

#### SPIRAMYCIN RESIDUES IN CHICKEN MEAT AND GIBLETS

Fatma, H. Amro<sup>a</sup>, Hassan, M. A. <sup>b</sup>. and Mahmoud, A. H.<sup>c</sup>

<sup>a</sup> Residues Analysis unit, Animal Health Research Institute, Dokki, Egypt.

<sup>b</sup> Department of Meat Hygiene, Faculty of Veterinary Medicine, Benha University, Egypt.

<sup>c</sup> Departement of Biotechnology, Animal Health Research Institute, Dokki, Egypt.

#### ABSTRACT

Spiramycin residues in chicken tissues "thigh, gizzard and liver" have an important role in the hazards effect of residues for consumers. The antibiotics residues were detected in the examined samples by using HPLC .The mean values of spiramycin residues in the examined samples were  $0.153\pm0.01$ ,  $0.152\pm0.01$  and  $0.664\pm0.05\mu g/g$  in the thigh, gizzard and liver, respectively. It was cleared that the highest incidence of this antibiotic residues in chicken liver was (40%) and the lowest in muscles and gizzard was (10%). Accurately, 92.5%, 85% and 100% of the examined samples of thigh, liver and gizzard were accepted according to codex alimentarieus commission [9]. Also, our study declared that boiling of positive samples can degrade the spiramycin residues.

**KEY WORDS:** Spiramycin -Gizzard- HPLC- Liver - Residues - Thigh.

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#### 1. INTRODUCTION

recent decades, The use of n the antimicrobial agents in either treatment of poultry or to improve their performance. stimulated many investigators to reveal their physiological, pharmacological effect on living birds as well as the residues of these drugs in edible parts of the poultry [16]. Spiramycin is a macrolide antibiotic; It has been isolated by [32] from cultures of Streptomyces ambofaciens. It is active against Grampositive organisms, some Gram-negative bacteria and other organisms, including Mycoplasma pneumoniae, Chlamvdia trachomatis. Toxoplasma gondii, Legionella pneumophila, and spirochetes and also used as a growth promoter.

Antibiotic resistance is an emerging public health problem especially due to the continuous use of antibiotics that selects more aggressive and resistant species [27]. The control of the antibiotic residues in meat and fish was done by giving the drugs to the animals after sensitivity test, by the accurate dose and prevent slaughtering in the withdrawal time [19].

The purpose of this work to estimate the tissue residues of Spiramycin in broiler chicken's (tissues and organs) by High Performance Liquid Chromatography (HPLC).

Codex Alimentarius Commission, [7] mentioned that the maximum permissible limit of Spiramycin is 0.2 mg/kg of chicken meat. EOSQC, [12] stated that the MRL of veterinary drugs used in chickens shall be evaluated according to the international standard limits that reported by FAO and/or EU those set MRLs for Macrolides in food and feed. Wang [41] discussed that Incorrect use of Macrolides or insufficient withdrawal time after treatment can possibly lead to the presence of these residues in food, which increases the potential risk to consumers because of allergic reactions which associated with increase in blood histamine.

Zorraquino et al. [45] stated that antibiotic residues can cause serious problems for heat treatment consumers. So may diminish the antimicrobial activity of these antibiotic residues. Three concentrations of spiramycin: 100, 200, and 400 µg/liter; To measure the loss of antimicrobial activity, a bioassay based on the growth inhibition of Micrococcus luteus was done. The results indicated that treatment at 120 °C for 20 min. produces inactivation percentages of 64% while treatment at 140 °C for 10sec results in generally lower percentages 35%. The lowest loss or lowest reduction of antimicrobial activity 13% spiramycin was obtained by treatment at 60 °C for 3 min

Codex Alimentarius Commission , [8,9] determined that MRL of Spiramycin in chicken muscle is 200  $\mu$ g/kg , in liver is 600  $\mu$ g/kg, in kidney is 800  $\mu$ g/kg & in fat is 300  $\mu$ g/kg.

Ray *et al.* [33] detected that during 5 days of Azithromycin therapy, there was a small absolute increase in cardiovascular deaths, which was most pronounced among patients with high baseline risk of cardiovascular disease.

