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Research Article
Kinetics of the Antimicrobial Activity Appearance in Ross 208 Broilers Chicken Tissues Following an Oxytetracycline Supplemented Diet

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Abstract

Background and Objective: Tetracyclines are currently among the most widely used antibiotics for growth promotion in the broiler breeding sector. The current study seeks to evaluate the presence of antimicrobial activity in tissue of broiler chickens that have been fed a classic diet supplemented with the antibiotic oxytetracycline. **Materials and Methods:** A four-plate test was used to compare antimicrobial activity in tissues of chickens that had and had not been fed an antibiotic. **Results:** The results of this test showed that antimicrobial activity first appeared in the kidneys, followed by the liver and then adipose tissues. The most intense antimicrobial activity was observed in adipose tissue, whereas no activity was detected in muscle tissues. **Conclusion:** The inclusion of oxytetracycline in broiler chickens' diet as an antibiotic growth promoter induced the appearance of antimicrobial activity in the kidneys and adipose tissue with an increasing intensity according to the time of the breeding period.

Key words: Antibiotics growth promoters, breeding, broiler, oxytetracycline, the four-plate test

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antibiotics are used on a large scale in modern breeding of poultry and especially for broiler chickens. Although some antibiotics are used for therapeutic purposes to maintain animal welfare, others are given for prophylactic purposes, such as when animals are exposed to a risk factor. Still other antibiotics are used as, antimicrobial growth promoters (GAP) to improve feed conversion performance and efficiency[1]. Tetracyclines (TC) and beta-lactams are the most commonly used antibiotics in animal farming and sulfonamides, lincosamides and macrolides are also used[2]. The amount of antibiotics used for veterinary applications has risen in parallel with global increases in broiler chicken production[3]. Some studies have shown that environmental contamination by residues of antibiotics can be caused either by inappropriate or uncontrolled use of these drugs. Such residues of antibiotics can induce direct negative effects on consumers[4]. Antibiotics that are administered to broiler chickens can accumulate in various tissues, as has been demonstrated by several studies Kan and Petz[5]. The presence of antibiotic residues in broiler chicken tissues is a very serious concern for public health agencies around the world[6]. The aim of the current study was to detect the appearance and intensity of antimicrobial activity in tissues of broiler chickens fed a diet supplemented with the antibiotic oxytetracycline, as a function of breeding time.

MATERIALS AND METHODS

Birds housing and diets

Experimental diets: The reference feed for chickens during this experiment was a basic diet consisting of corn, soybean meal and mineral-vitamin supplements. The composition and gross energies of the experimental foods are shown in Table 1.

Experimental design: A total of 180 one day-old, male Ross 208 chicks were collected for this study from "HAD SOUALEM", a local commercial hatchery located in the Casablanca region of Morocco. Breeding conditions, food, temperature and lighting were implemented in accordance with Ross 208 guidelines. The birds were given feed and water *ad libitum* throughout the experimental trial. All birds in the group were from the same breeding conditions and were divided into two experimental groups of 90 chicks each. The control group (T group) was fed a conventional basic diet (Table 1) and the experimental group (ATB group) was fed the same diet supplemented with 0.005g oxytetracycline (OTC, purity 97.0% Sigma-Aldrich, St. Louis, MO) per 100 g food. In this study, the breeding period lasted for 48 days with 22 and 26 days for the start-up and growth phases, respectively. During the start-up phase both groups of birds were fed the conventional basic diet as crumbles. During the growth phase, the ATB group was given the experimental diet as pellets.

Detection of antimicrobial activity in tissues

Screening test: A four-plate test was used to detect the presence of residues of antibiotics in broiler chicken tissues[7]. Trypticase soy (TS) agar was prepared for the plates at three different pH levels: pH 6, pH 7.2 and pH 8 with the pH adjusted using sodium hydroxide and hydrochloric acid. The use of culture media having different pH values is essential to increase the detection limits for antibiotic activity[8]. *Micrococcus luteus* ATCC9341 and *Bacillus subtilis* 10649 were used as reference bacterial strains for screening and each were incubated for 24 h in 10 mL TS broth. The turbidity of the bacterial suspensions was then adjusted using McFarland standards to yield a cell density of approximately 3×10^8 cells mL^{-1}. To detect the presence of different families of antibiotics in tissues, four different conditions were used in the screening test. *Bacillus subtilis* was seeded on pH 6 and pH 8 TS agar plates to detect the presence of

