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DETECTION OF ANTIBIOTIC RESIDUES IN LOCAL BROILERS WITH SPECIAL REFERENCE TO NORFLOXACIN

(With 3 Tables and 2 Figures)

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تقدير بقايا المضادات الحيوية فى الدواجن المحلية مع مرجعية خاصة للنورفلوكساسين

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تم جمع ٥٠ عينة دجاج مذبوح من محلات الدواجن بمحافظة الجيزة، وقد تم فحصها لوجود المضادات الحيوية باستخدام الطريقة الميكربيولوجية عند درجة حموضة ٦، ٨ باستخدام ميكروب الباسلس ستلس وتم تحضينها عند ٣٠ درجة مئوية لمدة ٢٤ ساعة. ولقد أوضحت النتائج تواجد المضاد الحيوى فى الدجاج المذبوح بنسبة ٢٢%، ٢٠% على التوالى. لم يكتشف وجود بقايا من النورفلسوكاسين بعد ٧٢ ساعة من الحقن العضلى (٢,٥ مجم / كجم من وزن الدجاج) من النورفلوكساسين لمدة ٥ أيام متتالية فى العضلات، القوانص، القلب، الجلد والدهن بينما قد أختفت بعد ١٢٠ ساعة، ٩٦ ساعة فى الكبد والكلى على التوالى باستخدام طريقة HPLC. ولقد كان تأثير المعاملة الحرارية على بقايا النورفلوكساسين بالغليان والشوى والقلى عند درجة ثقة أقل من ٠,٠٥ قد نقص فى كل من العضلات، الكبد والكلية بينما اختفى فى القونصة، القلب والجلد والدهن. ولقد نوقشت أهمية بقايا المضاد الحيوى فى لحم الدجاج وكذلك وقت اضمحلال المضاد الحيوى من الأنسجة.

SUMMARY

A total of fifty slaughtered chickens were collected from chicken shops in Giza governorate. All samples were examined for the presence of antibiotic residues using microbiological inhibitory assay (MIA). The freshly prepared *Bacillus subtilis* plates (pH 6 and pH 8) were used and incubated at 30°C for 24 hrs. The results revealed that the incidence of antibiotic residues in the chicken carcasses was 22% and 20%,

respectively. The residue levels of norfloxacin, using HPLC, after a single intramuscular injection of 2.5 mg/ kg body weight of norfloxacin for 5 successive days were not detected after 72 hours in muscle, gizzard, heart, skin and fat whereas, norfloxacin residues could not be detected after 96 and 120 hours in liver and kidney samples, respectively. Norfloxacin residues were decreased significantly ($P<0.05$) after heat treatments (boiling, roasting and frying) in muscles, liver and kidney samples. Such residues could not be detected after boiling, roasting or frying of gizzard, heart, skin and fat samples. The importance of antibiotic residues in chicken meat and the withdrawal time as well as the time of slaughter were discussed.

Key words: *Broilers, antibiotic residues, norfloxacin*

INTRODUCTION

Norfloxacin is a flouroquinolone antibacterial agent that is extensively used in veterinary medicine practice (Prescott and Baggot, 1993; Sarkozy *et al.*, 2004; Martinez *et al.*, 2006; Cho *et al.*, 2008). Norfloxacin (1-ethyl–6–fluoro–1, 4 – diydro –oco – 7 – {1- Piperazinyl} – 3 – qunoline carboxylic acid), is a fluorinated quinolone that has broad –spectrum antibacterial activity and widespread distribution in most tissues and body fluid (Downs *et al.*, 1982; Brown, 1996). Norfloxacine has high antimicrobial activity in vitro against a wide variety of gram negative and gram positive bacteria including, gentamicin – resistant *Pseudomonas aerugenosa* (Downs *et al.*, 1982; Sham and Koburoc, 1989). The antimicrobial spectrum of norfloxacin makes the drug attractive in veterinary therapy (Stefan *et al.*, 1994). Norfloxacin is essentially non- toxic as very large doses are necessary to give toxicity even as minor symptoms in mammals or birds, this is because of innate low toxicity similar to other quinolones and to fast absorption and elimination (Sugimoto *et al.*, 1981).

