DETERMENATION OF SOME ANTIBACTERIAL RESIDUES IN CHICKEN GIBLETS

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A B S T R A C T

This study was conducted to determine some antibacterial residues (amoxicillin and sulphaquinoxaline) in chicken giblets (heart, gizzard and liver), and their hazard on public health. A total of 90 random samples of chicken giblets represented by heart, gizzard and liver (30 of each) were collected from different slaughter poultry shops at Gharbia governorate. The results showed that 6.67%, 16.67% and 20% of heart, gizzard and liver were positive for presence of amoxicillin by High Performance Liquid Chromatography (HPLC), respectively. Accurately, 3.33%, 10 % and 13.33% of the examined samples of heart, gizzard and liver were exceeded the maximum permissible limit of amoxicillin, respectively. Concerning sulphaquinoxaline residue, it was found that 3.33%, 13.33% and 13.33% of the heart, gizzard and liver samples were positive by HPLC, respectively. Moreover, none of the examined heart samples exceeded the maximum permissible limit of sulphaquinoxaline while 10% and 13.33% of the examined gizzard and liver samples exceeded the maximum permissible limit, respectively, according to FAO, WHO (1999). The public health significance of such antibacterial agents and some recommendations to control their presence in chicken giblets were discussed.

KEY WORDS: Amoxicillin, Sulphaquinoxaline, Residues, Chicken giblets.

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1- I N T R O D U C T I O N

The widespread use of drugs however, is the problem associated with drug residues, which may ultimately become a part of the human diet. Improper use or insufficient withdrawal period of these antimicrobial drugs may lead to drug residues in edible parts of the food producing animal systems (Thomas, 1999). Sulphonamides are widely used for therapeutic and prophylactic purposes in human being (Kim and Park, 1998) and most commonly used in veterinary medicine for prophylaxis and therapeutic purposes (Al-Nazawi and Homeida, 2005).

Sulphonamides are sometimes used as additives in animals feed because prolonged ingestion of sulphonamides may have a growth promoting effect (Long *et al.*, 1990).

If the proper withdrawal periods are not observed before slaughtering or milking of the medicated animals, meat and milk from these animals may be contaminated with residual sulphonamides (Sachenbrecker and Fish 1980, Franco *et al.*, 1990 and McEvoy *et al.*, 1999).

Antibiotic resistance is an emerging public health problem especially due to the continuous use of antibiotics that selects more aggressive and resistant species (Messano, Petti, 2011).

Good cooking and freezing were used for removal of great part of the antibiotic residues; high heat followed by sudden cold, which used during industrial processing may also remove the residues (Adams, 1993).

Cooking processes do not guarantee full elimination of these drugs present in condemned animals and it can only decrease its amounts. The various

agents affecting antibiotic residues after the cooking process, cooking time and temperature can play a major role in antibiotic residue reduction while cooking food.

2. MATERIAL AND METHODS:

2.1. Collection of samples:

Ninety random samples of chicken giblets represented by heart, gizzard and liver (30 of each) were collected from different slaughter poultry shops at Gharbia. The giblets of each chicken carcass were identified and numbered for determination of their contents of amoxicillin and sulphaquinoxaline.

2.2. Application of HPLC technique:

HPLC has become the most widely used technique for the determination of antibacterial residues in chicken meat and giblets. It has been used most frequently as it provides a sensitive and specific, but very laborious and expensive analytical method. It is suitable for confirmation of contamination rather than for screening a large numbers of test samples. Quantitative analysis of antimicrobial agent in the examined samples of chicken giblets were done according to Pieckova and Van Peteghem (2001) and Oka et al. (2003). Accurately, 5gm of each sample and 10 gm of anhydrous sodium sulfate were blended with 20 ml of ethyl acetate and then centrifuged. The supernatant was evaporated and dried under reduced pressure at 40oC.The residue was dissolved in 5ml of ethy1 acetate-n-hexane and the solution was applied to a bond 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 0.05 M ammonium format. The preparation was injected into HPLC system (model LC – 10A series equipped with constant flow pump and variable wavelength U/V detection, Kyoto, Japan.

Accordingly, antibacterial residues were estimated by using their standard solutions specific for each of them. Operating conditions for analysis of amoxicillin were; eluant at 35oC, flow rate 1 ml/min., injection volume, 10ul; detection wave length 216/ nm. While, the operating conditions for analysis of sulphaquinoxaline were; eluant at 30oC, flow rate 1 ml/min., injection volume, 20/ul; detection wave length 272nm.

