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QUALITY EVALUATION OF POULTRY MEAT CARCASS IN EL- GHARBIA GOVERNORATE MARKETS

(With 8 Tables)

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تقييم جودة لحوم الدواجن المذبوحة بأسواق محافظة الغربية

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اشتملت الدراسة علي عدد ثمانون عينة من الأوراك والصدور (٤٠) عينة من كل نوع وأيضاً مائة عينة من (الكبد - الكلي - القلب - الطحال - القونصة) عشرون عينة من كل نوع وقد أسفرت الدراسة عن النتائج الآتية : أولاً: الفحص البكتريولوجي : (١) متوسط العد الكلي للميكروبات الهوائية للأوراك $1.37 \times 10^6 \pm 0.35 \times 10^6$ وللصدر $6.26 \times 10^6 \pm 0.19 \times 10^6$ ، (٢) متوسط العد الكلي لميكروب العنقودي الذهبي في حالة الأوراك $8.9 \times 10^3 \pm 0.34 \times 10^3$ ، $1.0 \times 0.17 \pm 1.0$. وكانت النسبة الإيجابية لوجود ميكروب العنقودي الذهبي للأوراك ٣٨,٧% وللصدر ٥١,٥% كما تم عزل أربع عترات سالمونيلا (٢) عترة من الأوراك سالمونيلا تيفوميوريم وسالمونيلا انترتيدرس و (٢) عترة من الصدور (سالمونيلا كينتاكي- وسالمونيلا تيشنجوى. كما تم عزل سبعة عشرة عترة من الميكروب القولوني المعوى الشيريشياكولاي ثلاثة عشرة من الأوراك وأربعة من الصدور وكان التصنيف المصلي لهذه العترات كالاتي: من الأوراك (٤) عترة من $O_{55}: K_{56} (B_5)$ ، (١) عترة من $O_{119}: K_{69} (B_{19})$ ، (٣) عترة من $O_{124}: K_{72} (B_{17})$ ، (٣) عترة من $O_{86}: K_{61} (B_{17})$ ، ومن الصدور كانت كالاتي : (٢) عترة من $O_{126}: K_{71} (B_{16})$ ، (١) عترة من $O_{119}: K_{69} (B_{19})$ ، و(١) عترة من $O_{124}: K_{72} (B_{17})$. كما تم عزل بعض الميكروبات سالبة جرام من الستروباكتز والانثيروباكتز والكابسيللا والبروتين والسرتينيا نسب مختلفة. ثانياً الفحص الكيميائي: وذلك بقياس الأس الهيدروجيني فكان متوسط القياس للأوراك 6.37 ± 0.05 وللصدر 5.9 ± 0.03 ثالثاً الفحص عن مدي تواجد بقايا المضادات الحيوية: فكانت للأوراك ٤٢,٥% ، وللصدر ١٢,٥% ، والكبد ٨٥% ، والكلي ٨٠% ، والقلب ٦٠% ، والطحال ٧٥% ، والقونصة ٤٥% وقد أكدت تلك الدراسة عن مدي التلوث بمختلف أنواعه الذي عرض له لحوم الدواجن وقد تم مناقشة الأهمية الصحية لكل من الميكروبات المعزولة ومدى وخطورة تواجد بقايا المضادات الحيوية وقد أظهرت

الدراسة الأهمية في اتباع القواعد الصحية الواجب توافرها في مجازر الدواجن والاشترائط الصحية للعاملين بها. كما أوضحت الدراسة ضرورة تقنين استخدام المضادات الحيوية والالتزام بالمدة المحددة للتخلص من هذه العقاقير قبل الذبح.

SUMMARY

Eighty random samples of poultry thigh and breast (40 of each) as well as 100 random samples of poultry giblets (liver, kidney, heart spleen and gizzard, 20 of each) were collected from different retail shops in El- Gharbia Governorate. The collected thigh and breast samples were subjected to bacteriological examination as well as determination of pH and antibiotic residues, while the giblets were examined only for detection of antibiotic residues. The bacteriological examination revealed that the mean value of aerobic plate count of the examined chicken thigh was $1.4 \times 10^6 \pm 4 \times 10^5$ and $6 \times 10^5 \pm 2 \times 10^4$ for the examined chicken breast. While the mean values of total *Staphylococcus* count of the examined chicken thigh and breast samples were $8.9 \times 10^3 \pm 0.3 \times 10^3$ and $2.7 \times 10^3 \pm 1.7 \times 10^4$, respectively. The incidence of coagulase positive staphylococci in the examined chicken thigh and breast samples was 38.7 % and 51.5%, respectively. *Salmonellae* were isolated from both thigh and breast (2% each). *E. coli* was isolated from 32.5 % and 10% of the examined chicken thigh and breast samples, respectively. Isolated *E. coli* was serotyped into O₅₅ : K₅₉ (B₃), O₈₆ : K₆₁ (B₇), O₁₁₉ K₈₉ (B₁₉), O₁₂₄ : K₇₂ (B₁₇) and O₁₂₆ : K₇₁, (B₁₆) serovars from 13 samples of thigh muscles while four samples of O₈₆: K₆₁ (B₇), and breast samples belonged to serovars O₁₁₉ : K₆₉ (B₁₉), O₁₂₄ : K₇₂ (B₁₇). Determination of pH values declared that the mean values of the examined chicken thigh and breast samples were 6.4 ± 0.05 and 5.9 ± 0.03 , respectively. Regarding antibiotic residues, 42.5% and 12.5% of the examined chicken thigh and breast samples contained antibiotic residues, respectively. Higher incidence of antibiotic residues was detected in poultry giblets as liver (85%), kidney (80%) heart (60%) spleen (75%) and gizzard (45%) which was considered of a great health hazard problems.

