibiotic residues in the resulting animals, since antibiotics are not completely removed from the animals or destroyed, as the animals receive the drug without waiting for the legal waiting period of the drug (55).

Tetracycline, oxytetracycline and chlortetracycline are commonly used in the tetracycline group due to their low toxicity and broad spectrum in animal breeding (55).

Various methods are used in the analysis of antibiotic residues. In our study, the purpose of using the MeRA test was to avoid unnecessary testing of antibiotic-free samples by separating samples with and without antibiotics in our samples. At the same time, the ability of the MeRA test to qualitatively identify many antibiotic residues is one of the reasons for using this kit (60).

In our study, the MeRA test provided easy interpretation with color change and easy to get results after 4 hours of incubation. The purpose of our Elisa test kit is to be a competitive enzyme immunoassay test for quantitative analysis of milk, milk powder, cheese, butter, kefir, cream, sour cream, honey, meat, sausage, eggs and fish.

We chose the chicken liver because it is the liver storage organ. However, since tetracycline group antibiotics are widely used in food animals, we aimed to investigate antibiotic residues of tetracycline group on chicken liver. We have quantitatively completed 100 chickens in our work with elisa test and qualitative with MeRA test. First, we separated the chicken livers with and without antibiotic residues by MeRA test, and we aimed to study elisa test with chicken livers containing antibiotics. In case of the MeRA test, we found antimicrobial agent concentrations above the limits for all of the samples when we concluded that the maximum residue limits of the ethane were obtained. Therefore, we have tested the face sample that we can not eliminate in the MeRA test. As a result of the Elisa test, the tetracycline levels of our 16 samples were above the acceptable maximum levels, 3 were at the borderline level and 84 were within acceptable limits. In the MeRA test, antimicrobial agent levels were found high in all samples because only tetracycline was not observed with the MeRA test kit. In conclusion, we concluded that the results of MeRA test and Elisa test overlap each other.

In 2011, residues of Norfloxacin group antibiotics (Norfloxacin, Ciprofloxacin, Enrofloxacin, Sarafloxacin, Ofloxacin, Perfloxacin, Lomefloxacin and Danofloxacin) on chicken liver were investigated by Jiang Jinqing et al. Norfloxacin (NFLX) is a synthetic antimicrobial agent. The reason for using NFLX in this study is that these antibiotic residues are not cooked or heat affected. As in our study, Elisa test kit and NFLX residue in chickens were searched. As a result of this study, 38% ciprofloxacin and 33% Sarafloxacin were detected in the samples (25).

In 2008, Alexander Pavlov and his colleagues worked on antibiotic remnants in chicken meat and offal, working on 115 chickens, 192 chicken livers and 155 chicken kidneys. Of these 462 examples, 245 were collected between November and March, and 217 between May and October. This study was conducted using a four-plate agar diffusion test. In the winter period, 75 samples of the total sample amount and 170 samples of the meat samples are examples of discomfort. Antibiotic residues were found to be positive in only 2 (4%) of the samples taken from the meat, whereas positive results of the liver and kidney samples were found to be much higher than the meat. The peak of the results was found in the chicken kidney with a positive result of 33%. Antibiotic residues were positive in 8 of the 45 chicken liver samples (17%). In the case of repeated studies in summer, no antibiotic residues were found in 30 chicken meat samples; antibiotic residues were positive in 3 out of 40 samples (7.5%) in chicken liver and 7 out of 40 chicken kidney samples (17.5%). As a result of the work done, the amount of antibiotics used in chickens during the winter months was determined more than in the summer months. The relatively low level of antibiotic treatment may be considered to be below or below the MRL. These values may also cause allergic reactions or antibiotic resistance to humans (52). The samples collected in our study were collected on August-September-October. Antibiotic residues were positive in 16 (16%) and antibiotic residues in 3 (3%) of the poultry samples we collected.

