RESEARCH ARTICLE

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Presence of Tetracycline-Group of Antibiotics in the Eggs Coded According to the Cultivation Method

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In the present study, the presence and levels of tetracycline-group antibiotics in the eggs coded according to the cultivation method (0-3) and those not coded were investigated. For this purpose, total 60 eggs (30 in each group) were used as sample. ELISA method and commercial test kits were used in the analyses. According to the results of the analysis , tetracycline residuals were found in 66.7% (n=40) of the egg samples. The level of tetracycline ranged between 2.40–83.74 ppb (mean: 37.59±25.11) in all the eggs samples. Only 33.3% (n=20) were found to contain no tetracycline residual. No tetracycline residue was detected in any of the organic egg samples. Tetracycline residue levels determined in egg samples were found to comply with the legal limits stated in the European Union Commission and Turkish Food Codex Egg Communiqué.

Key Words: *Egg, antibiotic, tetracycline, public health*

Yetiştirme Yöntemine Göre Kodlanmış Yumurtalarda Tetrasiklin-Grubu Antibiyotiklerin Varlığı

Bu çalışmada, yetiştirme metoduna göre kodlanmış (0-3) ve kod numarası olmayan yumurtalarda tetrasiklin grubu antibiyotiklerin varlığı ve düzeyi araştırılmıştır. Bu amaçla toplam 60 yumurta (her gruptan 30 adet olmak üzere) örneği materyal olarak kullanılmıştır. Analizlerde ELISA yöntemi ve ticari test kitleri kullanılmıştır. Analiz sonuçlarına göre, yumurta örneklerinin %66.7'sinde (40 örnek) tetrasiklin kalıntısı tespit edilmiştir. Tetrasiklin düzeyi tüm örneklerde 2.40-83.74 ppb (ortalama 37.59±25.11) arasında belirlenmiştir. Örneklerin %33.3'ünde (20 örnek) tetrasiklin kalıntısına rastlanmamıştır. Organik yumurta örneklerinin hiçbirinde tetrasiklin kalıntısı tespit edilmemiştir. Yumurta örneklerinde belirlenen tetrasiklin kalıntı düzeyleri AB Komisyonu ve Türk Gıda Kodeksi Yumurta Tebliği'nde bildirilen yasal limitlere uygun bulunmuştur

Anahtar Kelimeler: *Yumurta, antibiyotik, tetrasiklin, halk sağlığı*

Introduction

The definition of egg covers the unshelled eggs, which are obtained from "*Gallus gallus domesticus*" species of chicken and are suitable for directly putting into use of humans or for preparing egg products by processing, other than broken, incubated, or cooked eggs (1).

Turkey ranks 9th in the world production of chicken eggs. According to the data of Turkish Statistical Institute (TUİK), Turkey's total egg production in year 2019 was 1.1 million tons (1.1 billion units). In year 2018, the total number of organic egg production was 1.250.000. The egg consumption per capita in our country in year 2019 was 214, while it was 333 in Japan, 307 in China, 305 in Russia, and 280 in Argentina (2).

The provisions of the Regulation on Maintaining the Minimum Standards for Protecting the Layer Hens are taken as basis (1, 3). Before the company and coop numbers, the code of cultivation method is automatically assigned as 0: for organic cultivation, 1 for free range cultivation, 2 for cultivation in coop without cage, and 3 for cultivation in coop with cage (i.e.: 0TR060000060000-01 for organic cultivation). The date of laying might be labeled on the A-Class eggs. The code of cultivation method and the company and coop numbers are written in the coop or in the sorting or packaging facility of the same company. The A-Class egg, on which the code of cultivation method and the numbers of company and coop are not written, cannot the introduced to the market in wholesale or retail form. The code of cultivation method is explained in the label info of A-Class egg (Example: 0: Organic cultivation) (1).

Antibiotics are pharmacologically active materials that are widely used in veterinary practice in order to cure and prevent diseases or accelerate the healing process. However, the unconscious use of antibiotics (i.e.: unlicensed drug use) and not following the instructions on the label cause the problem of residuals on the tissues and organs having nutrient value and the foods obtained from them (meat, milk, offal, egg, honey) (4-6).

