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Hazards Analysis And Control Points In Chicken Meat

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Abstract:
Chicken is one of the fountain sources of amino acids, vitamins (such as vitamin A and B12), and minerals. This study's objective was to analyze the chemical and microbiological hazards and the application of treatment methods to reduce hazards. The data 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 effect on microorganisms. The data also reflect that all raw chicken samples contain residues of norfloxacin antibiotic with a range from 1.20 to 8.10 µg/kg and significant differences between samples, while oxytetracycline was detected in 22.2% only of tested samples. Also, all samples had residues of estradiol and estrogen residues. Boiling, grilling, and freezing can reduce antibiotic and hormone residues. The elements concentrations in the livers were higher than in muscle samples. The potential chemical risk assessment of the investigated sample may lead to severe toxicological implications.

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

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I. Introduction

Chicken and chicken products are the main animal protein sources in the human diet. In addition to the low level of cholesterol, compared with beef meat, poultry meat also contains the required essential amino acids, some vitamins (such as vitamin A), 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 the user if the product is not handled carefully. **(Mohammed, 2020)**

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

Salmonella, Esch. coli O157:H7, Staph. aureus and *List. monocytogenes* were 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. *Salmonella* infection is still a major global public health concern. The expenditures of disease prevention, treatment, and surveillance add to 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 important 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 characteristics of antibiotic resistance**. (Baruah *et al.*, 2023)**

Antibiotic resistance and toxicological consequences are the two main health risks associated with the residual of antibiotics in food. The progressive of resistant strains and failure the antibiotic therapy can result from a modest and constant 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 are a kind of growth promoter used to increase the rate of meat production on animal and poultry farms, such as estradiol, progesterone, testosterone, zeranol, trenbolone, and melengestrol, which adding to feed or implanted growth promoters are substances added to feed or implanted in animals 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

(USFDA) has approved the restricted use of certain hormones derived from natural sources for fattening animals. **(Kamaly & Sharkawy, 2023)**

Poultry feed contains a variety of toxic metals both as feed additives and as trace elements. 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 bacteriological and chemical hazards in chicken meat. Also, use some methods to reduce these hazards.

II. Materials And Methods

Materials: -

The materials used in this investigation included 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 one to 3 min, defeather manually, eviscerated, washed with tap water, and placed 15 min on a metal grid then raw chicken meat and chicken liver.

Saved at 4ºC ± 1 in foam dishes and examined for bacterial and chemical analysis as follows: Raw chicken meat, cooked chicken meat by boiling at 100ºC for 10 minutes, grilled chicken meat for 10 minutes, refrigerated chicken meat at 4ºC ± 1 for 7 days, frozen chicken meat at -18ºC ± 1 for 3 months, and dipping in lactic acid (2%) for 10 min.

Analytical Methods: -

Microbiological Analysis:

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 so on up to $1:10^2$ dilution so on up 10^5.

Analysis:

Psychrophilic bacteria were counted according to **Da Silva *et al.*, (2018)** as follows: A 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 allowing the plates to harden and incubating them for 8 days at 7°C ± 1, the findings are shown as log CFU for each gram of material. In **(Da Silva *et al.*, 2018).**

Detection of *Staphylococcus aureus* using Baird Parker agar, *Salmonella* spp. by Brilliant green agar (BGA) and *E. coli* using MacConkey agar according to methods described by **(Da Silva *et al.*, 2018).**

Chemical Methods:

Antibiotics residues:

Calibration standards were made using Oxytetracycline (Sigma) and Norfloxacin (Sigma) concentrations of 0.5,1 and 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.

