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DETERMINATION OF SULPHAMETHAZINE RESIDUES IN RABBIT CARCASSES AT KALYOBIA GOVERNORATE

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A B S T R A C T

Ninety samples of rabbit meat and liver (45 of each) were collected randomly from different localities at Kalyobia governorate represented by rural, suburban and urban areas (15 of each) for the presence of sulphamethazine using Four Plate Test (FPT) and High Performance Liquid Chromatography (HPLC). Also, the effect of boiling as a method of cooking on these residues was studied. The results showed that 13.33%, 13.33% and 26.67% of rabbit muscle samples were positive for the presence of sulphamethazine residues by using FPT method, respectively; however 13.33%, 20.00% and 33.33% were positive for such residues by using HPLC method for muscle samples collected from rural, suburban and urban areas, respectively. Concerning to rabbit livers, the positive samples for sulphamethazine in samples from rural, suburban and urban areas were 20.00%, 26.67% and 40.00% by using FPT and 26.67%, 26.67% and 46.67%, respectively. Moreover, 6.67% 6.57% and 13.33% of the examined muscle samples and 13.33%, 20.22% and 33.33% of the examined liver samples were exceeded the maximum residual limit of sulphamethazine. On boiling, the sulphamethazine were completely disappeared in muscle samples containing 0.19 1.04 mg/kg. However, 85.64% of sulphamethazine was destructed in samples containing 2.67% mg/kg. Concerning rabbit liver samples, 80.43% and 71.86% of sulphamethazine concentration were disappeared by boiling of samples containing 1.89 and 3.16 mg/kg. On the other hand, complete destruction of sulphamethazine was occurred in samples containing 0.33mg/kg. The public health significance of the determined sulphamethazine residues in rabbit muscle and livers and some recommendations to control their presence in rabbit muscle and livers were discussed.

KEY WORDS: Sulphonamide, Sulphamethazine, Rabbit, Residues

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1. INTRODUCTION

In Egypt, the rabbit meat has got a high degree of popularity as protein food. Many people prefer to consume rabbit meat due to its high nutrition value; higher percentage of protein 20.80% less fat 11-20% and less caloric value 795 cal. Rabbit meat is suitable for special diets, such as those for heart disease patients, diets for the aged, low sodium diets, weight reduction diets. The rabbit production depends on many chemotherapeutic agents as food additives for prophylactic and treatment of many diseases or used as growth promoters to increase the yield of protein for human consumption [21]. These chemotherapeutic agents include antibiotics and sulphonamides which are used in a wide scale.

Sulfonamides are synthetic antibacterial with a wide spectrum against most Gram-positive and many Gram-negative organisms. They inhibit bacteria, Chlamydia, toxoplasma and other protozoan agents especially coccidian in poultry, rabbit and other economically important animal species [20]. They inhibit

multiplication of bacteria by acting as competitive inhibitors of p-aminobenzoic acid in the folic acid metabolism cycle [8]. Sulphonamides are widely used for therapeutic and prophylactic purposes in human being [12] and most commonly used in veterinary medicine for prophylaxis and therapeutic purposes [1].

Current legislation establishes the maximum residual limit of sulphonamides (all compounds of sulphonamides) in food of animal origin at the level of 0.1mg/kg. Due to the fact that sulphonamides are used in food producing animals [16] and their potential carcinogenic characters [15], it is necessary to ensure that all foods sold in the markets contain a safe level of sulphonamides that has been restricted to 0.1 µg/g of food producing animal [5, 14].

Therefore, the aim of the current study is to determine the level of sulphamethazine residues in meat and livers of rabbits by using Four Plate Test (FPT) and High Performance Liquid Chromatography (HPLC). Also, study the effect of boiling as a method of cooking on these residues.

2. MATERIALS AND METHODS

A total of ninety random samples of rabbit meat and livers (45 of each) were collected randomly from different localities at Kalyobia governorate represented by rural, suburban and urban areas (15 of each). All collected samples were transferred as rapidly as possible to the laboratory for determination of their levels of sulphamethazine residues by FPT and HPLC technique.