Since the poultry industry is an important protein source for the human, cultivation and production of healthy broiler and layer hens is of significant importance. For this

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reason, the use of antibiotics for curing, protecting, or growth-supporting purposes is very wide in the poultry industry (7). The tetracycline-group antibiotics, especially tetracycline, chlortetracycline, and doxycycline are the antimicrobial agents that are preferred by veterinary physicians for the poultry thanks to their wide spectrum of activity and low costs (8). Besides their important benefits, like many other antibiotics, the tetracycline agents may also cause problems such as allergic reactions, bacterial resistance, liver damage, tooth yellowing, and gastrointestinal disorders among the consumers since the main compounds and degradation products are found in the animal products (9).

Tetracycline is used in chickens and turkeys for treating the chronic respiratory disease (CRD), blue comb, coryza, cholera, pullorum, infectious synovitis and sinusitis, hemorrhagic septicemia, and various streptococcus and staphylococcus infections. Considering the regulations in our country, the washout period for tetracycline residuals was set to be 7 days for poultry meat and 14 days for egg (10). In Turkey, there are two tetracycline-containing medications licensed for veterinary medicine and one of them is used in poultry (11).

In organic farming, the use of chemically synthesized veterinary medical products or antibiotics as preventive agents is prohibited. However, in case of any compulsory situation, the controlled use of these agents is allowed. In order for a product to be classified as organic, the extraction period (half-life) of medication is taken into consideration (1).

Precise determination of tetracycline residues in animal foods is very important for public health. The European Union (EU) commission has reported the maximum residue limit (MRL) of 200 µg/kg of tetracycline group antibiotics in poultry, chicken tissues and eggs. Prepared by the Ministry of Agriculture and Forestry within the framework of harmonization with the EU commission; In the Turkish Food Codex Regulation on Classification of Pharmacologically Active Substances That May Be Found in Animal Food and Maximum Residue Limits, the MRL value for tetracyclines in eggs offered for consumption is determined as 200 µg/kg (3, 12).

Within the scope of the present study, 60 egg samples produced as "coded and without code" according to the cultivation method in varying quality classes were examined in terms of tetracycline residues. The results obtained were analyzed in terms of food safety and public health by considering the standard values.

Materials and Methods

For this research, approval certificate was obtained from Cumhuriyet University Animal Experiments Local Ethics Committee (Decision No: 25.12.2020/466).

Material: In this study, 30 egg samples coded according to the cultivation method (0, 1, 2, 3) were used in different brands. Additionally, 30 egg samples were used as packaged and without code and without packaging. Thus, a total of 60 egg samples offered for consumption were used as material. The egg samples were periodically collected in June 2020 from different sales points in Sivas province (market, district bazaar, and wholesales market). The collected eggs samples were selected among the parties offered for sale. The collected egg samples were taken to the laboratory under appropriate conditions by preventing exposure to sun and heat. For 1 sample to be analyzed, 2 eggs were taken from the sales point and then homogenized before the use. Fifty-gram of each samples prepared in this way was taken and stored until analysis at –18 °C. Before the analysis, they were heated to room temperature (21 °C) (13).

Preparation of Samples: The level of tetracycline-group antibiotics in the egg samples was determined using ELISA (Enzyme Linked Immunosorbent Assay) method. Sinogeneclon Tetracycline ELISA (SG-4021) test kit was used for analyses.

Egg samples were brought to room temperature before starting analysis. From the homogenized egg specimens, 1g was taken into a tube and mixed by adding 2 mL trichloroacetic acid. Then, the mixture was centrifuged at 4000 rpm for 10 minutes. After the centrifuge process, 100 µL supernatant was taken into the Eppendorf tube and diluted using 1900 µL solvent solution. The prepared specimens were mixed for 30 seconds (using vortex) and then applied into microtiter plates.

Test procedure: From each of standard (0.05, 0.15, 0.45, 1.35, 4.05 ppb), samples, and antibody solutions, 50 µL were added into the microplate wells. Then, by covering the top of microplate, it was incubated kept at 37°C for 30 minutes in the incubator. After this process, the wells were rinsed 5 times using a rinsing solution. After the rinsing process, 100 µL enzyme conjugate was added into each well. Then, by covering the top of microplate, it was incubated kept at 37°C for 30 minutes in incubator. Then, the rinsing process was repeated (5 times). After the rinsing process, 50 µL substrate A and B solutions were added into each well. Then, by covering the top of microplate, it was incubated kept at 37°C for 30 minutes in incubator. After this process, 50 µL stop solution was added into each well and the transition of blue color into yellow color. Finally, the absorbance levels of standard and sample were recorded at 450 nm wavelength using ELISA device. The resultant absorbance values were calculated using a calibration curve and the levels of tetracycline corresponding to the absorbance values of samples were calculated as ppb. The calibration curve is presented in Figure 1.

