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The Effect of Cooking Techniques on Chicken Breast in Antibiotic Residue Level

MASTER THESIS

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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 26/08/2019 tarih ve 2019/14-14 sayılı kararı ile onaylanmıştır.

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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

ADI	Acceptable Daily Intake
AGP	Antimicrobial Growth Promoters
AMR	Antimicrobial Resistance
EFSA	European Food Safety Authority
EU	Europian Union
ELISA	Enzyme-Linked Immunosorbent Assay
IMS	Immuno-Magnetic Seperation
IPM	Integrated Production Model
MeRA	Meat Rapid Antibiotic
MFAL	Ministry of Food, Agriculture and Livestock
MRL	Maximum Residue Level
NOEL	Non Observable Effect Level
PBP	Penicillin Binding Proteins
PCR	Polymerase Chain Reaction
PFA	Phytogenic Feed Additives
SPP	Species
UK	United Kingdom
WHO	World Health Organization

ABSTRACT

Doğan, N. G. (2019). The Effect of Cooking Techniques on Chicken Breast in Antibiotic Residue Level. Yeditepe University, Institute of Health Science, Department of Nutrition and Dietetics, MSc Thesis, İstanbul.

Poultry meat has important role of the nutrition because of its nutritional value. In poultry husbandry, antibacterial agents have been used in order to meeting the increasing demand and preventing the diseases. Chloramphenicol usage is forbidden in poultry meat. This study investigates detection of chloramphenicol in chicken breasts.

Chicken samples were bought from 10 different brands in İstanbul. For each brand, 100 grams of chicken meat were calculated and seperated from 5 pieces as 20 grams. First 20 grams of chicken were mixed with blender and were put into sterilized plastic bag and labeled. Second group was boiled in steel pan for 15 minutes. They were mixed with blender and were put into sterilized plastic bag and labeled. Third group was baked in oven for 16 minutes in 200 degrees celsius. They were mixed with blender and were put into sterilized plastic bag and labeled. Fourth 20 grams samples were grilled in for 5 minutes. They were mixed with blender and were put into sterilized plastic bag and labeled. The last group was fried for 5 minutes in sunflower oil that was heated before. Samples were mixed with blender and were put into sterilized plastic bag and labeled. These steps were applied on each brands of chicken meat.Firstly antibiotic residues were determined qualitatively with MeRA test. According to results of MeRA test, 8 samples were negative. Then residue levels were detected with ELISA quantitatively. According to ELISA test results, samples were negative with chloramphenicol. This study is done in İstanbul. Further researches are needed with more samples and other countries.

Keywords: Antibiotics residues, antibiotics, MeRa test, ELISA test.

ÖZET

Doğan, N. G. (2019). Pişirme Tekniklerinin Tavuk Göğüs Etinde Antibiyotik Kalıntı Düzeyine Etkisi. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Beslenme ve Diyetetik Bölümü, Yüksek Lisans Tezi, İstanbul.

Beslenmede içeriği sayesinde önemli bir yeri bulunan tavuk eti yetiştiriciliğinde artan talebi karşılamak ve oluşabilecek hastalıkları önlemek adına antibakteriyel ajanlar kullanılmaktadır. Kloramfenikol kullanılması kesinlikle yasak olan bir antibiyotik türüdür. Bu çalışma tavuk göğüs eti örneklerinde kloramfenikol taranması amacıyla yapılmıştır. Örnekler İstanbul'da 10 farklı markadan alınıp, 100'er gram olarak ayrılmıştır, 100 gramlık örnekler 20 gram olarak 5 parçaya bölünmüştür. İlk 20 gramlık grup çiğ olarak blenderdan geçirilip steril poşete konulup etiketlenmiştir. İkinci grup 15 dk tencerede haşlanıp, blenderdan geçirilip etiketlenmiştir. Üçüncü grup 16 dakika 200 derece fırında pişirilip,blenderdan geçirilip etiketlenmiştir. Dördüncü grup 5 dk ızgara bekletilip, blenderdan geçirilip etiketlenmiştir. Son grup önceden ısıtılmış yağda 5 dk kızartılıp, blenderdan geçirilip etiketlenmiştir. Öncelikle örneklerdeki antibiyotik kalıntıları kalitatif olarak MeRa test ile tespit edilmiştir. MeRA test sonucuna göre 8 örnek negatif çıkmıştır. Ardından antibiyotik kalıntıları kantitatif olarak ELISA test ile belirlenmiştir. Sonuçlar bütün örneklerde negatif çıkmıştır. Bu çalışma İstanbul bölgesinde yapılmıştır. Başka şehirlerden daha fazla örneklem ile çalışmalar yapılmalıdır.

Anahtar kelimeler: Antibiyotik kalıntıları, antibiyotikler, MeRa test, ELISA test.

1. INTRODUCTION

Consumption of chicken meat is important in point of nutrition and economical both world and Turkey. However, studies in recent years and social media creates negative perception about chicken meat consumption.

Investigation of antibiotic residues at eatable parts of chicken is significant for public health. The aim of this research is investigation of chloramphenicol residues and the effect of cooking techniques on chicken breast in antibiotic residue level.

2. GENERAL INFORMATION

2.1. Chicken Meat in Nutrition

In an average adult, the 16% of the body consists of protein. Amount of resorvoir protein is low in the body and is composed of mostly cells that have special mission. Protein is used for growth, renewing tissues and organs, also structures of some enzymes and hormones that participates immune system.

In plant origin foods, digestion of essential aminoacid is low. In plant origin protein some of essential aminoacid (lysin, cysteine, cystine) are not enough than requirements (1).

Knowledge of purchasing choice of animal origin foods is important that determining models of nutrition. Poultry meat is one of significant animal origin food that is needed for physically and mental devolepment, healthy and balanced nutrition (2).

If the chicken meat is viewed by nutritional value, it has lower saturated fat and higher protein amount (3). In addition, it is rich of vitamins and minerals, easy to prepare, ingredients of various recipes and most importantly it is cheaper than red meat. These features causes to be prefered chicken meat (4).Nutritional Values of Chicken Meat is given below (Table 1).

In Turkey, there are some researches about chicken meat consumption. According to results of one of these researches, individual consumption in a year is 16,67 kgs. Another research in done at Erzurum, found individual consumption in a year is 10,8 kgs that was higher than red meat and fish consumption (4).

