ERCİYES ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ
Journal of Faculty of Veterinary Medicine, Erciyes University
Araştırma Makalesi / Research Article
15(3), 247-252, 2018
DOI: 10.32707/ercivet.477315

Determination of Some Antibiotic Residues by HPLC Method in Chicken Meats Prepared for Consumption*,**

Emre ARSLANBAŞ[1], Seyda ŞAHİN[2], Recep KALIN[3], Mahmut Niyazi MOĞULKOÇ[3], Hüseyin GÜNGÖR[1]

[1]Cumhuriyet University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Sivas-TURKEY
[2]Cumhuriyet University, Faculty of Veterinary Medicine, Food Hygiene and Technology Department, Sivas-TURKEY
[3]Cumhuriyet University, Faculty of Veterinary Medicine, Department of Microbiology, Sivas-TURKEY

Summary: In this study, it is aimed to search residues of enrofloxacin, doxycycline and tylosin which are widely used in poultry farming in fresh and packaged chicken meat samples taken from five brands being sold nationwide, through HPLC method. Enrofloxacin, doxycycline and tylosin levels in the samples of chicken meat were evaluated according to "The Regulation on Classification of Active Pharmacologic Substances that might be Available in Food of Animal Origin in Turkish Food Codex and Maximum Residue Limits" and international limits. Maximum residue limit (MRL) of enrofloxacin, doxycycline and tylosin in muscle tissues of chickens must be 100 µg/kg according to applicable regulations and international limits. It was found in totally 300 analyzed samples (whole chicken, drumstick and breast meat) that 11 (3.6%) of them had enrofloxacin, doxycycline and tylosin levels higher than MRL (between 100-150 µg/kg). Residue levels were less than allowed limits in other samples. Six (2%) of these 11 samples had enrofloxacin, 3 (1%) had doxycycline and 2 (0.6%) had tylosin residues. Since the results of this study showed that the majority of samples were lower than allowed limits, these results were considered as positive. However, as long as medicines do continue to be used for animals' health, there will always be a risk of existence of medicine residues in food of animal origin. Many stakeholders have a role in preventing residue risk arising out of veterinary medicines and all these persons must pay necessary attention to residue purification duration, training programs and keeping regular records of animals.
Key words: Chicken meat, doxycycline, enrofloxacin, residue, tylosin

Tüketime Sunulan Tavuk Etlerindeki Bazı Antibiyotik Kalıntılarının HPLC Yöntemi ile Belirlenmesi

Özet: Bu çalışmada, kanatlı hayvan yetiştiriciliğinde yaygın olarak kullanılan enrofloksasin, doksisiklin ve tilosin kalıntılarının, satışa sunulan beş farklı ulusal firmaya ait paketlenmiş taze tavuk eti örneklerinde HPLC yöntemiyle araştırılması amaçlanmıştır. Tavuk eti örneklerindeki enrofloksasin, doksisiklin ve tilosin düzeyleri, "Türk Gıda Kodeksi Hayvansal Gıdalarda Bulunabilecek Farmakolojik Aktif Maddelerin Sınıflandırılması ve Maksimum Kalıntı Limitleri Yönetmeliği"ne ve uluslararası limitlere göre değerlendirilmiştir. İlgili yönetmelikte ve uluslararası limitlerde enrofloksasin, doksisiklin ve tilosin için tavuk kas dokuda maksimum kalıntı limiti (MKL) 100 µg/kg olarak verilmiştir. Analizi yapılan toplam 300 örneğin (bütün tavuk, but eti ve göğüs eti) 11 (%3.6)'inde farklı örneklerde ve düzeyde MKL üzerinde (100-150 µg/kg arasında) enrofloksasin, doksisiklin ve tilosin bulunmuştur. Diğer örneklerde ise izin verilen limitlerin altında belirlenmiştir. Antibiyotik kalıntısı tespit edilen 11 örneğin 6 (%2)'sı enrofloksasin, 3 (%1)'ü doksisiklin ve 2 (%0.6)'si tilosin olarak saptanmıştır. Çalışmadan elde edilen sonuçların, büyük oranda izin verilen limitlerin altında kalması olumlu bir sonuç olarak değerlendirilmiştir. Ancak, hayvan sağlığı için ilaç kullanımı söz konusu olduğu sürece, hayvansal gıdalarda ilaç kalıntılarının bulunması riski muhtemeldir. Veteriner ilaçlarından ileri gelebilecek kalıntı riskinin önlenmesinde pek çok paydaşın rolü bulunmaktadır ve tüm ilgililer ilaç kalıntı arınma süresi, eğitim programları, hayvan kayıtlarının düzenli tutulması gibi konularda gereken hassasiyeti göstermelidir.
Anahtar kelimeler: Doksisiklin, enrofloksasin, kalıntı, tavuk eti, tilosin