Oxytetracycline residues

2 grams of minced chicken meat were weighed into a polypropylene centrifuge tube (50 ml) and mixed for 2 minutes, in that sequence, 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. Centrifuge for 10 minutes at 5300 rpm after vortex for thorough mixing and 15 minutes of ultra-sonication. 25 µl of sample injection in HPLC (Chromeleon (c) Dionex Version 7.2.10.23925) after filtration using a 0.45 µm nylon filter for analysis. The mobile phase was pumped at a flow rate of 1.5 ml/min, including 85% acetonitrile, and distilled water 15% **Fathy *et al.*, (2015),** and data calculation using the equation as following in **Fig, 1**

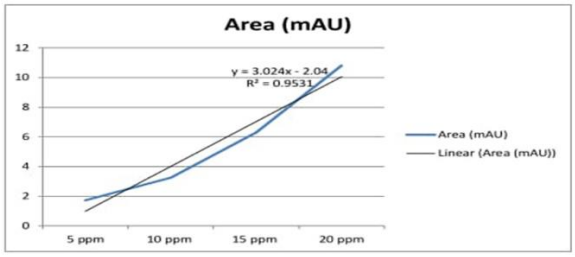

Figure (1): Calculation of Oxytetracycline

Norfloxacin residues

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 (Chromeleon (c) Dionex Version 7.2.10.23925). Sulphate is an ion-pairing agent and was composed of water/methanol (65:35, v/v, pH 3 adjusted with H_3PO_4), according to **kowalski *et al.*, (2005)** and data calculation using the equation as following in **Fig,2**

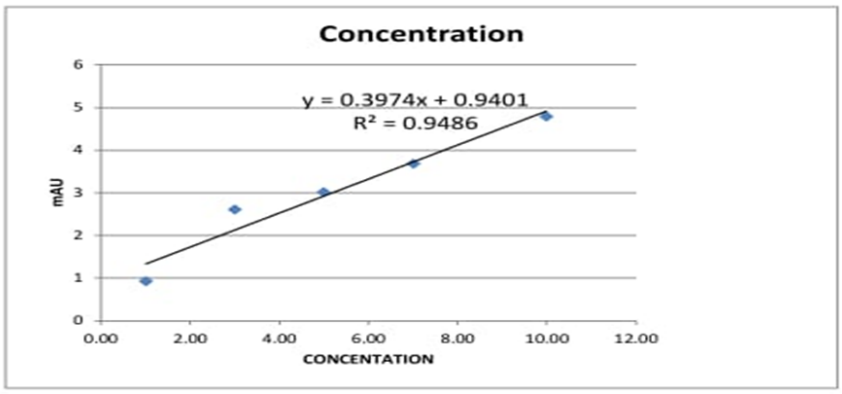

Figure (2): Calculation of Norfloxacin

Hormones residues

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.

Estradiol hormone residues

The Estradiol ELISA test kit from **Monocent Company (2019)** 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 absorbance at 450 nm.

Estrogen hormone residues

Estrogen hormone residues were determined using ELISA technique from **Bioassay Technology Lab (2019).** 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.

Heavy metals determination

Heavy metals determination according to the method described by **(Elsharawy & Elsharawy, 2015)** using atomic absorption spectrophotometer (Thermo-electron, S Series, S4 AA system, S. No. GE 711838, Thermo-electron Corp.)

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

Microbiological analysis:

Products made from chicken meat carry a risk of contamination from 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. *Staph. aureus* (log CFU/g) counts in **Table (1)and Figure (3)**, showed that 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 significant difference ($P \leq 0.05$).

Staphylococcus aureus counts obtained were higher than those of the data recorded by **(Elmelegy *et al.*, 2015)** who found that *Staph. aureus* were isolated from chicken was 13.33% of the examined chicken. On the other hand, the results of *Staphylococcus aureus* that obtained lower than of the data recorded by **Tagar & Qambrani, (2023)** who mentioned that *Staph. aureus* isolated from chicken meat were 8.1, 16.2 and 10.8 CFU/g. The obtained results agree with **Al-Jasser (2012)** who found that *Staph. aureus* increased during cold storage after 3 days.

One of the most harmful bacteria that is 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 significant differences ($P \leq 0.05$).

Results of *Salmonella* spp. obtained were 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. **EL-Gammal & Yassin (2016),** found that 18% of the chicken samples examined were positive for *Salmonella* spp.

Table (1): *Staphylococcus* aureus and *Salmonella* spp. Count (mean ± S.D) of chicken samples collected from the local market during cold storage 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 ($P \leq 0.05$)
Samples from A to I fresh samples were collected from the different local market.

