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Hazards Analysis And Control Points In Chicken Meat

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Abstract:
Chicken is a fundamental source of animal protein in the human diet; meanwhile, it is a rich food matrix that promotes the growth of microorganisms, including pathogen bacteria. This present study aimed to determine some microbiological and chemical hazards in chicken meat. In addition, the impact of some control measures on reducing these hazards was evaluated. The results showed that all samples were positive for pathogenic bacteria, with counts ranging from 5.39 to 6.68, 3.26 to 4.21, 6.07 to 7.30, and 6.0 to 7.46 CFU/g of staph. aureus, salmonella spp., E. coli, and psychrophilic bacteria, respectively. Lactic acid has a bactericidal impact on pathogenic microorganisms. The data also reflects that all raw chicken samples contained residues of norfloxacin antibiotic ranging from 1.20 to 8.10 µg/kg and significant differences between samples. At the same time, oxytetracycline was detected in 22.2% only of tested samples. All samples contained residues of estradiol and estrogen residues. The boiling, grilling, and freezing treatments reduced antibiotic and hormone residues. Regarding heavy metals, all samples of chicken liver and muscle tissues did not contain Cadmium, while they contained variable amounts of Lead, Copper, and Zink. However, the levels of chemical food safety hazards did not exceed the safety limit.

Key Words: *pathogenic bacteria, lactic acid, oxytetracycline, norfloxacin, estradiol, estrogen*

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I. Introduction

Chicken and chicken products are primary animal protein sources in the human diet. In addition to the low cholesterol level, compared with beef meat, poultry meat also contains the required essential amino acids, some vitamins (such as vitamin B12), and some minerals such as iron, sodium, calcium, phosphorus, and iodine. Poultry meat is known to have a significant amount of bacteria that are harmful to humans and is an excellent substrate for the growth of bacteria. These usually arise in areas with poor sanitation, and they only endanger food handlers and consumers if the product is not handled carefully. **(Mohammed, 2020)**

Bacterial infections caused by poultry processing equipment and surrounding areas may be found in slaughterhouse settings and could contaminate chicken meat. The causes of contamination can include the tissue itself, the surrounding air, and surfaces that are not cleaned before and after slaughtering birds and preparing. **(Tagar, & Qambrani , 2023)**

Salmonella, Esch. coli O157:H7, Staph. aureus and *List. monocytogenes* are the pathogenic bacteria most frequently linked to foodborne illnesses worldwide. Poultry, ground beef, fish, fruits, and vegetables are the food items outbreaks. **(Hashemi *et al.*, 2024)**

The importance of *Salmonella* species as food-borne bacterial diseases is growing worldwide, causing major global public health concerns. The cost of disease prevention, treatment, and surveillance stresses the economic burden of both developed and developing nations. Salmonellosis is a global issue that significantly reduces productivity and health. It is widespread throughout most nations, causing significant financial damage. Also, one of the essential sources of food-borne disease is thought to be *Staphylococcus* species. Entero-toxic strains of the bacterium are frequently found contaminated in meats, dairy products, and other foods. Moreover, there are now more *S. aureus* strains with antibiotic-resistance characteristics**. (Baruah *et al.*, 2023)**

Antibiotic resistance and toxicological consequences are the two main health risks associated with the residual of antibiotics in food. The progress of resistant strains and failure of antibiotic therapy exposure to antibiotics in food. Resistance to antibiotics has increased to hazardous rates globally because of the emergence and spread of multidrug-resistant bacteria, or "superbugs." **(Zhixin, *et al.*, 2023)**

Hormones, such as estradiol, progesterone, testosterone, zeranol, trenbolone, and melengestrol, are growth promoters used to increase the meat production rate on animal and poultry farms. They are added to feed or implanted growth promoters to increase milk or meat production. While all hormones or hormone-like substances are prohibited from being used as growth promoters by the European Economic Community (EEC),

the United States Food and Drug Administration (FDA) has approved the restricted use of certain hormones derived from natural sources for fattening animals. **(Kamaly & Sharkawy, 2023)**

Poultry feed may contain a variety of toxic metals. Heavy metal poisoning can cause weight loss, organ failure, and even death in chickens. Exposure route, duration, and absorbed dosage—acute or chronic—all influence the toxicity of metals **(Aljohani, 2023)**.

This work aimed to investigate *Staphylococcus aureus, Salmonella, E. coli*, and *Psychrophilic*. In addition, chemical hazards, including antibiotic residues such as oxytetracycline and norfloxacin and hormone residues such as estradiol and estrogen, were determined in chicken meat. The impact of dipping in lactic acid (2%), boiling, grilling, and freezing for 3 months on reducing these hazards was evaluated.

II. Materials And Methods

1. Materials: -

Nine samples of broiler chicken (approximately 2 kg for each) were collected from local markets in 3 different zones from The Ismailia Government. The chickens were slaughtered, allowed to bleed for 5 min, scaled at 60ºC for 3 min, defeathered manually, eviscerated, washed with tap water, and placed for 15 min on a metal grid. Both chicken meat and chicken liver were stored at 4ºC ±1 in foam dishes and examined for microbiological and chemical analysis. Also, chicken meat was cooked by boiling at 100ºC for 10 minutes, grilled chicken meat for 10 minutes, refrigerated chicken meat at 4ºC ±1 for 5 days, frozen chicken meat at -18ºC ±1 for 3 months, and dipping in lactic acid (2%) for 10 min before analysis.

2. Chemicals:

Nutrient agar was purchased from LabM, UK, Baird Parker agar purchased from LabM, UK, Brilliant green agar purchased from LabM, UK and MacConkey agar purchased from LabM, UK. Oxytetracycline was purchased from Sigma-Aldrich and Norfloxacin was purchased from Sigma-Aldrich. Other chemicals from Alpha Chemika.

3. Microbiological Analysis:

3.1. Preparation and dilution of homogenate:

A sterile conical flask holding 90 ml of peptone water was filled with ten grams of the mixed sample. The suspension was prepared for a 1:10 dilution, and the flask was well-sheathed. To make a 1:10 dilution, one milliliter from the $1:10^1$ dilution was added to nine milliliters of sterile peptone water, and dilution so on up 10^5.

