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Effect of Various Cooking Methods on Some Antibacterial Residues in Imported and Local Frozen Dressed Broilers and their Giblets in Egypt

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Abstract

A total of 120 local fresh and imported frozen broilers breast, liver, kidney and gizzard were collected from different places in Al-Menofia Governorate, Egypt for estimation of their contents of Oxytetracycline (OTC) and Ampicillin residues by using high performance liquid chromatography (HPLC).

Results revealed that 55%, 70%, 50%, 70%, 10% and 15% of the examined samples of local broilers meat, liver, gizzard, kidneys, imported broiler meat and kidneys were positive for residues of OTC with mean values of 471.4 ± 33.8, 773.9 ± 49.5, 11.6 ± 0.9, 2676.0 ± 117.5, 114.7 ± 8.3 and 546.7 ± 41.2, respectively. While 50%, 55%, 35%, 50%, 35% and 45% of the same samples were positive for residues of Ampicillin with mean values of 329.2 ± 20.7, 853.3 ± 65.1, 164.5 ± 9.8, 531.6 ± 37.4, 177.9 ± 15.4 and 323.8 ± 26.6, respectively.

Application of various cooking methods; boiling, frying and grilling on ten samples of chicken muscles indicated that cooking had an effect in reducing the concentration of OTC residues with mean reduction % of 84.52%, 93.62% and 96.58% after boiling, frying and grilling and 81.22%, 90.54% and 94.5% after boiling, frying and grilling for concentrations of ampicillin residues.

Presence of antibiotic residues in chicken carcasses possesses a health risk to consumers such as antibiotic resistance, teratogenicity, carcinogenicity, hepatic and renal failure. Veterinary control of withdrawal times in poultry farms and post-mortem inspection of slaughtered carcasses for antibiotic residues are important measures to reduce the incidence of antibiotic residues in poultry meat to improve the quality of chicken meat and to safeguard consumers.

Introduction

In poultry, antibiotics are being excessively used for various purposes as prophylactic, control of diseases and as growth stimulants, therefore antibiotic usage had facilitated their efficient production and enhanced the health and wellbeing of poultry [1,2]. Oxytetracycline (OTC) and Ampicillin are the most common routinely used in veterinary medicine for prevention and control of diseases in poultry [3].

Unfortunately, the use of antibiotics in food-producing animals may leave residues in food stuffs of animal origin like meat due to the failure to observe the withdrawal periods of each drug, extra-label dosages for animals, contamination of animal feed with the excreta of treated animals and/or the illegal use of unlicensed antibiotics [4]. The presence of Oxytetracycline residues in edible animal tissues has harmful effects on consumer's health such as allergic reactions, spreading of drug-resistant microorganisms, liver damage, yellowing of teeth, gastrointestinal disturbance and possible mutagenic and/or carcinogenic effects [5]. While the most common adverse effects caused by consuming of penicillin residues in meat were hypersensitivity reactions, especially skin rashes and gastrointestinal disturbances including diarrhea, nausea and sometimes vomiting [6]. Monitoring of antibiotic residues in poultry meat is important in controlling quality and safety of foods, consequently several analytical techniques are available for determination of antibiotic residues in poultry tissue, the most powerful and sensitive is the technique of high performance liquid chromatography (HPLC) [7]. Relatively simple and rapid typical detection for the presence of multi-residues in tissues samples could be achieved by using HPLC technique [8].

To ensure human food safety, WHO and FAO have set standards for maximum residue limits (MRLs) in foods. Additionally, the European Union (EU) has set own MRLs [9]. The acceptable MRLs for OTC residues are 200ug/kg in muscle, 600ug/kg in liver and 1200 ug/kg in kidney, while acceptable MRLs for ampicillin residues are 50ug/kg in muscle, liver and kidney according to the Joint FAO/WHO Expert Committee on Food Additives [10-12]. Control of the antibiotics residues in poultry meat could be achieved by giving the drugs to the birds after sensitivity test, by the accurate dose and prevents slaughtering in the withdrawal time [13]. Also, 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 [14].

The objective of this study is to through the light on safety of the local and imported frozen broilers tissues and giblets through monitoring the antibiotics residues (Oxytetracycline and Ampicillin) by using the technique of High Performance Liquid Chromatography (HPLC), with special references to the effect of the most common cooking procedures (boiling, frying and grilling) on the antibiotic residues level.

