



Investigation of Beta-Lactam and Tetracycline Group Antibiotic Residues in Bovine Liver, Kidney and Muscle Tissue

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ABSTRACT

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In this study, beta-lactam and tetracycline antibiotic residues were investigated in cattle liver, kidney and muscle samples. For this purpose, a total of 75 bovine tissue samples (each of 25 from liver, kidney, muscle) taken from 25 cattle slaughtered in a local slaughterhouse in Sivas were used as materials. ELISA method was applied in the analysis and it was studied with commercial test kits. According to the results of the analysis; beta-lactam and tetracycline residues were detected in all bovine tissue samples. Beta-lactam level was determined between 0.75-1.07 ppb (mean 0.94 ± 0.01) in liver samples, 0.67-1.05 ppb (mean 0.81 ± 0.01) in kidney samples and 0.70-2.57 ppb (mean 0.97 ± 0.07) in muscle samples. Tetracycline level was detected in the range of 4.48-8.50 ppb (mean 6.14 ± 0.17) in liver samples, 1.73-6.39 ppb (mean 4.90 ± 0.24) in kidney samples and 3.31-7.45 ppb (mean 5.67 ± 0.25) in muscle samples. The residue levels determined in the examples complied with the legal limits reported in the European Commission and the Turkish Food Codex Communiqué.

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Introduction

Protein deficiency is a nutritional problem that affects every age group in the world and in our country. The World Health Organization (WHO) has determined the daily protein requirement of a healthy individual to be 0.8-1 g/kg (body weight). This amount varies according to age, gender, health, physiological status and daily activity. Which is important for a healthy diet that 40-50% of the protein taken daily is of animal origin (Tayar and Yarsan, 2014).

Meat and meat products are an important food group in terms of animal proteins. Meat is food which rich in proteins, minerals (iron, zinc, phosphorus, sodium, potassium, magnesium) and vitamins (B1, B2, B6, B12) with high biological value. Essential amino acids that cannot be synthesized in the body, which must be taken with nutrients, are found in sufficient quantities and proportions in the composition of meat (Tayar and Yıldırım, 2020).

Varieties of antibiotics are widely used in farm animals to treat and prevent diseases or accelerate growth. However, uncontrolled use of these drugs poses a risk to both consumer and animal health. After the last application, the “legal waiting period” of the animals applied to antibiotics is sent to slaughter before the completion, edible tissues and organs and animal food obtained from them causes the formation of residues. The same situation applies to dairy animals treated with antibiotics and other animals whose meat is suitable for human consumption (such as poultry, fish) (Tayar and Yarsan, 2014; Kaya, 2018).

Antibiotic residues in foods can causes such as susceptible individuals, drug allergy (allergic reactions, hypersensitivity, anaphylactic shock), antibiotic-resistant bacteria, the development of a disruption of the intestinal flora, sex characteristics a change in suppression of the immune system on endotoxic shock (septic shock), blood clotting, spoilage, food poisoning, some organs (liver,

kidneys, organs of hearing and balance) irreversible damage, permanent staining of the teeth; carcinogenic, mutagenic and teratogenic effects; in the food industry, especially fermented products (fermented sausage, bacon, yogurt, cheese, butter), such as bacteria and preventing the development of starter cultures in the production of fermentation that enables manufacturing defects, environmental pollution and economic losses (Shea, 2003; Nisha, 2008; Landers et al., 2012; Darwish et al., 2013; Mensah et al., 2014; Beyene, 2016; Jayalakshmi et al., 2017; Tadesse and Tadesse, 2017; TÜBA, 2017; Kaya, 2018; Tesfaye, 2019).

The aim of this study was to determine the presence and level of beta-lactam and tetracycline group antibiotics in bovine liver, kidney and muscle tissue and to evaluate the findings in terms of public health.

Material and Method

Material

In this study, 75 bovine tissue samples (each of 25 from liver, kidney, muscle) were used as materials. Tissue samples were taken periodically from animals slaughtered

in the slaughterhouse in September-October 2018, and were brought to the laboratory in a cold chain with sterile stomacher bags and analyzed on the same day. Samples were kept in the refrigerator (+4°C) during this process.

Method

Beta-lactam and tetracycline levels were determined by ELISA (enzyme Linked Immunosorbent Assay) in bovine liver, kidney and muscle samples. Commercial test kits as Randox Food Diagnostic (REF BL3448) for beta-lactam and Sinogeneclon (REF SG4021) for tetracycline were used in the analyses.