### 2. MATERIALS AND METHODS

In the present work the residual effect of spiramycin was studied in broiler chickens. Standard drugs were from sigma company. *2.1. Field survey tissue sampling plane* 

A total of 120 poultry thigh, Liver and gizzard were collected from special farm in Banha Governorate as fresh samples (40 samples for each). Each sample was represented by 20 grams for fresh samples. The samples were placed in plastic bags and transferred to the laboratory without delay in an ice box. Then, 9 samples of highly positive fresh samples were boiled for detecting the boiling effect on spiramycin residues.

# 2.Samples preparation before solid-phase extraction:

Chicken tissues (thigh, liver, gizzard) were minced and homogenized in the mincer for 1min.10 gm of homogenate was accurately weighted into a polypropylene centrifuge tube. 40 ml of 0.5 M phosphoric acid and methanol (3:7) was added. The solid residue was discarded. The water phase was washed 2 times 40 ml n-hexane. Centrifugation at 8000 rpm for 10 min. Evaporation at 45°c water bath to3 ml. The 3 ml sample was subjected to SPE cleanup. Analytical method was done by estimation of macrolide antibiotic spiramycin by performance HPLC (high liquid chromatography) in the collected tissue samples according to Lee, et al.[24]

3. Liquid chromatography operating conditions Spiramycin:

Injection volume, 50 µl; flow rate, 1 mL/min; wave length, 232 nm; column temperature,  $35^{\circ}$ c; stop time, 15 min; post time, 6 min.; mobile phase 0.05 M phosphoric acid : acetonitrile = 75:25 (PH 3.0 v/v).

#### 3. Statistical Analysis:

The obtained results were statistically evaluated by application of Analysis of Variance (ANOVA) test according to Feldman et al. [15].

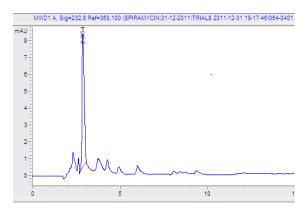


Fig. (1): Chromatogram showing 1 ppm of spiramycin standard.

#### **3. RESULTS**

Chicken tissues	No. of examined samples (120)	Positive samples		Negative samples	
	-	No.	%	No.	%
Thigh	40	4	10	36	90
Gizzard	40	4	10	36	90
Liver	40	16	40	24	60

Table 1 Incidence of spiramycin as antibiotic residue in the examined samples of chicken meat and giblets.

Table 2 Statistical analytical results of spiramycin residues ( $\mu g/g$ ) in the examined samples of chicken meat and giblets (n=40).

Chicken tissues	Min.	Max.	Mean $\pm$ S.E <sup>*</sup>
Thigh	0.080	0.250	0.153 ± 0.01
Gizzard	0.099	0.210	$0.152 \pm 0.01$
Liver	0.070	1.890	$0.664\pm0.05$

\*S.E = Standard error of mean

Means showing highly significantly different at (P < 0.01)

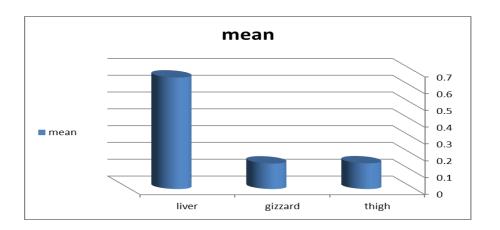


Fig. (2) Mean values of spiramycin residues in the examined samples of chicken meat and giblets.

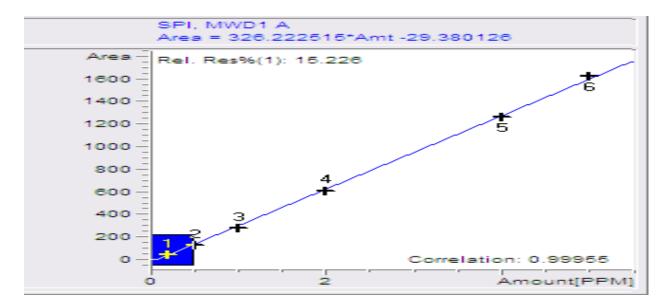
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Chicken tissues	Maximum Permissible Limit (ug/g)*	Accepted samples		Unaccepted Samples	
		No.	%	No.	%
Thigh	0.2	37	92.5	3	7.5
Gizzard	0.6	40	100	-	-
Liver	0.6	34	85	6	15

Table 3 Acceptability of the examined samples of chicken meat and giblets based on their levels of spiramycin (n=40).

