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DETERMINATION OF OXYTETRACYCLINE RESIDUES IN CHICKEN MEAT

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A B S T R A C T

Ninety random samples of chicken breast and thigh (45 of each) were collected from 3 different chicken processing plants A, B, C (30 of each) located in Kalyobia Governorate. Thus, each plant was represented by 15 thigh and 15 breast samples. The collected samples were analyzed for detection of their contents of oxytetracycline residues using four plate test (FPT) and high performance liquid chromatography (HPLC). The results showed that the positive samples of chicken breast and thigh samples were 26.67% & 40.00% , 26.67% & 33.33% and 13.33% & 26.67% for processing plants A, B and C, respectively concerning that the unaccepted samples of breast and thigh were 13.33% and 26.67 % for plant A, 13.33% and 20.00% for plant B and 6.67% and 6.67% for plant C, respectively. Finally, the public health significance of these serious residues and sources of their presence in chicken meat as well as some recommendations to avoid them in such food items were discussed.

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1. INTRODUCTION

Poultry meat constitutes an excellent source of high quality animal proteins required for nutrition of infants, young children, adults and convalescents. In addition, vitamins especially B complex and minerals such as potassium, magnesium and phosphorus are present in considerable amounts in the poultry meat (4).

Antibiotics are widely used in poultry farms as dietary supplements. Tetracycline is one of these antibiotics, which have abroad spectrum action on a variety of infection (18).

About 60% of an ingested dose of tetracycline was absorbed from the gastrointestinal tract of human being and then widely distributed in the body, particularly liver, kidney, bones and teeth. However, the use of this compound may result in residues in poultry meat especially if proper withdrawal times have not been used (6)

Tetracyclines are broad-spectrum antibiotics because they are active against Gram positive and Gram-negative bacteria. They also act against some pathogenic agents unaffected by other antibiotics e.g. rickettsiae, certain large viruses belonging to psittacosis group in animals and lympho-granuloma venerum group in humans, tetracyclines have activity against mycoplasmas, spirochetes and actinomycetes. Tetracycline undergo metabolism to various degrees. The most frequently identified substance in urine, faces and tissue is the parent tetracycline. As much as 30% is excreted unchanged in feces. Tetracyclines are reversibly bound to plasma proteins and are widely distributed. Tetracyclines diffuse throughout the body and are found in highest concentrations in

kidney, liver, spleen and lungs. They are also deposited at active sites of ossification (16).
Oxytetracycline used as feed additive to broiler chicken from 1-8 weeks ago. Tissue samples including muscles, liver and kidney were collected. The results of tissue examination for the drug residues were not detected after one-week of the withdrawal period of the drug, and this was attributed to the route and duration of drug administration. The presence of other antibiotic may alter the antibiotic elimination rate due to the deposition of the drug in the bone and its elimination and re-precipitaition in the tissues, (20). The maximum residues limit of oxytetracycline is 0.1 mg/kg of chicken meat (5).
The tetracycline which added to the feed are absorbed and deposited in organs and tissues to considerable amount, the freezing at-20^{O}C slightly affect on the residues after 4months of storage. The heat treatment decrease the antibiotic concentration but degradated products may still be lift in the tissue. These may be toxic for human beings, (10). The risks associated with drug residues in meat above the established tolerance, may lead to carcinogenic, mutagenic and teratogenic effects which are of the possible public health consequences that may occur as a result of exposure to illegal residues, in addition to hypersensitivity to such drugs may be occur red. (14).
The use of antibiotics causes serious disturbances of biological and biochemical equilibrium and killing of the non pathogenic bacteria which are necessary for break down of waste products. Also using low levels of antibiotics will increase the drug resistance for unfavorable microorganisms and drug failure for these antibiotics (21). The antibiotic residues in meat may develop urticaria, dermatitis and allergic conditions (22).
Therefore, many programs include mainly educational programs, widespread testing of foods for antibiotic residues and financial penalties. Implementation of quality assurance programs to protect public health against adverse effect of antibiotic is a major challenge for developing countries, where there is a veterinary misuse of such drugs and Currently, there are no major quality assurance programs in place in the country to protect public health against the adverse effect of antibiotic used in animal husbandry, which is partly due to the lack of research data to inform policy (7).