In veterinary medicine, the potential usefulness of norfloxacin for treatment of common infection in poultry requires detailed information on the pharmacokinetic properties, bioavailability, metabolism, distribution and elimination of norfloxacin in birds, to establish the orally administrated dose for maintaining bactericidal drug concentrations in the body (Anadon *et al.*, 1992). The therapeutic use of antimicrobial drugs has played an important role in animal husbandry by controlling diseases, improvement of growth and efficiency of feed –

conversion. Although the importance of the withdrawal times of antibiotics as a safe way for avoiding residues in human foods of animal and poultry origin was discussed by several authors (Anadon *et al.*, 1990, Mignot *et al.*, 1993; Daoud and Yanny, 2000). Antibiotics are still used in large scale in veterinary field and poultry farms with little amount of control.

The present study was, therefore, planed to evaluate the incidence of antibiotic residues in chickens as well as to determine norfloxacin residues in chicken tissues after parentral administration. The effect of different heat treatments on norfloxacin residues was also scrutinized.

MATERIALS and METHODS

Fifty slaughtered chickens were purchased from chicken shops in Giza Governorate. The average weight of the dressed chicken was about 1–1.5 kg b.wt. The antibiotic residues was determined by the microbiological assay technique.

1. Detection of antibiotic residues in dressed chickens:

Chicken samples, *Bacillus subtilis* spore suspension, medium and test plates were prepared according to the technique recommended by Levetzow and Wise (1979).

Procedure:

From each sample a cylinderical piece was removed by a sterile cork borer, then pieces were diagonally placed on the surface of the freshly prepared *B. subtilis* plates (pH 6 and pH 8). The plates were incubated at 30°C for 24 hours.

2. Norfloxacin residues in chicken meat:

2.1. Experiment 1:

Thirty chickens weighed about 2 kg were kept in cages and fed antibacterial free ration two weeks before norfloxacin administration to be sure of complete clearance of their bodies from any antibacterial residues. All chickens were I.M. injected with norfloxacin (2.5 mg/ kg) for 5 successive days. Three chickens were slaughtered after 2, 4, 6, 8, 10, 12, 24, 72, 96 and 120 hours post-administration. Samples from muscle (breast and thigh), liver, kidney, gizzard, heart, skin and fat were prepared for estimation of norfloxacin residue.

Preparation of sample for HPLC analysis:

Five grams from each sample were mixed with 20 ml acetonitrile, then homogenized and centrifuged. The sediment was discarded and the supernatant was resuspended in 10 ml of petroleum ether, then centrifuged. The top layer was discarded and the bottom layer was evaporated at 40°C. The residue was resuspended in 10 ml saline and concentrated by solid phase extract column. The purified extract was evaporated and the residue dissolved in 1 ml ethanol. The norfloxacin residues were analyzed using HPLC as described by Ellerbroak (1993).

2.2. Experiment 2:

Effect of heat treatment on the norfloxacin residues in muscle tissue:-

Twelve broiler chicken weighed about 2 kg were (free from any antibiotic residues); divided into 4 groups (3 of each) and I.M. injected with 2.5 mg/ kg b.wt of norfloxacin for 5 successive days. After the last dose the chicken were slaughtered and chicken muscles (breast and thigh), liver, kidney, gizzard, heart, skin and fat were prepared for the following: the 1st group was considered as a control before treatment. The 2nd group was boiled for 30 min., the 3rd group was roasted at 150°C for 30 min., and the 4th group was fried for 5 min in cotton seed oil. Chicken samples were analyzed for norfloxacin residues as described by Ellerbroak (1993) using HPLC.

Statistical analysis: Data obtained were statistically analyzed using ANOVA test according to SPSS 14 (2006).

RESULTS

Table 1: Incidence of antibiotic positive chicken carcasses.