\* according to codex alimentarieus commission (9).

Fig (3): Calibration curve of spiamycin residues in the examined samples of chicken meat and giblets by HPLC.



Chicken tissues	Before boiling	After boiling	Reduction%
Thigh			
1	0.080	$ND^*$	100
2	0.113	ND	100
3	0.250	0.032	91.20
Gizzard			
1	0.099	ND	100
2	0.110	ND	100
3	0.210	0.014	93.33
Liver			
1	0.070	ND	100
2	0.868	0.155	82.14
3	1.890	0.472	75.03

Table 4 Effect of boiling on spiramycin residues ( $\mu g/g$ ) in the examined samples of chicken meat and giblets.

\*ND= Not detected

#### 4. DISCUSSION

Antibiotic residues are on the top of priority for the public health authorities all over the world. Heavily & incorrect use of Macrolide antibiotics in poultry field as feed additives for growth promotion and in the treatment of some respiratory & enteric diseases, more over slaughtering birds before sufficient withdrawal time after treatment can possibly lead to the presence of these antibiotic residues in its edible tissues. Generally residues became an essential part of our food, and we can explain that these residues were consisted of the parent compound or compounds derived from the parent drug (or both) including metabolites & residues bound to macromolecules[42]. Concerns has been expressed about possible harmful effects on humans though the use of drugs as follows: increases of microbial drug resistant, drug residues in food, allergic sensitization reactions & to antimicrobials.[4,43]

The presence of chemical residues in food, particularly meat, over the past years has assumed a much higher profile for consumers and has dominated public health concerns about the regulation of food safety [40]. This issue remains the cornerstone of quality assurance system that are being developed by the meat industry to assure consumers of the high quality of poultry meat. With the exception of environment contaminants, chemical residues in meat generally arise from misuse of chemicals and drugs or failure to observe the prescribed with holding period between last treatment and slaughter. [29] In most cases, failure to comply, with the requirements for use of the chemical or drug is unintentional, though the consequences for the poultry market can be serious.

From the results reported in table (1) it is obvious that the Spiramycin residues were detected in 10%, 10% & 40% of the examined samples of chicken Thigh, Gizzard and Liver respectively. Therefore 90%,90% & 60% of such examined samples.

Results achieved in table (2) and figure (1) declared the level of spiramycin residues ( $\mu$ g/g). The examined samples of chicken thigh, gizzard and liver ranged

from 0.080 to 0.250 with mean average of 0.153  $\pm$ 0.01 for chicken gizzard , from 0.099 to 0.210 with mean average of 0.152 $\pm$ 0.01 for chicken liver from 0.070 to 1.890 with mean average of 0.664 $\pm$ 0.05 for liver .

Difference associated with the examined samples of chicken "thigh, gizzard and liver" as a results of (P < 0.01) were highly significantly according to their contents of spiramycin residues.

Acceptability of the examined samples of chicken & giblets based on their levels of spiramycinis shown table in (3). Accurately , 92.5% , & 85% of the examined samples of chicken thigh and liver were accepted respectively according to , Codex Alimentarius Commission [9]. in the other words 7.5 % & 15% of the examined samples of chicken thigh and liver were unaccepted . While, all examined of gizzard were accepted where they did not exceeded the permissible limits.

It was clear that the highest incidence of antibiotic was in chicken Liver (40 %) and the lowest incidence was in chicken muscles & chicken Gizzard which (10%) Concerning the tissue residues of spiramycin in normal chicken following repeated oral administration, the drug was detected in most tissues and organs. Higher spiramycin residues were detected in Liver and kidney, 24 hour after the stop of administration, while the lowest concentrations were found in breast and thigh muscles. Spiramycin was completely disappeared from serum and all tissues 120 hours after the stop of administration. At the same time [23] reported that spiramycin residues (about 0.1 ppm) were found only in liver and its presence in other organs, expressed in spiramycin activity, was therefore lower than (0.01-0.02 ppm) so the highest concentration of spiramycin was found in liver while the lowest concentration was detected in muscles. In other investigation, [39] recorded that spiramycin concentration in Liver, Kidney, Spleen, and Heart and

epically Lungs were high. The drug had along retention time in these tissues [38,36] The long retention in tissues was caused by its relatively slow metabolism and by tissue binding.[3]

The obtained results were similarly near to those which obtained by [5,28] who from their studies on spiramycin residues in poultry tissues, found that the liver is indicated as the target tissues for Spiramycin residues.