Materials and Methods

Collection of samples

Total of 120 broilers breast, liver, kidney and gizzard were collected from poultry shops of different places in Al-Menoufia Governorate, Egypt as frozen local and imported samples for determination of their residues of oxytetracycline and ampicillin).The samples were collected in clean sterile

polyethylene bags and transferred directly to the laboratory without delay in an ice box.

Determination of antibiotic residues in examined samples

Determination of oxytetracycline and ampicillin residues in examined samples was applied by using High Performance Liquid Chromatography (HPLC) technique.

Determination of oxytetracycline residues in examined samples

All samples were finely diced with scissors after trimming of the external fat and fascia. 2 g of each organ to be analyzed were weighed using digital balance and then cut into very small pieces and subsequently ground into fine powder using Sartorius mincer, then homogenized in a blender for 2 min. and then 0.1 gm of citric acid was added. One ml of nitric acid (30%), 4 ml methanol and 1 ml deionized water were added to this mixture, respectively. The suspension with solid particles was put in a vortex for good mixing, kept in an ultrasonic bath for 15 min and centrifuged for 10 min at 5300 rpm. After filtering through a 0.45 um nylon filter, 20 ul of solution was injected into HPLC for analysis according to Senyuva et al. [15].

Determination of ampicillin residues

Each sample was prepared for extraction of the drug with a specific solvent

Extraction: Accurately, 5 ± 0.01 g of each sample were put into 50 ml capped polypropylene centrifuge tube and 15 ml of acetonitrile/water (15:2) were added. Complete homogenization of the sample for 1 minute and centrifugation at 4000 rpm was carried out. The supernatant was taken for repeating the homogenization and centrifugation one additional time. Furthermore, the combined supernatants were placed into a round bottom flask and the acetonitrile was evaporated at 37°C. At least, approximately 6 ml of remaining supernatant should be found in the flask. The total volume of supernatant should reach 20 ml using phosphate buffer (pH 8.5). The supernatant was filtered through regenerated cellulose, 25 mm, 45 um syringe filter (Agilent pin 5185-5831). Actually, 10 ml of the extract were loaded onto the Agilent Sampli Q OPT 6 mL/150 mg cartridge [16].

Purification: The cartridge was washed with 0.1% formic acid in water and then pH 8.5 potassium phosphate buffer. Finally, the sample was eluted with 3 ml acetonitrile. The sample was filtered with a 13 mm, 45 ml poly tetrafluoro ethylene (PTFE) syringe filter (Agilent pin 518-5836). The eluent was dried under nitrogen at room temperature. The residue was re-suspended in mobile phase to1.0 ml. The sample was vortexed for 2 minutes and then transferred to a 2 ml, auto sampler vial (Agilent pin 5182-0864) [17].

Solid-phase extraction and final sample preparation: The SPE cartridge was placed into vacuum manifold system with SPE cartridge effluent going to a solvent trap. Accurately, 25 ml of methanol and then 25 ml of water and then 40 ml 0.01 M calcium hydroxide were added. Flow rate is not important for these steps. Furthermore, 3ml of the sample was applied to the cartridge with a flow rate not more than 2 drops/s. The cartridge was not allowed to dry at this step and the cartridge was flushed with 40 ml distilled water and then 10 ml acetonitrile. Elution was performed successively with 40 ml of 2.5% acetic acid including 50% methanol. The collected elute was evaporated using rotator evaporator at 45°C till complete dryness. The dried residue was reconstituted in 3 ml of the mobile phase. The samples were mixed and filtered through 0.2 µm filters before injection into the LC system.

Chromatographic conditions

Oxytetracycline: Include a mobile phase of methanol and formic acid 0.1% using a gradient method with a flow rate of 1.5 ml/min. at 25°C. The separation was done on Hypersil gold C18 (10 um, 100 × 4.6 mm) columns with mobile phase as described above. Detection was performed with PDA detector set at 350 nm wave length. Quantification of residues in samples was obtained and calculated from areas under curves extrapolated automatically by the software (Chromo Quest 5).

Ampicillin: High performance liquid chromatography (HPLC) used for antibiotic determination was an Agilent 1100 HPLC system at Animal Health Research Institute in Dokki, Agilen Technologies, Waldbronn, Germany, equipped with quaternary pump model G 1311A, UV detector (Model G 1314A) set at 254 nm wavelength, auto sampler (model G1329A VP-ODS) and Shim pack (150 × 4.6 mm) column (Shimadzu, Kyoto, Japan).