Introduction

Antibacterial medicines are used in poultries for treatment and protection purposes as well as increasing efficiency, and growth factors (10). However, since most of these antibiotics that have been used as growth factors and to accelerate development have caused quick reproduction of some resistant strains among some pathogenic bacteria types (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp.) in humans and animals, they are no longer being used. European Union (EU) has

Geliş Tarihi/Submission Date : 02.10.2017
Kabul Tarihi/Accepted Date : 25.04.2018

* This study was supported by the Scientific Research Project Fund of Cumhuriyet University under the project number V-016
** This study was presented as a oral presentation in the I. International Scientific and Vocational Studies Congress, October, 2017; Nevşehir-Turkey.

widely prohibited to use antibiotic originated growth factors in poultry farming in 1998, and 1999 and has prohibited the usage of all growth factor antibiotics in the feeds of poultries in 2006. Rules applied in EU are also effective in our country in accordance with harmonization with EU legislations (6,22).

Inappropriate, illegal, uncontrolled and insensible utilization of antibiotics in poultries causes residues in food of animal origin (23). Resistant bacteria populations increase and accumulate in various organs and tissues, particularly in liver and kidneys as a result of insensible and uncontrolled utilization of antibiotics. On the other hand, these antibiotics may penetrate animal products and cause intoxications from mild allergies to anaphylactic shocks, teratogenic, mutagenic and carcinogenic effects, reproduction disorders and adverse effects on digestion tract flora in humans that consume such products, based on the type and amount of antibiotics available in these products (1,6,13). Increase of multi-resistant features of pathogenic microorganisms in antibiotics and utilization of similar antibiotic groups in treatment of humans and animals has led us to think that the number of antibiotics that can be used in treatment of humans will be significantly decreased in the future. The effectiveness of antibiotic, which causes the occurrence of resistant bacteria, will also decrease and, thus, the medicine consumption will increase (6,20). Antibiotic residues penetrating humans kill non-resistant and harmless bacteria in the body and cause the reproduction of strong and harmful bacteria, which also renders antibiotics ineffective. These residues also prevent reproduction of starter cultures in food industry, particularly in the production of yogurt, cheese and sausage, which, in turn, causes the occurrence of production faults and economic losses (12). Medicine residues in foods that are exceeding tolerated levels cause various threats to the human health and such food must not be consumed. Tolerance levels show allowable levels of medicine residues in raw tissues according to applicable legislations. However, most of the food of animal origin, such as meat, milk and egg, are consumed after they are cooked through different methods, and such different cooking methods cause differences in the solubility or quantities of medicine residues (2,18).

Tetracycline group antibiotics (doxycycline), which are widely used for poultries since they are broad spectrum antibiotics with low toxic effects, broad spectrum fluoroquinolones (enrofloxacin) and narrow effect spectrum macrolides (tylosin) are the antibiotics that are used widely in poultry farming (4,6,16). Therefore, detection of enrofloxacin, doxycycline, and tylosin residues in chicken meats is highly important for the protection of the health of those who are the end consumers of these chicken meats.

Possible antibiotics residue levels in the food of animal origin must be determined through highly selective and sensitive chemical methods. Today various advanced analytic methods capable of quantitative measurement, such as ELISA, Charm II, GC and HPLC, LC-MS and LC-MS-MS are used to detect antibiotic residues in different foodstuff and meats (14,23). High performance liquid chromatography (HPLC) is preferred not only because of its capability to analyze thermolabile compounds, but also because it is a specific, certain and sensitive method (19).