Muscle samples Examined	+ve antibiotic residues			
	pH 6		pH 8	
	No.	%	No.	%
50	11	22	10	20

Table 2: Residue levels (μg/g) of norfloxacin in broiler chickens after a single IM injection of norfloxacin 2.5 mg/kg b.wt. for 5 consecutive days (mean ± SE).

	Hours after treatment									
	2	4	6	8	10	12	24	72	96	120
Muscles	1.16 ± 0.007	1.26 ± 0.007	1.18 ± 0.007	1.08 ± 0.007	0.83 ± 0.006	0.35 ± 0.006	0.07 ± 0.006	U.D.	U.D.	U.D.
Liver	1.28 ± 0.006	1.36 ± 0.006	1.49 ± 0.006	1.62 ± 0.007	1.06 ± 0.007	0.93 ± 0.006	0.54 ± 0.205	0.14 ± 0.007	0.06 ± 0.006	U.D.
Kidney	1.27 ± 0.067	1.31 ± 0.006	1.42 ± 0.007	1.60 ± 0.058	0.97 ± 0.006	0.89 ± 0.006	0.49 ± 0.200	0.05 ± 0.006	U.D.	U.D.
Gizzard	0.97 ± 0.006	1.18 ± 0.006	0.84 ± 0.007	0.36 ± 0.007	0.16 ± 0.007	0.08 ± 0.006	0.04 ± 0.007	U.D.	U.D.	U.D.
Heart	1.28 ± 0.112	1.26 ± 0.013	1.17 ± 0.067	0.85 ± 0.006	0.32 ± 0.006	0.12 ± 0.007	0.02 ± 0.007	U.D.	U.D.	U.D.
Skin	1.19 ± 0.007	1.37 ± 0.067	1.22 ± 0.006	1.13 ± 0.007	0.98 ± 0.006	0.38 ± 0.006	0.10 ± 0.001	U.D.	U.D.	U.D.
Fat	1.17 ± 0.006	1.28 ± 0.006	1.20 ± 0.058	1.09 ± 0.006	0.89 ± 0.006	0.40 ± 0.007	0.12 ± 0.006	U.D.	U.D.	U.D.

UD = undetectable.