2. MATERIAL AND METHODS

2.1. Samples

A total of 90 random samples of chicken meat products represented by breast and thigh (45 of each) were collected from there different chicken processing plants (30 of each) located in Kalyobia Governorate to determine their toxic residues.
Each sample was kept in a separate sterile plastic bag and transferred to the laboratory in an insulated ice box as quickly as possible. All collected samples were examined for detection of their contents of oxytetracycline residues.
Four plate test (FPT) (Microbiological method): The method recommended by (3) was carried out. Each sample was divided and applied to four plates of antibiotic agar medium, three of which were inoculated with Bacillus subtilis spores at PH 6, 7.2 and 8. Moreover, trimethoprim was incorporated into the medium at PH 7.2 to enhance the test for sulfonamide residues. Diffusion of the active sulphamethazine was detected by the formation of inhibition zones on one or more plates after incubation at 37^{O}C overnight, The sensitivity of the test was monitored by applying 6mm-diameter discs containing standard quantities of known antimicrobial agents in each run.

2.2. Application of HPLC technique:

Quantitative analysis of antimicrobial agent in the examined samples of chicken thigh

and breast were done according to (15) and (13).

Accurately, 5gm of each sample and 10gm of anhydrous sodium sulfate were blended with 20ml of ethy1 acetate and then centrifuged. The supernatant was evaporated and dried under reduced pressure at 40 ^{O}C. The residue was dissolved in 5ml of ethy1 acetate-n-hexane and the solution was applied to abond Elute previously washed by 5ml n-hexane, The cartridge was washed with 3ml n-hexane and air-dried by aspiration. The surveyed antimicrobial residues were eluted from the cartridge with 5ml acetonitrile (20%) and 0.05M ammonium format. The preparation was injected into HPLC system (model LC – 10A series equipped with constant flow pump and variable wave length U/V detection, Kyoto, Japan). Accordingly, antibiotic residues were estimated by using their standard solutions specific for each of them. Operating conditions for analysis of tetracycline were eluant at 35C, flow rate 1 ml/min., injection volume, 10ul; detection wave length 216nm, while, the operating conditions for analysis sulfamethazine were eluant at 30C, flow rate 1ml/min, injection volume, 20ul; detection wave length 272nm.

2.3. Statistical analysis

Statistical analysis of the obtained results was done by application ofanalysis of variance " ANOVA" according to (17).

3. RESULTS

Table (1): Oxytetracycline levels (mg/kg) in the examined samples of chicken meat (n=15).

Chicken Meat Samples / Processing Plants	Breast			Thigh		
	Min.	Max.	Mean ± S.E*	Min.	Max.	Mean ± S.E*
A	0.06	0.17	0.11 ± 0.02	0.08	0.35	0.19 ± 0.03
B	0.03	0.16	0.09 ± 0.01	0.07	0.23	0.14 ± 0.02
C	0.01	0.12	0.06 ± 0.01	0.02	0.11	0.08 ± 0.01

S.E*= Standard error of mean.

Table (2): Oxytetracycline levels in the examined chicken breast and thigh samples.

Source of Variance	DF	S.S	M.S	F.value
Total	89	0.2608		
Between Chicken Cuts (C)	1	0.0095	0.0095	3.80 +
Between Plants (P)	2	0.0379	0.0189	7.59 ++
(P) × (C) interaction	2	0.0033	0.0017	0.67 NS
Error	84	0.2101	0.0025	

D.F = Degrees of freedom, + = Significant differences (P<0.05), S.S = Sum squares ++ = High significant differences (P<0.01), M.S = Mean squares NS = Non significant differences

Table (3): Acceptability of the examined samples of chicken breast and thigh based on their levels of oxytetracycline (n=15).

