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Effect of thermal processing on antibacterial drug residue of tetracycline and sulfonamide in fresh beef meat and Iraqi processed meat

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Abstract---This study has demonstrated the presence of antibacterial drugs, residue from fresh beef meat, and Iraqi processed meat (minced meat and kebab meat) . One hundred and sixty samples were collected 80 fresh meat and 80 Iraq processed meat (40 minced meat and 40 kebabs) (February to September) randomly for the detection of antibacterial drugs residue of tetracycline and sulfonamide by high-performance liquid chromatography (HPLC) .The positive sample was processed with heat treatment (roasting and boiling) at 100 °C/30 min and to determine the effect of heat treatment to decrease the levels of tetracycline and sulfonamide drug residue in fresh beef meat and Iraqi processed meat. The present study shows that over 160 beef samples, 29 (18.12%), and 19(11.87%) appear positive to antibacterial drug residue in tetracycline, and sulfonamide. There were a significant reduction of antibacterial drug residue of tetracycline levels from 1572.99±120.93 ppb, 1253.70±249.15 ppb, and 1606.33±161.52 ppb before heat treatment to 33.43±11.99 ppb, 21.40±3.18 ppb and 45.23±11.52 ppb for boiling methods, and 15.69±5.05 ppb, 9.23±3.83 ppb, and 18.44±5.84 ppb respectively for roasting method in fresh meat and minced meat and kebab. Also, there is significant reduction of antibacterial drug residue of sulfonamide levels from 65.20±7.78 , 41.03±5.56 and 131.34±24.44 ppb in before heat treatment to 33.43±11.99 ppb, 21.40±3.18 ppb, and 45.23±11.52 ppb for boiling methods, and 15.69±5.05 ppb, 9.23±3.83 ppb, and 18.44±5.84 ppb respectively for roasting method in fresh meat and minced meat and kebab. conclusion of this study, the heat treatment (boiling and roasting methods) reduced the levels of tetracycline and sulfonamide drug residue in fresh meat and Iraqi processed meat. To safeguard

consumers from the risk of such residues proper use of antibiotics through good diagnosis of the diseases by experienced veterinarians and creation of legislation of compulsory following the rules of withdrawal time of the antibiotic.

Keywords---meat, antibacterial drug residue, thermal processing, fresh beef meat.

Introduction

Meat can be considered as a valuable food since it is composed of different nutrients among which are proteins, minerals, fats, vitamins, small amounts of carbohydrates, among other bioactive components, and given its composition can be part of a balanced diet (Obaidi,2016 and Odhaib, *et al.*,2021)

Antibacterial is used largely for three purposes in animals, therapeutic use to treat sick animals, prophylactic use to prevent infection in animals and as growth, promoters to improve feed utilization and production for their growth-promoting properties they are routinely used at sub-therapeutic levels as animal feed additives. Growth promoters are antimicrobials that, when administered in low doses in animal feed, have a preventive effect against certain bacterial infections and modify the composition of the intestinal microbiota, improving feed assimilation. The impact of these protective effects on animal production is to accelerate livestock growth (Tadesse, and Tadesse, 2017). The main antibiotics used in animal husbandry include sulfonamides and tetracyclines (Tian, *et al.*,2017 and Correa, *et al.*,2018).

Antibiotic residues in foods of animal origin may be the cause of numerous health problems in humans (Mangsi, *et al.*,2014). These problems include were allergic reactions anemia, disturbances in the stability of the gut microflora, and antibiotic resistance and its transfer to the human (Al-kurashi, and Najim,2017). The presence of residues may be the result of failure to monitor the withdrawal periods, illegal or off-label use of drugs, and incorrect dosage levels or dosing schedules. Unauthorized antibiotic use and lack of good veterinary practices may result in residues of these substances in milk and tissues (Paturkar, *et al.*,2005).