3.2. Analysis:

The microbiological analyses were carried out according to procedures discribed by **Da Silva *et al.* (2018).** For *Psychrophilic* bacteria: one milliliter (ml) was taken from each of the previously indicated dilutions (in duplicate). The total plate count of agar media was put over the plates. After hardening and incubated for 8 days at 7°C ±1, the results are expressed as log CFU for each gram of chicken meat. Detection of *Staphylococcus aureus* 0.1 ml from each of the previously prepared serial dilutions was spread over a duplicate plate of Baird Parker agar using a sterile rod glass spreader. The inoculated plates were incubated at 37°C for 48 h., *Salmonella* spp. Pre-enrichment: 25 gram of the sample were weighed out and homogenized in 225 ml of buffered peptone water in a conical flask to give a dilution of 1:10 flasks were closed properly, labelled, and incubated overnight (18–20 h) at 37°C. Selective enrichment: Rappaport-Vassiliadis soya peptone (RVS) was prepared. 10 ml were poured into test tubes. 0.1 ml of the pre-enrichment was taken using a sterile pipette and transferred into the test-tube containing the Rappaport-Vassiliadis soya peptone broth. Test tubes were marked, capped with corks, and incubated for 18 to 24 h at 41.5°C ± 0.5°C. Selective agar plating: A 10 µl wire loop was used to pick a loop volume from the RVS broth and inoculated into prepared and solidified Brilliant green agar (BGA). Plates were spread out, labelled and incubated at 37°C overnight (18–24 h). *E. coli* 0.1 ml of dilutions for every sample, it was respectively plated on McConkey agar (MCA) and spread out using a glass rod spreader. Plates were labelled and incubated overnight (18–24 hours) at 37°C.

4. Chemical Analysis:

4.1. Antibiotics residues:

Standard calibration curve was made using Oxytetracycline and Norfloxacin concentrations of 0.5 to 5.0 mg/L in eluent and spiked samples with the same concentration. The daily-prepared stock solution was used to prepare these standards and spikes, which were then handled accordingly.

4.1.1. Determination of oxytetracycline residues

HPLC analysis was carried out according to **Fathy *et al.*, (2015).** 2 grams of minced chicken meat and mixed for 2 minutes, with 0.1 grams of citric acid, 1 ml of 30% nitric acid, 4 ml of methanol 70%, and 1 ml of deionized water were added into a polypropylene centrifuge tube (50 ml). Then, vortexed for thorough mixing,

centrifuge for 10 minutes at 5300 rpm after ultra-sonicated for 15 minutes. 25 µl of sample was filtered using 0.45 µm nylon filtered before injection to HPLC (Ultimate 3000 HPLC system, Thermo scientific, Germany) using column C18 (150 × 4.6) mm, particle size 5µm. the detection was performed at wavelength 270 nm using DAD detector. The mobile phase (85% acetonitrile, and distilled water 15%) was pumped at a flow rate of 1.5 ml/min, results of oxytetracycline residues were calculated using the equation as presented in **Fig, 1** as µg/kg (wet weight)

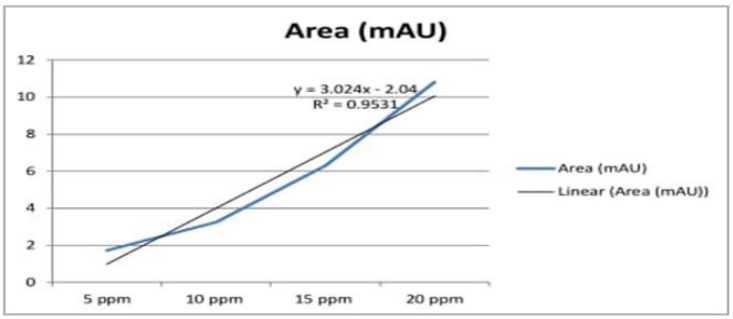

Figure (1): Calibration standard curve of Oxytetracycline

4.1.2. Determination of norfloxacin residues

HPLC analysis was carried out according to **kowalski *et al.*, (2005).** 2 grams of minced chicken meat were weighed into a 50 ml polypropylene centrifuge tube and homogenized for 2 minutes. Centrifuge for 5 minutes at 14000 rpm after adding 8 ml of TCA (5%) and vortexing and filtration using a nylon filter (A 0.45 µm). Norfloxacin determination using HPLC HPLC (Ultimate 3000 HPLC system, Thermo scientific, Germany) using column C18 (150 × 4.6) mm, particle size 5µm. the detection was performed at wavelength 270 nm using DAD detector. Sulphate is an ion-pairing agent and was composed of water/methanol (65:35, v/v, pH 3 adjusted with H_3PO_4), and the results calculation using the equation as follows in **Fig,2** by µg/kg (wet weight)

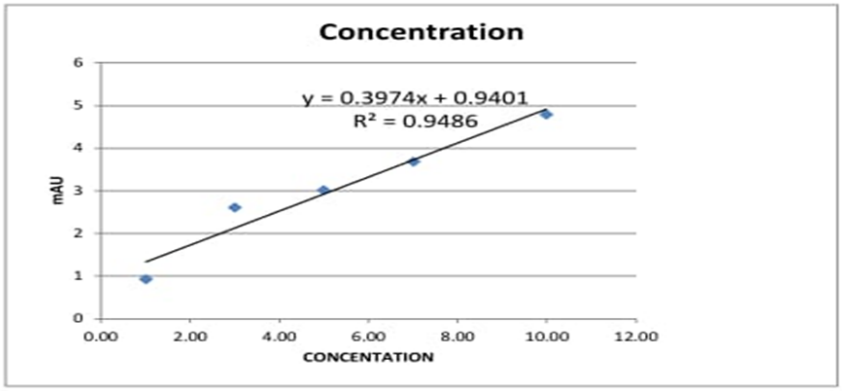

Figure (2): Calibration standard curve of Norfloxacin

4.2. Hormone residues

4.2.1. Sample preparation

Before homogenizing the tissues, weigh them and thoroughly rinse them in ice-cold PBS (pH 7.4) to eliminate any excess blood. Using a homogenizer, mince the tissues and homogenize them in PBS (tissue weight (g): PBS (mL) volume 1:9). Following that, the homogenates are centrifuged at 12,000 RPM for 15 minutes.

4.2.2. Determination of estradiol hormone residues

The Estradiol ELISA test kit from **Monocent Inc. Company (2019) 9025 Eton Ave. Ste C, Canoga Park, CA 91304, USA** explains how to do it. Before using, bring all reagents to a temperature of 20 to 25 °C. Insert the required number of coated wells into the holder. Once the appropriate wells have been filled, add 25µl of standards, specimens, and controls. Next, add 50µl of the Estradiol Biotin Reagent working solution to each well. Shake well and permit it to sit for 10 to 20 seconds. After 45 minutes of incubation at 20 to 25°C, add 100µl of Estradiol Enzyme Reagent to each well. (Note: Put immediately over the top of the Biotin.) Shake vigorously for 10 to 20 seconds. Next After 45 minutes of incubation at 20–25°C, remove the liquid from each well. Use 300 µl of 1X wash buffer three times to wash the wells. After blotting on paper towel or absorbance paper, pour 100µl of TMB Reagent into each well. After 20 minutes of incubation at 20 to 25°C, stop the reaction by adding 50 µl of Stop Solution to each well and gently mixing for 30 seconds. Ensuring that the entire blue color changes to yellow is critical. In 15 minutes, use a microplate reader to read at 450 nm.