Table 1: Nutritional Values of Chicken Meat

	Chicken Breast	Chicken Leg
Energy (kkal)	116	126
Protein (g)	21,8	19,1
Fat (g)	3,2	5,5
Sodium (mg)	72	89
Potassium (mg)	330	300
Calcium (mg)	10	11
Magnesium (mg)	27	22
Iron (mg)	0,5	0,9
Copper (mg)	0,14	0,25
Zinc (mg)	0,7	1,6
Vitamin B6 (mg)	0,53	0,3
Folic acid (mcg)	8	12
Biotin (mcg)	2	3
Pantoneic acid (mcg)	1,2	1,3
Thiamin (mg)	1,1	0,11
Riboflavine (mg)	0,1	0,22

2.2. Antibiotics

Antibiotics are chemical or natural materials that is produced fungi or similar microorganisms, block growth of microorganisms and other organisms and even kill them. The term of antibiotics originated from Greek words “anti” means against and “bios” means life. It is also defined as common name of ‘a material that is produced from plants especially fungus or artificially which blocks growing bacteria or other microorganisms or destroy them’. Antibiotics that biologically or artificially obtained are very efficient bioactive materials. There are a lot of antibiotics depending their effects and effected microoganisms. They are used for human and animal health, protection of foods in food sector, health and growth of aquatic creatures like fish and scientific researches in hospitals and drug industry (5) .

The most popular drug that is used all around the wolrd is antibiotics.

Unfortunately, it's usage is mistake and excessively among developing countries. Except benefits of antibiotics, it should be keep in mind that they also have harmfull effects. That's why, decreasing usage of antibiotics in Turkey should be provided with precautions (5) .

Antibiotics those supress growth of microorganisms or kill them are very effective bioactive materials which have biological origin or synthetically obtained. There are many antibiotics according to the effect manner of them and the microorganisms they effect. Antibiotics are used for human and animal health, food protection in food industry, health and growth of aquatic livings as fish and in hospitals and scientific activities in pharmaceutical industry. Antibiotics are the widely used pharmaceuticals in whole world. Antibiotics are used wrongly and excessively in developing countries. Furthermore, antibiotics could be sold in pharmacies even in markets without prescription. It should be born in mind that antibiotic usage has damages together with benefits. Therefore, required cautions have to be taken for the decrement of antibiotic consumption in our country (5) .

Antibiotics that is originated naturally are produced and released by most of mold and bacteria. Up to 1950, half of antibiotics that known today have been discovered and 'golden age' for them has been came through. Antibiotics still conserve their importance for modern medicine. Their usage as therapeutics against infectious disease, as prophylactic for cancer treatment and transplantation surgeries is proceeding (6) .

2.2.1.Classification of Antibiotics

There are many ways to classify antibiotics. On the basis of chemical structure, origin, range of activity (spectrum of activity), mode of action, effects of their activity and route of administration (6).However, the most common classifications are on the basis of effects of activity and on the basis of mode of action (7). In this study, antibiotics are classified as effects of their activity. It brances two groups in itself according to concentration of body liquids and effect on microorganisms; bacteriostatics and bactericidals (7).

Bakteriostatics inhibit growing and reproduction of bacteria. Bacteriostatics are supposed to require phagocytic cells to kill bacteria.So they aremore ineffective with an inefficient immune response. This situation causes illness.

Therefore immunosuppressed patients with bacterial infections should be treated with bactericidal antibiotics (8).

Tetracyclines, Macrolides, Sulphonamides, Amphenicols, Lincosamides, Metronidazoles and Myconazoles are in this group. The indicator of power of bacteriostatic effect is Minimum Inhibatory Concentrations (MCIs) (7). MICs are considered to gold standard to determine the susceptibility of organisms (9).

Bactericidalskill bacteriaand have more powerful action. Aphagocyteindependent killing with bactericidal drugs is generally recommended under such circumstances (8).Beta-lactams,Polypeptides, Fluoroquinolones, Vancomycins, Rifamycins and Teicoplanins are in this group. The indicator of power of bactericidal effect is Minimum Bactericidal Concentration (MBC)(7).

2.2.1.1. Tetracyclines

Tetracyclines are broad spectrum antbiotics that affect either gram positive or gram negative bacteria. Because of increase of resistance, tetracyclines usage decreases. However tetracyclines are used for acnes, infections of urinary tract and respiratory system (6).Tetracyclinesprevent binding of t-RNA of the cells to the A site (10).

2.2.1.2. Macrolides

Macrolides generally affect gram positive and intracellular pathogens. Macrolides destroy protein synthesis in ribosome. Their effects are bigger than penicillines. Even some species of bacteria develop resistance to macrolides, they are most second prescribed group of antibiotics (6).

2.2.1.3. Sulphonamides

Sulphonamides are discovered at 1930 which generally used with trimetoprims. Both two antibiotics affect folic acid metabolism which is required for synthesis of nucleic acid. At the present time, usage of sulphonamides is restriced that may cause developing bacterial resistance and liver damage (6).

2.2.1.4.Amphenicols

This group includes chloramphenicol which has importance of this study (6). Amphenicols block to bind t-RNA to the A site (10). In this way, synthesis of protein

stops. Chloramphenicols are broad-spectrum antibiotics that are found either naturally or produced with chemical synthesis (6).

Usage of chloramphenicols are in the treatment of penicillin-allergic or resistant patients with bacterial infections caused by resistant to other antibiotics (11).

Chloramphenicols are administered orally, intravenously, or intramuscularly. Oral preparations are quickly absorbed from the gastrointestinal tract with peak blood levels reached with two hours. Chloramphenicols are spreaded to most body compartments, tissues, cerebrospinal fluid, the eye, and joints (11).

Chloramphenicols are toxic and usage of chloramphenicols is not prevalent thathave side effects include aplastic anemia, thrombocytopenia, or leucopenia (6), (11).

In developed countries, chloramphenicols are used just vital situations. In developing countries, prescription of chloramphenicols is more common because of its cheap and easy to reach. In Turkey, usage of this group of antibiotics is restricted and not allowed to asfind residues in foods(6).

2.2.1.5.Lincosamides

Lincosamides are derived from galactosides that includes aminoacids an done sulphuride. It is a natural antibiotic that generated from Streptomyces Linconensis.
Lincosamides bind 50S ribosome therefore synthesis of protein is inhibated (6).