Enrofloxacin, doxycycline and tylosin are widely used antibiotics in poultry farming. Insensible and uncontrolled utilization of antibiotics causes resistant bacteria population to increase, their residues penetrate animal products and cause intoxications and undesired effects, ranging from mild allergies to anaphylactic shocks on humans that consume these products. HPLC is the preferred method in calculating the levels of antibiotic residues in food of animal origin since it is a specific, certain and sensitive method. Therefore, detection of enrofloxacin, doxycycline, and tylosin residues in chicken meats by HPLC method is highly important for protection of public health.

Material and Methods

300 packaged fresh chicken meat samples, 100 of which were whole chicken, 100 were drumstick and the last 100 were chicken breast, taken from five different brands being sold nationwide for consumption, were used in this study. These samples were quickly brought to the laboratory without breaking the cold chain (at +4°C). Samples were analyzed by HPLC method in order to detect enrofloxacin, doxycycline and tylosin residues. The chemicals used in the study were enrofloxacin (33699, HPLC reagent), doxycycline (33429, HPLC reagent) and tylosin (32298, HPLC reagent) standards, methanol, ethylenediaminetetraacetic acid (EDTA) (Sigma-Aldrich; St. Louis, Missouri, USA), for-

mic acid (Scharlau; Barcelona, Spain) and acetonitrile (Merck; Darmstadt, Germany) (All chemicals used in the study were used as HPLC grade). 0.1 mg/ml stock standard solutions of enrofloxacin and doxycycline standards were prepared in methanol and tylosin standard was prepared in acetonitrile.

The method suggested by Chico et al. (8) was used for analysis after it was modified and adapted for laboratory conditions. When preparing the samples, initially muscle tissues that were separated from fat and skin tissues were cut into small pieces. Then these pieces were minced and 3 g of each were put into polypropylene centrifuged tubes of 50 ml before adding 200 µl 0.1 M EDTA and 10 ml methanol: ultra distilled water (70:30, v/v) mixture. The mixture was mixed for 30 seconds with vortex and mechanically for 15 minutes and then centrifuged at 2507×g for 5 minutes. 500 µl of extract was diluted with 2 ml ultra distilled water and this dilution was passed through 0.45 µm membrane filter (Sartorius; Göttingen, Germany). Finally 20 µl of filtrate was applied to HPLC system.

Analyses were performed in HPLC device (Flexar HPLC-Perkin Elmer; Massachusetts, USA) by using photodiode array detector and UV detector. Bio C_{18} (5 µm, 150 mmx4.6 mm) was used as column (Brownlee Bio C_{18}; Shelton, USA). The data received from the device were processed with Chromera software. The study was performed isocratic by using mobile phase, composed of acetonitrile and 0.1% formic acid. Study standards, diluted at different concentrations of 5, 10, 20, 50 and 100 µg/kg from standard stock solution, were prepared for calibration graphic. These solutions that were prepared in different concentrations were studied in 3 separate times to form calibration graphics and their LOD (limit of detection) and LOQ (limit of quantification) values were calculated. Recovery rates were calculated by adding the standard solutions in 3 different concentrations to the samples, antibiotic content of which is known. Regression analysis was performed with NCFF statistic software.

Results

Standard calibration formula, found for enrofloxacin, doxycycline, and tylosin are given in Table 1 with their R^2 (R-squares) and recovery rates as well as LOD and LOQ values. The results showed that the highest R^2 rate is seen in tylosin with a value of 0.99 and the highest recovery rate is seen in enrofloxacin with a rate of 100.14% (doxycycline and tylosin recoveries respectively; 99.03%, 100.78%). The lowest LOQ value of 1.104 µg/kg was found in tylosin (enrofloxacin LOQ value 1.292 µg/kg; doxycycline LOQ value 4.450 µg/kg).