Plant / Chicken Meat	Maximum Permissible Limit (mg/kg)*	Total positive samples		Unaccepted Samples	
		No.	%	No.	%
Plant (A):					
Chicken breast	0.1	4	26.67	2	13.33
Chicken thigh	0.1	6	40.00	4	26.67
Plant (B):					
Chicken breast	0.1	4	26.67	2	13.33
Chicken thigh	0.1	5	33.33	3	20.00
Plant (C):					
Chicken breast	0.1	2	13.33	1	6.67
Chicken thigh	0.1	4	26.67	1	6.67

* FAO/WHO (1999)

4. DISCUSSION

Residues of veterinary drugs in food of animal origin represent a risk to human health and they have negative impact on the technological processes in the food industry. Accordingly, the improper use of veterinary drugs may result in drug residue in rabbit tissues causing allergic reactions in the sensitive individuals consuming such foodstuff. The results achieved in table (1) declared that the concentration of oxytetracycline (mg/kg) in the examined samples of chicken breast were 0.06 to 0.17with an average 0.11 ± 0.02 for plant A,

0.03 to 0.16 with an average 0.09 ± 0.01 for plant B and 0.01 to 0.12 with an average 0.06 ± 0.01 for plant C. While, the mean value of oxytetracycline levels in the examined thigh samples were 0.19 ± 0.03, 0.14 ± 0.02 and 0.08 ± 0.01 for plant A, B and C, respectively. Difference associated with the examined chicken meat samples were significant at (P<0.05)for chicken cuts. Also, the processing plants had highly significant effect at (P<0.01) on oxytetracycline levels. However, the inter action between chicken cuts and processing plants were not significant as shown in table (2). Table (3) indicated that the acceptability of the examined samples of chicken breast and thigh based on their level of oxytetracycline (9) stipulated that the permissible limit of oxytetracycline in chicken meat was 0.1 mg/kg. Therefore, the unaccepted samples of breast and thigh were 13.33% & 26.67% for plant A, 13.33% & 20.00% for plant B and 6.67% & 6.67% for plant C, respectively. These results nearly similar to those obtained by (11). While, higher results were obtained by (12, 19, 8, 2 and 1).

The present study allows confirming the habhazard using of antibiotics inside the poultry farms and the failure in monitoring the withdrawal periods of such drugs, Accordingly, the concerned authorities must take extra efforts for corrective use of veterinary drugs for solving the problems of such residues in chicken meat.

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قياس متبقيات الأكسي تتراسيكلين في لحوم الدواجن

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الملخص العربي

أجريت هذه الدراسة لمعرفة مدي تواجد متبقيات الأكسي تتراسيكلين في لحوم الدواجن من عينات الصدور والأوراك حيث تم تجميعها من ثلاثة مصانع مختلفة A و B و C في محافظة القليوبية وقد تم تمثيل كل مصنع بعدد (15) عينة من الصدور وعدد (15) عينة من الأوراك وذلك باستخدام الطريقة المكروبيولوجية (FPT) للتقدير الكيفي واستخدام جهاز (HPLC) للتقدير الكمي علي تركيز هذا الدواء. وقد كشفت النتائج أن متوسط تركيز الأكسي تتراسيكلين (مجم /كجم) في عينات الدجاج التي تم فحصها هي 0.11 ± 0.02، 0.09 ± 0.01، 0.06± 0.01 لعينات الصدور و 0.19 ± 0.03، 0.14 ± 0.02، 0.08 ± 0.01 لعينات الأوراك في مصنع A، B، C علي الترتيب وقد كانت نسبة العينات الغير مقبولة من الصدور والاوراك هي 13.33%، 26.67% لمصنع A و 13.33%، 20% لمصنع B و 6.67% ، 6.67% لمصنع C علي الترتيب. وقد اهتمت الدراسة ببيان الأهمية الصحية لوجود متبقيات الأكسي تتراسيكلين في لحوم الدواجن ومدي تأثيرها على صحة الإنسان، مع وضع بعض التوصيات اللازمة للسيطرة على هذا المتبقيات السامة.

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