Lincosamides are effected on gram positive bacteria and anaerobic bacteria. Species of Pseudomonas and Acinetobacter are resistant to Lincosamides (12).

Lincomycin which is subgroup of Lincosamides is used for treatments of cattles however it is not suggested due tos ide effects (13).

Lincomycin is used at poultry farms as increasing performance and inhibating bacterial enteric infections (6).

2.2.1.6. Metronidazoles

Metronidazoles have been used for 45 years. They are used forthe treatment of trichomoniasis, amoebiasis, and giardiasis. They are low cost and have insginificant side effects (14).

2.2.1.7.Beta-lactams

This is the most important and most preferred group of medication at worldwide. More than 70 years, Beta-lactams have been used efficiently for infections caused by gram negative coccus. This group is quite wide and includes narrow spectrum penicillines, Cephalosporins and Manobactams which are only affect gram negative pathogens (6).

Beta-lactams primary target is the Penicillin binding proteins (PBP). Betalactams affect cell wall that inhibate cross link of the D- alanyl D- alanine side of peptide chain that is normally bound by PBP when peptidoglycan chains are created
(10).
Penicillins and Cephalosporins are not used treatment of just human also used pets (6).

2.2.1.8.Polypeptides

Bacittracin A, Polymyxin B and cholistin are the part of polypeptide group (6).The glycopeptides binds to D- alanyl D- alanine side ofthe chain and prevents binding with the PBP, therefore, inhibits synthesis ofcell wall (10).

2.2.1.9.Fluoroquinolones

Fluoroquinolones broke the replication of DNA through DNA gyrase inhibator (12). Fluoroquinolones are broad spectrum antibiotics. Especially they affect on gram negative bacteria, species of mycoplasma and some species of gram positive bacteria (Staphylococcus). They can not make an effect at place without oxygen.
Fluoroquinolones may deform to progress cartiloginous tissue at offsprings. Therefore, using of this group of antibiotics is avoided on offsprings and pregnants (13).

2.3. Poultry Husbandry

First of all poultry house must be clean well with high pressure water and is waited drying. Growing poultry is needed place that is important to be clean, bright and low humidity (15).

In Turkey, integrated production model (IPM) is used to manufacture poultry. IPM includes mix feedmill, stocks, hatcheries, rearing stations, slaughter house, rendering stations, filtration station of effluent wastewater and marketing network. (16).

Chickens for breeding are fed for 24 weeks under appropriate temperature and humidity cicumstances. After 24 weeks animals start to lay eggs. These eggs are sended brood machine that is similar to original brood. After 21 days, broiler chicks which are showed up from these eggs, are controlled by veterinarians and are done vaccines, after that are send to broiler coop for 40-45 days. (16). Afterwards, chickens who achieve to 2-2.5 grams are send to slaughterhouses (16).

The requirements which animals should be managed are the keys to good standarts or principles that are the quality and class of stock, confining the birds which provides protection from predators and reduces the labour and productioncosts, protection from a harsh environment, welfare needs, maintaining good health which involves prevention, early recognition and treatment of disease, nutrition for economic performance, practice of good stockpersonship ‘’the harmonious interaction between the stock and the person responsible for their daily care’’ having a right attitude, the maximum use of management techniques, keeping records and lastly marketing (17).

2.4. Use of Antibiotics in Broiler

Since 1950, antibiotics are used for healthy development of digestive system and control of subclinical diseases. Beside the goal of treatment, antibiotics are also used for growth in feed stuff. However, when it was understandable that continuous usage of antibiotics led to bacterial resistance and found resiudes at broiler chickens, usage of antibiotics on the purpose of growth was forbidden since 2006.

Animals who takes antibiotics with the purpose of treatment, must not slaughter before legal washout period (18).

Antibiotics do not affect against fungal and viral pathogens. Antibiotics treat infectious diseases arising from bacteria (19). For instance, intestinal infections such as colibacillosis, necrotic enteritis, and other diseases caused by Salmonella, Clostridium spp. or E. coli (20).

In 1940, anabolic effects of antibiotics are discovered at USA while gaining weight is observed with addition of antibiotics in feed of chicks (21).

It is important that usage of veterinarian medicines controlly and consciously and observing residue levels. The reasons of residues are slaughter of animals without waiting for an enough time, applying high dosage or frequent intervals of antibiotics and choosing unlicensed drugs. There are few terminologies to explain evaluation of residue levels (22).

Acceptable daily intake (ADI)

It can be defined as the maximum amount of a chemical to that a person can be exposed, on a daily basis over an extended period of time, with minimal or zero risk of adverse effects, and if the ingestion exceeds, this amount may cause toxic effects. The ADI is in the food, per kilogram of body weight per day (mg kg_ 1 day_ 1), (23).

Non observable effect level (NOEL) and safety factor must be known to determinateADI for a chemical. Experiments are done with a drug or a chemical that is not an anticarcinogenic, on at least two mammals. Safety factor calculates according to these experiments. People are considered sensitive 10 times than mammals and differences between humansso another 10 times are added. Therefore safety factor is identified. (22).

Maximum residue level (MRL)

It is the highest level of a pesticide residue which is legally tolerated in or on food or animal feed when pesticides are applied correctly and also it has 4 types. The most type is 0 tolerance. It means that some matters must not be found in foods like stilbenes, steroids, beta-agonists, chloramphenikol, dimetridazole and metronidazole (22).
In recent years, researchers try to alternative growing matters replacing antibiotics. Probiotics, organic acids and enzymes have examined(21).

2.5. Detection of Antibiotics

Microbiological methods are separeted as traditional and rapid methods. At the present time, tradititonal methods are accepted as gold standards. Rapid methods are used for identification and enumeration of bacteria, fungus,virus and protozoa, isolation of their metabolites and resistance of antibiotics (24).

Anticor-antigen reaction is important that understanding of characterization of microorganisms. Immunologic methods are preferred to define the food contaminants, pesticides or veterinary medicines (25).

Latex aglutination test , Enzyme-Linked İmmunosorbent Assay (ELISA), Lateral Migration İmmunoassay and İmmuno-Magnetic Seperation (IMS) tests are included in the mentioned methods (24).