Results of analyses were evaluated according to "The Regulation on Classification of Active Pharmacologic Substances that might be Available in Food of Animal Origin in Turkish Food

Table 1. Standard calibration formula, R^2 and recovery rates and LOD and LOQ values of antibiotics

Antibiotics	Standard calibration formula (y=ax+b)			Recovery rate (%)	LOD (µg/kg)	LOQ (µg/kg)
	a	b	R^2			
Enrofloxacin	9840.2	252071	0.89	100.14	0.426	1.292
Doxycycline	3466.5	108675	0.97	99.03	1.469	4.450
Tylosin	15238	94842	0.99	100.78	0.364	1.104

Table 2. Antibiotic residue amounts in chicken meat samples

Sample	Enrofloxacin [µg/kg]	Doxycycline [µg/kg]	Tylosin [µg/kg]	Total
Drumstick	2 [111.9; 115.5]	1 [121.4]	1 [105.4]	4 (1.3%)
Breast	3 [124.3; 129.7; 144.1]	1 [137.6]	1 [109.2]	5 (1.6%)
Carcasses	1 [130.2]	1 [126.8]	-	2 (0.6%)
Total	6 (2%)	3 (1%)	2 (0.6%)	11 (3.6%)

Codex and Maximum Residue Limits" (21) and international (EU 37/2010 EC directives) (9) limits. Aforesaid regulation and international limits require maximum amount of enrofloxacin, doxycycline, and tylosin must be MRL 100 µg/kg in chicken's muscle tissue. The amount of enrofloxacin, doxycycline, and tylosin found in 11 (3.6%) of total 300 analyzed samples, taken as whole chicken, drumstick and breast, was higher than MRL (between 100-150 µg/kg). Residue levels were less than allowed limits in other samples. 6 (2%) of these 11 samples had enrofloxacin, 3 (1%) had doxycycline, and 2 (0.6%) had tylosin residues. More than allowed amounts of antibiotics were found in 5 (1.6%) chicken breast meat, 4 (1.3%) drumstick and 2 (0.6%) whole chicken meat, which were among analyzed samples (Table 2).

Discussion and Conclusion

When the results achieved from this study were evaluated according to "The Regulation on Classification of Active Pharmacologic Substances that might be Available in Food of Animal Origin in Turkish Food Codex and Maximum Residue Limits" (21) and international (EU 37/2010 EC directives) (9) limits, it was found that enrofloxacin, doxycycline, and tylosin levels (100-150 µg/kg) were over the allowed limits (MRL should be 100 µg/kg in chicken muscle tissue for enrofloxacin, doxycycline and tylosin) in only 11 (3.6%) of 300 analyzed samples in total. Antibiotic levels of other samples were found less than MRL (100 µg/kg in chicken muscle tissue for enrofloxacin, doxycycline and tylosin).

As the results were mostly lower than allowed limits, the results of this study were found in compliance with the results of some previous studies (3,5,11,14). In a study, fluoroquinolone group antibiotic (ciprofloxacin, enrofloxacin, balofloxacin) residues in broiler meats in Argentina were found to be less than MRL (11). Bousova et al. (5) analyzed 36 different antibiotics, such as enrofloxacin, tylosin, doxycycline, chlortetracycline, ciprofloxacin, danofloxacin, josamycin, kanamycin, lincomycin, neomycin, oxytetracycline, spiramycine, and tilmicosin, in samples of chicken meat and it was found as a result of this study that antibiotics are less than maximum residue limits. In another study conducted by Lopes et al. (14) the residue analysis of 20 veterinary medicines, including quinolones, sulphonamides, macrolides, anthelmentics, avermectins, diamino derivatives, and benzathine penicilines, was performed in 11 samples of chicken meat taken from local supermarkets in Spain (Almeria), and it was found that results were lower than MRL. Also the data retrieved from erythromycin, tylosin, spiramycine, josamycin, roxcytrhromycin, troleandomycine and tilmicosin analyses on beef, chicken and pig meats as well as seabream and trout meats, taken from various supermarkets in Spain, were found to be lower than MRL (3).

On the other hand, in a study performed on 98 poultry samples (61 chicken meat and 37 turkey meat) in Portugal to research fluoroquinolone group antibiotics (enrofloxacin, ciprofloxacin, norfloxacin, and sarafloxacin) it was found that 44.2% of chicken meat and 37.8% of turkey meat was contaminated with antibiotics (16). In another study, 270 samples (chicken muscle, liver and kidneys) taken from a Tehran slaughterhouse in Iran, were analyzed for residues of enrofloxacin through HPLC method and the residues in 8 muscle, 12 liver and 22 kidney samples were found to be higher than MRL (17).