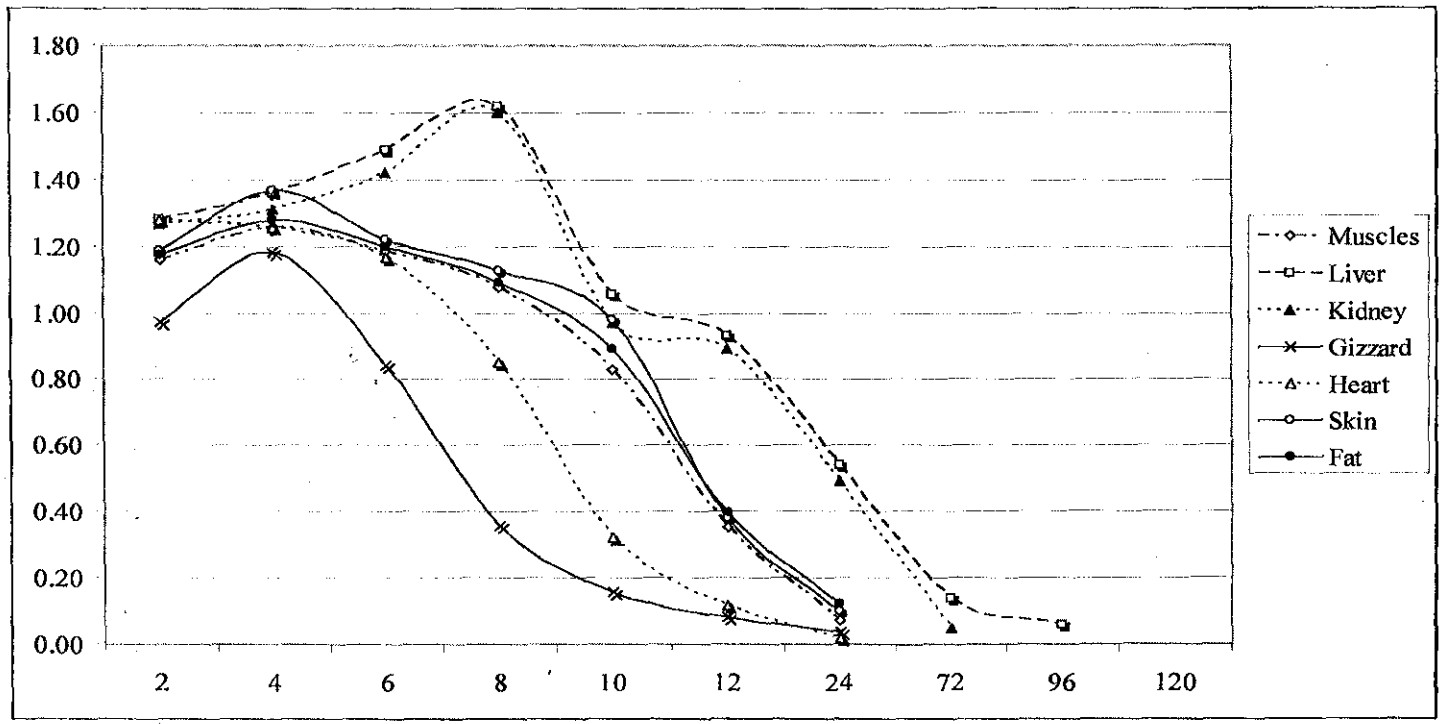

Fig. 1: Residue levels (µg/g) of norfloxacin in broiler chickens after single IM injection of norfloxacin 2.5 mg/kg b.wt. for 5 consecutive days (mean ± SE).

Table 3: Effect of heat treatments on norfloxacin residues in different chicken tissues (mean ± SE).

	Before heat treatment	After boiling 30 min	After roasting 30 min at 150°C	After frying/ 5 min in cotton seed oil	F-calculated	Probability
Muscles	1.150 ± 0.006 a	0.127 ± 0.001 b	0.107 ± 0.007 b	0.080 ± 0.006 c	12.987*	0.0057
Liver	1.287 ± 0.007 a	0.297 ± 0.007 b	0.270 ± 0.006 b	0.257 ± 0.007 b	11.254*	0.0043
Kidney	1.230 ± 0.006 a	0.167 ± 0.001 b	0.157 ± 0.001 b	0.145 ± 0.001 c	10.543*	0.0071
Gizzard	0.970 ± 0.006	U.D.	U.D.	U.D.		
Heart	1.167 ± 0.007	U.D.	U.D.	U.D.		
Skin	1.180 ± 0.006	U.D.	U.D.	U.D.		
Fat	1.140 ± 0.006	U.D.	U.D.	U.D.		

* Significant at $P < 0.05$

Means in rows with different alphabetical superscruipts are significantly different at $P < 0.05$ using Duncan Multiple Range Test for comparative of means.