In 2006, when four samples of plaque test and 47 samples of chicken meat were examined for antibiotic residues in 2006, 17 samples (36.17%) were found to have residues and more beta-lactam and tetracycline residues were found in samples. In our study, 16 out of 100 (16%) antibiotic residues were above the MRL.

In a study, the antibiotics (tetracycline, oxytetracycline and chlortetracycline) of the tetracycline group with HPLC technique and 200 chicken meat and 200 poultry liver samples collected from many cities of Turkey. As a result of the study, in chicken meat, 8.1% oxytetracycline, 7% tetracycline and 5.5% chlortetracycline; while 47% of oxytetracycline, 47% of tetracycline and 5.5% of chlortetracycline were found in chicken livers. In our study, tetracycline residues were found out of the maximum residue limits 16% of 100 samples, but in our MeRa test results, we detected antibiotic residues in all of our 100 samples (20).

In Iran (2006), 270 muscles, lungs, and kidneys were collected from Tehran slaughterhouse and enrofloxacin analysis was performed by HPLC. 8 muscles, 12 lungs, and 22 kidneys were encountered in excess of MRLs.

In 2012, Çetinkaya and his colleagues investigated the tetracycline group by liquid chromatography-dual mass spectrometry in the case of 60 chickens obtained from supermarkets in Bursa. In the case of 6 chickens, doxycycline between 19.9 and 35.6 µg kg-1 was determined. One chicken sample contained 17,2 µg kg_1 tetracycline. Oxytetracycline and chlorotetracycline were not found in any chicken sample.

In a study conducted by Muriuki et al. In Kenya in 2001, 250 tetracycline group of cattle were investigated antibiotics by HPLC method. In this study; Oxytetracycline was found in 110 (44%) and chlorotetracycline was in 4 of the samples. The identified tetracycline residues were found to be on the MRL.

In China, Chen-Hao (Andy) Zhai published tetracycline by HPLC method in their work published in 2008 and as a result of the experiments performed, tetracycline residues in chickens were found to be below the MRL values.

The use of antibiotics by chickens in our country is not seen as a problem for food inspectors. However, after using antibiotics, antibiotics are an important question for society and environmental health, because these chickens are cut out without compliance with the cutting period and are not controlled only by animals, but for prevention or larger animals.

The goal of producers to achieve maximum efficiency with minimal cost, minimum loss, gives more antibiotics to chickens, resulting in an unhealthy but big image in chickens.

In this case the producer will be offering a bigger, more muscular animal. Antibiotics can be given to chickens at the time of illness by continuous or parenteral routes by mixing them with oral routes. Antibiotics given with feed can not wait for at least 10 days required to stop after using antibiotics since they are consumed as much as chickens. The antibiotics that enter the chickens' bodies are digested and consumed before the consumer is thrown out. More serious sanctions, punishments, inspections, and antibiotic residues in chickens we consume can be reduced to the producers. In our country, there are no healthy persons related to the usage amount of veterinary medicines. This is especially possible with the application of data matrix and traceability schemes in medicines. However, the number of licensed medicines will give an idea on this point.

As a result, it is observed that antibiotic usage continues to be uncontrolled in chicken production in our country. More careful checks must be carried out by authorities in response to problems that will result in the presence and persistence of antibiotic residues in food for environmental and human health. Otherwise, it will be difficult to treat the bacterial strains that will be formed in the human body. The penetration of antibiotic residues spreading around the earth into the human body with water and water again is an important question that will negatively affect both us and future generations.

Since Elisa test and MeRA test were compared; The MeRA test has proven to be cheaper, more practical and quicker in terms of evaluation and operation.

In this study, it is aimed to make producers and consumers more conscious. Although the study we conducted shows similarity with the results of similar studies, it is necessary to repeat both antibiotic residue amounts used in different seasons by increasing the number of samples and comparatively expanding the antibiotic residue amounts used in different seasons.

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