Arithmetic mean, percentage, standard error, minimum, maximum values of beta-lactam and tetracycline detected in the samples were analyzed in the SPSS 22.00 package program (SPSS).

Results

Beta-lactam and tetracycline levels and % distributions determined in bovine liver, kidney muscle samples are given in Table 1 and Table 2.

Table 1. Beta-lactam levels (ppb) and % distribution in bovine liver, kidney and muscle samples

| Beta lactam (ppb) | | 0.46-0.92 | 0.92-4.60 | Total |
|-------------------|---------|-----------|-----------|-----------|
| Liver | | 10 | 15 | 25 |
| Kidney | n | 21 | 4 | 25 |
| Muscle | | 13 | 12 | 25 |
| Liver | | 40% | 60% | 100.0% |
| Kidney | % | 84% | 16% | 100.0% |
| Muscle | | 52% | 48% | 100.0% |
| Liver | | 0.75 | 0.93 | 0.75 |
| Kidney | Min. | 0.67 | 0.94 | 0.67 |
| Muscle | | 0.70 | 0.93 | 0.70 |
| Liver | | 0.92 | 1.07 | 1.07 |
| Kidney | Max. | 0.89 | 1.05 | 1.05 |
| Muscle | | 0.92 | 2.57 | 2.57 |
| Liver | | 0.85±0.01 | 0.99±0.01 | 0.94±0.01 |
| Kidney | Mean±SE | 0.78±0.01 | 0.97±0.02 | 0.81±0.01 |
| Muscle | | 0.82±0.02 | 1.12±0.13 | 0.97±0.07 |

Table 2. Tetracycline levels (ppb) and % distribution in bovine liver, kidney and muscle samples

| Tetracycline (ppb) | | 1.35-4.05 | >4.05 | Total |
|--------------------|---------|-----------|-----------|-----------|
| Liver | | - | 25 | 25 |
| Kidney | n | 7 | 18 | 25 |
| Muscle | | 3 | 22 | 25 |
| Liver | | - | 100.0% | 100.0% |
| Kidney | % | 28% | 72% | 100.0% |
| Muscle | | 12% | 88% | 100.0% |
| Liver | | - | 4.48 | 4.48 |
| Kidney | Min. | 1.73 | 4.19 | 1.73 |
| Muscle | | 3.31 | 4.13 | 3.31 |
| Liver | | - | 8.50 | 8.50 |
| Kidney | Max. | 3.83 | 6.39 | 6.39 |
| Muscle | | 3.99 | 7.45 | 7.45 |
| Liver | | - | 6.14±0.17 | 6.14±0.17 |
| Kidney | Mean±SE | 3.37±0.28 | 5.49±0.17 | 4.90±0.24 |
| Muscle | | 3.69±0.20 | 5.94±0.23 | 5.67±0.25 |

According to the results of the analysis, beta-lactam and tetracycline residues were detected in all of the bovine tissue samples (liver, kidney, muscle) which examined. Tetracycline levels were higher in all samples. Beta-lactam levels (mean) were determined as 0.94±0.01 ppb in liver samples, 0.81±0.01 ppb in kidney samples and 0.97±0.07 ppb in muscle samples. Tetracycline levels (mean) were found to be 6.14±0.17 ppb in liver samples, 4.90±0.24 ppb in kidney samples and 5.67±0.25 ppb in muscle samples.

Discussion

Antibiotics are widely used in farm animals to treat and prevent diseases or accelerate growth and development. However, in addition to their positive effects, their improper use without the knowledge of the veterinarian poses a potential danger to human and animal health. This is also leads to economic losses.

In this study, the presence and level of beta-lactam and tetracycline group of antibiotics were investigated in cattle liver, kidney and muscle samples in Sivas province.

According to the results of the analysis, beta-lactam and tetracycline group antibiotic residues were detected in all of the bovine tissue samples which examined. Beta-lactam levels were determined between 0.75-1.07 ppb in liver samples (mean 0.94±0.01), 0.67-1.05 ppb in kidney samples (mean 0.81±0.01) and 0.70-2.57 ppb in muscle samples (mean 0.97±0.07). Tetracycline values were found between 4.48-8.50 ppb in liver samples (mean 6.14±0.17 ppb), 1.73-6.39 ppb in kidney samples (mean 4.90±0.24 ppb), in muscle samples 3.31-7.45 ppb (mean 5.67±0.25 ppb) (Table 1-2).

The levels of antibiotics and residues detected in various tissue samples in studies conducted in Turkey and other countries are given in Table 3 and Table 4.