Results and Discussion

From the results reported in table (Table 1) and figures (Figure 1 and 2) it is obvious that the oxytetracycline residues were detected in 55%, 70%, 50%, 70%, 10% and 15% of the examined samples of local broilers meat, liver, gizzard, kidneys, imported broiler meat and kidneys with mean values of 471.4 ± 33.8, 773.9 ± 49.5, 11.6 ± 0.9, 2676.0 ± 117.5, 114.7 ± 8.3 and 546.7 ± 41.2, respectively. The current results come in accordance with those reported by Salehzadeh et al. [18], who detected mean concentrations of oxytetracycline (OTC) residues in chicken liver and kidney of 576.657 ± 201.908 and 517.56 ± 186.64 ug/kg, respectively. The obtained results are being higher than those obtained by Cetinkaya et al. [19] who determined levels of oxytetracycline residues in chicken tissues of 17.2 ug/kg. The current results are being lower than those recorded by Salama et al. [20] who reported a maximum concentration of oxytetracycline residues in fresh chicken breast, thigh and liver samples of 5812, 6010 and 8148 µg/kg, respectively.

Otherwise, the ampicillin residues were detected in 50%, 55%, 35%, 50%, 35% and 45% of the examined samples of local broiler meat, liver, gizzard, kidneys, imported broiler meat and kidneys with mean value of 329.2 ± 20.7, 853.3 ± 65.1, 164.5 ± 9.8, 531.6 ± 37.4, 177.9 ± 15.4 and 323.8 ± 26.6, respectively. The incidence of ampicillin residues in examined samples substantiated what have been recorded by Verdon et al. [21] who used HPLC method to determined ampicillin residues in kidney, liver and muscle tissues and the limit of detection was approximately 3-11 ug/kg and the limit of quantitation was evaluated down to 25ug/kg.

Acceptability of the examined samples of local and imported broiler meat and giblets based on their levels of oxytetracycline residues was shown in figure 3. Accurately, 65%, 50%, 50% and 100% of the examined samples of local broiler meat, liver, kidneys and gizzard were accepted, respectively. While all examined samples of imported meat and kidneys were accepted where they did not exceed the permissible limits. This according to MRL of oxytetracycline (200 ug/kg for muscle, 600 ug/kg for liver and gizzard and 1200 ug/kg for kidney) which stipulated by US code of federal regulations [22], FAO/WHO [23], Egyptian Organization of Standardization and Quality Control "EOSQC" No. 3692 [10] and Codex Alimentarius Commission [12]. Otherwise, according to MRL of oxytetracycline residues (100 ug/kg for muscle, 300 ug/kg for liver and gizzard and 600 ug/kg for kidney) which estimated by FAO/WHO (1998) [24] and European community (EC) [25], the accepted samples of local broiler meat, liver, kidneys and gizzard based on their levels of oxytetracycline residues were 45%, 45%, 40% and 100%. The accepted samples of imported broiler meat and kidneys based on their levels of oxytetracycline residues were 90% and 95%, respectively figure 3. Oxytetracycline (OTC) residues could be detected with different percentage in samples of chicken tissues by Al-Ghamdi et al. [26] who detected that 87% and 100% of poultry muscle and liver samples had OTC residues respectively.

Items	Local samples				Imported samples	
	Meat	Liver	Gizzard	Kidneys	Meat	Kidneys
OxyTC No. of +ve samples	55 (11%)	70 (14%)	50 (10%)	70 (14%)	2 (10%)	3 (15%)
Mean ± S.E*	471.4 ± 33.8	773.9 ± 49.5	11.6 ± 0.9	2676 ± 117.5	114.7 ± 8.3++	546.7 ± 41.2
Ampicillin No. of +ve samples	10 (50%)	11 (55%)	7 (35%)	10 (50%)	7 (35%)	9 (45%)
Mean ± S.E*	329.2 ± 20.7	853.3 ± 65.1	164.5 ± 9.8	531.6 ± 37.4	177.9 ± 15.4++	323.8 ± 26.6

Table 1: Statistical analytical results of Oxytetracycline and Ampicillin residues (Ug/Kg) in samples of local and imported frozen broiler meat and giblets (n=20).
S.E*-Standard error of mean ++-*t*-test indicated high significant differences (P<0.01)