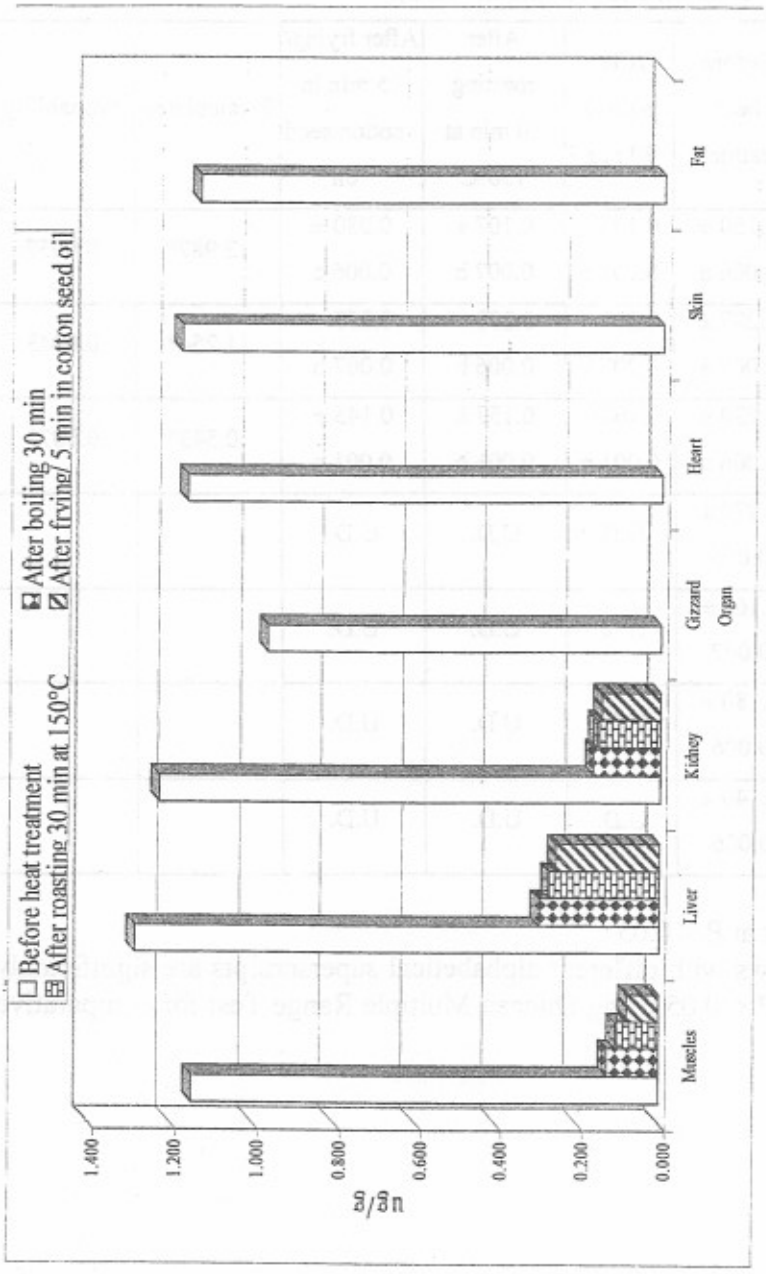

Fig. 2: Effect of heat treatments on norfloxacin residues in different chicken tissues.

DISCUSSION

It is documented that the extensive use of antibiotic in poultry farms for increasing growth rates, prophylaxis and/or therapy gives rise to the problem of drug residues. Therefore, information regarding antibiotic elimination from chicken meat and organs are crucial to save guard consumer against the hazards which resulted from consumption of such residues.

The results of this study revealed that the incidence of antibiotic residues in chicken carcasses was 22% and 20% at pH 6 and pH 8, respectively (Table, 1). Higher results were recorded by Gab Allah *et al.* (2002) in broiler carcasses, (36.7%) at pH 6, and (33.3%) at pH 8. However, lower values were recorded by thin layer chromatography and microbial inhibitory assay in chicken edible tissue, 8% and 12%, respectively (El-Shater and Hassan, 2003). The elimination of antibiotics from poultry and animal tissues has important public health significance.

The peak of norfloxacin residue (μg/g) after a single I.M. injection (2.5 mg/ kg b. wt) for 5 consecutive days were 1.26, 1.18, 1.26, 1.37 and 1.28 in muscles, gizzard, heart, skin and fat, respectively after 4 hours post-administration. However, the corresponding peaks in the liver (1.62 μg/g) and kidney (1.60 μg/g) were recorded after 8 hours of the last injection.

After 24 hours, norfloxacin residues were obviously declined to 0.07, 0.54, 0.49, 0.04, 0.02, 0.1 and 0.12 μg/ g, in the foregoing organs, respectively (Table 2 and Fig., 1). On the other hand, norfloxacin residues could not be detected after 72 hours in muscle, gizzard, heart, skin and fat. At the same interval, the residues in the liver and kidney were 0.14 and 0.05 μg /g, respectively. Moreover, norfloxacin residues were not detected in liver and kidney after 120 and 96 hours, respectively (Table 2 and Figure 1). Nearly similar results were recorded by Ibrahim (1998) who reported that norfloxacin residues reached their maxima after 8 hours in the liver (1.42 μg / g), kidney (1.27 μg / g), muscles (1.15 μg / g), heart (1.28 μg/g) and gizzard (1.17 μg/g).