Table 1: Feed shape and composition during the chick rearing period

Breeding phase	Feeds shape	Feeds composition	(%)	Gross energies (Joule kg^{-1})
Start-up (day 1-22)	Crumb	Maize	61	$1.18.10^7$-$1.22.10^7$
		Soyabean meal	32	
		Bran	2.5	
		Vitamin and mineral mix[a]	1.5	
		CaCO3	1.5	
		Ca2PO4	1.5	
Growth (day 22-48)	Granule	Maize	65	$1.18.10^7$-$1.22.10^7$
		Soyabean meal	28	
		Bran	4	
		Vitamin and mineral mix[a]	1.5	
		CaCO3	0.5	
		Ca2PO4	1	

[a]Providing the following per kg of diet; Vitamin A: 8 000 IU, Vitamin B1: 1 mg Vitamin B2: 3 mg, Vitamin B6: 1 mg, Vitamin B12: 0.01 mg, Vitamin D3: 600 IU, Vitamin E: 16 mg, Vitamin K3: 1 mg, Vitamin B3: 16 mg, Pantotenic acid: 7 mg, Mn: 70 mg, Zn: 50 mg, Cu: 4 mg, Se: 0.1 mg, Fe: 30 mg, Co: 0.2 mg, I: 1 mg, Phytase: 300 units, Choline: 240 mg

tetracycline/beta-lactams and aminoglycosides, respectively. *M. luteus* was seeded on pH 7.4 and pH 8 TS agar to detect the presence of sulfa drugs and macrolides, respectively[9].

Sampling: Every three days during the 48 day experimental period (i.e., Day 21, 24, 27, 30, 33, 36, 39, 42, 45 and 48) two animals from each group were randomly sampled for the screening test. The selected animals were subsequently euthanized and kidney, muscle, fat and liver tissues were recovered under sterile conditions. The tissues were frozen at -80°C and sliced into thin sections from which 4 mm discs were removed using a sterile punch. The tissues discs were then placed on the surface of each of the four types of plates described above. A sterile Whatman paper disc soaked in dihydrostreptomycin (0.5 µg/100 µL) or sulfamethazine (5 µg/100 µL) served as a positive control. After placement of the discs, the dishes were incubated at 37°C for either 18-24 or 48 h for *B. subtilis* or *M. luteus*, respectively.

RESULTS

Detection of antimicrobial activity in tissues: Results of the four plate test indicated that antimicrobial activity was detectable in tissues of broiler chickens in the ATB group but not the control group. All positive samples in the current study were detected on pH 6 TS agar plates seeded with *B. subtilis*. A positive result was indicated by a zone of complete growth inhibition on the agar surface around the tissue discs that was at least 2 mm in diameter[7]. Similar zones of inhibition were measured for the positive control plates. The results are summarized in Table 2. The screening test results showed that antimicrobial activity was detected in the kidneys beginning on Day 5 of the growth phase (Day 27 of the experimental period) and persisted through the end of the breeding period. The zone of inhibition diameters ranged from 11-11.5 mm. Meanwhile, antimicrobial activity in adipose tissue appeared on Day 33 of the breeding period and the zone of inhibition diameter reached maximum values (15.5 mm) by Day 45. For liver tissues, antimicrobial activity was detected three times during sampling, on Days 30, 42 and 45. No antimicrobial activity was detected in muscle tissue.