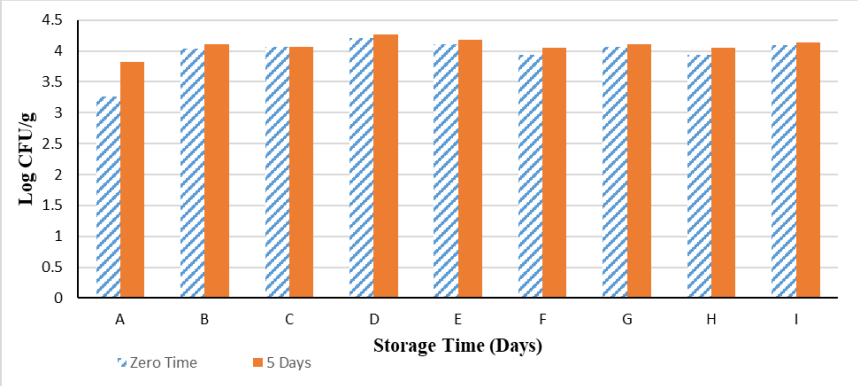

Figure (3): *Staphylococcus Aureus* Counts Of Chicken Sample

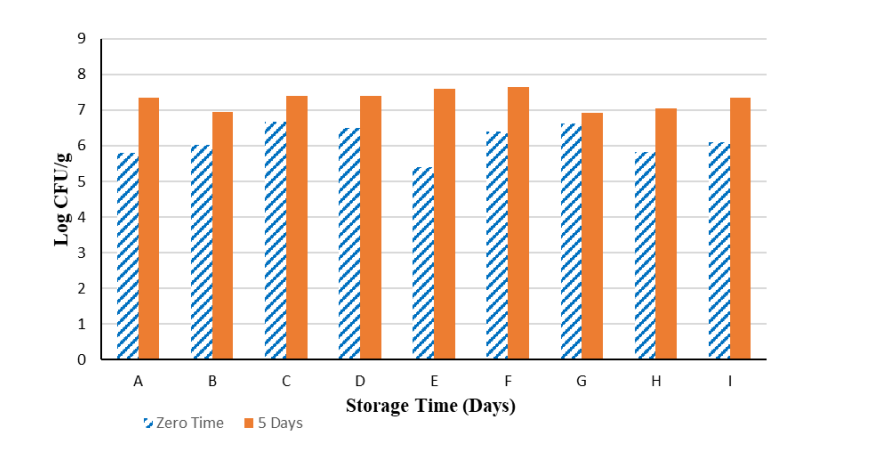

Figure (4): *Salmonella* Sp. Of Raw Sample And During Storage At 4º C±1 For 5 Days

The presence of *E. coli* in chickens is sign poor sterile practices in abattoirs or exchanging areas. *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 microbiological (***E. Coli***) samples were significant differences ($P \leq 0.05$).

The results of *E. coli* that obtained lower than of the data recorded by **(Tagar & Qambrani, 2023)**. **Al-Jasser, (2012)** found that the coliform group increased during cold storage after 3 days.

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.

Table (2): *E. coli* and *Psychrophilic bacteria* counts (mean ± S.D) of chicken samples collected from the local market:

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)

Results of *Psychrophilic* bacteria that obtained higher than those of the data recorded by **Sheir *et al.* (2020)**, while the results obtained by **(Morshedy & Sallam, 2020),** show that the count was 4.62 log CFU/g for the examined chicken meat that is lower than the obtained results. Also, **Mashishi *et al.* (2019),** who reported that *Psychrophilic* bacteria isolated from chicken meat was 3.23, 3.00, and 3.13 CFU/g.