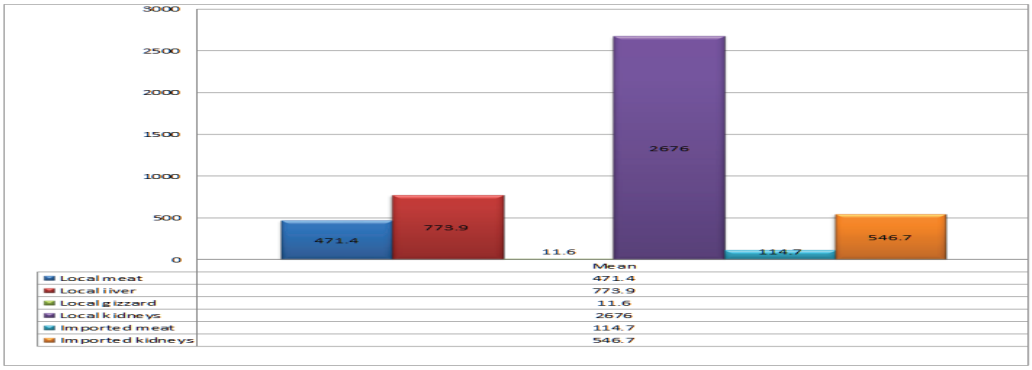

Figure 1: Mean values of oxytetracycline concentrations (ug/kg) in the samples of local and imported frozen broiler meat and Giblets.

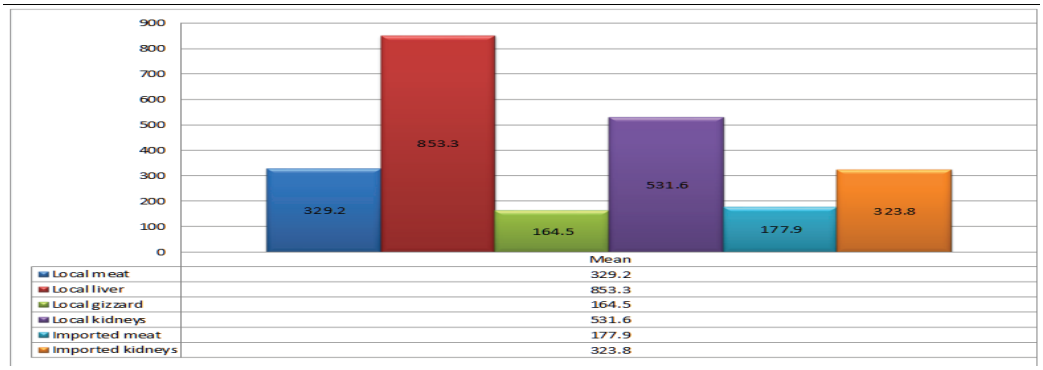

Figure 2: Mean values of ampicillin concentrations (ug/kg) in the samples of local and imported chicken meat and giblets.

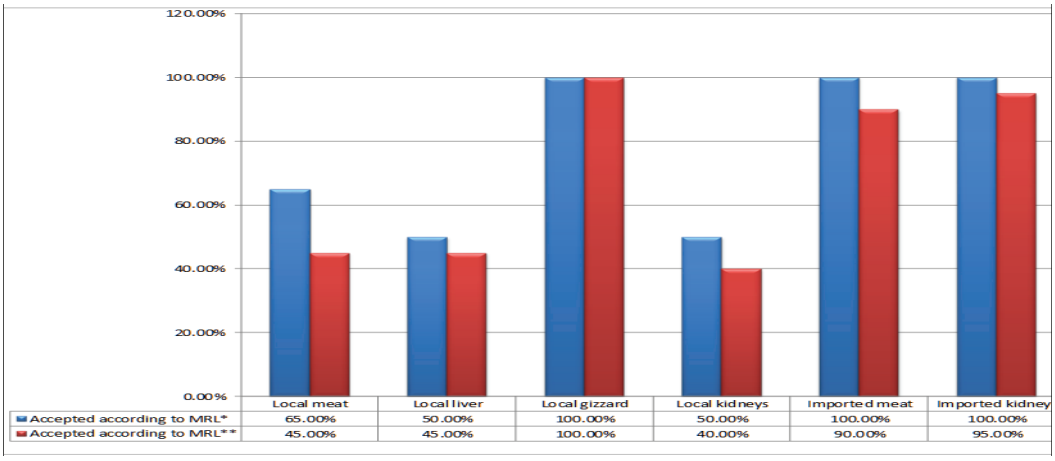

Figure 3: Acceptability of the examined samples of local and imported frozen broiler meat and giblets according to their contents of oxytetracycline residues. (*MRL according to US code of federal regulations, 2003, FAO/WHO, EOSQC, 2008 and Codex Alimentarius Commission, 2012 and **MRL according to EC, 2010)