Kaya et al. (1992), were found antibiotic residue in 21% of liver and kidney samples and 9% of muscle samples in an analysis of 255 bovine tissues (muscle, liver, kidney). Yuksek (2001) was found oxytetracycline residue in 7

kidney samples in an analysis of 50 bovine tissues (muscle, liver, kidney, spleen). Koc (2006) reported that quinolone residue was not found in any of the samples in an analysis of 240 samples of beef and mutton. Oruc et al. (2007), in an analysis of 60 beef samples, in 4 samples (6%) streptomycin (mean 38.7 µg / kg), 1 sample (1.5%) of sulfamethazine (mean 12 µg / kg). Erdogdu et al. (2009), were found tetracycline residue in 13 samples in an analysis of 275 samples of beef and mutton. Residue level was determined between 275-2540 µg/kg. Er et al. (2013), identified quinolone residue in 60 samples (57.7%) in an analysis of 104 beef samples. Residue level was determined as 6.64 µg / kg (Table 3).

In this study, the mean tetracycline level determined in bovine muscle samples were found lower than the reported value by Erdogdu et al. (2009). There weren't any research papers about beta-lactam residue in bovine tissue in a literature review.

They determined in studies conducted in other countries were examined; Muriuki et al. (2001) determined tetracycline level between 524-1.046 µg/kg in bovine tissue (muscle, liver, kidney); Abbasi et al. (2012) found 226.3 ng/g in bovine muscle, 651.3 ng/g in liver and 672.4 ng/g in kidney samples. The level of tetracycline in the tissue samples they examined as; Abbasi et al. (2009) 33.8 µg/kg (bovine muscle); Vragovic et al. (2011) 1.62 µg/kg (beef); Abdullah et al. (2012) 26.04-282.21 µg/kg (beef) and 26.12-102.89 µg/kg (mutton); Ramatla et al. (2017) 48.6 µg/kg (bovine muscle), 92.3 µg/kg (bovine liver), and 192.2 µg/kg (bovine kidney); Baghani et al. (2019) 0.2 ng/g (beef); Biswas et al. (2007), 0.08 µg/g (beef); Olatoye and Ehinmowo (2010), 51.8 µg/kg, 372.7 µg/kg and 1197.7 µg/kg, respectively, in muscle, kidney and liver samples; Al-Gendy et al. (2014), 109.5 µg/kg in meat samples, 584.7 in liver samples and 1115.2 µg/kg in kidney samples; ampicillin levels in the same order as 17.5, 50 and 72.3 µg / kg; Emiri et al. (2014), 57-100 µg/kg (beef) (Table 4).

Table 3. Antibiotic residues in animal tissue samples in studies conducted in Turkey.

| City | n | Tissue | Antibiotic | n ₁ (%) | Residue level | Reference |
|------------------|------------|--|--------------------------------|--------------------------|---------------------------|-----------------------|
| Ankara | 255 | Beef tissue Muscle Liver Kidney | Different type | (9%) (21%) (21%) | | Kaya et al. (1992) |
| Ankara | 50 | Beef tissue Muscle Liver Kidney Spleen | Oxytetracycline | 7 (kidney) | | Yukse (2001) |
| Ankara | 240 | Beef Mutton | Quinolone | | | Koc (2006) |
| Bursa | 60 | Beef | Streptomycin Sulfamethazine | 4 (6%) 1 (1.5%) | 38.27 µg/kg 12 µg/kg | Oruc et al. (2007) |
| Different cities | 275 | Beef Mutton | Tetracycline | 13 | 275-2540 µg/kg | Erdogdu et al. (2009) |
| Ankara | 104 127 | Beef Chicken | Quinolone | 60 (57.7%) 58 (45.7%) | 6.64 µg/kg 30.81 µg/kg | Er et al. (2013) |

n: sample count n₁: positive sample

Table 4. Antibiotic residues in animal tissue samples in studies conducted in various countries