2.1. *Four plate test (FPT) (Microbiological method)*

The method recommended by Bogarets and Wolf [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 6 mm-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 samples was done according to Oka *et al.*, [17] and Pieckova and Van Peteghem [18]. Accurately, 5g of each sample and 10 g of anhydrous sodium sulfate were blended with 20 ml of ethy1 acetate and then centrifuged. The supernatant was evaporated and dried under reduced pressure at 40°C. The residue was dissolved in 5 ml 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 5 ml 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 wave length U/V detection, Kyoto, Japan.

Accordingly, sulfamethazine residues were estimated by using their standard solutions specific for each of them. Operating conditions for analysis of tetracycline were; eluent at 35°C, flow rate 1 ml/min., injection volume, 10 µl; detection wave length 216 nm. While, the operating conditions for analysis of sulfamethazine were; eluent at 30°C, flow rate 1ml/min., injection volume, 20 µl; detection wave length 272nm.

2.3. *Statistical analysis*

Statistical analysis of the obtained results was done by application of analysis of variance (ANOVA) according to Rosner [19].

3. RESULTS AND DISCUSSSION

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 the rabbit tissues causing allergic reactions in the sensitive individuals consuming such food stuff. Table (1) revealed that the sulphamethazine residue in the examined samples of rabbit meat and liver by using FPT were detected in 13.33% and 20% for rural area, 13.33% and 26.67% for suburban area and 26.67% and 40% for urban area; respectively. In general, 17.78% and 28.89% of rabbit meat and liver samples contained sulphamethazine regardless to the localities; respectively. Regarding to HPLC, the sulphamethazine residues were detected in 13.33% and 26.67% for rural area, 20.00% and 26.67% for suburban area and 33.33% and 46.67% for urban area, respectively. Regardless to the localities, the sulphamethazine residues in rabbit meat and liver samples were 22.22% and 31.11%; respectively by using HPLC. Results in table (2) declared that the sulphamethazine residue concentrations ranged from 0.01 to 1.06 with a mean value of 0.53 mg/kg, 0.01 to 1.88 with a mean value of 0.72 mg/kg and 0.04 to 2.67 with a mean value of 1.29mg/kg for rural, suburban and urban areas respectively. While for rabbit liver, the sulphamethazine concentrations ranged from 0.04 to 2.53 with average 1.17, 0.05 to 3.16 with average 1.95 and 0.07 to 5.02 with average 2.38 mg/kg for rural, suburban and urban areas, respectively. The current results come in accordance with those reported by former authors [2, 4, 10].

It is evident from the results recorded in table (3) that 6.67%, 6.57% and 13.33% of the examined samples of rabbit meat and liver at rural, suburban and urban areas; respectively exceeded the permissible limit (0.1mg/kg) recommended by FAO/WHO [6]. While that for rabbit liver samples, was 13.33%, 20% and 33.33% at rular, suburban and urban areas, respectively exceeded the permissible limit (0.1mg/kg). In general, 8.89% and 22.22% of rabbit meat and liver exceeded the permissible limit (0.1mg/kg) regardless to the localities.

Effect of boiling on sulphamethazine concentration in the examined samples of rabbit meat and liver was shown in table (4). In regard to rabbit meat samples containing 0.19 and 1.04 mg/kg, the sulphamethazine were completely disappeared after boiling at 100°C for 30 minutes, however, 85.64% of sulphamethazine levels were destructed in these samples containing 2.67%mg/kg.

Concerning rabbit liver samples, 80.43% and 71.86% of sulphamethazine concentration were disappeared by boiling in the samples containing 1.89 and 3.16 mg/kg; respectively. On the other hand, complete destruction of sulphamethazine was occurred in samples containing 0.33mg/kg. The obtained results were in agreement with those reported by previous authors [7, 9, 11, 13].

Table 1 Incidence of sulphamethazine in the examined samples of rabbit meat and livers (15 of each).

Localities	No. of examined samples	Rabbit meat				Rabbit livers			
		FPT*		HPLC**		FPT*		HPLC**	
		No.	%	No.	%	No.	%	No	%
Rural	15	2	13.33	2	13.33	3	20.00	4	26.67
Suburban	15	2	13.33	3	20.00	4	26.67	4	26.67
Urban	15	4	26.67	5	33.33	6	40.00	7	46.67
Total (45)	45	8	17.8	10	22.22	1	28.89	14	31.11

*FPT: Four Plate Test. ** HPLC: High Performance Liquid Chromatography. No. : Number of positive samples.