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Acceptability of the examined samples of local broiler meat and giblets based on their levels of ampicillin residues was shown figure 4. Accurately, 50%, 45%, 70%, 50%, 70% and 55% of the examined samples of local broiler meat, liver, gizzard, kidneys, imported broiler meat and kidneys were accepted, respectively. This according to MRL of ampicillin (50 ug/kg for muscle, liver, gizzard and kidney) recorded by FAO/WHO (1998) [24], Egyptian Organization of Standardization and Quality Control "EOSQC" No. 3692 (2008) [10], European community (EC) (2010) [25] and Codex Alimentarius Commission (2012) [12]. Also, ampicillin residues could be detected with different percentage in tissue samples by Darwish et al. [27] at 18%.

The obtained results showed that the highest incidence of oxytetracycline and ampicillin residues was recorded in broiler liver. These results are consistent with those reported by Pavlov et al. [28] who mentioned that the highest residue concentrations were observed in liver and kidney and residue concentrations in skin fat, abdominal fat and muscle were very low.

Difference associated with the examined samples of local and imported broiler meat and giblets were highly significant ($P<0.01$) as a results of their contents of oxytetracycline and ampicillin residues as shown in table 2.

Results achieved in figure 5 declared the effect of different cooking methods (boiling, frying and grilling) on oxytetracycline (OTC) and ampicillin residues in the examined chicken meat samples. Before boiling, frying and grilling the concentration of OTC residues in chicken meat were with mean value of 446.16 ug/kg. While, the reduction % in the concentrations of OTC residues was with mean value of 84.52%, 93.62% and 96.58% after boiling, frying and grilling, respectively.

Otherwise, the concentrations of ampicillin residues in chicken meat were with mean value of 253.7 ug/kg before boiling, frying and grilling. While, the reduction % in the concentrations of ampicillin residues was with mean value of 81.22%, 90.54% and 94.5% after boiling, frying and grilling, respectively. From these results, it was concluded that cooking methods (boiling, frying and grilling) have positive effects on the oxytetracycline and ampicillin residues in total and partial degrading of these residues. Thus, application of heat treatment like boiling, roasting, frying and autoclaving may lead to destroying of all drug residues [29]. Therefore, use of proper cooking processes that have a higher temperature and longer time can lead to the most reduction in antibiotics residues in foodstuff and it can provide an additional margin of safety for consumers [30]. Thus, good cooking was used for removal of great part of the antibiotic residues such as oxytetracyclin and ampicillin [14].

Conclusions and Recommendations

The obtained results allow to conclude that the examined samples of local and frozen imported broilers giblets and tissues proved to be contaminated with residues of oxytetracycline and ampicillin especially in the liver samples followed by kidney, muscle and gizzard, thus may constitute health hazards in both animals and human. Furthermore, the application of different cooking methods (boiling, frying and grilling) have a positive effects on the residues of oxytetracycline and ampicillin in degrading the concentrations of residues in such examined samples.

Therefore, to improve the hygienic quality of poultry meat and to safeguard consumers from being adversely affected from the antibiotic residues in such meat the ante-mortem examination should be done in abattoirs to exclude the diseased poultry and suspected samples should be tested for residues by using easy, simple, sensitive and rapid method as HPLC test, active surveillance programs to monitor drug residues in food should be established and Heat treatment of meat should be done to inactivate antibiotic contaminants in feed stuffs.