| Country | n | Tissue | Antibiotic | n ₁ (%) | Residue level | Reference |
|--------------|----------|----------------|--|--------------------|-----------------|--------------------------------|
| Kenya | 250 | Bovine tissue | Tetracycline Oxytetracycline Chlortetracycline | 114 | 524-1.046 µg/kg | Muriuki et al. (2001) |
| | | Muscle | | (45.6%) | | |
| | | Liver | | 110 (44%) | | |
| | | Kidney | | 4 (1.6%) | | |
| India | 122 | Beef | Oxytetracycline | 5 | 0.08 µg/g | Biswas et al. (2007) |
| Iran | 500 | Bovine tissue | Tetracycline Oxytetracycline Chlortetracycline | 380 (74%) | 33.8 µg/kg | Abbasi et al. (2009) |
| | | Muscle | | | 52.2 µg/kg | |
| | | Liver | | | 125.2 µg/kg | |
| | | Kidney | | | | |
| Nigeria | 180 | Bovine tissue | Oxytetracycline | 17 | 51.8 µg/kg | Olatoye and Ehinmowo (2010) |
| | | Muscle | | 48 | 372.7 µg/kg | |
| | | Liver | | 33 | 1197.7 µg/kg | |
| | | Kidney | | | | |
| Croatia | 75 | Beef | Streptomycin | (91%) | 44.14 µg/kg | Vragovic et al. (2011) |
| | | | Tetracycline | (96%) | 1.62 µg/kg | |
| Iran | 100 | Bovine tissue | Tetracycline | 66 | 226.3ng/g | Abbasi et al. (2012) |
| | | Muscle | | 12 | 651.3 ng/g | |
| | | Liver | | 22 | 672.4 ng/g | |
| | | Kidney | | | | |
| Iraq | 23 22 | Beef Mutton | Tetracycline | 14 | 26.04-282.21 | Abdullah et al. (2012) |
| | | | | (60.86%) | µg/kg | |
| | | | | 11 (50%) | 26.12-102.89 | |
| | | | | | µg/kg | |
| Egypt | 90 | Sheep tissue | Oxytetracycline | (13.33%) | 109.5 µg/kg | Al-Gendy et al. (2014) |
| | | Muscle | | (20%) | 584.7 µg/kg | |
| | | Liver | | (33.33%) | 1115.2 µg/kg | |
| | | Kidney | | | | |
| Albania | 37 | Beef | Oxytetracycline | 4 | 57-100 µg/kg | Emiri et al. (2014) |
| Tanzania | 60 | Bovine tissue | Oxytetracycline | 60 (71.1%) | 2604.1 µg/kg | Kimera et al. (2015) |
| | | Muscle | | | 3434.4 µg/kg | |
| | | Liver | | | 3533.1 µg/kg | |
| | | Kidney | | | | |
| Tanzania | 171 | Bovine tissue | Oxytetracycline | 87 (51%) | 0-11.72mg/kg | Bilashoboka (2016) |
| | | Muscle | | | 0.19-3.0 mg/kg | |
| | | Liver | | | 0.01-1.67 mg/kg | |
| | | Kidney | | | | |
| Egypt | 30 | Beef | Oxytetracycline | 11 | 17.5-601.8µg/kg | Elbagory et al. (2017) |
| | | | Penicillin | (36.67%) | 5.9-71.3 µg/kg | |
| | | | | 7 (23.33%) | | |
| Tanzania | 60 | Beef | Oxytetracycline | 21 (35%) | 0.69 ng/g | Mgonja et al. (2017) |
| Cameroon | 404 | Bovine tissue | Penicillin G Oxytetracycline | | 17.58 µg/kg | Vougat et al. (2017) |
| | | Muscle | | | 240 µg/kg | |
| | | Liver | | | | |
| South Africa | 50 | Bovine tissue | Tetracycline | 3 (20%) | 48.6 µg/kg | Ramatla et al. (2017) |
| | | Muscle | | 5 (29.4%) | 92.3 µg/kg | |
| | | Liver | | 5 (27.7%) | 192.2 µg/kg | |
| | | Kidney | | | | |
| Zambia | 224 | Beef | Oxytetracycline | 77 (34.4%) | 196.6 ng/g | Nchima et al. (2017) |
| | | | Sulfamethazine | 39 (17.4%) | 86.5 ng/g | |
| Ethiopia | 250 | Beef | Oxytetracycline | 191 | 100 µg/kg | Agmas and Adugna (2018) |
| | | | Beta-lactam | (76.4%) | 5 µg/kg | |
| | | | Sulfonamide | | 100 µg/kg | |
| Tanzania | 171 | Bovine tissue | Oxytetracycline | 137 | 6 mg/kg | Bilashoboka et al. (2019) |
| | | Muscle | | 20 | 0.25 mg/kg | |
| | | Liver | | 14 | 1.28 mg/kg | |
| | | Kidney | | | | |
| Iran | 41 | Beef | Ciprofloxacin | 41 (100%) | 0,8 ng/g | Baghani et al. (2019) |
| | | | Tetracycline | 31 (75%) | 0.2 ng/g | |