4.2.3. Determination of estrogen hormone residues

Estrogen hormone residues were determined using ELISA technique from **Bioassay Technology Lab (2019) 501 Changsheng S Rd, Nanhu Dist, Jiaxing, Zhejiang, China.** As prescribed, prepare all the reagents, standard solutions, and samples. Before using it bring all of the reagents to room temperature. It is recommended to conduct the assay at room temperature. Following that, establish how many strips are needed for the assay. For use, place the strips inside the frames. The unused strips should be set in a blank well with no solution and kept at 4°C for a maximum of one month. For each negative control well, add 50 ul of negative control, and for each positive control well, 50 ul of positive control. Mix thoroughly after adding 10 ul of the sample and 40 ul of the sample diluent. After removing the sealant, use the wash buffer to wash the plate five times. For each wash, soak thoroughly in 300ul of wash buffer for 30 to 60 seconds. Aspirate every well and use the wash buffer to wash five times for automated washing. Using paper towels or another absorbent material, blot the plate. Each well (except from the blank well) should get 50ul of HRP. After covering with a plate sealer, incubate at 37°C for 30 minutes. After that, wash as described previously after removing the sealer. Fill each well with 50 ul of substrate solution A, followed by 50 ul of substrate solution B. Stir thoroughly. When you add 50ul of stop solution to each well of a plate sealed with a fresh sealer and incubate it for 10 minutes at 37°C in the dark, the blue hue will instantly turn yellow. Within 15 minutes of applying the stop solution, use a microplate reader set to 450 nm to determine the optical density (OD value) of each well.

4.3. Determination of heavy metals

The determination of lead (Pb), copper (Cu), zinc (Zn), and cadmium (Cd) was carried out according to the method described by **Elsharawy & Elsharawy (2015)** using an atomic absorption spectrophotometer (Thermo-electron, S Series, S4 AA system, S. No. GE 711838, Thermo-electron Corp.).

5. Statistical analysis: Each experiment was analyzed with triplicated samples. Significant differences between the mean values of the estimated tests and the standard deviation (SD) were determined using SPSS statistic 22 for Windows. Differences were considered significant at $P < 0.05$.

III. Results And Discussion

1. Microbiological analysis:

Chicken meat and Products may carry a variety of pathogens, which can arise during preparation and processing as well as during additional processing that occurs through packaging, marketing, and storage. These food items could become unsafe to consumers or unsuitable for human consumption as a result of contamination **(Shaltout *et al.*, 2019).**

Staph. aureus is one of the dangerous types of bacteria that can be found in chicken meat. Results presented **Table (1) and Figure (3)**, showed that all chicken meat samples were positive for *Staph. aureus.* Whereas, sample A had range in zero time of 5.80 increased to 7.34 log CFU/g with an increasing ratio of 26.6% during five days at refrigerated temperature, while the count in sample B at zero time was 6.02 and increased to 6.96 after 5 days of cold storage at 4ºC ±1 by ratio 15.6%, Sample C at zero time was 6.68 then increased to 7.41 log CFU/g by ratio 10.9%, The data also showed that the counts were 6.49, 5.39, 6.40, 6.62, 5.83, and 6.11 log CFU/g at zero time for samples D, E, F, G, H and I, respectively, while these counts increased to 7.39, 7.59, 7.64, 6.62, 6.93, 7.06 and 7.36 log CFU/g after storage at cold storage at 4º C ±1 for 5 days by increasing ratio 13.9%, 40.8%, 19.4%, 4.7%, 21.1% and 20.5% respectively. From the same table, it can be noticed all determined microbiological samples were different significantly ($P \leq 0.05$).

Staphylococcus aureus counts obtained from present study were higher than those of the data reported by **Elmelegy *et al.* (2015)** who found that *Staph. aureus* were isolated from chicken was 13.33% of the examined chicken. Also, **EL-Gammal & Yassin (2016),** found that 20% of the chicken samples examined were positive for *Staph. aureus* spp. On the other hand, the obtained results agree with **Al-Jasser (2012)** who found that *Staph. aureus* increased during cold storage after 3 days at 4 ±1°C.

One of the most harmful bacteria frequently found in chicken meat is *Salmonella* spp. It is particularly prevalent in poultry meat and can be transmitted by handling raw poultry carcasses and products or by eating undercooked poultry meat **(Adeyanju& Ishola , 2014).** The data tabulated in **Table (1) and Figure (4)** reflects that samples had a count of *Salmonella* spp. in zero time 3.26, 4.11, 4.06 log CFU/g for samples A, B, and C and increased after 5 days of cold storage by increasing ratio 17.2%, 2% and 0.2% respectively. The same trend was observed for another sample with different significantly ($P \leq 0.05$). *Salmonella* was isolated from chicken meat was 100% of the examined chicken meat samples.

Results of *Salmonella* spp. obtained from the present study are higher than those of data recorded by **Akermi *et al.* (2020)** who reported that *Salmonella* was isolated from 31.42% of the chicken samples examined. Also, **EL-Gammal & Yassin (2016),** found that 18% of the chicken samples examined were positive for

Salmonella spp. The results of *Salmonella* obtained were lower than the results recorded by **Tagar & Qambrani, (2023)** mentioned that *Salmonella* was present by 97.3% in chicken meat samples.

Table (1): *Staphylococcus aureus* and *Salmonella* spp. count (mean ± SD) of chicken meat stored at 4ºC±1:

Chicken Samples	*Staphylococcus aureus* (Log CFU/g)		Increasing Ratio%	*Salmonella spp.* (Log CFU/g)		Increasing Ratio%
	0 day	5 days		0 day	5 days	
A	$5.80^{cd} \pm 0.199$	$7.34^{b} \pm 0.037$	26.6%	$3.26^{d} \pm 0.259$	$3.82^{d} \pm 0.011$	`17.2%
B	$6.02^{c} \pm 0.062$	$6.96^{cd} \pm 0.010$	15.6%	$4.03^{b} \pm 0.055$	$4.11^{bc} \pm 0.104$	2%
C	$6.68^{a} \pm 0.059$	$7.41^{b} \pm 0.018$	10.9%	$4.06^{b} \pm 0.051$	$4.07^{c} \pm 0.013$	0.2%
D	$6.49^{b} \pm 0.028$	$7.39^{b} \pm 0.035$	13.9%	$4.21^{a} \pm 0.031$	$4.27^{a} \pm 0.032$	1.4%
E	$5.39^{d} \pm 0.088$	$7.59^{a} \pm 0.010$	40.8%	$4.11^{b} \pm 0.029$	$4.18^{b} \pm 0.122$	1.7%
F	$6.40^{b} \pm 0.060$	$7.64^{a} \pm 0.009$	19.4%	$3.94^{c} \pm 0.008$	$4.05^{c} \pm 0.042$	2.8%
G	$6.62^{a} \pm 0.026$	$6.93^{d} \pm 0.018$	4.7%	$4.07^{b} \pm 0.054$	$4.11^{bc} \pm 0.113$	1%
H	$5.83^{cd} \pm 0.128$	$7.06^{c} \pm 0.030$	21.1%	$3.93^{c} \pm 0.029$	$4.05^{c} \pm 0.033$	3.1%
I	$6.11^{c} \pm 0.034$	$7.36^{b} \pm 0.009$	20.5%	$4.10^{b} \pm 0.024$	$4.13^{b} \pm 0.029$	0.7%

Means of the same columns with same superscripts are not significantly different at (P≤ 0.05)
Samples from A to I fresh samples were collected from the different local market.