Statistical Analysis: The descriptive statistics of the level of tetracycline found in samples were analyzed using SPSS 22.00 package software.

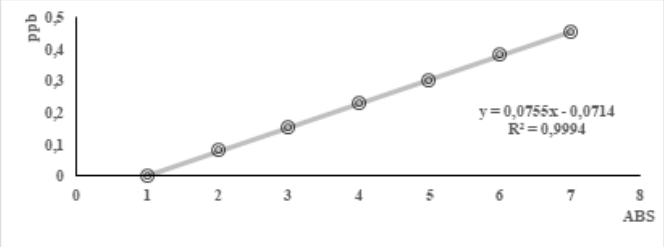

Figure 1. Calibration curve (450 nm)

Results

The levels of tetracycline determined in analyzed egg samples are presented in Table 1, whereas the results of statistical analyses are shown in Table 2.

When the analysis findings were evaluated, tetracycline residues were detected in 66.7% (40 samples) of the egg samples. Tetracycline ratio was determined as 16.6% (10 samples) in different brands and packaged egg samples coded according to the cultivation method. This ratio; it was determined to3.3% (2 samples) in the samples with code number 1; 5% (3 samples) in the samples with code number 2, and 8.3% (5 samples) in the samples with code number 3. As shown in Figure 2, the average tetracycline levels of coded egg samples are as follows; code 1; 3.06±0.93 ppb, code 2; 8.72±2.06 ppb and code 3; 11.82±1.93 ppb (Figure 2).

Table 1. Tetracycline levels in coded and without coded egg samples (ppb)

Sample number	Tetracycline	Sample number	Tetracycline	Sample number	Tetracycline	Sample number	Tetracycline	Sample number	Tetracycline	Sample number	Tetracycline
1	29.36	**11**	10.35*	**21**	45.71	**31**	70.31	**41**	35.41	**51**	ND
2	31.24	**12**	11.98*	**22**	9.40*	**32**	6.40*	**42**	2.40*	**52**	10.74*
3	10.61*	**13**	ND*	**23**	ND*	**33**	ND*	**43**	ND*	**53**	ND
4	ND*	**14**	65.47	**24**	75.36	**34**	62.43	**44**	41.35	**54**	64.31
5	ND*	**15**	ND*	**25**	34.45	**35**	49.87	**45**	54.16	**55**	35.12
6	15.12*	**16**	63.71	**26**	37.41	**36**	ND*	**46**	ND*	**56**	3.71*
4	ND*	**17**	ND*	**27**	ND*	**37**	38.21	**47**	ND*	**57**	ND
8	45.61	**18**	24.14	**28**	45.62	**38**	ND*	**48**	28.43	**58**	26.37
9	83.74	**19**	34.17	**29**	48.10	**39**	65.31	**49**	10.67*	**59**	32.70
10	ND*	**20**	55.23	**30**	55.46	**40**	33.40	**50**	ND*	**60**	ND

ND: Not detected, *: Coded

Table 2. Statistical findings of tetracycline levels in coded and without coded eggs (ppb)

Tetracycline	n	%	Min.	Max.	Mean±SD
0	20	33.3	-	-	-
0-0.05	10	16.7	2.40	15.12	9.14± 3.87
0.05-0.15	4	6.7	24.14	26.37	27.08± 2.31
0.15-0.45	9	15	31.24	38.21	34.68±2.18
0.45-1.35	6	10	41.35	49.87	46.04±2.87
1.35-4.05	3	5	54.16	55.46	54.95±0.69
4.05>	8	13.3	62.43	83.74	68.83±7.35
Total	60	100	2.40	83.74	37.59±25.11

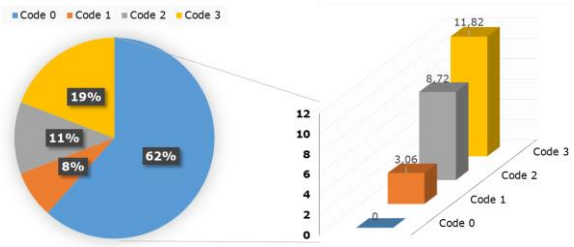

Figure 2. Tetracycline ratio (%) in coded egg samples and average tetracycline levels (ppb)

Discussion

Tetracycline ratio was determined as 33.3% (30 samples) in egg samples sold without code and without packaging. Tetracycline level was determined between 2.40-83.74 ppb (mean 37.59±25.11) in all samples. No tetracycline residue was detected in 33.3% of the egg samples (20 samples). No tetracycline residue was detected in any of the organic egg samples. The absence of antibiotic residuals in these samples was found to be in compliance with organic farming specification (1).