Regarding the studies in Turkey, Akar (1) researched erythromycin, chloramphenicol, monencyn and tylosin residues on 175 chicken meat samples, taken from markets and companies operating in Ankara market. As a result of the study, erythromycin residues were found in 2.3% and tylosin residues were found in 1.14% of the samples and it was stated that 5.7% of chicken meats contain 3 of 4 antibiotics (except monencyn) in different levels. In another study, Yıldırım et al. (24) examined 40 chicken meat samples being sold in Istanbul in order to investigate enrofloxacin and danofloxacin residues, which are fluoroquinolone derivatives, and the results showed that there was not any enrofloxacin and danofloxacin residues within 50 µg/kg. Öbekçi (15) has researched tetracycline group antibiotics (tetracycline, oxytetracycline and chlortetracycline) residues in 200 chicken meat samples through HPLC method. The results showed that there were 8.1% oxytetracycline, 7% tetracycline, and 5.5% chlortetracycline residues in the samples. Another study was performed in 2012 on 60 chicken meat samples, taken from supermarkets in Bursa, by analyzing tetracycline group antibiotics with liquid chromotography-double mass spectrometer and doxycycline was found in 4 and tetracycline was

found in 1 of the samples. No oxytetracycline and chlortetracycline was found in any of the samples (7). It is seen that the results of this study are similar, in terms of MRL, to the results of the studies, performed by Yıldırım et al. (24) and Cetinkaya et al. (7).

As the results of this study, in which enrofloxacin, doxycycline, and tylosin residues were researched in chicken meats, were mostly lower than allowed limits, they were considered as positive results for risk evaluation. However, as long as medicines continue to be used for animals' health, there will always be a risk of existence of medicine residues in food of animal origin and penetration of this residues to consumers at different levels. But the most important issue at this point is to have residue levels under the tolerance limits.

In conclusion, the stakeholders, namely the veterinary physician who issues the prescription or makes the treatment, the farmer or keeper of animals, and other organizations, have a role in preventing of veterinary medicine residues in food of animal origin. It is also highly important to keep regular records of animals and monitor medicine consumption. It might be useful to inform and give training to farmers and their employees, which aim proper and timely utilization of veterinary medicines in order to prevent residues by taking social and economic status of farmers and practices on the field into consideration. Manufacturers must comply with medicine residue purification duration, take preventive actions to protect farming equipment from contamination, and differentiate the animals, which have received medicines, from others either by marking them individually or through group records. So it might be possible to be protected from residues, which are important for public health, and to reduce economic risks.

References

1. Akar F. Investigation on various antibiotic residues in chicken meat and liver. AÜ Vet Fak Derg 1994; 41(2): 199-207.
2. Baydan E, Filazi A, Kum C, Sekkin S. Etlik piliçlerde pişirme ve dondurma işlemlerinin ilaç kalıntıları üzerine etkileri: 1. enrofloksasin üzerine pişirme ve farklı sürelerde soğukta depolamanın etkisi. Türk Vet Hek Derg 2000; 71: 19-22.
3. Berrada H, Borrull F, Font G, Marcé RM. Determination of macrolide antibiotics in meat and fish using pressurized liquid extraction and liquid chromatography–mass spectrometry. J Chromatogr A 2008; 1208 (2008): 83-9.
4. Berrada H, Moltó JC, Mañes J, Font G. Determination of aminoglycoside and macrolide antibiotics in meat by pressurized liquid extraction and LC-ESI-MS. J Sep Sci 2010; 33(4-5): 522-9.
5. Bousova K, Senyuva H, Mittendorf K. Quantitative multi-residue method for determination antibiotics in chicken meat using turbulent flow chromatography coupled to liquid chromatography-tandem mass spectrometry. J Chromatogr A 2013; 1274: 19-27.
6. Can HY, Çelik TH. The residue risk and use of antibiotic in poultry breeding. Vet Hekim Der Derg 2008; 79(4): 35-40.
7. Cetinkaya F, Yibar A, Soyutemiz GE, Okutan B, Ozcan A, Karaca MY. Determination of tetracycline residues in chicken meat by liquid chromatography-tandem mass spectrometry. Food Addit Contam Part B 2012; 5 (1): 45-9.
8. Chico J, Rubies A, Centrich F, Companyo R, Prat MD, Granados M. High-throughput multiclass method for antibiotic residue analysis by liquid chromatography–tandem mass spectrometry. J Chromatogr A 2008; 1213(2008): 189-99.
9. Commission Regulation. Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-5/reg_2010_37/reg_2010_37_en.pdf. Erişim tarihi:02.10.2017.
10. Filazi A. Kanatlılarda akılcı antibakteriyel ilaç kullanımı. Mektup Ankara 2009; 7(2): 3-8.
11. Garcia-Ovando H, Gorla N, Weyers A, Ugnia L, Magnoli A. Simultenous quantification of ciprofloxacin, enrofloxacin and balofloxacin in broiler chicken muscle. Arch Med Vet 2004; 36(1): 93-8.
12. Kaya S, Şahal M. Drug residues in foodstuffs, their tolerance levels and withdrawal times for sloughtering animals or discarded times of milk from animals given drugs. AÜ Vet Fak Derg 1989; 36(2): 390-403.
13. Lee JB, Chung HH, Chung YH, Lee KG. Development of an analytical protocol for detecting antibiotic residues in various fo-