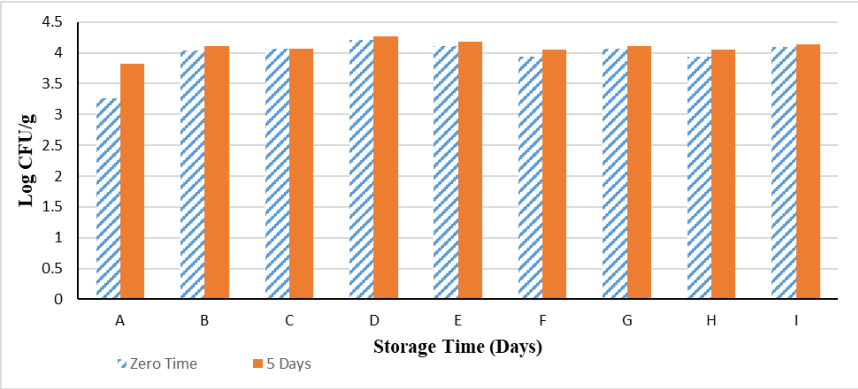

Figure (3): Load of *Staphylococcus aureus* counts (Log CFU/g) in chicken meat stored at 4º C±1 for 5 days

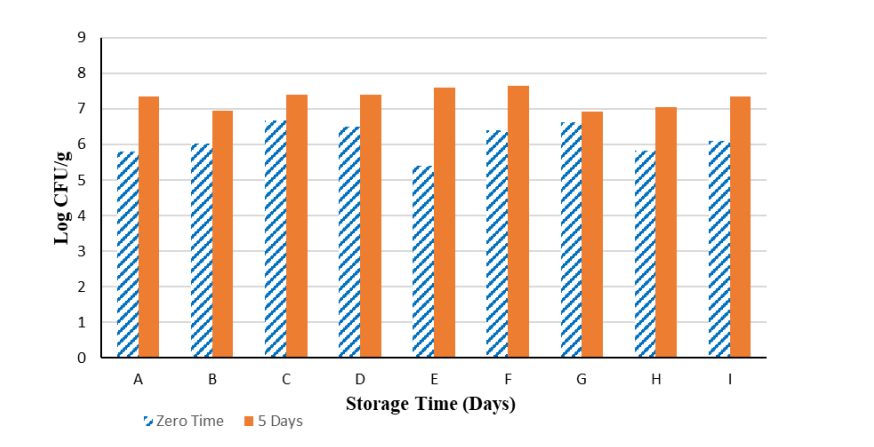

Figure (4): Load Of *Salmonella* sp. counts (Log CFU/g) in chicken meat stored at 4º C±1 for 5 days

The presence of *E. coli* in chickens is a sign of poor sterile practices in sanitation and hygiene practices. *E. coli* (log CFU/g) The counts in **Table (2) and Figure (5)**, show that the mean counts of *E.coli* were 7.00 log CFU/g while increased to 7.13 after 5 days of cold storage with an increasing ratio of 5.9%, 2.5%, 1.1%, 0.6%, 0.3%, 0.4%, 1.3%, 1.4%, and 3.5% for samples A, B, C, D, E, F, G, H, and I respectively. From the same (**Table)**, it can be noticed that all determined samples were different significantly ($P \leq 0.05$).

However the microbial load of *E. coli* obtained in these present study are lower than of that reported by **Tagar & Qambrani (2023)** mentioned that *E. coli* was present by 97.3% in chicken meat samples. Also **EL-**

Gammal & Yassin (2016), found that 12% of the chicken samples examined were positive for *E. coli*. **Al-Jasser (2012)** found that the coliform group increased during cold storage after 3 days at 4±1°C.

Psychrophilic bacteria are **Table (2) and figure (6)**, show that sample (A) had a count in zero time 7.00 then increased to 7.44 log CFU/g after five days of storage at 4ºC ±1 with an increasing ratio 24%. From the same Table, it can be noticed that all other samples had the same phenomena at zero time and after cold storage.

Results of *Psychrophilic* bacteria that obtained higher than those of the data recorded by **Sheir *et al.* (2020)** reported that the *psychrotrophic* count in examined samples of chicken meat ranged from 2.9×10^3 to 3.1×10^4 CFU/g. While the results obtained by **Morshedy *et al.* (2023)** showed that were 36.67% of the examined chicken product samples contaminated by *Pseudomonas* spp. that is lower than the obtained results. Also, **Mashishi *et al.* (2019)** reported that *Psychrophilic* bacteria isolated from chicken meat range from 3.60 to 4.13 CFU/g.

Table (2): *E. coli* and *Psychrophilic bacteria* counts (mean ± SD) of chicken meat stored at 4ºC±1:

Sample	*E. coli* (Log CFU/g)		Increasing Ratio%	*Psychrophilic* bacteria (Log CFU/g)		Increasing Ratio%
	0 day	5 days		0 day	5 days	
A	$6.07^{d} \pm 0.111$	$6.43^{d} \pm 0.032$	5.9%	$6.00^{g} \pm 0.151$	$7.44^{b} \pm 0.010$	`24%
B	$7.11^{b} \pm 0.022$	$7.29^{ab} \pm 0.016$	2.5%	$6.55^{f} \pm 0.073$	$7.56^{a} \pm 0.018$	15.4%
C	$6.99^{c} \pm 0.023$	$7.07^{c} \pm 0.056$	1.1%	$7.27^{bc} \pm 0.023$	$7.29^{c} \pm 0.014$	0.3%
D	$7.03^{c} \pm 0.011$	$7.07^{c} \pm 0.040$	0.6%	$7.14^{d} \pm 0.004$	$7.31^{c} \pm 0.010$	2.4%
E	$7.02^{c} \pm 0.034$	$7.04^{c} \pm 0.033$	0.3%	$6.85^{e} \pm 0.013$	$7.60^{a} \pm 0.007$	10.9%
F	$7.30^{a} \pm 0.023$	$7.33^{a} \pm 0.033$	0.4%	$7.31^{b} \pm 0.013$	$7.49^{b} \pm 0.022$	2.5%
G	$7.18^{ab} \pm 0.020$	$7.27^{ab} \pm 0.012$	1.3%	$7.37^{b} \pm 0.028$	$7.58^{a} \pm 0.018$	2.9%
H	$7.24^{a} \pm 0.022$	$7.34^{a} \pm 0.020$	1.4%	$7.46^{a} \pm 0.014$	$7.57^{a} \pm 0.010$	1.5%
I	$7.07^{c} \pm 0.035$	$7.32^{a} \pm 0.028$	3.5%	$7.33^{b} \pm 0.010$	$7.34^{c} \pm 0.024$	0.1%