The present results were agreed with [17] who found that the concentration of Spiramycin in liver is much higher in comparison with the concentration in other organ.

Results achieved in table (4) declared the effect of boiling on spiramycin residues in the examined chicken samples (Thigh & giblet) and showed that the concentration of spiramycin residues in chicken Thigh was 0.080  $\mu$ g/g, 0.113  $\mu$ g/g and 0.250 $\mu$ g/g before boiling and after boiling the first second concentrations and were disappeared completely as they below the permissible limits but the third concentration partially reduced to 0.032  $\mu g/g$  by 91.20% as it exceed the permissible limits according to Codex Alimentarius Commission [9], spiramycin residues in chicken Gizzard was 0.099  $\mu g/g$ , 0.110  $\mu g/g$  and 0.210  $\mu g/g$  before boiling and after boiling the first and second concentration were completely reduced as they below the permissible limits but the third concentrations partially reduced to 0.014  $\mu$ g/g by 93.33% as it permissible exceed the limits and spiramycin residues in chicken Liver was  $0.070 \ \mu g/g$  which after boiling disappeared , but 0.868  $\mu$ g/g & 1.890  $\mu$ g/g which after boiling partially reduced to 0.155  $\mu$ g/g & to 0.472  $\mu$ g/g by 82.14% & 75.03% as they exceed the permissible limits.

From these results we can concluded that boiling have a positive effects on the Spiramycin residues in degrading the concentrations of residues so boiling will act as minimal safeguards in destroying the antibiotic residues that are present in edible tissues [25].

The obtained results agreed with those reported by [45] who found that the treatment of spiramycin residues at 120 °C for 20 min produces inactivation percentages of 64% while treatment at 140 °C for 10 sec. results in generally lower percentages 35%. The lowest loss or lowest reduction of antimicrobial activity 13% spiramycin was obtained by treatment at 60 °C for 30 min.

Also, these results agree, , with those obtained by [21] who determine that effects different the of cooking processes like boiling, on Deoxycline residues in muscle, liver and gizzard tissues of broiler chickens. The results showed а reduction in the concentration of Deoxcycline residue after different cooking processes and a part of the residue in the boiling process were excreted from the tissue to the cooking fluid. Between the various agents affecting antibiotics residue after the cooking process. cooking time and temperature can play a major role in antibiotic residue reduction while cooking food. results, they conclude Regarding the that cooking processes do not full elimination of guarantee these drugs and it can only decrease its amounts.

higher The detected initial spiramycin residues concentration of in some organs may be responsible for the persistence of some residues as in liver. These results are compatible with those of [20,30,34] who reported that heating or boiling, decreased the antimicrobial activity in chicken meat or organs. Moreover, antibiotic can be refractory for heat degradation in animal tissues unless high temperature levels are maintained for considerable period. [37,44,31]

These results are in harmony with those previously reported by [30,18]

who reported that coocking of meat decreased its content of antibiotic residues.

However, some antibiotics are heat stable as chloramphenicol while others are polymerized at higher temperature  $(200C^{\circ})$ and produce toxic or mutagenic products.[6] Finally, residues of antibacterial drugs in the edible tissues of food producing animals represent a serious problem human being consuming such for tissues [2].

Hypersensitivity, or even toxicity and development of bacterial resistance strains are among the hazards of antimicrobial residues. [10,1,6,26]

The use of HPLC method for detecting the antibiotic residues in this study was confirmed by [35,26,11] who used microbial and HPLC methods for the detection of the antibiotic residues. Moreover,[14,22] mentioned that HPLC could identify the antibiotic at level below the MRL.

## 5. CONCLUSION

The present results allow concluding that the spiramycin residues in broiler chicken tissues were recorded at highest concentrations in the examined samples of liver followed by muscle and gizzard. Furthermore, the application of boiling have a positive effects on the Spiramycin residues in degrading the concentrations of residues in such examined samples.

Veterinarians might be careful in prescribing any antibiotic as there are many rules for the selection of certain drugs, putting in consideration public health importance of the drug residues as toxicity, carcinogenic effect, development drug resistant bacteria drug of • compatibility and interaction, synergism, effect of the antibiotic on the normal flora, risk of such antibiotic on birds and immunosuppressant.