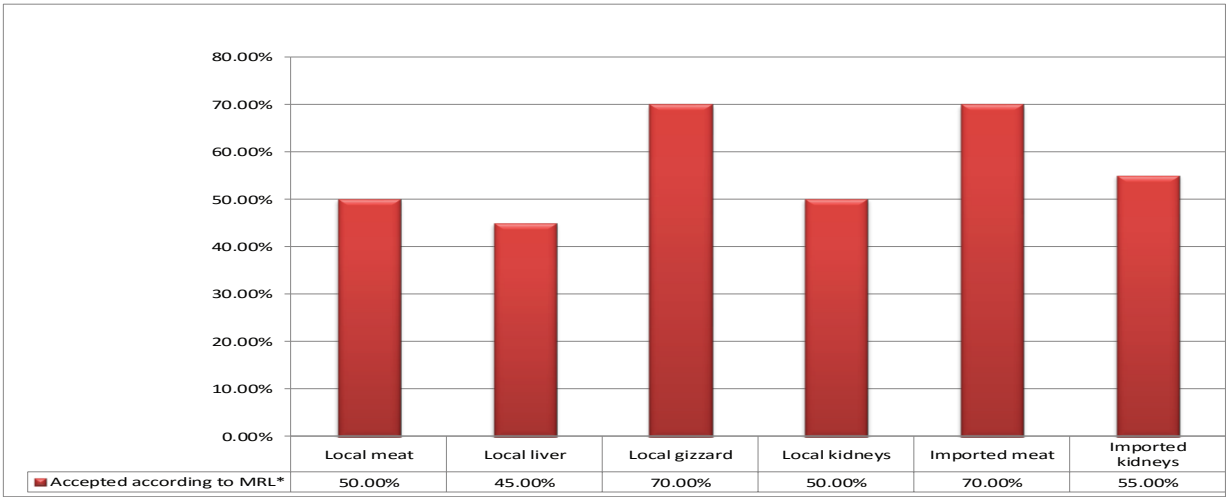

Figure 4: Acceptability of the examined samples of local and imported frozen broiler meat and giblets according to their contents of ampicillin residues. (*MRL according to US code of federal regulations, 2003, FAO/WHO, EOSQC, 2008, EC, 2010 and Codex Alimentarius Commission, 2012)

Source of Variance	Oxytetracycline				Ampicillin			
	D.F	S.S	M.S	F. value	D.F	S.S	M.S	F. value
Total	79	149.803			79	68.439		
Between Tissues (T)	3	75.934	25.311	26.04 **	3	18.132	6.044	9.13 **
Error	76	73.869	0.972		76	50.307	0.662	

Table 2: Analysis of Variance (ANOVA) oxytetracycline and ampicillin residues in the samples of local broiler meat and giblets.
++-*t*-test indicated high significant differences ($P<0.01$)

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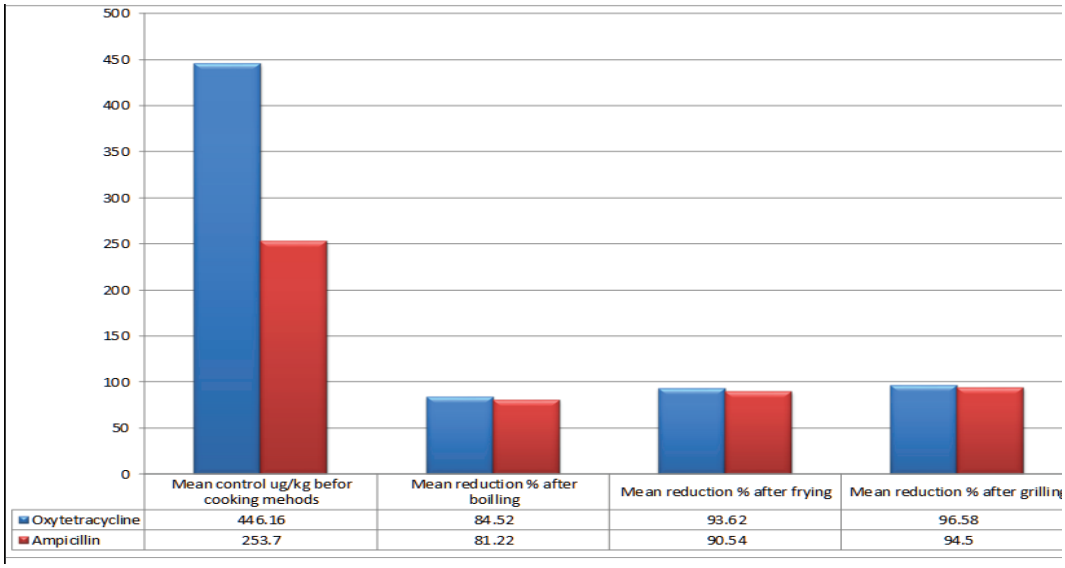

Figure 5: The effect of different cooking methods (boiling, frying and grilling) on oxytetracycline and ampicillin residues in chicken meat (Reduction %).

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