Mean in the same columns with different superscripts are significantly different at (P≤0.05)

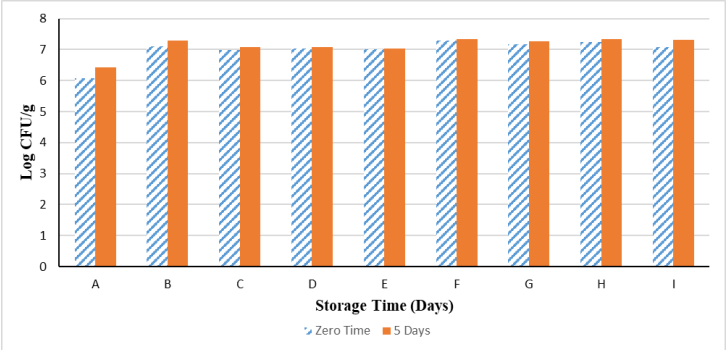

Figure (5): Load of *E. coli* counts (Log CFU/g) in chicken meat stored at 4º C±1 for 5 days

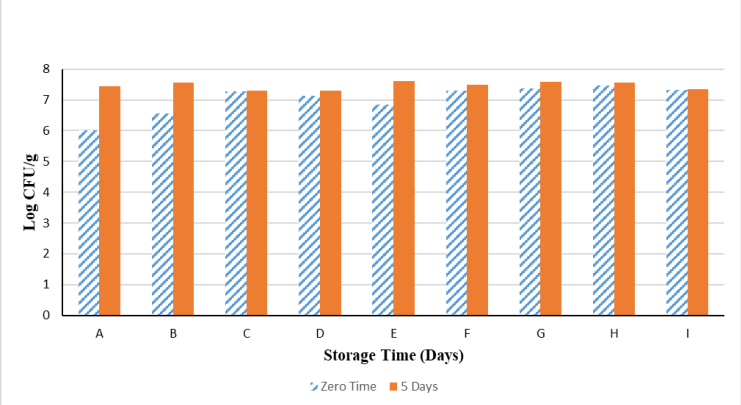

Figure (6): Load of *Psychrophilic* bacteria count (Log CFU/g) in chicken meat stored at 4º C±1 for 5 days

2. Reducing of microbial load by dipping in lactic acid (2%)

According to **Ramirez-Hernandez *et al.* (2018)**, lactic acid acts through cytoplasmic membrane penetration, intracellular pH reduction, and outer membrane disruption of bacteria. **Table (3) and Figure (7)** show that *Staphylococcus aureus* was not found at one day and after 5 days of cold storage in sample (A), while the counts in samples B and C at zero time were 5.15 and 5.69 CFU/g respectively and decreased to 0.00 after 5 days of cold storage with a decreasing ratio 100%. The data also showed that the counts were 3.33, 6.09, 5.97, 6.14, 6.46, and 0.00 CFU/g at zero time for samples D, E, F, G, H, and I. Respectively, while counts decreased to 0.00 CFU/g for 5 days by decreasing ratio 100% for all treated samples. From the same table it can be noticed that all determined samples were different significantly ($P \leq 0.05$). Load of *Staph. aureus* obtained achieved after dipping in lactic acid 2% agree with the results recorded by **(Abu-Ghazaleh, 2013)** and **(Rosengren *et al.*, 2013).**

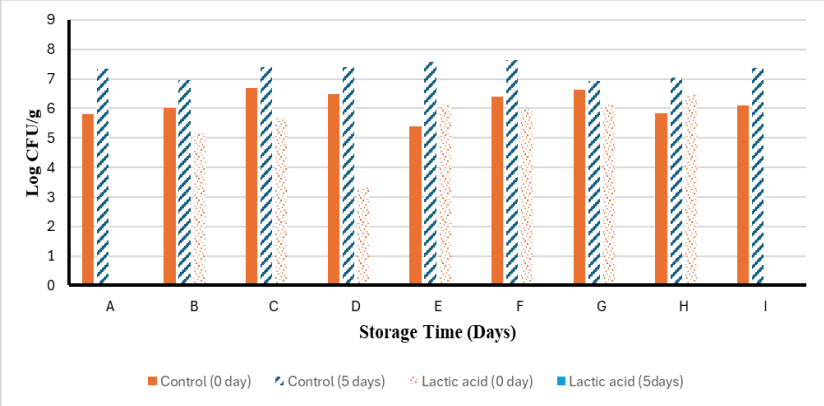

Figure (7): Effect of dipping in lactic acid (2%) on *Staphylococcus aureus in chicken meat* stored at 4º C±1 for 5 days

Similar to results obtained regarding *Staph. aureus*, dipping in lactic acid (2%) exhibited bactericidal effect on *Salmonella*. Results presented in **Table (3) and Figure (8)** showed that *Salmonella spp.* counts (log CFU/g) after dipping chicken meat in lactic acid (2%) decreased in some samples to 0.00 CFU/g after 5 days. Sample A had count in zero time 3.07 decreased to 3.00 CFU/g after 5 days of cold storage and dipping lactic acid, while the count in sample B at zero time was 3.84 CFU/g and decreased to 3.22CFU/g after 5 days of cold storage and dipping lactic acid. The same phenomenon was observed for the other samples according to the data tabulated in **Table (3)**. From the same Table, it can be noticed all determined microbiological samples were significantly different ($P \leq 0.05$). **Ramirez-Hernandez *et al.*, (2018)** reported that lactic acid and buffered lactic acid treatments produced the greatest reductions in Salmonella counts**.** Also, **(El-Khawas *et al.*, 2020),** found that *Salmonella* spp. reduced the count by about 1.5 log CFU/g treatment with lactic acid 1%.