ods. Food Chem 2007; 105: 1726-31.
14. Lopes RP, Reyes RC, Romero-González R, Frenich AG, Vidal JLM. Development and validation of a multiclass method for the determination of veterinary drug residues in chicken by ultra high performance liquid chromatography-tandem mass spectrometry. Talanta 2012; 89: 201-8.
15. Öbekçi J. Tavuk eti ve karaciğerlerinde tetrasiklin grubu antibiyotiklerin HPLC ile saptanması. Uzmanlık Tezi. Tarım ve Köyişleri Bakanlığı. 2002.
16. Pena A, Silva LJG, Pereira A, Meisel L, Lino CM. Determination of fluoroquinolone residues in poultry muscle in Portugal. Anal Bioanal Chem 2010; 397(6): 2615-21.
17. Salehzadeh F, Salehzadeh A, Rokni N, Madani R, Golchinefar F. Enrofloxacin residue in chicken tissues from Tehran slaughterhouses in Iran. Pakistan J Nutrition 2007; 6(4): 409-13.
18. Sever E, Baydan E. The effect of various cooking and freezing processes on the levamisole residues in broiler tissues. Kafkas Univ Vet Fak Derg 2013; 19(2): 239-44.
19. Sırmagül B. Terapötik ilaç düzeyi izleminde analiz yöntemleri ve çalışma ilkeleri. Türk Farmakoloji Derneği XVII. Farmakoloji Eğitim Sempozyumu. 28 Mayıs 2010. Lefkoşa-Kıbrıs.
20. Tang HP, Ho C, Lai SS. High-throughput screening for multi-class veterinary drug residues in animal muscle using liquid chromatography/tandem mass spectrometry with on-line solid-phase extraction. Rapid Commun Mass Spectrom 2006; 20(17): 2565-72.
21. The Official Gazette of the Republic of Turkey. The regulation on classification of active pharmacologic substances that might be available in food of animal origin in Turkish food codex and maximum residue limits, T.C. Başbakanlık, Mevzuatı Geliştirme ve Yayın Genel Müdürlüğü Mevzuat Bilgi Sistemi, 04.05.2012, http://www.resmigazete.gov.tr/eskiler/2012/05/20120504-9.htm, Erişim tarihi: 11.10.2017.
22. Tuncer Hİ. To banned usage of hormones, antibiotics, anticoccidials and drugs in compound animal feed. Lalahan Hay Arast Enst Derg 2007; 47(1): 29-37.
23. Yıbar A, Soyutemiz GE. Antibiotics use in food-producing animals and possible residual risk. Atatürk Üniversitesi Vet Bil Derg 2013; 8(1): 97-104.
24. Yıldırım M, Demirel H, Bakırel T. The investigations on the residues of the some fluoroquinolone antibacterials in chicken meat marketed in Istanbul. İstanbul Üniv Vet Fak Derg 1996; 22(1): 63-7.

Corresponding Author
Asst Prof Emre ARSLANBAŞ
Cumhuriyet University,
Faculty of Veterinary Medicine,
Department of Pharmacology and Toxicology,
Sivas-TÜRKİYE
E-mail: earslanbas@cumhuriyet.edu.tr