2.2.1. Reagents and Chemicals:

All reagents were analytical or HPLC grade. Methanol (MeOH) was purchased from Merck KGaA (Darmstadt, Germany), while acetonitrile (ACN), acetone and glacial acetic acid (HOAc) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Sodium acetate (NaOAc) was from Saarchem Analytical (Krugersdorp, South Africa). Fluorescamine (98%) and sulfonamide drugs including the internal standard were purchased from Sigma-Aldrich (St. Louis, MO, USA). The water used was from a MilliQ system (Milford, Mass, USA).

2.2.2. Solutions and Standards:

A stock solution of 0.05 M sodium acetate was prepared by dissolving 4.1 g NaOAc in 1.0 L of ultrapure water and filtered through a Whatman membrane filter (47 mm diameter and 2 µm pore size). The pH was adjusted using HOAc. Fluorescamine reagent (0.02%) was prepared by dissolving 20 mg Fluram in 10 mL of acetone. The solution was stored at 4 °C. A 1% HOAc in ACN solution was prepared by diluting 10 mL HOAc to 1.0 L with ACN.

Standard and internal standard primary stock solutions (1 mg/mL) were prepared in ACN and stored at –20 °C. From the primary stock solution, 10 µg/mL standard mixtures also in ACN were prepared for the calibration curves. All working solutions were prepared daily by serial

dilution in 0.05 M NaOAc (pH 3.5). All the solution vials were wrapped with aluminum foil because some of the sulfonamide drugs are light-sensitive.

2.2.3. Equipment and Material:

The analysis was performed on an Agilent 1200 Series HPLC (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a binary pump and a fluorescence detector (FLD) set at λex = 405 nm and λem = 495 nm. Separation of the compounds was achieved on an Agilent ZORBAX Eclipse plus C18 column (4.6 mm × 75 mm, 3.5 µm, p/n 959933-902). The data was processed by HPLC 2D Chemstation software.

Extraction and cleanup were carried out with an Agilent SampliQ Buffered QuEChERS AOAC Extraction kit, p/n 5982- 5755 and an Agilent SampliQ QuEChERS AOAC Dispersive SPE kit, p/n 5982-5158, (Agilent Technologies).

A Jenway 3510 pH meter (Jenway, London, UK) monitored the pH of the solutions, and a Kenwood grinder (Kenwood, Grahamstown, South Africa) homogenized the chicken sample.

The concentration of each antibacterial residue was estimated and recorded.

3. RESULTS:

Results given in table (1) declared that the incidence of amoxicillin in the examined samples of chicken heart, gizzard and liver was 6.67%, 16.67% and 20%, respectively. Besides, the concentrations (ug/kg) of amoxicillin in the examined samples of chicken giblets varied from 24 to 71 for heart, 25 to 180 for gizzard and 35 to 210 for liver. However, FAO WHO (1999) recommended that the maximum permissible limit of Amoxicillin in chicken heart, gizzard and liver should be 50,100 and 100 ug/kg. Therefore 3.33%, 10 % and 13.33% of the examined samples of heart, gizzard and liver were unaccepted and unfit for human consumption.

Table (2) revealed that the incidence of Sulphaquinoxaline in the examined samples of chicken heart, gizzard and liver was 3.33%, 13.33% and 13.33%, respectively. Moreover, the concentrations (ug/kg) of Sulphaquinoxaline in the examined samples of chicken giblets were 65 as an average for heart, 40 to 255 for gizzard and 45 to 305 for liver. However 10.00% and 13.33% of the examined samples of gizzard and liver, respectively were unaccepted and unfit for human consumption. Moreover, none of the examined heart samples were exceeded the maximum permissible limit of sulphaquinoxaline. This is based on the FAO WHO (1999) recommendation who stated that the maximum permissible limit of Sulphaquinoxaline in either chicken heart, gizzard or liver should not exceed 100 ug/kg.

4. DISCUSSION:

The mean values of amoxicillin residues were 47.50 ± 3.08, 106.83 ± 7.41 and 133.17 ± 8.92 for heart, gizzard and liver samples, respectively.

The greatest dangers to human health deriving from the ingestion of foodstuffs of animal origin containing antibiotic residues are allergic phenomena, sensitization and antibiotic resistance (Dan, 2003).

Antibiotic residues have very dangerous health hazards; these hazards can be one of the followings; transfer of antibiotic resistant bacteria to the human, immunopathological effects, autoimmunity, carcinogenicity, mutagenicity, nephropathy, hepatotoxicity, reproductive disorders, bone marrow toxicity or allergy (Nisha, 2008).

Concerning sulphaquinoxaline residues, the mean values were 65, 127.49 ± 9.38 and 153.86 ±11.26 for heart, gizzard and liver, respectively.