Latex Aglutination Tests are used mostly finding toxins. ELISA is the most used test in present days to react antigen with anticor. In this method, antigen or anticor is marked with an enzyme and immunological reactions is measured as a result of

enzymetical activity. Lateral Migration Immunoassay test kit has 3 reaction areas. First area includes anticors that bond with color particules. Second area has another anticors who cover first group that causes blue line. Over-anticors collapse over the third region. All the procedure lasts 10 minutes. IMS, metal particules are covered with anticors and mixed. After the reaction, test tube is placed in magnetical power source. IMS system can be combined with other diagnostic methods (24).

The most used methods for the determination of antibiotics are chromatographic, electrophoresis, diode array or ELISA. For instance, fluoroquinolones are detected by microdialysis–liquid chromatographic system (26). For the detection of bacteria, some methods based on nucleic acid like Polymerase Chain Reaction (PCR) or immunologic reactions have been developed (27). Meanwhile PCR can give reliable and correct result of diagnosis of mycoplasma species (28). Determination of chloramphenicol residues in the food, high performance liquid chromatography (HPLC) is used (25).

2.6. Impacts of Antibiotic Residues

As all chemicals or medicals, antibiotics can cause side effects too. It can be examined on healthof human and animal, on the environment and meat quality.

2.6.1. Impacts of Residues on People

Some researches show that clenbuterol causes food poisoning and muscle tremors, palpitations and tachycardia at times. Chloramphenicol causes potential problems (19).

Some studies show that colchicine and chloramphenicol have multiple effects on neutrophil function and indicates that factors related to the ingested particle exert significant influences on the metabolic response of the neutrophil to phagocytosis (29).

Antibiotic residues can cause type I drug allergy. Especially beta-lactams can cause this type of allergy. Penicillin and Sulphonamides can responsible for the most serious allergy (21). Chloramphenicol can suppress bone marrow that the result of allergic reaction (19).

Long term usage of antibiotics can have carcinogenic effect for human health. Chloramphenicol, nitrofurans, imidazole compounds, some sulfonamids are in the list of carcinogenic effects (25).

Cholistin, chloramphenicol, streptomycin and sulphonamides can cause paraesthesia that is investigated in neurotic impacts (25).

Tetracycline, sulphonamides, chloramphenicol, cephalosporins, ampicillin, isoniazid and quinolone can cause gastro-intestinal disorders like nausea, vomit, diarrhea, infection and decreatin of synthesis of vitamin K and B (25).

Lastly, usage or indirect usage of antibiotics causes antibiotic resistance that defines as not interaction between drugs and bacteria (21).

2.6.2 Impacts of Residues on Animals

Some studies show that usage of some species of antibiotics causes lactobacillus in poultry intestines, this situation may decrease intestinal activity that affects the immune system of chickens (19). In addition to this effect, usage of antibiotics can cause antibiotic resistance and functional problems at poultry as well (21).

2.6.3. Impacts of Residues on the Environment

Over usage of antibiotics results in bioaccumulation, contamination of aquatic ecosystem and the soil. This spread can cause epidemic diseases and threats public health (19).

2.7. Legislative Regulations of Antibiotics in Poultry

There are numerous regulations about using antibiotics in poultry.

2.7.1. In the World

It can be classified as degrees of limitation of antibiotics.

2.7.1.1. Restricted Countries

European Union

In European Union (EU), firstly usage of avoparcin was forbidden in 1997. In 1999, some antibiotics that used for growth factors were forbidden. In 2006, all antibiotics that used for growth were banned (30).

Taiwan

Taiwan allows some kind of antibiotics for growth (30).

Netherlands

Olaquindox and carbadox were forbidden because of the effect of toxicity and carcinogenic in 1997. In Netherlands , veterinary prescriptions are necessary (30).

Germany

In 2008, Germany legislated national antibiotic resistance strategy is named Deutsche Antibiotika-Resistenzstrategie or "DART". DART controls antimicrobial resistance (AMR) and creates datas (30).

Denmark

In Denmark, in 1995, avoparcin was forbidden and the veterinarians were inspected on the purpose of profits from antibiotic sales. In 1998, industry of poultry voluntarily stopped using AGPs (30).

Sweden

In 1986, Sweden was the first country to forbid AGPs in food animal production. Sweden allows usage of antibiotics in case of curing or preventing disease (30).

2.7.1.2. Semi-restricted Countries

United Kingdom

Since 2012 the United Kingdom (UK) poultry meat industry has usen measures proposed by the British Poultry Council on the use of antibiotics classed by the WHO as 'critically important'. Since that time, the industry has phased out use of the modern cephalosporins completely and use of the fluoroquinolones for disease prevention in one-day-old chicks (31).

Mexico

Federal Law of Animal Health in Mexico, restricts most of AGP use in animal feed and requires a veterinary prescription excluding avoparcin, vancomycin, bacitracin, tylosin, virginiamycin, avilamycin, bambermycin, spiramycin, salinomycin, and monensin (30).

Japan

Food Safety Commission of Japan restricts some uses of antibiotics (30).

South Korea

South Korea wants a veterinary prescription to use antimicrobials. In 2010, South Korea announced a ban that limits the amount of drugs added into premixed animal feed (30).

Hong Kong

Agriculture, Fisheries and Conservation Department of Hong Kong restricts AGPs. Veterinary prescription is also required by law (30).

2.7.1.3. Unlimited Countries

Canada, China (excluding Hong Kong), Australia, Brazil and Ukraine do not have any formal national restrictions on AGPs and only have limited requirements to obtain veterinary prescriptions (30).

2.7.2. In Turkey

Since 2006, antibiotic use for growth promotion has forbidden. Antibiotic use for treatment on food animals, has to complete legal washout period before present consuming. Antibiotics that are allowed to use for treatment are take license from Ministry of Food, Agriculture and Livestock (MFAL) (18).

MFAL is the authority in the fields of food safety, veterinary and phytosanitary and responsible for developing policy, legislation and the enforcement for food and feed safety, animal health, animal welfare and plant health in Turkey (32).

Turkey has National Food Codex Commission consists of seven members that represent MFAL with two members, Ministry of Health with one member, Turkish Standard Institution with one member, Universities with two members and NGO's with one member. This Commission is responsible for preparing Turkish Food Codex which includes legislation technic and hygienic properties of foodstuffs (32).