The complete withdrawal of norfloxazin residues, as reported by Ibrahim (1998), were 12 hours from gizzaed, 24 hours from heart, 3 days from muscle and lung, 4 days from kidney and lastly from the liver. The higher tissue concentrations of norfloxacin were reported in the liver and kidney than other tissue samples. This was anticipated because norfloxacin or most drugs are eliminated from the body via the bile and

the same extent via the kidney (Scheer, 1990). Moreover, the higher norfloxacin residues in liver and kidney could be attributed to the role of liver in drug metabolism and detoxication of the drug by its microsomal enzymes as well as to the role of kidney in the filtration and clearance of blood from any undesirable constituents.

The concentration of norfloxacin in chicken plasma were determined up to 12 hours and were not detected in all chickens after 24 hours post single oral and intravenous administrations (Abu–Basha *et al.*, 2008). The bioavailability of nofloxacin in broiler chicken was 57.0% (Anadon *et al.*, 1992). The pharmacokinetic parameters of norfloxacin in broiler chickens were different from other animals, such as sheep, dogs and goats (Kivisto *et al.*, 1992; Song and Chen, 1995, Albarellos *et al.*, 1996; Gonzalez *et al.*, 1997).

The optimal dose range of the drug has been suggested to be 5 – 22 mg / kg body weight in dog, calves, laboratory animals, pigs and chickens (Laczay *et al.*, 1998). The optimal dose has been suggested to be based on the pharmacokinetic- pharmacodynamic relationship (Schentag, 1999).

Before heat treatments norfloxacin residues in muscles, liver, kidney, gizzard, heart, skin and fat (μg / g) were 1.15, 1.287, 1.230, 0.970, 1.167, 1.80 and 1.140, respectively. The norfloxacin residues were significantly ($P < 0.05$) decreased by boiling, roasting and frying muscles, liver and kidney.

On the other hand, the gizzard, heart, skin and fat norfoxacin residues were not detected after the difference heat treatments. Moreover, there was no significant different between the boiling and roasting methods in the examined organs whereas, there were significance difference between boiling for 30 min, roasting for 30 min against frying for 5 min in cotton seed oil.

The present results were in partial agreement with Hassan (1998) who reported that boiling, roasting and frying could destruct the quinolone residue (enrofloxacin) in gizzard, heart, lung, skin and fat while the heat treatments were significantly ($P < 0.05$) decreased the enrofloxacin residues in liver, kidney and muscles. On the other hand, the same author mentioned that the heat treatments could destruct the danofloxacin residues in all the examined organs except the liver.

Unfortunately the amount of residues after boiling, roasting and frying muscles, liver and kidney ranged around 0.080 – 0.297 μg/g which exceed the maximum residue limit (safe residue limit) for

norfloxacin (50 ng/ g) recommended by European union (Brown, 1996) for chicken edible tissues.

The heating or boiling decreases the antibacterial residues in chicken meat and organs. Moreover, antibiotics can be refractory for heat degradation in animal tissues unless high temperature levels are maintained for considerable periods (Peric and Dakic, 1973; O'brien *et al.*, 1981; El-Zeini and Atta, 1995). This proved that the temperature and the duration time resulted in disappearance of antibiotic residues in all edible tissues except some organs. However some antibiotics are heat stable such as chloramphenicol (Hamman *et al.*, 1978), while others are polymerized at higher temperature (200°C) and produce toxic or mutagenic products (Booth and McDonald, 1988).

The presence of antibiotic residues after cooking represents serious problems for human beings consuming such tissues. Hypersensitivity or ever toxicity and development of bacterial resistant strains are among the hazard of antimicrobial residues (Corry *et al.*, 1983; Andrews *et al.*, 1988; Booth and Mc Donald, 1988). Therefore, when the recommended dose of antibiotic or antibacterial agents were used together with proper withdrawal time as well as effective heat treatment, residue problem can be overcome and/or disappear.

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