n: sample count n₁: positive sample

In the studies were determined oxytetracycline levels as Kimera et al. (2015), 2604.1 µg/kg in meat samples, 3434.4 in liver samples and 3533.1 µg / kg in kidney samples; Bilashoboka (2016), between 0-10.72 in meat samples (mean 0.60), in liver samples 0.19-3.0 (mean 1.28) and 0.01-1.67 mg/kg in kidney samples (mean 0.26); Elbagory et al. (2017), 17.5-601.8 µg/kg in beef; Mgonja et al. (2017), ort. 0.69 ng / g; Vougat et al. (2017), 240 µg/kg (et); Nchima et al. (2017), 196.6 ng/g in beef; Agmas and Adugna (2018), 100 µg/kg in beef; Bilashoboka et al. (2019), 6.0 mg/kg in muscle samples, 0.25 mg/kg in liver samples and 28 mg/kg in kidney samples (Table 4).

In this study, the mean level of tetracycline determined in bovine muscles did not parallel the findings of these researchers. In the same way, tetracycline values detected in liver and kidney samples were found to be lower than by some researcher's results (Abbasi et al., 2012; Ramatla et al., 2017).

The differences between the research findings can be explained by the fact that the studies were conducted in different years, the number of samples and the methods applied were different. In addition, regional differences have probably been influential in this regard.

Tetracycline group antibiotics (chlortetracycline, oxytetracycline, tetracycline) the maximum residue limit (MRL) in bovine muscle, liver and kidney, respectively, in the 200, 600, and 1200 mg/kg as determined by the Codex Alimentarius Commission (CAC, 2018). MRL values for amoxicillin and benzyl penicillin (penicillin G) group of Beta-lactam antibiotics were determined as 50 µg/kg in muscle, liver and kidney (bovine). The tetracycline group determined the MRL value of antibiotics in muscle, liver and kidney as 100, 300 and 600 µg/kg, respectively in all food-produced species by Commission of the European Union (EU) (EU, 2010). MRL values for amoxicillin and benzyl penicillin were determined as 50 µg/kg in muscle, liver and kidney (bovine).

MRL values related to veterinary drugs in animal foods are regulated in the "Turkish food Codex regulation on the classification and Maximum Residue Limits of pharmacological active substances that can be found in animal foods (TGK, 2017)", prepared in accordance with EU legislation in Turkey. MRL value for tetracycline (the sum of the main substance and 4 epimers) in all food-produced/producing species was determined as 100 µg/kg, 300 µg/kg and 600 µg/kg in muscle, liver and kidney respectively in the regulation. MRL values for amoxicillin and benzyl penicillin in all food-produced species have been reported as 50 µg/kg in muscle, liver and kidney.

In this study, tetracycline levels detected in bovine tissue samples (muscle, liver, kidney) conformed to the limit values reported in European standards and the Turkish Food Codex (EU, 2010; TGK, 2017; CAC, 2018).

Conclusion

When the research findings were examined, beta-lactam and tetracycline group antibiotic residues were detected in all the samples which analyzed. However, the values determined were found to be in accordance with the legal limits. At the same time, the presence of antibiotic residue in all samples indicates that these antibiotics are widely used. In solving the residue problem caused by

antibiotics, public organizations, veterinarians, animal breeders, food manufacturers, pharmaceutical companies, units related to the distribution and sale of drugs have important responsibilities. In order to prevent or limit drug residues in animal foods, the veterinarian should be aware of the responsibility and equipped with information, the diagnosis of the disease, drug selection, dose of the drug, method of administration and duration of treatment should be done correctly. Animal owners or animal breeders should be trained in relation to the subject. Animals with food value should not be sent to slaughter until the legal waiting period is complete following the administration of antibiotics. The drugs used in veterinary or animal by animal breeders must record and the records should include the type, of animal, drug type, dose, dose interval, application type, and duration of use, side effects and the treatment result information. Veterinary drugs should not be used as a growth factor in animals with food value. This practice was banned in Turkey in 2006 in line with the EU. However, this restriction does not apply in some countries (USA, Canada, Brazil, China). Waste belonging to animals treated with antibiotics should not be used as feed additives. Pharmaceutical companies should be aware of responsibility and animal owners should be properly informed. Chip earrings should be used in animals and drug tracking system (DTS) should be used by applying square code for the traceability of veterinary drugs. Analysis methods and studies should be revised and improved as part of the residue monitoring and control program maintained by the Ministry of Agriculture and Forestry. Necessary legal actions should be taken by the authorized institutions or organizations if residues above the tolerance level are detected.

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