Veterinary residues are conformed with provisions that published at 7/3/2017 and 30000 official gazette at Turkish Food Codex Regulation on Classification and Maximum Residue Limits of Pharmacologically Active Substances in Foodstuffs of Animal Origin (33). This regulations are enclosed herewith 1 and also unpermitted pharmacological active matters are demonstrated (Table 2)

Table 2: Unpermitted Pharmalogical Active Matters

Pharmacological Active Matter	Maximum Residue Level
Aristolochia spp.	Can not be seen
Dapsone	Can not be seen
Dimetridazole	Can not be seen
Chloramphenicol	Can not be seen
Chlorpromasine	Can not be seen
Colchicine	Can not be seen
Nitrofurans	Can not be seen
Nitrofurans	Can not be seen
Ronidazole	Can not be seen

2.8. Ways to Decrease Antibiotic Residues

Cooking and freezing operations are significant in inactivation of antibiotic residues. The heat treatment of animal origin foods may inactivate antibiotics. Most of the studies have demonstrated that degradation of Beta-lactams, tetracyclines,quinolones, sulfonamides, macrolides and aminoglycosides are temperature-dependent. Therefore, prolonged heating helps to induce degradation. Activated charcoal, resin and UV irradiation might be useful inactiviation of antibiotics. Easy and economic field tests may be developed to identify antibiotic residues in animal foods and ethno-veterinary practices may be promoted (34).

2.9. Alternative Options for Antibiotics

In recent years, doubts against antibiotics prompt researchers to find other choices instead of antibiotics. Some research has been done less harmful but more harmless agents for growth. Most accentuated agents are phytogenic feed additives, essential oils, probiotics, prebiotics, organic acids, enzymes, immunostimulants, bacteriocins, bacteriophages, phytocides and nanoparticles (19).

Phytogenic feed additives (PFA) derived from plants, herbs and spices are examined to develop poultry. Some researchers show positive effects of growth and immune system that were done with cinnamon, Lippia javanica, mixture of garlic and

black pepper, Cratoxylum formosum, fennel, melissa, peppermint, anise, oak, clove, thyme, pennyroyal, neem and black seed (19).

Essential oils are plant derived aromatic compunds. Some studies show that thymol, transcinnamaldhyde, carvacrol, oregano and eugenol can increase growth, improve chicken production and immune system (19).

Recent researches demonstrate that use of probiotics such as Enterococcus faecium, Streptomyces species and Bacillus subtilis strength immune system. As these probiotics mannose and mannoproteins that are the kind of prebiotics can also increase intestinal health thus immune system. Bacillus subtilis has an effect for growth. Some probiotics such as Aspergillus awamori, Bacillus licheniformis and Saccharomyces cerevisiae have positive effects to increase poultry meat quality (19).

Organic acids can be produced by microbial processes. Most of organic acids are natural products of microorganisms (35).

Some studies showed that organic acids like citric acid, formic and propionic acid promote growth, prevent Campylobacter and E. Coli infection (19).

Lastly, other options to antibiotics such as milk and propolis, also show same effects (19).

2.10. Cooking Techniques

Cooking techniques varies from countries to countries and different cooking techniques are used providing desired taste and smell (36).

The purpose of cooking is to make chicken meat palatable, digestible and microbiologically safe. Meat products undergo many changes during cooking, such as weight loss, water holding capacity, texture, and color and aroma. The characteristics of quality are affected by the composition and type of the muscle, cooking method, and cooking time and temperature (37).

In the worldwide, blanching, boiling, braising, deep frying, shallow frying, glazing, grilling, poaching, roasting, pot-roasting, simmering, steaming, stewing, tandoori, baking, infrared, sous-vide and microwave are the methods of cooking that most common used (36).

2.10.1 Boiling

Boiling means cooking foods in boiling water. Boiling effects meat getting soft via the fiber and the connective tissue dissolving (38).

2.10.2 Baking

Baking is cooking food in an oven with dry heat. It meets same meaning with roasting for meats and vegetables in some countries. Over time cookingcauses losing moisture (38).

2.10.3.Grilling

Grilling is a fast, dry method of cookery which uses the intense heat radiated by an electrical element,gas flame,glowing charcoal or an open wood fire.The heat source can be either above or below the food (38).

2.10.3 Frying

Deep frying involves fully immersing food in hot oil. It is a really fast cooking method, and it is classified as a dry cooking method although the use of liquid oil, is best however it does not involve liquid water.Pan-frying and stir-frying include similar coatings of fat, but are less visually striking (38).

3. MATERIAL and METHOD

3.1. Material

10 different brands of chicken were bought from meat counter of one of grocery store chain in İstanbul (Picture 1). Chicken meats were reached to laboratory with cold chain.

Picture 1: Chickens at meat counter undercondition of cold stage.

For every brands, 100 grams of chicken meat were calculated with precision scales and they were seperated from 5 pieces as 20 grams. They were named uncooked, boiled, baked, grilled and fried respectively.

Firstly, 20 grams of raw meat were mixed with blender and were put into sterilized plastic bag and were labeled and were waited in the refrigetor (Picture 2).

Picture 2: Samples were put in plastic bag and labeled.

Second 20 grams samples were boiled in steel pan for 15 minutes. They were mixed with blender and were put into sterilized plastic bag and labeled. Third 20 grams samples were baked in oven for 16 minutes in 200 degrees celsius. They were mixed with blender and were put into sterilized plastic bag and labeled. Fourth 20 grams samples were grilled in for 5 minutes. They were mixed with blender and were put into sterilized plastic bag and labeled. Lastly, fifth 20 grams samples were fried for 5 minutes in sunflower oil that was heated before. Samples were mixed with blender and were put into sterilized plastic bag and labeled.

These steps were applied on each brands of chicken meat.

3.2. Method

In the first place, antibiotic residues in the samples were determined qualitatively with MeRA test. Thereafter, chloramphenicol levels of the samples were detected with ELISA test quantitatively.

3.2.1 MeRA Test

MeRA test is the microbiological test that includes Geobacillus stearothermophilus spores to detect residues of antimicrobial agents in meat products (39).

Each package of MeRA test contains 50 tubes containing liquid culture medium and 50 discs containing spores of Geobacillus sterothermophilus (Picture 3).

Picture 3: MeRA test package.

The test detects residues of antibiotics and sulphamides through their inhibition action against the growth in vitro, at 64 ± 0,5 °C (40).

