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The Overuse Of Tetracycline Compounds In Chickens And Its Impact On Human Health

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Abstract. Antibiotics have been extensively involved in food biotechnology and have been used in animals for prophylaxis and growth promotion. However, its effect on human health and environment remains in question. Chickens samples from intestine, liver and kidney were analyzed using a tandem quadruple mass spectroscopy high performance liquid chromatography (HPLC/MS/MS). Standard solutions of chlorotetracycline (CTC), deoxycycline (DTC), oxytetracycline (OTC) and tetracycline (TC) were used and the signal intensities were compared with tetracyclines in organ samples. In total, tetracycline values were significantly higher in liver followed by intestine and kidney. TC values were significantly higher in liver and kidney over the intestine while DTC values were significantly higher in liver and intestine over the kidney. OTC values were significantly higher in kidney and intestine over the liver while CTC liver values were significantly higher than in the kidney and intestine. Although the values of the four antibiotics were within the average range of the recommended maximum residue limit (MRL), their impact on environment and human health is a public concern if this trend of antibiotic overuse continues.

Keywords: Bioengineering, food technology, tetracycline, residue, chickens, organ.

1. Introduction

Antibiotics have been used extensively since the 1950s for a better treatment of microbial diseases in humans, and also improve food quality production. Antibiotics have been used in animal health, prophylaxis and growth promotion especially in poultry [1]. The most common used antibiotic at farms is tetracycline. The main organs involved in food uptake and metabolic processes are intestine, liver and kidney. They are used in small dosages which increase protein and decrease fat components [2]. However, the use of antibiotics may also affect the normal flora which resulted in antibiotic resistance [3]. Further spread of resistance to the environment occurs as a result of agricultural runoff and contaminated effluents which would affect the surrounding water [4], [5]. Even though there are advantages in the use of those antibiotics, there is also concern regarding the overuse of antibiotics [6]. Failure of farm management to adhere to a recommended period of withdrawing the drug before marketing the animal causes an escalation of the problem. Regulations must be adhered to control the usage of antibiotics and therefore stopping the emergence of multiple resistant strains.

Bedsides the microbial treatment, tetracyclines are used as an anti-inflammatory [7] and tumor growth inhibition, and growth inhibitor [8], [9]. However, tetracycline compounds have been reported to affect growth of teeth and bones in children [3].

Because of several health and environment concerns regarding the use of antibiotics for better food quality and production, the main objectives of this investigation is to assess tetracycline compounds in animal organs (intestine, liver and kidney).

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2. Materials and Methods

Ten samples from each organ (intestines kidneys and livers) from chickens were collected in phosphate buffer saline (PBS) and stored at -20 ^oC.

An internal TC standard was prepared from kidney and liver samples from chickens not fed with antibiotics according to the method of Van Eeckhout [10]. An amount of 120 µL of the internal TC standard was added in 10 mL of McIlvain-EDTA buffer containing 2g of homogenized intestine, kidney and liver samples. Calibration standards for analysis of OTC, CTC, DTC and TC were prepared (Sigma Aldrich, UK). The McIlvain-EDTA buffer contained 12g citric acid monohydrate, 10.9g Na_2HPO_4 and 37.2g EDTA in 1L water and the was set at pH3.8. All solutions were vortexed vigorously for 1 min, and sonicated for 10 min. The samples were then centrifuged at 16,000 X g for 10 min at 4 ℃. The supernatant was filtered and 10 µL of the filtrates were injected using an autosampler onto analytical separation column (Xterra C18, 2.1x50mm, 3.5 µm; Waters Corp., MA, USA) on a tandem quadruple spectroscopy, High Performance Liquid Chromatography system (HPLC MS-MS) (Quattro Ultima Pt, Waters Corp., MA, USA). The signal intensity was compared with standard solutions of OTC, CTC, DTC and TC at concentrations of 1mg/mL, 100ng/ µL, 1ng/uL and 100pg/uL.

The OTC, CTC, DTC and TC calibration standards at 1, 5, 10, 50, 100, and 500 (pg/ µL) concentrations were used (Fig 1). Quality control assessment for precision and accuracy of the analytical method were made at 3, 30, and 300 (pg/ µL) concentrations and 1ng/ µL of OTC, CTC, DTC and TC standard solution was used to tune the mass spectrometer for optimum sensitivity (Fig. 2). The collision energy of the cone was set at 18eV-50V.

OTC, CTC, DTC and TC separation by HPLC (Agilent 1100 Palo Alto, CA, USA) was used. Acetonitrile/water (70/30, v/v) was used as mobile phase (Sigma Aldrich, UK), at a flow rate of 0.3mL/min and an Xterra C18, 2.1x50mm, 3.5 µm column (Waters Corp., MA, USA).