The residues of a range of veterinary drugs have varying degrees of stability during cooking; the cooking influences the level of risk posed by such residues. For many years several studies were conducted for evaluating whether antibiotic residues can be destroyed by cooking procedures. the cooking influences the level of risk posed by such residues. most food-producing animals are always cooked before consumption and the variations in antibiotics levels in the tissue are dependent on the type of cooking (Shaltout, *et al.*,2019). In this study, the aims were to determine the levels of antibacterial drug residues (ppb) of tetracycline and sulfonamide in the meat and meat product as well as to evaluate the effect of different commercial heat treatments processes such as boiling and roasting methods at 100°C\30 mint. on positive samples to decrease the levels of antibacterial drug residues in meat and meat product.

Material and Methods

Samples collection

One hundred and sixty samples were collected randomly from 80 samples from fresh beef meat 80 from Iraq processed meat (40 minced meat and 40 kebabs) samples from the different supermarkets. This study was designed to determine the level of antibacterial residual by high-performance liquid chromatography (HPLC). The positive sample was processed with heat treatment (roasting and boiling) at 100 °C/30 min and to determine the effect of heat treatment to decrease the levels of antibacterial drug residue in fresh meat and Iraq processed meat (minced and kebab meat) (Shaltout, *et al.*,2019).

Preparing samples for detection antibacterial:

The method of analysis used a weight of 10 g (from the fresh meat, minced meat, and kebab meat taken from the model and placed in a mortar containing quartz sand and added (20 ml) from buffer suitable for antibiotic extraction (2%) pepsin solution in citrate- muraiticer with (PH = 1.5) mix for 30 minutes and incubate for 1 hour and a half. The model is 3000 r/min for 20 minutes. The hydrolyzed model is used in Petri dishes. In an incubator at 29°C for 18 hours and become proper to detect antibacterial residue by HPLC (Abdul-Razaq, *et al.*,2016).

The samples were collected and prepared for quantitative analysis using HPLC with solvent A (Acetonitrile) and solvent B (20 Mm² PO₄, pH 3.5) as mobile phase. The standard of active material was separated on the Fast Liquid Chromatographic (FLC) column, linear gradient from 0%b-100% B in 12 min. Detection UV of tetracycline 310 nm , Em = 420 nm , Flow rate = 1.2 ml / min and sulfonamide the detector UV- 240 nm, Injection volume: 100μL. The separation occurred on liquid chromatography Shimadzu 10AV-LC equipped with delivery pump model LC-10A Shimadzu, the eluted peaks were monitored by UV-Vis 10A-SPD spectrophotometer. The measurements took place in isocratic mode (Al-kurashi, and Najim,2017).

Statistical analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). Two-way ANOVA and Least significant differences (LSD) post hoc test were performed to assess significant differences among means. P < 0.05 is considered statistically significant (SAS.2010).

Result and Discussion

The present study, that the overall percentage levels of the antibacterial residual of Tetracycline, and Sulfonamide (18.12% and 11.87% respectively) using the HPLC technique during the examines demonstrate that there was a significant (p<0.05) difference in percentage levels between the fresh beef meat and Iraqi processed meat) show in (Table 1). The percentage of antibacterial drug residue level of tetracycline in kebab meat was recorded significantly (p≤ 0.05) highest contamination their average levels (22.5%) compared with minced meat 17.5%

and fresh meat (16.5%). The percentage of antibacterial drug residue level of sulfonamide in kebab meat was recorded significantly ($p \leq 0.05$) highest contamination their average (17.5%) compared with minced meat (12.5%) and fresh meat (8.75%). These results were in agreement with (Ramatla, *et al.*,2017) obtained that (14.6%) of the samples contained residues. Also (Fahim, *et al.*,2019) showed that the positive samples of beef for antibiotic residues were (32%). In another study, (Abbasi, *et al.*2012) found 25.8% contaminated with antibacterial drug residue of tetracycline in muscle samples. Additionally, (Al-mashhadany,2019) found that the occurrence rate of antibiotic residues in beef samples was 10.8%. (Elbagory, *et al.*,2017) revealed that oxytetracycline residues were detected in 10% and 36.67% of the examined samples of imported and local beef, respectively.