Key words: Broilers carcasses, poultry meat, giblets

INTRODUCTION

The unsanitary conditions during scalding, evisceration, washing and lack of refrigeration after dressing can lead to increase in the

microbial load of the carcasses (Thomas *et al.*, 1987). So, hygiene play an important role during processing in decreasing the initial bacterial contamination of poultry carcasses (Smulders, 1987, Narasimba Rao *et al.*, 1998). Contamination of poultry carcasses by *Staphylococcus aureus* indicates its contamination from handlers and inadequately cleaned equipments. While *Salmonella* which is the major cause of foodborne illness contaminate the carcasses through faecal polluted water during dressing and washing or through cross contamination in displaying and handling in kitchen. *Escherichia coli* and coliform group predominate among aerobic commensal flora present in the gut of man and animals. So, their presence in poultry meat are indication of faecal contamination. The pH value of meat products varied greatly according to the state and type of meat. However, the pH value of raw meat varied depending upon the amount of glycogen present at the time of slaughter and the subsequent changes after that, a high pH value favour microbial growth. Hudson and Roberts (1984) recorded positive correlation between pH value and total aerobic count.

Antibiotic residues in meat may develop an allergic reaction such as urticaria, eczema and other dermatitis as well as increasing resistance of pathogenic microorganisms in man, in addition to their bad effects on the normal microflora which consequently produce vitamins (El-mossalami *et al.*, 1985). Moreover, Grossklaus (1977) recorded that antibiotic residues may interfere or mask the bacteriological quality of meat and impair its fitness for manufacture. Therefore the aim of the present study was planned to cover the following items:

- 1- Evaluation of the microbiological status of dressed broilers (thigh and breast) through:
 - a) Total Aerobic Plate count (TAPc).
 - b) Total *Staphylococcus* count
 - c) Incidence of *salmonellae*
 - d) Incidence of *Escherichia coli*
- 2- Determination of pH value
- 3- Detection of antimicrobial residues in broilers thigh and breast muscles as well as poultry giblets (liver, kidney, heart, spleen and gizzard).

MATERIALS and METHODS

Collections of samples :

Eighty random samples of poultry thigh and breast (40 of each) as well as one-hundred random samples of poultry giblets (liver, kidney,

heart, spleen and gizzard, 20 each) were collected from different retail shops in El-Gharbia Governorate. The collected samples were transferred to the laboratory under complete aseptic conditions without delay in an insulated ice box. The collected thigh and breast samples were subjected to bacteriological examination as well as determination of pH and antibiotic residues while giblets samples were examined only for detection of antimicrobial residues.

I- Bacteriological examination:

Preparation of the samples (Harrigan, 1998):

Twenty five gm were taken aseptically from both breast and thigh muscles and put into a sterile homogenizer bottle containing 225 ml sterile peptone water 1%. Homogenization for 2.5 minutes at 3000 r.p.m to provide a dilution of 10^{-1} was done then one ml of the homogenate was transferred with a sterile pipette into another sterile test tube containing 9 ml of sterile peptone water 1% to give 10^{-2} dilution from which decimal serial dilutions were prepared. Then the following examinations were done:

- (1)- Total Aerobic Plate Count (TAPc) according to Harrigan, (1998).
- (2)- Total *Staphylococcus aureus* count and its identification according to Harrigan, (1998).

A loopful of suspected isolates was inoculated into tubes containing 5 ml brain heart infusion broth Inoculated tubes were incubated at 37°C for 18 hours and the growth cultures were identified according to Harrigan, (1998).

1. Morphological examination by Gram' stain and motility test,
2. Biochemical identification by catalase test, mannitol fermentation test and coagulase test (tube method).