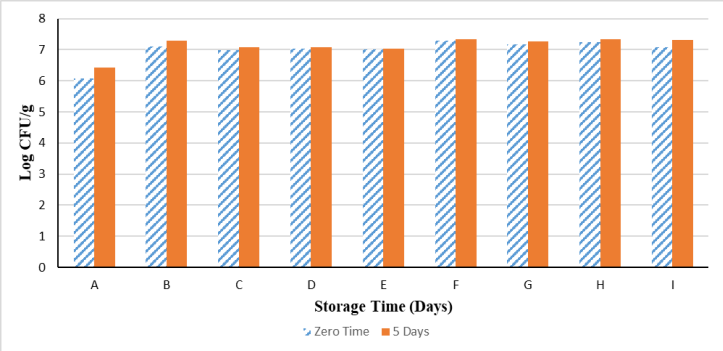

Figure (5): *Escherichia Coli* Counts Of Chicken Samples During Storage At 4°c±1

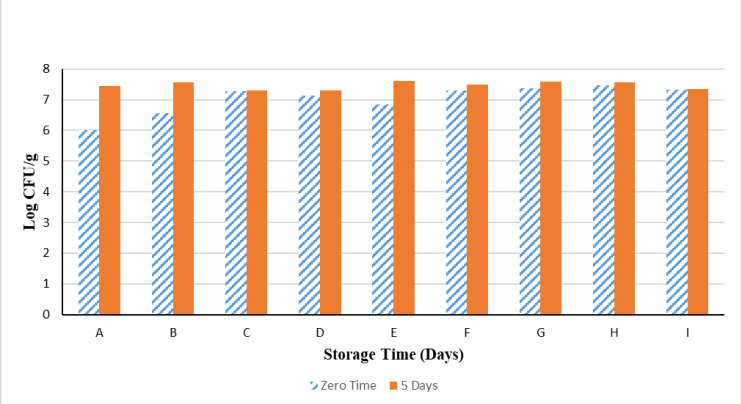

Figure (6): *Psychrophilic* Bacteria Count Of Chicken Samples During Storage At 4°c±1

Reduce microbial level using Lactic Acid (2%) as a control point

According to **Ramirez-Hernandez *et al.* (2018)**, The separated form of LA 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 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 after storage at cold storage at 4° C±1 for 5 days by decreasing ratio 100% for all treated samples. From the same table it can be noticed that all determined microbiological samples were significant difference ($P \leq 0.05$). *Staph. aureus* that was obtained after dipping in lactic acid 2% agree with the data recorded by **(Abu-Ghazaleh, 2013)** and **(Rosengren *et al.*, 2013).**

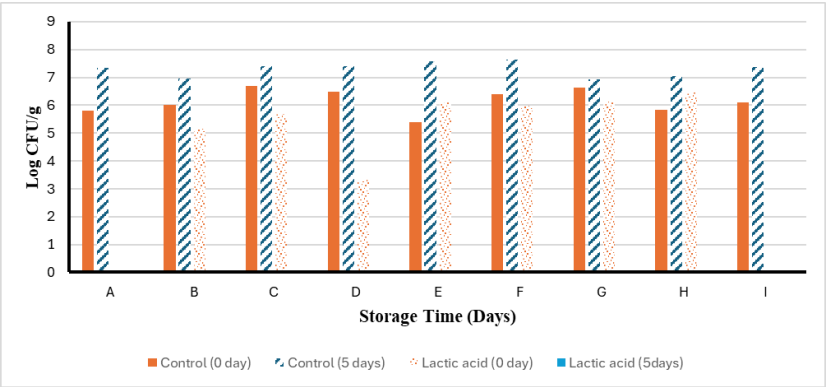

Figure (7): Effect of lactic acid 2% on *staphylococcus aureus* during cold storage

Salmonella (log CFU/g) counts after dipping chicken meat in lactic acid (2%) in **Table (3) and Figure (8),** were lower than sensitive for lactic acid (2%) compared with *Staph. aureus*. Therefore, the data showed that 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)** and **(El-Khawas *et al.*, 2020),** found that *Salmonella* spp. was reducing from 5 to 3.7 log CFU/g after treatment with lactic acid 1%.