Samples	No. of examines sample	Tetracycline		Sulfonamide	
		No. of a positive sample	Percentage	No. of a positive sample	Percentage
Fresh meat	80	13	16.25%	7	8.75%
Minced meat	40	7	17.5%	5	12.5%
Kebab meat	40	9	22.5%	7	17.5%
Total samples	160	29	18.12%	19	11.87%

Table 1: Percentage of Antibacterial Drug Residue in Fresh Beef Meat and Iraqi Processed Meat

The data demonstrated that the mean value of antibacterial drug residue of tetracycline (ppb) before heat treatment and the positive sample between boiling and roasting was non-significantly ($P \geq 0.05$) between fresh meat and Iraq processed meat (minced meat and kebab) was 1572.99 ± 120.93 ppb, 1253.70 ± 249.15 ppb, and 1606.33 ± 161.52 ppb respectively. the positive sample between boiling and roasting in fresh meat and Iraq processed meat (minced meat and kebab) was 33.43 ± 11.99 ppb, 21.40 ± 3.18 ppb and 45.23 ± 11.52 ppb for boiling methods, while was 15.69 ± 5.05 ppb, 9.23 ± 3.83 ppb, and 18.44 ± 5.84 ppb respectively for roasting method.

The statistical analysis showed a significant ($p \leq 0.05$) difference in antibacterial drug residue levels of tetracycline between before and after heat treatment (boiling and roasting) in fresh meat and Iraq processed meat (minced meat and kebab). The means value of tetracycline levels before heat treatment was 1572.99 ± 120.93 ppb, 1253.70 ± 249.15 ppb, and 1606.33 ± 161.52 ppb respectively compared after processing heat treatment was 33.43 ± 11.99 ppb, 21.40 ± 3.18 ppb, and 45.23 ± 11.52 ppb for boiling methods, while was 15.69 ± 5.05 ppb, 9.23 ± 3.83 ppb, and 18.44 ± 5.84 ppb respectively for roasting method. These results were in agreement with (Kimera, *et al.*,2015) detected that the mean level of oxytetracycline in muscle meat was 1126.7 ± 204.3 $\mu\text{g/kg}$ in Kilosa, Tanzania. These results were similar to those (Ramatla, *et al.*,2017) who found that tetracycline residue concentrations range from 65.2–952.2 $\mu\text{g/kg}$ by HPLC in meat. This study disagrees with. (Elbagory, *et al.*,2017) found that level of

oxytetracycline in local beef was $212.5 \pm 14.96 \mu\text{g}$ in Egypt. A low level was found of oxytetracycline in beef meat $16.17 \pm 5.52 \mu\text{g/kg}$ by (Adesokan, et al., 2013). (Abbasi, et al., 2012) who detected that the mean value of the antibacterial drug of tetracycline residues in muscle samples was $176.3 \pm 46.8 \mu\text{g/kg}$. Also (Muriuki, et al., 2001) recorded that the tetracycline residue levels in the meat samples in Athi River, Kenya were $280 \mu\text{g/kg}$. (Fahim, et al., 2019) detects that the heat processing treatment (boiling and roasting) on reduction for Oxytetracycline residues in rabbit muscle 87.97% and 73.98%, respectively. (Mgonja et al., 2017) found a reduction of OTC with boiling up to 91% in 30 minutes in meat. (Abou-Raya, et al., 2013) cooking breast meat for 40 min by boiling and roasting led to a reduction of OTC contents by 91.5 and 72.1%, respectively.