The chromatogram peak area in the 10pg/mL OTC, CTC, DTC and TC standards solutions in water/acetonitrile were compared to a standard, at the same concentration, of spiked solutions and the recovery efficiency was 98%. The back-calculated concentrations from antibiotic extraction method were used to evaluate the tetracycline concentrations in intestine, liver and kidney extracts and were analyzed statistically.

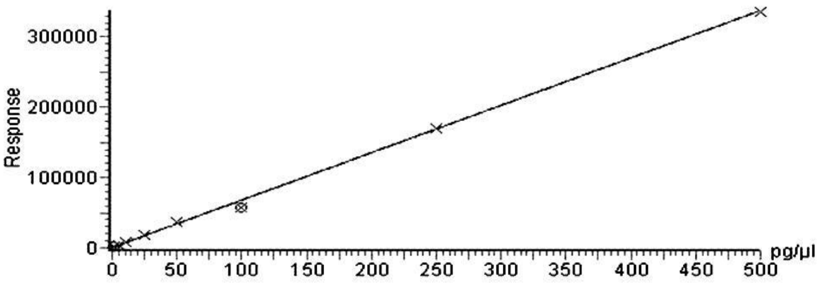

Fig. 1: The standard concentration curve for calibration of tetracycline compounds.

3. Results

The total concentration of tetracycline compounds is illustrated in Fig.ure 3. The liver has the highest concentrations over the intestine and kidney ($P < 0.05$).

The antibiotic tetracycline concentrations in the three organs (liver kidney and intestine) varied in the four tetracycline compounds (TC, CTC, DTC and OTC), (Fig. 4).

In TC, the concentrations were significantly higher than in liver and kidney over the intestine ($P < 0.05$). In DTC the concentrations were significantly higher than in liver and intestine over the kidney ($P < 0.05$). In OTC, the concentrations were significantly higher in kidney and intestine over the liver ($P < 0.05$). In CTC the liver concentrations were significantly higher than in kidney and intestine ($P < 0.05$).

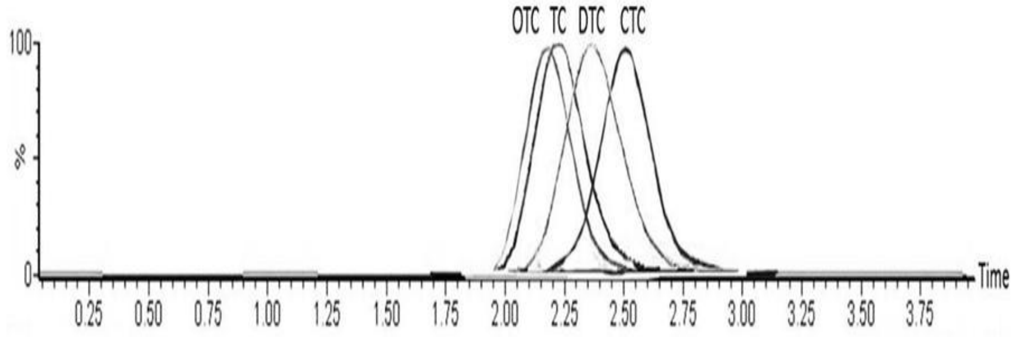

Fig. 2: Retention time of the four types of tetracycline compounds

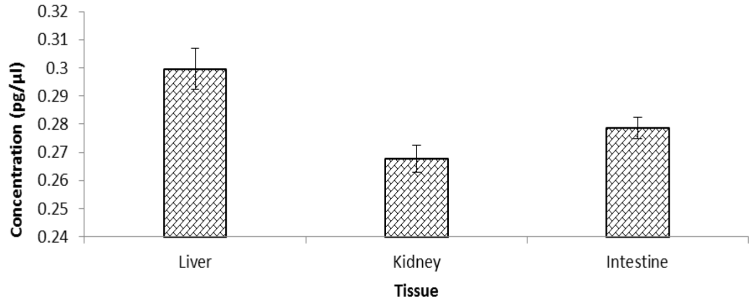

Fig. 3: Average concentrations of tetracycline compounds in chicken organs.