DISCUSSION

Treatment of poultry with antibiotics and inclusion of antibiotics in poultry diets is a major source of antibiotic contamination in foods for human consumption[10]. In the screening tests performed for this study, antimicrobial activity was first seen in kidney tissue of broiler chickens fed a diet containing antibiotics beginning on Day 27 of the breeding period. Antimicrobial activity was also observed in adipose tissues of these birds beginning on Day 33 and this activity persisted throughout the remainder of the experimental period. These results are consistent with the metabolism of semi-synthetic cyclins, particularly oxytetracycline, in kidneys. These antibiotics have a bioavailability that can reach 90% and a half-life of between 18 and 22 h. Cyclins also have good diffusion which explains the appearance of antimicrobial activity in liver tissues for some samples from the group fed a diet containing antibiotics. Although the excretion rate of sulfonamides and tetracyclines is generally between 40 and 90%, this rate can vary according to the nature of the administered drug, its mechanism of action and the species to which the drug is administered[11,12]. The subsequent presence of significant antimicrobial activity in adipose tissue is likely due to the accumulation of oxytetracycline or its metabolites. After administration, residues of antibiotic can persist in the body of broiler chickens for long periods, even if no additional antibiotics are given. In addition to the risk for acquisition of resistance to certain antibiotics, the presence of residues of antibiotics in livestock tissues can induce severe anaphylactic reactions in consumers who may have allergies to these

Table 2: The antimicrobial activities distribution by organ on TS medium seeded with *B. subtilis* at pH 6 for ATB group

	Inhibition diameter (millimeter) according to the different tested organs for ATB group			
Sampling age (days)	Fat	Kidney	Liver	Muscle
21	-	-	-	-
24	-	-	-	-
27	-	11±0.00	-	-
30	-	11±0.50	11±0.00	-
33	11±0.00	11±0.00	-	-
36	11±0.00	11±0.50	-	-
39	12±0.50	11±0.00	-	-
42	13±1.00	11±0.50	11±0.00	-
45	15±0.50	11±0.00	11±0.00	-
48	15±0.00	11±0.50	-	-

substances. Moreover, antibiotic resistant bacterial strains could be transmitted to consumers through direct contact with animals or through the food chain[13]. GAPs act primarily on some populations of intestinal bacteria and can reduce the total number of microorganisms (including pathogenic strains) that colonize the digestive tract by creating a more favorable balance between beneficial and non-beneficial microorganisms[14]. Some parameters of immune status in broilers can be modified following administration of diets supplemented with certain GAPs. Indeed, Lee *et al.*[15] noted an increased number of Ig As in ileal cells from broilers fed a diet containing oxytetracycline. On the other hand, these amounts decreased in broilers administered with the thiopeptide antibiotic Nosiheptide relative to controls fed diets lacking this GAP. Chickens fed GAP-supplemented diets also tend to have low levels of ileal intraepithelial lymphocytes (IELs) and high levels of duodenal IELs compared to untreated chickens[16]. GAPs have also been shown to increase the expression of interferon-c (IFN-c) in the small intestine of chickens[17]. In vitro studies on neutrophils and human monocytes showed that antibiotics can modulate the mRNA expression levels of genes that encode cytokines[18,19]. Thus, underlying immunomodulation could be, in part, related to changes in intestinal microbiota following administration of GAP-supplemented diets to broiler chickens[17]. Upon hatching, the digestive tract of chicks is sterile but colonization by microorganisms begins immediately after hatching and depends either on diet, water and/or the environment[20]. The compositional structure of intestinal microbiota may change according to diet components. Whereas some bacterial populations may disappear or emerge in the gut, others may persist throughout the life of the bird. Studies have shown that broiler performance may be due in part, to differences between bacterial strains that constitute the intestinal microbiota and the function of these strain[21]. The results of this study suggest that uncontrolled or inappropriate use of antibiotics in livestock, particularly broilers, can result in increased amount of residues of antibiotics in tissues from these animals. Such residues of antibiotics in livestock represent a challenging public health concern. Findings by previous studies confirmed the presence of tetracycline, aminoglycosides and macrolides in commercial eggs[22], in addition to the passive attitude of consumers towards the presence of residues of antibiotics[23], emphasize the need for better programs that provide surveillance and control strategies to reduce the incidence of residual antibiotics, as well as the rate of antibiotic resistance arising from livestock applications.

CONCLUSION

These findings indicate that the inclusion of oxytetracycline in broiler chickens' diet as an antibiotic growth promoter induced the appearance of antimicrobial activity in the kidneys beginning on Day 27 of the experimental period and persisted through the end of the breeding period. Antimicrobial activity appeared in adipose tissue on Day 33 with an increasing intensity according to the time of the breeding period. The intensity reached maximum values by Day 45. This work will help to get a clear idea and a better comprehension of how these substances spread as well as improving their capacity to target the most affected tissues.

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