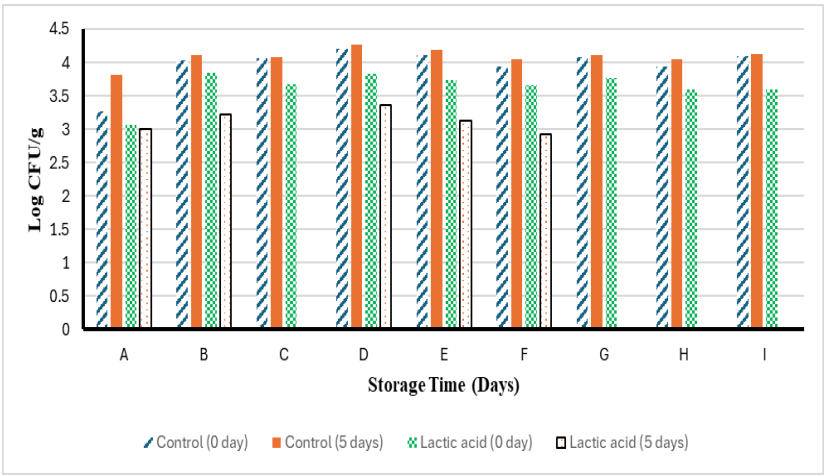

Figure (8): *Salmonella* spp. counts of chicken samples treated by dipping in lactic acid (2%) stored at 4ºC±1 for 5 days

Table (3): *Staphylococcus* aureus and *Salmonella* spp. Counts (log CFU/g) of treated chicken samples during cold storage at 4°C ±1 (mean ± S.D):

Chicken Samples	Treatments							
	Control		Lactic acid 2%		Control		Lactic acid 2%	
	Staph. aureus		*Staph. aureus*		*Salmonella* spp.		*Salmonella* spp.	
	(0)	(5)	(0)	(5)	(0)	(5)	(0)	(5)
	Days		Days		Days		Days	
A	$5.80^{d} \pm 0.199$	$7.34^{b} \pm 0.037$	$0.00^{f} \pm 0.0$	0.00 ± 0.0	$3.26^{e} \pm 0.259$	$3.82^{d} \pm 0.011$	$3.07^{d} \pm 0.017$	$3.00^{d} \pm 0.044$
B	$6.02^{cd} \pm 0.062$	$6.96^{d} \pm 0.010$	$5.15^{e} \pm 0.151$	0.00 ± 0.0	$4.03^{c} \pm 0.055$	$4.11^{bc} \pm 0.104$	$3.84^{a} \pm 0.056$	$3.22^{b} \pm 0.141$
C	$6.68^{a} \pm 0.059$	$7.41^{b} \pm 0.018$	$5.69^{d} \pm 0.088$	0.00 ± 0.0	$4.06^{bc} \pm 0.051$	$4.07^{c} \pm 0.013$	$3.67^{bc} \pm 0.014$	$0.00^{f} \pm 0.0$
D	$6.49^{b} \pm 0.028$	$7.39^{b} \pm 0.035$	$3.33^{d} \pm 1.443$	0.00 ± 0.0	$4.21^{a} \pm 0.031$	$4.27^{a} \pm 0.032$	$3.83^{a} \pm 0.029$	$3.36^{a} \pm 0.038$
E	$5.39^{e} \pm 0.088$	$7.59^{a} \pm 0.010$	$6.09^{b} \pm 0.088$	0.00 ± 0.0	$4.11^{b} \pm 0.029$	$4.18^{bc} \pm 0.122$	$3.74^{ab} \pm 0.059$	$3.13^{c} \pm 0.095$
F	$6.40^{b} \pm 0.060$	$7.64^{a} \pm 0.009$	$5.97^{bc} \pm 0.023$	0.00 ± 0.0	$3.94^{d} \pm 0.008$	$4.05^{c} \pm 0.042$	$3.66^{bc} \pm 0.065$	$2.93^{a} \pm 0.026$
G	$6.62^{a} \pm 0.026$	$6.93^{d} \pm 0.018$	$6.14^{b} \pm 0.031$	0.00 ± 0.0	$4.07^{bc} \pm 0.054$	$4.11^{bc} \pm 0.113$	$3.77^{ab} \pm 0.064$	$0.00^{f} \pm 0.0$
H	$5.83^{d} \pm 0.128$	$7.06^{c} \pm 0.030$	$6.46^{a} \pm 0.016$	0.00 ± 0.0	$3.93^{d} \pm 0.029$	$4.05^{c} \pm 0.033$	$3.59^{c} \pm 0.099$	$0.00^{f} \pm 0.0$
I	$6.11^{c} \pm 0.034$	$7.36^{b} \pm 0.009$	$0.00^{f} \pm 0.0$	0.00 ± 0.0	$4.10^{b} \pm 0.024$	$4.13^{bc} \pm 0.029$	$3.59^{c} \pm 0.115$	$0.00^{f} \pm 0.0$

Mean in the same columns with different superscripts are significantly different.

Data also shows that the count of *E. coli* in chicken meat samples was more sensitive to lactic acid (2%) than *Staph. aureus* and *Salmonella*. Whereas *E. coli* was not detected in all treated samples either before storage or after storage at 4°C±1 for 5 days.

These results agreed with those reported by **El-Khawas *et al.* (2020)** and **Fang *et al.* (2022)** who found that the count of E. *Coli* decreased by treating with lactic acid. Also, **Abu-Ghazaleh (2013)** reported that lactic acid (0.03% or 0.1%) alone inhibited the growth of *E. coli.*

3. Impact of treatments on antibiotic residues

A first-generation synthetic antibacterial drug called norfloxacin is used to treat both simple and complex urinary tract infections. Additional uses include ocular preparations for the management of conjunctival infections and prostatitis caused by *E. coli* **(Meena *et al.*, 2020)**.

Results of norfloxacin residues in chicken meat were detected in all samples **(data not tabulated)** where sample (A) was 7.2 µg/kg after boiling decreased to 0.88 µg/kg, while not detected after grilling, and dipping in lactic acid (2%). The result shows that norfloxacin was detected in raw sample B (5.02 µg/kg) and was not detected after boiling, grilling, and dipping in lactic acid. After 3 months of freezing storage norfloxacin was detected in sample A after the first month at level 0.06 µg/kg then not detected. However, sample B does not detect norfloxacin residues within the three months. Sample C was 1.99 µg/kg and was not detected after boiling, grilling, and dipping in lactic acid. Norfloxacin residues not detected in B, C and D samples within the three months except sample D showed residues of norfloxacin 3.02 µg/kg at zero time and not detected after boiling, grilling and dipping in lactic acid. Sample E had norfloxacin residues 1.2 µg/kg and was not detected after boiling, grilling, and dipping in lactic acid. After freezing for 3 months sample E does not detect norfloxacin residues within three months. Residues of norfloxacin in sample F was 5.28 µg/kg after boiling decreased to 0.1 µg/kg and were not detected after grilling and dipping in lactic acid. Norfloxacin was detected in sample G at a concentration 8.1 µg/kg after boiling decreased to 1.45 µg/kg and was not detected after grilling and dipping in lactic acid. After 3 months of frozen storage norfloxacin sample G was 0.48 µg/kg after the first month then not detected after three months. Sample H was 7.44 µg/kg after boiling became 0.82 µg/kg, and not detected norfloxacin residues after grilling, and dipping in lactic acid. After freezing for 3 months sample H in the first month was 0.12 µg/kg while not detected after three months. The data also showed that sample I contains 5.19 µg/kg of norfloxacin and was not detected after boiling, grilling, and dipping in lactic acid. After 3 months of freezing norfloxacin was not detected in sample I within three months. **Jeong *et al.* (2011)** reported that the content of norfloxacin in poultry meat was 3 µg/kg.