(3)- Screening of *salmonellae*:

Two hundred and twenty five ml of peptone water 1% as a pre-enrichment broth recommended by Edel and Kamplmacher (1973) were inoculated by 25 gram of the original samples under examination and incubated at 37°C for 18 hours.

One ml of the inoculated broth was transferred into 9 ml Rappaport Vassiliadis enrichment broth and incubated at 43°C for 24 hours (Rappoport *et al.*, 1956, Harvey, Price, 1981).

Loopfuls from inoculated tubes were streaked over Xylose Lysine Deoxycholate agar (XLD) medium plates and then incubated at 37°C for 24 hours. Suspected colonies appear as red colonies with or without black center were picked up for further confirmation according to Harrigan, (1998) by:

- 1- Microscopical examination using Gram's stain.
- 2- Biochemical identification by motility, IMViC reaction, hydrogen sulphide, urease, gelatin hydrolysis test and fermentation of sugars.
- 3- Serological examination:

Isolates proved biochemically to be *Salmonella* microorganisms were subjected to serological identification for presence of O and H antigen by slide or plate agglutination test with polyvalent sera in the laboratory of the Ministry of Health .

(4) - Isolation and Identification of *Escherichia coli* (Harrigan, 1998):

One ml from the originally prepared dilution was inoculated into MacConkey broth tube supplemented with inverted Durham's tube, then the tube was incubated at 44 ± 0.5 °C for 24 hours. The positive tube shows acid and gas production

One ml from positive MacConkey broth was transferred into Brilliant Green Bile broth 2% and incubated at 44 ± 0.5 °C for 18 hours then a loopful from positive Brilliant Green Bile broth 2% tube was streaked on Eosine Methylene Blue agar (EMB) and incubated at 37°C for 24 hours. The typical colonies appear as greenish with metallic sheen and dark purple center were picked up and purified for further identification according to the procedures outlined by Harrigan (1998) as follows:

- 1- Microscopical staining.
- 2- Biochemical examination.

The following tests were done: Motility test, indole production, methyl red reaction, Voges proskaure, gelatin hydrolysis, urease test, hydrogen sulphide production test, citrate utilization test and fermentation of sugars.

3- Serological Identification:

Isolates proved biochemically to be *E. coli* microorganisms were subjected to serological identification using diagnostic sera "Welcome *E. coli* agglutinating sera" for diagnosis of enteropathogenic serotypes in the Health Ministry laboratory.

II- Determination of pH value (Chamber *et al.*, 1976):

Approximately 10 grams of each sample were blended and mixed with 10ml of distilled water in a beaker and left for about 10 minutes at room temperature, then the electrodes of pH meter (C- D 620 digital pH) were placed in the meat extract and the pH value was recorded.

III- Detection of antibiotic

Detection of antibiotic residues in the poultry tissues and organs of the examined samples was carried out according to Arret *et al.*, (1971) using *Bacillus subtilis* (Atec 6633).

Tissue extraction:

The technique recommended by Scheer, (1987) was followed where the nutrient agar was divided and tested simultaneously in acid and alkaline medium at pH (6.0 and 8.0) as some antibiotics (as penicillin) have a greater effect at lower pH others as (streptomycin) at higher pH. The media were tempered at 45°C to 50°C to which one ml of spore suspension were added, thoroughly mixed, then poured about 15 ml on a levelled flat bottom Petri-dishes till complete solidification. The agar layer was pored 4 wells by sterile stainless steel cylinder with sharp edges in which tissues extract was put in them. Plates were left for 3 hours at room temperature, then the inoculated as well as the control plates were incubated at 37°C for detection of presence of inhibitory zone or not. In order to test the sensitivity of the plates ready for use a paper disk with diameter of 6mm containing 0.01 I.U penicillin on pH (6.0) test plate and paper disk containing 0.5 mg streptomycin on a pH (8.0) test plate were used. The results for penicillin and streptomycin must be an inhibition zone of about 6mm, and inhibition zone around the sample of 2mm or more was evaluated as positive result.

RESULTS

Table 1: Statistical analytical results of total aerobic plate count and *Staphylococcus* count in the examined chicken thigh and breast (n = 40 each).

Microbial count	Chicken thigh Mean ± S.E	Chicken breast Mean ± S.E
Total aerobic plate	$1.4 \times 10^6 \pm 3.5 \times 10^5$	$6.3 \times 10^5 \pm 1.9 \times 10^4$
<i>Staphylococcus aureus</i>	$8.9 \times 10^3 \pm 3.4 \times 10^2$	$2.7 \times 10^3 \pm 1.7 \times 10^2$

Table 2: Incidence of coagulase positive *Staphylococcus aureus* in the examined chicken thigh and breast (n = 64 each).