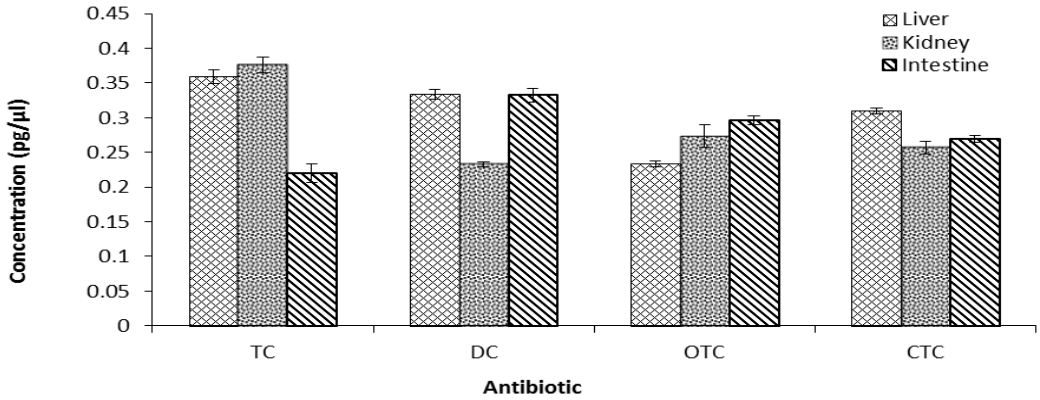

Fig. 4: Tetracycline concentrations in chicken organs.

4. Discussion

Tetracycline compounds are increasingly being used in farm animal production as growth promoters and for treatments of diseases. For economic reasons local poultry producers add this drug to water which may enhance the absorption of the drug and ultimately penetrates the tissues [11]. Over the years, the question of antibiotic residue from farm animals and their effect on human health has been a major concern. However, the use of tetracycline compounds in animals varies in different countries. Due to the fact that assay methods are much improved today, the same level of antibiotic detected now may not been shown several years ago. The MRL depends on the time allowed between the use of the antibiotics and the time of slaughter, in which sometimes the presence of the antibiotic could drop to an undetectable level. In fresh liver 95.5% of the antibiotic residue-positive had mean concentration of at least one tetracycline compounds exceeding MRL residue. All four types of tetracycline compounds were detected at the time of slaughter which indicated that they had been used throughout the production cycle [12]. In our study the four types of tetracyclines (TC, CTC, DTC and OTC) were present in the samples. All residues were below MRL.

CTC and OTC have been approved as therapeutic drugs in the USA for nutrition and prophylactic. TC and DTC are used only as therapeutic agents. All tetracycline compounds are distributed throughout body tissue but found in high concentration in excretory organs. Nhiem et al. [13] in Vietnam reported that 5.5% of farm animal tissue contained tetracycline residue and muscle samples which exceeded MRL values. Oral and intramuscular administration of tetracycline compounds to poultry resulted in significant residue in the organs. In comparison, random samples were collected from local markets in Egypt for antibiotic residue. Among the samples, 10% of chicken kidney and 4% of liver contained antibiotics [14].

It was reported that tetracycline compounds are heat labile for a specific temperature and that its consumption is acceptable. However, if it is not destroyed by heat it causes resistance to bacteria that leads to other environmental problems [12]. Chlortetracycline and oxytetracycline residues were found to be destroyed by roasting, frying, and autoclaving poultry tissue. On the other hand, poaching and scrambling eggs did not destroy the antibiotics [15].

Antibiotic residue in foods is serious health and environmental concerns to the consumer. It is not known which persistent concentration in nanogram levels can lead to toxicity. Low levels of antibiotics act as a persistent pressure for the selection of resistant bacteria to colonize animal tissues and cause normal flora disturbance [16]. It is well established that the use of any antibiotics for prophylaxis and growth promoters will eventually develop resistance of microbes to antibiotics. Transfer of antibiotic resistant strains to humans remain a very important issue and of a great concern to our health. Antibiotic resistant bacteria can contaminate meat and food products consumed by humans.

Prolongation of elimination of tetracycline half-life is related to extensive intrahepatic circulation and, thus, it stays in the body for a longer period after the secession of drug administration. Contamination of food with antibiotic residue may present some health problems, such as immunological response in a susceptible person causing disorder of intestinal normal flora. Therefore, it is important to carry out thorough investigations to determine adequacy of the antibiotic withdraw period. The major problem of antibiotic resistance is the transfer of resistant microbes to humans via contaminated food. It becomes a serious issue in treating the infected individuals. Public awareness is an important factor for the reduction of unnecessary and overuse of antibiotics. It requires education and cooperation of both health care professionals and the public.

In conclusion, it is essential to monitor the overuse of antibiotics in food biotechnology, set new guidelines and investigate the emergence of resistant bacteria.

Implementation and enforcement of stringent regulations regarding usage of antibiotics is urgently needed. Development of laboratory analysis and interpretation remain vital to the monitoring process in order to take immediate action and limiting environmental and human toxicity from antibiotic uptake.

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