Some antimicrobial agents are sensitive to heat therefore the molecules that members of these chemical class, are inactivated undercondition of growth heat of thermophilic bacteria. MeRA test includes pre-incubation step that provides growth and production of G. Stearothermophilus. There is an interaction between vegetative form of G.stearothermophilus andantibiotics in samples, if there is in (39).

Construction of MeRA test:

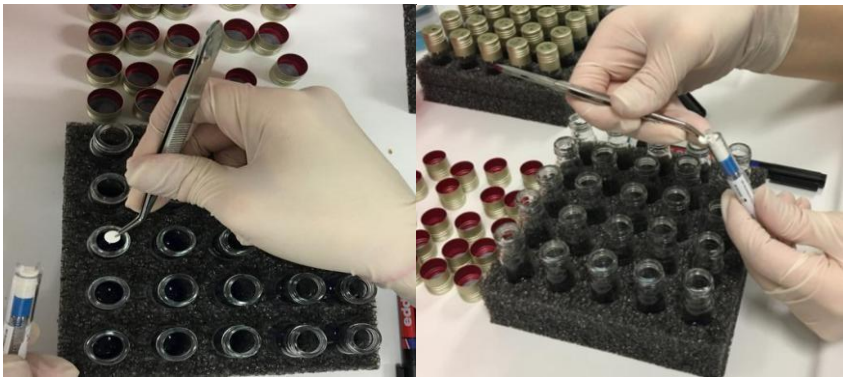
1. The chicken meat samples that were prepared before were taken 2 grams with pipette and they were tranferred into 10 mililiters test tube. 6 mililiters of distillate water was added into test tube. The ratio between chicken and water has to be 1:3 (Picture 4).

Picture 4: Preparation of samples.

2. The sample with the tube were homogenized with vortex.
3. Then the test tube was centrifuged at 4000 rpm for 15 minutes (Picture 5).

Picture 5:Centrifugion of samples.

4. 1 spor disc was put into the liquid culture medium (Picture 6).



Picture 6:Spor discs were put in the liquid culture medium.

5. The solution and spor disc were pre-incubated for 20 minutes at 64 °C.
6. Incubated solution was waited until room temperature.
7. 1 ml homogenized supernatant (the sample) was added into incubated solution. The solution was waited for 20 minutes at room temperature if there was an antimicrobial agent, it had been affected.
8. The sample was incubated at thermoblock at 64°C for 3 to 3,5 hours (Picture 7).

Picture 7: Samples at thermoblock.

9. After observing the colour change in the test tube, if there isn't colour change (Blue-Green Coloured): Antimicrobial agent residue in the sample is admitted as above the recognition limits (Picture 8). If there is colour change (Yellow

coloured): Antimicrobial agent residue in the sample is accepted as below the recognition limits or non-existent (39).

Picture 8:Yellow coloured and blue-green coloured.

3.2.2. ELISA Test

ELISA determines a quantitative level of the presence of chloramphenicol. This test has a sensitive detection systemdue to the use of anticor specifically developed for the targeted molecule. This test has easy preparation and short-time (41).

A typical ELISA kit is composed of 96 wells, coated by anticor developed against targeted veterinary medicine. Analysis is made with these wells, by adding enough sample extract and a range of reactive and after then by washing up. At the end of the analysis, with the help of the spectrophotometer amount is determine. Some liquid samples being diluted by buffer solution are directly analysed; solid samples (chicken) should be mixed with extraction solvent before ELISA and requires filtration.
(25).

Preparation of samples:

1. 4 grams of chicken meat subject was put into 50 ml falcon tube. 20 of 50 mm of succinic acid were added.
2. Falcon tube was put shaking incubator for 15 minutes at room temperature.

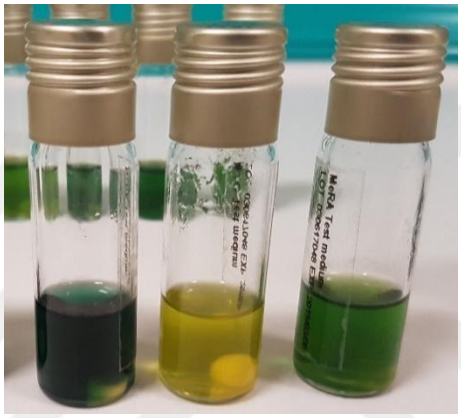
3. Then the tube was centrifuged at 4000 rpm for 15 minutes.
4. After centrifuge, supernatant has been diluted 1/10 (100 µl supernatant, 900 µl PBS- Phosphate buffered saline). 50 µl of the mixture were used in the experiment.
5. Concentrates standards were diluted. For each standard (50 µl), 450 µl sample buffer were diluted with 1.

Construction of ELISA test:

1. The solutions in the kit and plate were waited at room temperature.
2. Test wells at a number of sample and standard were replaced to plate.
3. Standards and samples were pipetted into the 50 µl wells, respectively.
4. 50 µl anti-chloramphenicol antibody were pipetted into each well.
5. It was incubated for 1 hour under room temperature.
6. In automatic ELISA washer, it has been washed 3 times for each wash to come 250 µl wash buffer.
7. To each well, 100 µl conjugate was added with the help of multi-channel pipette and shaken and incubated in room temperature for 15 min.
8. In automatic ELISA washer, it has been washed 3 times for each wash to come 250 µl wash buffer.
9. To each well, 100 µl substrate/chromogen was added and shaken and incubated in room temperature for 15 min.
10. 100 µl stop solution was added and analyzed at ELISA reader using 450 nm filter.
11. Chloramphenicol values were counted interms of ppb using formula of absorbents sample/zero standart ‘’absorbents x100’’.

4.RESULTS

As seen from Table 2, 8 out of 50 chicken samples that were analysed with MeRa tests are detected as negative. As seen in Picture 9, blue-green colour has taken positive that shows antimicrobial agent concentration is above the limit of detection.Yellow colour has taken negative that means there is not any antimicrobial agent or if there are antimicrobial agent that is below the limit of detection.Therefore, 42 chicken samples are blue-green coloured (positive) and they are determined above the limits.



Picture 9:Blue, yellow and green colour.

After ELISA test chloramphenicol levels of the positive resulting samples have been watched., Antimicrobial residues in all samplesbelow the 0.03 as negative.

4.1. MeRA Test Results

Results were calculated qulitatively with this test. 50 samples were analyzed. 8 samples were negative (yellow coloured) and 42 samples were positive (green-blue coloured).