Table 2 Statistical analytical results of sulphamethazine concentrations (mg/kg) in the examined samples of rabbit meat and livers (n=15)

Localities	Rabbit meat			Rabbit livers		
	Min.	Max.	Mean ± S.E	Min.	Max.	Mean ± S.E*
Rural	0.01	1.06	0.53±0.01	0.04	2.53	1.17±0.02 +
Suburban	0.01	1.88	0.72±0.02	0.05	3.16	1.95±0.04
Urban	0.04	2.67	1.29±0.06	0.07	5.02	2.38±0.04

*****S.E**:** Standard Error**. +**: Significant differences ($p < 0.05$).

Table 3 Acceptability of the examined samples of rabbit meat and livers according to their contents of sulphamethazine (n=15).

Localities	Maximum Residual Limit (mg/kg)*	Unaccepted rabbit meat		Unaccepted rabbit livers	
		No	%	No	%
Rural	0.1	1	6.67	2	13.33
Suburban	0.1	1	6.57	3	20.00
Urban	0.1	2	13.33	5	33.33
Total (45)	0.1	4	8.89	10	22.22

*FAO / WHO (1999)

Table 4 Effect of boiling (100°C/ 30 minutes) on sulphamethazine concentrations (mg/kg) in the examined samples of rabbit meat and livers.

Samples	Rabbit meat			Rabbit livers		
	Before boiling	After boiling	Reduction %	Before boiling	After boiling	Reduction %
Trail 1	0.19	ND	100	0.33	ND	100
Trail 2	1.04	ND	100	1.89	0.37	80.43
Trail 3	2.67	0.41	85.64	3.16	0.92	71.86

ND : Not Detected

The present study allows confirming the bad hazard using of sulphonamides inside the rabbit 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 problem of such residues in rabbit tissues.

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مجلة بنها للعلوم الطبية البيطرية
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قياس متبقيات السلفاميثازين فى ذبائح الأرانب فى محافظة القليوبية

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الملخص العربي

أجريت هذه الدراسة لمعرفة مدى تواجد السلفاميثازين فى عينات اللحوم والكبد للأرانب المذبوحة بمحافظة القليوبية فى محلات مختلفة من المناطق الريفية والضواحى والمناطق الحضرية باستخدام الطريقة الميكروبيولوجية للتقدير الكيفى(FPT) وكذلك استخدام جهاز الكروماتوجرافى السائل العالى الجودة للتقدير الكمى(HPLC) فى العينات، كما تم دراسة تأثير الغليان عند100° م/30 دقيقة على تركيز هذا الدواء. قد كشفت النتائج أن معدل انتشار متبقيات السلفاميثازين في عينات لحوم الأرانب والكبد كانت 17.78٪ و 22.22-28.89٪ و 31.11٪ باستخدامHPLC–FPT على التوالي بغض النظر عن أماكن تحصيلها. من ناحية أخرى كانت متوسط قيم متبقيات السلفاميثازين في عينات اللحوم والكبد 0.53±0.01 و 1.17±0.02 لمنطقة الريف، 0.72±0.02 و 1.95±0.04 لمنطقة الضواحي و1.29±0.06 و 2.38±0.04 مجم/ كجم بالنسبة للمنطقة الحضرية على التوالي. كما تم دراسة تأثيرالغليان عند 100 درجة مئوية لمدة ثلاثون دقيقة على تلك المتبقيات فى العينات الإيجابية فقد وجد أن غليان لحوم الأرانب وكبدها له تأثير جيد على التخلص من تلك المتبقيات. حيث أنه يحدث تكسير كامل للسلفاميثازين فى عينات اللحوم المحتوية على تركيز 0.19و1.04 ملجم / كجم وعينات الكبد المحتوية على0.33 ملجم/كجم. كما اهتمت الدراسة ببيان الأهمية الصحية لوجود متبقات السلفاميثازين في مذبوحات الأرانب مع وضع بعض التوصيات اللازمة للسيطرة على هذه المتبقيات الخطيرة.

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