Antibiotics are drugs that are used to treat and prevent bacterial infections. They function by obstructing vital bacterial functions, either eliminating the organism or delaying its growth. In veterinary medicine, the tetracycline antibiotic class is typically utilized to treat various illnesses, with oxytetracycline (OTC) being the most widely prescribed medication **(Verma *et al.*, 2021).**

Results of oxytetracycline residues in chicken meat samples were detected in some samples and the data not tabulated**.** Oxytetracycline residues were found in sample D and sample E and were not detected in the other samples. The residues of oxytetracycline in sample D were 4.81µg/kg after boiling decreased to 0.03 µg/kg and did not disappear after grilling or dipping in lactic acid. After three months of freezing oxytetracycline does not detect in samples during the storage period. At the same time, sample E content of the antibiotic was 4.8µg/kg while, after boiling became 0.01 µg/kg and was not detected after grilling or dipping in lactic acid (2%). Also, oxytetracycline was not detected during the frozen period and after boiling.

Across the globe, antibiotic substances in animal products above the MRL are seriously problematic. The overuse or abuse of antibiotics, as well as a lack of knowledge regarding prescription withdrawal times, result in the production of antibiotic residues in animals **(Murni *et al.*, 2016).**

Antibiotic residues will be built up in various animal and poultry body sections because of ongoing therapy. **Verma & Haritash (2020)** investigated the presence of antibiotics in chickens' kidneys, liver, muscle, and fat. Larger fenestrae (50–150 nm in diameter) on endothelial cells in the peritubular capillaries of the kidney and the hepatic sinusoids promote drug accumulation in the liver and kidneys.

Javadi *et al.* (2011) verified that cooking reduced enrofloxacin residues. According to **Hussein & Khalil (2013)**, who reported that frying and roasting lowered oxytetracycline levels to a moderate extent.

4. Impact of treatments on Hormone residues:

In livestock and poultry farms, hormones are one type of growth promoter used to increase the rate of meat production. The most widely utilized anabolic hormones to boost protein deposition and nitrogen retention in cattle are estradiol, estrogen, trenbolone, zeranol, progesterone, and testosterone **(Kamaly & Sharkawy, 2023).**

According to the result obtained of Estradiol residues in chicken meat samples by ELISA technique, data tabulated in **(Table, 4)** revealed that Estradiol residues of samples (A) ranged from (0.4) in fresh sample to 0.2 µg/kg after boiling while after grilling was 0.35 µg/kg. Also, the dipping in lactic acid led to a slight decrease in estradiol (0.32 µg/kg). After freezing for 3 months sample A had the content of estradiol 0.36 µg/kg of storage for one month then became 0.34 µg/kg after 3 months of freezing storage. The data also showed that sample B showed values of estradiol residues 0.7, 0.6, 0.64, and 0.6 µg/kg for fresh, boiling, grilling, and dipping in lactic acid respectively. After freezing for 3 months sample B in the first month had 0.63 and 0.6 µg/kg after 3 months. On the other hand, the same table reflects that estradiol residues values in sample C were 0.46 0.36, 0.4, and 0.36 µg/kg for fresh, boiling, grilling, and dipping in lactic acid respectively. After freezing for 3 months sample C after the first month was contained 0.44 µg/kg of estradiol then became 0.41 µg/kg after 3 months of frozen storage.

Sample D contains 0.57 µg/kg of estradiol after boiling became 0.47 µg/kg, after grilling became 0.52 µg/kg, and after dipping in lactic acid became 0.48 µg/kg. After freezing for 3 months sample D showed 0.53 after one month then became 0.5 µg/kg after 3 months of frozen storage while the residues of sample E were 0.7 µg/kg after boiling and became 0.62 µg/kg and after grilling and dipping in lactic acid became 0.59 and 0.61 µg/kg respectively. After freezing for 3 months sample E in the first month showed reduced 0.68 µg/kg then became 0.66 µg/kg after 3 months. Also, showed that sample F had a high residue 0.92 µg/kg compared to the other samples which after boiling decreased to 0.82, 0.83, and 0.84µg/kg after grilling and dipping in lactic acid. After freezing for 3 months of frozen storage sample F the residues of estradiol were 0.87 and 0.85 µg/kg after 1 and 3 months respectively.

Sample G showed also the second highest level of hormones (0.79 µg/kg), while became 0.72 and 0.7 µg/kg after grilling and dipping in lactic acid respectively. After freezing of 3 months sample G had a content of hormone 0.73 and 0.7 µg/kg after 1 and 3 months respectively. Sample H showed residues of hormone 0.59 µg/kg after boiling became 0.48 µg/kg, after grilling became 0.56 µg/kg, and after dipping in lactic acid became 0.54 µg/kg. From the same results, it can be noticed that residues of hormone in sample H were 0.57 µg/kg after one month of frozen storage then became 0.51 µg/kg after 3 months. Sample I was 0.41 µg/kg slight decrease was observed after boiling (0.34 µg/kg), after grilling the value became 0.33 µg/kg, and after dipping in lactic acid became 0.3 µg/kg. After freezing for one-month sample I contained 0.39 then became 0.35 µg/kg after 3 months. Estradiol residues were 0.5, 0.82, 0.63, 0.77, 0.87, 1.05, 0.92, 0.84, and 0.76 for samples A, B, C, D, E, F, G, H and I in liver.

According to the result obtained of Estrogen residues in chicken meat samples by ELISA in **Table (6)** the data reflect that all samples were positive for estrogen residues with a range from 0.38 for sample I to 2.1 µg/kg for sample F. The data also showed that all treatments had a decreased effect on the contents of estrogen in all tested samples by decreasing ratios 38%, 60%, 42%, 23%, 21%, 44%, 6%, 8%, and 26% for samples A, B, C, D, E, F, G, H, and I respectively after boiling were 25%, 48%, 41%, 32%, 33%, 68%, 42%, 38%, and 16% for samples A, B, C, D, E, F, G, H, and I respectively after grilling while were 30%, 22%, 36%, 19%, 28%, 51%, 13%, 37%, and16% for samples A, B, C, D, E, F, G, H, and I respectively after dipping in lactic acid and for samples A, B, C, D, E, F, G, H, and I respectively (The data not tabulated). On the other hand, the same

phenomenon was observed during freezing after one and three months. Estrogen residues of liver samples were 0.86, 1.41, 0.88, 0.83, 1.02, 4.04, 0.98, 0.77 and 0.59 µg/kg for samples A, B, C, D, E, F, G, H and I respectively and the results not tabulated.