As seen from Table 2, all samples in raw state are positive. When sample 2 was boiled and baked has became negative. When sample 3 was boiled became negative. For sample 4 , all states are negative except raw state. This means, for sample 4, cooking are effective to eliminate antibiotics. Lastly, Sample 4 became negative just in case of grilling.

Table3 : MeRA Test Results

Sample	Raw	Boiled	Baked	Grilled	Fried
1	Positive	Positive	Positive	Positive	Positive
2	Positive	Negative	Negative	Positive	Positive
3	Positive	Negative	Positive	Positive	Positive
4	Positive	Negative	Negative	Negative	Negative
5	Positive	Positive	Positive	Positive	Positive
6	Positive	Positive	Positive	Positive	Positive
7	Positive	Positive	Positive	Positive	Positive
8	Positive	Positive	Positive	Negative	Positive
9	Positive	Positive	Positive	Positive	Positive
10	Positive	Positive	Positive	Positive	Positive

4.1. ELISA Test Results

All samples were seenbelow 0.03. It can be said as negative. All results seen at table 4.

Table 4 : ELISA Test Results

Sample	Raw	Boiled	Baked	Grilled	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

Protein is used for growth, renewing tissues and organs, also structures of some enzymes and hormones that participates immune system.

Poultry meat is one of significant animal origin food that is needed for physically and mental devolepment, healthy and balanced nutrition (2). Besides including good amounts of proteins and micronutrients and rich in n-3 polyunsaturated fatty acids, poultry meat is low in fats and cholesterol, particularly when it is consumed without skin. Chicken meat also contains glutathione, taurine and anserine (43).

These days consumption of poultry meat changes according to development level of countries. Individual consumption in a year was 19,3 kgs in Turkey, 43,2 kgs in USA and 22,9 kgs in Russia at 2012 (44). But now, individual consumption of poultry meat is 16,67 kgs (4). Another research done at Erzurum, found individual consumption in a year is 10,8 kgs that was higher than red meat and fish consumption (4).

Another research was done at 2013, with 301 students in Artvin shows that individual consumption of chicken meat in a week is 1,3 kgs and this result was interpreted as high (44).Other research was done at 2013, in Amasya with 380 people found individual consumption in a year is 5,18 kgs. This result was interpreted as adequate consumption to sustain healthy nutrition (45).

All foods have to be safe. Producers, consumers, the government have important role to prepare, choose and keep safe foods. Keeping foods safe provides public health and belief to brands and the legislatative regulations. One of the suspicious issue according to public is veterinary residues.

If all the necessary precautions are not taken along the poultry production, marketing and processing chains, poultry meat can be contaminated by infectious agents. Poultry products can be contaminated with the antimicrobial and anti-parasitic agents(46).

Antibiotics are chemical or natural materials that is produced fungi or similar microorganisms, they have lots of species. They are used for human and animal health, in food sector, in hospitals and drug industry (5). Since 1950, antibiotics are used for healthy development of digestive system and control of subclinical diseases. Beside the goal of treatment, antibiotics are also used for growth in feed stuff (18). In 1940, anabolic effects of antibiotics are discovered at USA while gaining weight is observed with addition of antibiotics in feed of chicks (21). However, when it was understandable that continuous usage of antibiotics led to bacterial resistance and found resiudes at broiler chickens, usage of antibiotics on the purpose of growth was forbidden since 2006.

Animals who takes antibiotics with the purpose of treatment, must not slaughter before legal washout period (18). It is important to use controlly veterinarian medicines. The reasons of residues are slaughter of animals without waiting for an enough time, applying high dosage or frequent intervals of antibiotics and choosing unlicensed drugs (22).Penicillin, cephalosporin, tetracyclines, chloramphenicols, macrolides, spectinomycin, lincosamide, sulphonamides, nitrofuranes, nitroimidazoles, trimethoprim, polymyxins, quinolones and macrocyclines are used for different animals and several purposes (47).According to FDA, the widest category of use in animals is tetracycline at 43 percent of the total, and ionophores, at 29 percent (48).

Chloramphenicols which are members of Amphenicols are broad-spectrum antibioticswhich affect many aerobic and anaerobic gram positive and negative except Nocardia species (spp.)and theyare used just vital situations (49). In Turkey, usage of this group of antibiotics are not allowed to find residues in foods (6). Because of serious side effectslike bone marrow suppresion, fatal aplastic anemia, neurotoxcicity, optic neuritis, gray baby syndrome (49). Usage of chloramphenicol in human may cause fetotoxicity and aplastic anaemia (50).

However in this study, according to MeRa Test results 42 out of 50 chicken samples are detected as chloramphenicol positive. According to ELISA Test all of the samples are positive.

According to WHO report, haemotoxic effect of chloramphenicol in humans can be described in two forms. The first one is a common, dose-related and reversible bonemarrow depression, that develops during treatment and reversible. The second form is a severe aplastic anaemia, which is not dose-related and often irreversible (50).

According to European Food Safety Authority (EFSA) report is published in 2018, reversible anaemia with or without leukopenia or thrombocytopenia, aplastic anaemia and the most seen haematotoxicity are the reported side effecs of chloramphenicol (51).

Another study was done on the 37 pregnant women with using100 mg of transvaginal chloramphenicol results in chloramphenicolwas detected from all maternal plasma specimens, vaginalchloramphenicol transfers to blood and maternal plasma concentrationvaried was associated with the administration day, but not with neonatal health (52).

Other study which was done at hospitals in Israel , aims to evaluate reasons of use of chloramphenicol. They used surveys and landed up use of chloramphenicol is 83.3%, the most application reason is treatment of aspiration pneumonia. 22.2% of doctors thoght

that chloramphenicol is harmfull but they believed chloramphenicol has a role in the treatment of infections in hospitalized patients because of its broadspectrum quality (53).

If we search the studies that effects of chloramphenicol on animals, some of these show that causes antibiotic resistance (18). A study claims that chloramphenicol shows genotoxicity but, owing to the lack of data, the risk of carcinogenicity and adverse effects cannot be determined but the same study also said that causes reproductive and liver toxicity in animals(51).