Treatment Sample	Before treatment	After treatment	
	Mean $\pm$ SE	Boiling (Mean $\pm$ SE)	Roasting (Mean $\pm$ SE)
Fresh meat	A1572.99 $\pm$ 120.93a	A33.43 $\pm$ 11.99b	A15.69 $\pm$ 5.05b
Minced meat	A1253.70 $\pm$ 249.15a	A21.40 $\pm$ 3.18b	A9.23 $\pm$ 3.83b
Kebab	A1606.33 $\pm$ 161.52a	A45.23 $\pm$ 11.52b	A18.44 $\pm$ 5.84b
LSD	493.88		

Table 2: Concentration of Antibacterial Drug Residue Level of tetracycline (Ppb) in Fresh Meat and Processed Meat (Minced Meat and Kebab) and Effect Heat Treatment on tetracycline

The data demonstrated that the mean level of antibacterial drug residue of sulfonamide (ppb) before heat treatment was significantly ($p \leq 0.05$) high contamination in kebab 131.34 ± 24.44 ppb comparison between fresh meat and minced meat 65.20 ± 7.78 ppb and 41.03 ± 5.56 ppb respectively. While the mean level of antibacterial drug of sulfonamide (ppb) in the positive sample after heat treatment processed were non-significant significantly ($P \geq 0.05$) between boiling and roasting in fresh meat and Iraq processed meat (minced meat and kebab). The mean value of tetracycline was 1.55 ± 0.32 , 1.59 ± 0.33 , and 1.83 ± 0.49 for the boiling method, while 1.40 ± 0.48 , 0.30 ± 0.11 , and 1.02 ± 0.50 respectively for the roasting method.

The present study found that the mean value of sulfonamide in fresh meat before heat treatment was recorded significantly ($p \leq 0.05$) difference in contamination was 65.20 ± 7.78 ppb, 41.03 ± 5.56 ppb and 131.34 ± 24.44 ppb comparison with positive samples after heat treatment processed was 1.55 ± 0.32 , 1.59 ± 0.33 and 1.83 ± 0.49 for boiling method, while 1.40 ± 0.48 , 0.30 ± 0.11 and 1.02 ± 0.50 respectively for roasting method. These results were similar to that (Ramatla, et al., 2017) noticed that range of antibacterial drug residues levels of sulfonamide in meat $20.7\text{--}82.1 \mu\text{g/kg}$. This result was an agreement with (Mangsi, 2017) indicate that sulphonamides residue in meat reduces after boiling processing from $345.22 \pm 2.27 \mu\text{g/kg}$ to $2.26 \pm 0.11 \mu\text{g/kg}$; the residual level of sulphadimidine reduced to

96% at the boiling process of beef. (Furusawa and Hanabusa ,2002) found that the sulfonamides in the group of sulphadimidine drugs had a reduction of 45-61% by boiling, 38-40% by roasting; The more reduction observed for the present cooking method of boiling might be due to the transfer of drug residue from the muscle into the boiling water.

Also, (Tian, *et al.*2017) recorded that thermal processing results in a decrease in the concentration of parent antibiotic residues. Although antibiotic residues were degraded during boiling, we suggest doing other studies including toxicological experiments for the effect of degradation by-products on the human body. As some of these products were shown to be hazardous, further investigation is needed to determine their impact on food safety and human health, as well as, most of the residue in the boiling process was excreted from the tissue into cooking fluid.

Treatment Sample	Before treatment	After treatment	
	Mean± SE	Boiling (Mean± SE	Roasting(Mean±SE)
Fresh meat	B65.20±7.78a	A1.55±0.32b	A1.40±0.48b
Minced meat	B41.03±5.56a	A1.59±0.33b	A0.30±0.11b
Kebab	A131.34±24.44a	A1.83±0.49b	A1.02±0.50b
LSD	40.43		

Table 3: Concentration of Antibacterial Drug Residue Level of sulfonamide (ppb) in Fresh Meat and Processed Meat (Minced Meat and Kebab) and Effect Heat Treatment on sulfonamide

Conclusion

The most preferable methods of cooking for reducing antibiotic on meat were grilling followed by microwave and boiling .To safeguard consumers from the risk of such residues proper use of antibiotics through good diagnosis of the diseases by experienced veterinarians and creation of legislation of compulsory following the rules of withdrawal time of the antibacterial drug.

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