The maximum residual level of sulfonamide in all compound of sulfonamide group was 0.1mg / kg in food of animal origin, therefore in order to

overcome the problem of drug residues, the drug must be administered only in recommended concentrations and their withdrawal times must be observed together with the original 7 days withdrawal period must be in ceased to 15 days (Augsburg, 1988).

There are some toxic, allergic and teratogenic reaction were presented a public health hazards arising from the use of sulfonamides and antibiotics as prophylactic or therapeutic or growth promoters in animal or poultry (Mol, 1971).

The antibiotic and sulfonamide residues in meat cause implications for human health. These implications were brought by the subtherapeutic use of antibiotic drugs. The regulation governing the use of antibiotic and sulfonamides in poultry feed, the FDA and Food Safety inspection service (FSIS) cooperative program and antibiotic resistance and public health. Problems arising from the persistence of antibiotic and sulfonamide residues in meat following their inclusion in poultry feed are highly lighted with particular reference to the increased prevalence of drug resistant enteric organisms (possible human pathogens) favoured by such poultry feeding regimes (Franco *et al.,* 1990).

The current results in the present study allow to conclude that the examined samples of chicken giblets constitute, at times, public health hazard as a result of their contamination with antibacterial residues (Amoxicillin & Sulphaquinoxaline).

Further, the highest concentrations of these residues were found in liver samples, followed by gizzard and the lowest levels were detected in heart samples.

Table (1): Statistical analytical results of amoxicillin levels (ug/kg) in the examined samples of chicken giblets (n=30).

Chicken giblets	+ve samples		Maximum Permissible Limit (ug/kg)*	Unaccepted Samples		Min	Max	Mean ± S.E*
	No.	%		No.	%			
Heart	2	6.67	50	1	3.33	24	71	47.50 ± 3.08
Gizzard	5	16.67	100	3	10.00	25	180	106.83 ± 7.41
Liver	6	20.00	100	4	13.33	35	210	133.17 ± 8.92

S.E* = standard error of mean
* FAO/WHO (1999)

Table (2): Statistical analytical results of sulphaquinoxaline levels (ug/kg) in the examined samples of chicken giblets (n=30).

Chicken giblets	+ve samples		Maximum Permissible Limit (ug/kg)*	Unaccepted Samples		Min.	Max.	Mean ± S.E*
	No.	%		No.	%			
Heart	1	3.33	100	-	-	-	-	65
Gizzard	4	13.33	100	3	10.00	40	255	127.49 ± 9.38
Liver	4	13.33	100	4	13.33	45	305	153.86 ±11.26

S.E* = standard error of mean, * FAO/WHO (1999)

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مدي تواجد متبقيات بعض المضادات الحيوية في الأحشاء الداخلية للدواجن

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الملخص العربي

يعتبر تلوث الاحشاء الداخلية للدواجن ببعض المتبقيات الضارة مثل بعض المضادات الحيوية ذو أهمية كبيرة لما له من أثار سمية تراكمية تمثل خطورة بالغة على صحة المستهلك. لذلك أجريت هذه الدراسة على عدد تسعون عينة (90) من الاحشاء الداخلية للدواجن "القلب، القونصة، الكبد" بواقع ثلاثين عينة (30) من كل نوع من محلات بمحافظه الغربية وذلك لمعرفة مدى تواجد متبقيات الاموكسيسيلين وسلفاكينوكسالين بهم. فيما يخص الاموكسيسيلين كانت 6.67 %، 16.67 % و20% من عينات القلب، القونصه والكبد ايجابيه، على التوالي. وكان 47.50، 106.83 و133.17 متوسطات تركيز متبقيات الاموكسيسيلين في كل من القلب، القونصة، الكبد على التوالي وكان 3.33 ٪ و10٪ و13.33 ٪ من عينات فحص القلب، القونصة والكبد غير مقبولة حيث أنها تجاوزت الحدود القصوى المسموح بها للأموكسيسيلين، على التوالي. بينما كان 3.33%، 13.33% و13.33% من عينات القلب، القونصه والكبد ايجابيه لتواجد السلفاكينوكسالين، على التوالي. وكان 65، 127.49 و153.86 متوسط تركيز متبقيات السلفاكينوكسالين في كل من عينات القلب، القونصة، الكبد، على التوالي وكانت 10٪ و13.33٪ من عينات القونصة والكبد غير مقبولة على التوالي ولكن كانت كل عينات القلب مقبولة وضمن الحد المسموح به طبقاً لـ FAO, WHO (1999) وقد تمت مناقشة الخطورة الصحية لهذه المتبقيات الضارة في الاحشاء الداخلية للدواجن مع بيان المصادر المختلفة للتلوث وكذلك وضع بعض التوصيات للحد من خطورة هذه المتبقيات على الصحة العامة.

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