Usage of chloramphenicol is forbidden because of its side effects however some researches show that there are residues in food producing animals. The research done in Iran with 31 broilers shows 54.8% of broilers chloramphenicol has detected in kidney, liver and thigh muscle of chickens (55). Another research done in Iran shows 17.5% of 160 chicken contaminated with chloramphenicol mostly in liver (56). Other research also done in Iran, extrapolates 31.1 % of broilers contaminated withchloramphenicol (57). A research done in Philippines had resulted in 6 chicken breasts and 5 chicken livers out of 42 samples positive with chloramphenicol (58). Another research done in Turkey, 2 out of 25 chickens were contaminated with chloramphenicol but interestingly the research aims that consumption of chicken is not a public health problem according to results (59).

In this study, 10 different brands of chicken samples are contaminated with chloramphenicol.

Another important issue is that chloramphenicol whether pass or not to humans consuming foods. There are some researches address this problem. One of these said that undesirable effects of antibiotics such as poisoning, allergies and degradation of intestinal flora may be seen on people transferring residues from foods (54). Another study shows that antibiotic resistant bacteria may transfer to the humans (47). Another study said that consuming raw or low-cooked poultry meat has been referred as a potential risk factor for people cases of influenza H5N1 infection (46). Lastly, a research implies that theresidues of antibiotics or their metabolites in theanimal foods can cause adverse effects on health of the consumers, can occur antibioticresistance among human and can result in increasedhuman morbidity and mortality (60). However one of the research said that antibiotics may become ineffective in people if they are givento food producing livestock and poultry (48).

The most used methods for the determination of antibiotics are chromatographic, electrophoresis, diode array or ELISA.

The research that mentioned before residues, 31 broiler chickenswere homogenized, extracted using ethyl acetate and dried under N2 flow then ELISA was used (55).Another research with 160 chickens were analyzed with Four Plate Test, ELISA and HPLC for determining chloramphenicol (56). Other study done in Iran, ELISA was used to determinate amount of chloramphenicol in chickens (57). Other study that investigates chloramphenicol in chicken were analyzed with ELISA (58). A research done in Turkey, LC-MS/MS were used for analysis of concentrations and that reaches LC-MS/MS is usefull for detection of residues of multi-antibiotics (59).Amounts of antibiotics of chicken samples were detected by LC-MS/MS in Antakya (61). Another study done with eggs, chloramphenicol and tetracycline levelswere determined by ELISA method (21). Lastly, the research done with chickens, antibiotic residues were qualitatively detected with the MeRA Test. Positive samples were examined for tetracycline levels by ELISA (25).

In this research chicken samples were analysed qualitatively with MeRa tests in the first place then, chloramphenicol levels of the samples were detected with ELISA test quantitatively.

The aim of cooking is to make chicken meat palatable, digestible and microbiologically safe (37).

A study done at Argentina with chicken eggs examined drug residues in egg under different traditional cooking procedures. The eggs were cooked in boiling, microwaving and omelette making. The results of the study are albendazoleconcentrations were not affected by boiling and microwaving, flubendazole decreased with all methods and enrofloxacin and ciprofloxacin did not show important changes after any cooking method (62). Another study done at Spain, impacts of microwaving, roasting, boiling, grilling and frying were investigated. It bring to a concusion that cooking procedures did not affect enrofloxacin residues but there was a decrease in quinolone concentration (63). A review shows that degradation rates, for liquid matrix at 100 °C, β-lactams = tetracyclines (most heat-labile) > lincomycin > amphenicols > sulfonamides > oxfendazole> levamisole (most heat-stable) (64).

In this study, antimicrobial residues decreases by boiling the most. However the residues does not over. Baking is the second choice that shows decrease. Frying is the worst choice because there are some samples that the residues increases with frying. In

literature there are some studies claim that plants and probiotics can be used instead of antibiotics.

A review study explains thyme is a good alternative for antibiotics in poultry nutrition because it has qualities such as; kills pathogen microorganisms, inhibits toxins in feed stuffs, activates digestion enzymes and empoweres immune system. However dosage, chemical compositions, interactive relations and periods of availability can be considered(65).In literature, antihepatotoxic, antibacterial, antifungal, antioxidant and antimalarial qualities of absinth has mentioned. If the right dosage can be determined, absinth may be used for antibiotics (66).A research claims that with the optimal dose and correct strain of probiotic microorganism will be the best alternatives for antibiotics (67).Cinnamon and mixture of garlic and black pepper used on broilers that causes gaining weight.Oregano essential oil also causes gaining weight in broilers (19). Probiotics has so many effects such as; supports growth improves instestinal health and meat quality and increases protein amount. A significant amount of studies supports mentioned qualities of probiotics and implies usage of probiotics instead of antibiotics (19).

As a conclusion, in this study 10 different brands of chicken meat were bought. For each brand, 100 grams of chicken meat were calculated and seperated from 5 pieces as 20 grams. Totally 50 samples were mixed with blender, put into sterilized plastic bag and labelled uncooked, boiled, baked, grilled and fried respectively. Firstly antibiotic residues were determined qualitatively with MeRA test then chloramphenicol levels were detected with ELISA quantitatively. According to results of MeRA test, as seen from Table 2, 8 samples were negative and 42 samples were positive and they are determined above the limits. According to ELISA test results, all samples were negative.

In order to minimize the residues in chicken meats, use of antimicrobial agents may be conducted more and strict inspections. Nevertheless, inspections may be open to the general public via social media. Types of antibiotics may be indicated on food labels. Probiotics, phytogenic feed additives, essential oils, organic acids and enzymes may be used in poultry nutrition instead of antibiotics.

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APPENDIX 1. Curriculum Vitae

Personal Informations

Name	Nazlı Gülşah	**Surname**	DOĞAN
Place of Birth	Kocasinan	**Date of Birth**	26/05/1993
Nationality	Turkish	**TR ID Number**	32726158598
E-mail	ngulsahdogan@gmail.com	**Phone number**	+905052056132

Education

Degree	Department	The name of the Institution Graduated From	Graduation year
Master	Nutrition and Dietetics	Yeditepe University	
University	Nutrition and Dietetics	Yeditepe University	2016
University	Healthcare Management	Anadolu University	2016
High school		Mehmet Akif Ersoy Anadolu Lisesi	2011

Languages	**Grades (□)**
English	Good / YDS 65

Work Experience (Sort from present to past)

Position	Institute	Duration (Year - Year)
Dietician	Kaliteli Yaşam Polikliniği	2017 -

Computer Skills

Program	Level
Microsoft Office	Excellent
Estesoft	Excellent
SPSS	Excellent