Ibrahim *et al.* (2018) reported that a higher amount of estradiol residues were 0.782±0.07 ppb. Lower findings obtained by **Kamaly & Sharkawy (2023)** results were 0.49 ± 0.02, 0.55 ± 0.005, 0.78 ± 0.009, and 0.79 ± 0.005 for estradiol.

Khalafalla *et al.* (2010) reported that freezing for three months at -20ºC failed to do damage to the estradiol residues in the tissues. This is possible because the hormonal residues are highly resistant to the low temperatures and mentioned that freezing chicken meat at -20˚C is not an effective means to remove hormone residues in quantities that exceed the permitted limits. **Alqahtani *et al.* (2020)** reported that processed products yielded estrogen-positive results for all samples that were examined.

Table (4): Estradiol and Estrogen residues in chicken meat (µg/kg) before and after treatments

Chicken Samples	Treatments					
	Raw Chicken Sample	After Boiling	After Grilling	After 5 days of Dipping in Lactic Acid	After One Month of Freezing	After Three Months of Freezing
Estradiol						
A	0.4	0.2	0.35	0.32	0.36	0.34
B	0.7	0.6	0.64	0.6	0.63	0.6
C	0.46	0.36	0.4	0.38	0.44	0.41
D	0.57	0.47	0.52	0.48	0.53	0.5
E	0.7	0.62	0.59	0.61	0.68	0.66
F	0.92	0.82	0.83	0.84	0.87	0.85
G	0.79	0.66	0.72	0.7	0.73	0.7
H	0.59	0.48	0.56	0.54	0.57	0.51
I	0.41	0.34	0.33	0.3	0.39	0.35
Estrogen						
A	0.6	0.37	0.45	0.42	0.55	0.52
B	0.92	0.37	0.48	0.72	0.89^{b}	0.87
C	0.64	0.37	0.38	0.41	0.58	0.5
D	0.62	0.48	0.42	0.5	0.6	0.58
E	0.86	0.68	0.58	0.62	0.79	0.75
F	2.1	1.18	0.68	1.02	1.5	1.2
G	0.83	0.78	0.48	0.72	0.82	0.77
H	0.52	0.48	0.32	0.33	0.49	0.44
I	0.38	0.28	0.32	0.32	0.37	0.32

5. Trace heavy metals in chicken samples

Residues of lead in meat chicken and liver samples are presented in **Table (5)**. The average of lead values in tissues and liver of samples were 0.0804, ND, ND, 0.0075, 0.0024, ND, 0.0333 and 0.0685 µg/g for A, B, C, D, E, F, G, H and I in chicken samples respectively, and were 0.00136, ND, 0.0074, ND, 0.0104 respectively for liver samples and not detected in sample H and sample I, not exceeded the safe permissible limit recommended by **EOS (2005)** for lead in chicken meat products (0.5 ppm). Similar results observed by **Hamasalim & Mohammed (2013)** reported that lead residues in samples 1.19, 1.02, 1 and 0.52 µg/g. Lead can bioaccumulate in human tissues and organs, particularly in the bones, gizzard, and liver which can result in several illnesses.

The average of copper values in chicken meat and liver of samples were 0.0747, 0.1033, 0.0270, 0.0234, 0.0903, 0.2191, 0.0691, 0.0837 and 0.0935 µg/g for meat A, B, C, D, E, F, G, H and I respectively, and were 0.0932, 0.796, 0.1579, 0.1195, 0.1216, 0.1362, 0.1028, 0.1111, and 0.1113 µg/g for A, B, C, D, E, F, G, H and I samples respectively, not exceeded the safe permissible limit recommended by **EOS (2005)** for copper in chicken meat products (20 ppm). Similar finding was observed by **(Hamasalim & Mohammed, 2013)** and **(Hassanin *et al.*, 2014).**

The data also showed that the residues of zinc in all samples range from 0.6626 µg/g to 1.8612 µg/g in chicken meat samples and from 0.7626 µg/g to 1.1781 µg/g in liver samples, not exceeded the safe permissible limit recommended by **EOS (2005)** for copper in chicken meat products (150 ppm).

Iwegbue *et al.* (2008) reported that the zinc concentrations in turkey meat, chicken flesh, and chicken gizzard were 4.95–48.23, 6.12–33.21, and 10.19–37.03 mg.kg-1, respectively. The trace residues of cadmium in samples were not detected in all samples of chicken meat and liver.

Table (5): Heavy metal (µg/g) in chicken meat and liver of fresh samples collected from the local market

Sample	Muscle				Liver			
	Pb	Cu	Zn	Cd	Pb	Cu	Zn	Cd
A	0.0804	0.0747	0.9177	ND	0.0136	0.0932	0.8127	ND

B	ND	0.1033	0.9288	ND	ND	0.0796	0.7626	ND
C	ND	0.0270	0.9377	ND	0.0074	0.1579	1.1781	ND
D	0.0075	0.0234	0.8094	ND	ND	0.1195	1.0059	ND
E	0.0024	0.0903	0.9252	ND	0.0104	0.1216	1.0981	ND
F	ND	0.2191	1.8612	ND	ND	0.1362	1.1332	ND
G	0.0333	0.0691	0.8414	ND	ND	0.1028	1.0178	ND
H	0.0685	0.0837	0.6626	ND	0.0039	0.1111	0.8282	ND
I	0.0625	0.0935	1.1302	ND	ND	0.1113	0.8449	ND

ND = Not Detected

IV. Conclusion:

Results of this present study show that *Salmonella* spp., *Staph. aureus*, *E. coli*, and *Psychrophilic* bacteria are found in chicken meat. The results showed that treating chicken meat by dipping it in lactic acid (2%) effectively reduces the load of pathogens such as *Salmonella* and *Staph. aureus* and *E. coli*. The results obtained by this present study showed that norfloxacin residues are found in all chicken meat samples. Boiling chicken meat decreased the norfloxacin residues in some samples, while norfloxacin was not detected after grilling, dipping in lactic acid, and freezing for 3 months. All chicken meat samples were positive for both estradiol and estrogen residues. Treating the chicken meat by boiling, grilling, dipping in lactic acid (2%), and freezing can significantly decrease estradiol and estrogen residues. In addition, this study shows that all samples of chicken purchased from markets of the Ismailia government contain safe levels of lead, copper, cadmium, and zinc either in chicken meat or liver. However, more studies might be recommended to evaluate the impact of lactic acid on other pathogens, bacteria, and chemical hazards in chicken meats and organs.

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