Slaughtering broilers treated with antibiotics before withdrawal time should

be prevented. They should be aware of using drugs banned by the international authorities even if it is still permissible on our country.

#### **Proper using of antibiotics by:**

Good diagnosis to diseases by experienced veterinarians.Using of modern techniques laboratory tests for confirming the diagnosis should be adapted.

Using sensitivity tests for choosing the antibiotics should be carried out.

Completing the course of antibiotic (not less than 5 days).

Proper detection of antibiotic residues.

On presence of the antibiotic residues in broilers meat, they must be lower than the permissible limit of antibiotic residues.

Some antibiotic residues can be controlled by heat & cold treatment like " spiramycin , tilmicosin and oxytetracycline".

Some antibiotics have a great dangerous effect on public health so it must be prevented from usage as growth promoters. Finally, all measures must be taken for preventing or decreasing antibiotic residues.

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#### مجله بنها للعلوم الطبية البيطرية



# متبقيات الأسبير اميسين في لحم الدجاج والحوائج فاطمة حسين أمره ، محمد أحمد محمد حسن<sup>2</sup> ، أيمن حامد محمود<sup>3</sup>

<sup>1</sup> وحدة تحليل متبقيات الادوية - معهد بحوث صحة الحيوان بالدقى <sup>2</sup>قسم الرقابة الصحية على اللحوم والدواجن والأسماك ومنتجاتها- كلية الطب البيطري - جامعة بنها. <sup>3</sup>قسم البيوتكنولوجي – معهد بحوث صحة الحيوان بالدقى.

#### الملخص العربى

يعتبر الإسبيراميسين أحد المضادات الحيوية التي تستخدم بكثرة في علاج دجاج التسمين وقد تذبح هذه الطيور قبل انتهاء فترة نضوبها من أنسجتها مما قد يؤثر سلبياً على صحة المستهلك. كما تم الكشف عن بقايا الإسبيراميسين باستخدام جهاز (HPLC) . لقد تم تجميع 120 من دجاج التسمين " الفخذ ، الكبد ، الحوصلة "من مزرعة بمحافظة بنها كعينات طازجة (40 عينات لكل منهما) للكشف عن بقايا الأسبيراميسين. وتوزن كل عينة من عينات 20 جرام . كما أوضحت النتائج أن متوسط تركيز بقايا الأسبيراميسين (ميكروجرام / جرام) في دجاج التسمين في " الفخذ ، الحوصلة ،الكبد" كانت (10.0± 153 الفخذ)، ( 10.0± 10.0± (ميكروجرام / جرام) في دجاج التسمين في " الفخذ ، الحوصلة ،الكبد" كانت (10.0± 153 الفخذ)، ( 10.0± 10.0± ( ميكروجرام / جرام) في دجاج التسمين في " الفخذ ، الحوصلة ،الكبد" كانت ( 20.0± 10.5 الفخذ)، ( 10.0± 10.0± ( 20.0± 20.60 للكبد)، على التوالي . . كما أتضح أن أعلى نسبة وجود لبقايا المضاد الحيوي وجدت بالكبد بنسبة (40%) و أقل نسبة وجود بالفخذ و الحوصلة بنسبة (10%). ولذلك تم قبول ( 2.29٪ ) من عينات الفخذ ، ( 28٪ ) من عينات الكبد، من من عينات الحوصلة على التوالي طبقاً للمواصفات الدولية. وقد تم غلي 9عينات من العينات الايجابية للكشف عن بقايا هذا المضاد بها وقد كانت نسبة أخترالها تتراوح بين (10، 100%) لعينات الفخذ ، ( 93، 100%) لعينات الكبين عن الميات الكبد. لذا معاملة عينات الدجاج " الفخذ ، الكبد ،الحوصلة" الإيجابية حرارياً بالغليان لبقايا الأسبيراميسين لها تأثير كبير هذه البقاي وخصوصاً عندما يتم الكشف عنها في تركيزات منخفضة أقل من الحدود المسموح بها . هذا و يمثل الغليان درع الوقاية و الحماية من بقايا المضادات الحيوية التي تكون موجودة في الأنسجة الصالحة للاستهلك.

(مجلة بنها للعلوم الطبية البيطرية: عدد 24 (1)، يونيو 2013: 51- 61)