Of the analysis samples, 43.27% were packaged and with a code number (0, 1, 2, 3) (n=26). The tetracycline levels (ppb) of the packaged samples with code number were evaluated and the results are presented in Figure 2. In other words, no tetracycline residual was detected in 26.67% (code 0; n=16) of coded samples, whereas tetracycline residual was detected in 16.6% (code 1, 2, 3; n=10). The levels of residuals in the packaged samples ranged between 2.40 -15.12 ppb (mean 9.14±3.87 ppb).

Although the bacteria strains that are resistant to their effects arose, tetracycline-group antibiotics (especially the oxytetracycline) are among the antibiotics

most widely used in veterinary medicine in our country and their wide spectrum plays an important role in their wide area of use. Tetracycline-group antibiotics are used in poultry especially for diseases such as contagious coryza, erysipelas, CRD, and poultry cholera (*Pasteurella multocida*). For this reason, the tetracycline and its degradation products may be found in poultry meat and egg, as in other animal products, and they may cause important undesired effects (14, 15).

In a study examining the tetracycline levels in 72 egg specimens, the level of tetracycline was found to be 0-200 ppb in 61% (n=44), 200-400 ppb in 34.72% (n=25), 400-600 ppb in 2.78% (n=2), and 600-800 ppb in 1% (n=1) (16). When compared to the results obtained in the present study, it can be seen that the residual levels were lower. Difference of sampling regions, number of examined samples, and variables related with analysis kits used are among the reasons for these differences.

Besides ELISA method, also HPLC and LC-MS-MS methods were used in determining the levels of tetracycline residuals in egg samples. In another study, the tetracycline residual levels were determined using HPLC method in 204 samples obtained from 2040 eggs samples. The levels of chlortetracycline ranged between 135.82-229.53 µg/kg (17). When compared to the results obtained in the present study, it can be seen that the residual levels in their study were higher. The sensitivity of method used and the number of samples are believed to play a role in this difference.

For the tetracyclines with very high residual possibility, MRLs were determined for poultry, chicken tissues, and eggs in the EU and our country (1, 3). It is very important to sensitively determine the tetracycline residuals in animal products by considering these limits. In the present study, the residual levels determined in all the samples were found to be below the MRL values (200 µg/kg for egg).

In their study, Hind et al. (18) examined the levels of oxytetracycline in 180 egg samples. Oxytetracycline was determined in 50% (n=90) of analyzed samples. The residual levels ranged between 24.1±3.37 and 30.6±9.34 mg/kg. These findings suggest that misuse of oxytetracycline, which may endanger human health, could be very dangerous.

In previous studies, one of the methods used for determining the tetracycline residual levels is the antimicrobial imaging (Microbiological Four-Plate Test). In those studies, the analyses were performed taking the known recycling as basis. In a study examining the use and residuals of antimicrobials in commercial chicken eggs obtained from small-scaled poultry farms, the oxytetracycline residuals were found in 21.4% of 70 egg samples (19). The authors emphasized that the presence of antimicrobial residuals are very important for public health. In another study, it was reported that tetracycline residual levels of 40 egg samples ranged between 4.75-6.83 ng/g, that the findings were below the maximum residual limit (200 ng/g) specified by EFSA (European Food Security Agency), and that the level of tetracycline residuals decreased by 20% after boiling process (20). In another study, several antibiotic residuals were analyzed in 300 chicken eggs and tetracycline residuals were found in 39.5% (n=32) of samples (21).

In conclusion the presence of antibiotic residues that are illegal or higher than the legal value in animal foodstuffs creates a problem in terms of both technology and public health. Besides that, the use of antibiotics in human medicine, veterinary medicine, and agriculture results in the development of resistant bacteria. In addition, attention should be paid to rational use of antibiotics in terms of public health. The presence of antibiotics in animal-origin foods is always a risk factor. For this reason, excessive use of antibiotics should be avoided and the legal sanctions and audits should be increased.

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