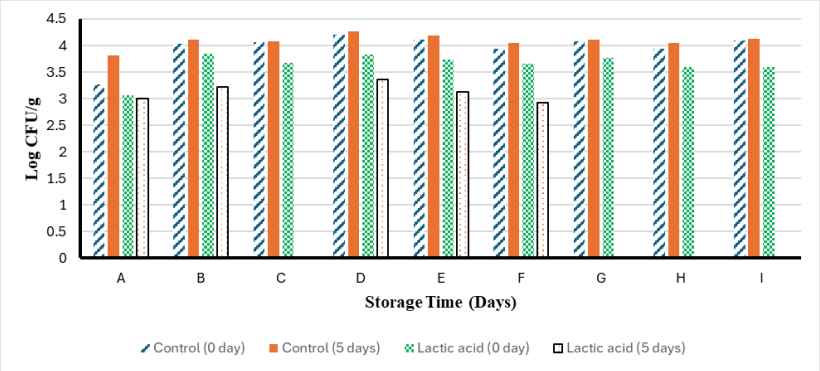

Figure (8): *Salmonella* spp. Counts of chicken samples treated by lactic acid (2%) during cold storage

Table (3): *Staphylococcus* aureus and *Salmonella* spp. Counts (log CFU/g) of treated chicken samples during cold storage at 4ºC ±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%). So, *E. coli* was not detected in all treated samples at any time during storage.

The results obtained agree with **(El-Khawas *et al.*, 2020)** and **(Fang *et al.*, 2022)** who found that the count of *E. Coli* decreased during chilling and was treated with lactic acid.

Antibiotic residues and control treatments

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 brought on 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 first, two, and three months. Also soup after boiling analysis and not detect norfloxacin residues. Sample C was 1.99 µg/g and was not detected after boiling, grilling, and dipping in lactic acid. Norfloxacin residues not detected in B, C and D samples within the first, two, and 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/g 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 the first, two, and three months. Also soup after boiling analysis and not detect norfloxacin residues. Residues of norfloxacin in sample F was 5.28 µg/g after boiling decreased to 0.1 µg/g 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/g 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 two and three months. Also soup after boiling analysis and not detect any residues. 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 two and three months. Also soup after boiling analysis and not detect any residues. 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 the first, two, and three months. Also soup after boiling analysis and not detect norfloxacin residues.

Pena *et al.* (2010) mentioned that the interval of sample collection and the duration of antibiotic removal in chickens caused differences in the findings. In samples of chicken meat, the limit of detection for norfloxacin was 0.028 µg/kg. However, the limit of quantification for norfloxacin in chicken meat samples was 0.01 µg/kg. A suitable and accurate limit of detection for norfloxacin using HPLC was indicated by the chromatographic technique used for the investigation, which produced good data in terms of limit of detection and limit of quantification. **Jeong *et al.* (2011)** determined the limit of norfloxacin in poultry meat as 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/g 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. Also, the antibiotic was not detected in the soup after boiling. 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 build up in various animal and poultry body sections because of ongoing therapy. This study 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 **(Verma & Haritash, 2020)**.

Boiling results in another investigation **(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.

Hormones' residues and control treatment

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 and not detected in soup. 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, 0.6, and zero µg/kg for fresh, boiling, grilling, dipping in lactic acid and soup 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, 0.36, and zero µg/kg for fresh, boiling, grilling, dipping in lactic acid and soup 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 and was not detected in soup, 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 were not detected in soup 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 while not detected in soup. 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 high level of hormones (0.79 µg/kg) and was not detected in soup, 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 and was not detected in soup, 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) and not detected in soup, 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), while not detected in soup. 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.

For three months freezing 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. **Khalafalla *et al.* (2010)**, 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 some 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

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, 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)**. Who 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, 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, 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:

All samples had counts of *Staph. aureus* ranged from 5.39 to 6.68 log CFU/g at zero time and from 6.93 to 7.64 log CFU/g after 5 days of cold storage. All samples were positive for *Salmonella* spp. The means count of *E.coli* were 7.00 log CFU/g increased to 7.13 log CFU/g after 5 days of cold storage. The data also showed that all other samples had the same phenomenon. The data showed that dipping chicken meat in lactic acid (2%) leads to decrease of all microorganisms group. The results of norfloxacin residues in chicken meat samples were detected in all tested samples. Boiling decreased the residues of norfloxacin in some samples with significant differences while was not detected after grilling, dipping in lactic acid and freezing for 3 months. All samples showed residues of estradiol and estrogen at zero time. Boiling, grilling, dipping in lactic acid (2%) and freezing treatment showed significant effects on the values of estradiol and estrogen residues. All samples had trace residues of lead, copper and zinc, while the trace residues of cadmium in chicken meat and liver samples were not detected.

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