Isolated strain	Chicken thigh			Chicken breast			Total		
	No.	+ve	%	No.	+ve	%	No.	+ve	%
Coagulase +ve	31	12	38.7	33	17	51.5	64	29	45.3
Coagulase -ve	31	19	57.5	33	16	48.5	64	35	54.7

Table 3: Incidence and serotyping of *Salmonella* in thigh and breast chicken samples.

Sample Incidence and serotyping	Chicken thigh			Chicken breast		
	No. of Samples	No. of +ve Samples	%	No. of Samples	No. of +ve Samples	%
Incidence of <i>Salmonella</i>	40	2	5	40	2	5
Serotyping of <i>Salmonella</i> serovars	<i>Salmonella typhimurium</i> O: 1,4(5), 12 H: 1,1,2			<i>Salmonella kentucky</i> O: 8,20 H: 1,Z,6		
	<i>S. enteritidis</i> O: 1,9,12 H: gm, (1,7)			<i>S. tshiongwe</i> O: 68 H L ehenz, 1,5		

Table 4: Incidence of *E. coli* isolated from the examined chicken thigh and breast samples.

Type of samples	No. of examined Samples	Positive samples	
		No.	%
Chicken thigh	40	13	32.5
Chicken breast	40	4	10
Total	80	17	21.25

Table 5: Seriological identification of the isolated *E. coli* from the examined chicken thigh and breast samples (n = 40 each).

E. coli serotype	Chicken thigh		Chicken breast		Total	
	No.	%	No.	%	No.	%
O ₅₅ : K ₅₉ (B ₃) (EPEC)	4	10	-	-	4	5
O ₈₆ : K ₆₁ (B ₇) (EPEC)	1	2.5	2	5	3	3.75
O ₁₁₉ : K ₆₉ (B ₁₉) (EPEC)	2	5	1	2.5	3	3.75
O ₁₂₄ : K ₇₂ (B ₁₇) (EPEC)	3	7.5	1	2.5	4	5
O ₁₂₆ : K ₇₁ (B ₁₆) (EPEC)	3	7.5	-	-	3	3.75

Table 6: Incidence of enteric bacteria isolated from the examined chicken thigh and breast samples (n=40 each).

Isolated strain	Chicken thigh		Chicken breast		Total (n=80)	
	No.	%	No.	%	No.	%
<i>Citrobacter freundii</i>	8	20	6	15	14	17.5
<i>Citrobacter diversus</i>	6	15	2	5	8	10
<i>Enterbacter aerogens</i>	6	15	14	35	20	25
<i>Enterobacter cloacae</i>	3	7.5	1	2.5	4	10
<i>Enterobacter hafniae</i>	2	5	-	-	2	2.5
<i>Enterobacter agglomerans</i>	2	5	-	-	2	2.5
<i>Klebsiella ozaenae</i>	4	10	8	20	12	15
<i>Klebsiella pneumoniae</i>	7	17.5	2	5	9	11.25
<i>Proteus mirabilis</i>	6	15	5	12.5	11	13.75
<i>Proteus morgani</i>	4	10	11	27.5	15	18.75
<i>Proteus rettgeri</i>	3	7.5	-	-	3	3.75
<i>Proteus vulgaris</i>	12	30	7	17.5	19	23.75
<i>Serratia liquefaciens</i>	3	7.5	1	2.5	4	5
<i>Serratia marcescens</i>	2	5	2	5	4	5
<i>Serratia rabidaea</i>	2	5	-	-	2	2.5

Table 7: Statistical analytical results of pH value in the examined chicken and thigh and breast sample.

Type of samples	No. of examined samples	Min.	Max.	Mean ±S.E
Chicken thigh	40	6.00	7.01	6.37±0.05 ⁺
Chicken breast	40	5.60	6.30	5.90±0.03 ⁺

(+) significant difference

Table 8: Microbial inhibition zones of the examined chicken thigh, breast and giblets as detected by microbiological inhibition test.

Type of sample	No. of examined samples	+ ve	%
Chicken thigh	40	17	42.5 ⁺
Chicken breast	40	5	12.5 ⁺
Liver	20	17	85
Kidney	20	16	80
Heart	20	12	60
Spleen	20	15	75
Gizzard	20	6	45

(+) significant difference

DISCUSSION

The results achieved in Table (1) revealed that the mean value of APC in the examined poultry thigh samples was $1.4 \times 10^6 \pm 3.5 \times 10^5$ CFU/gram, while that of breast samples was $6.2 \times 10^5 \pm 1.9 \times 10^4$. The higher microbial load of the thigh could be attributed to the technique of evisceration, as injury of the gut during manual evisceration and poor hygienic portioning process increases such type of contamination. According to the Egyptian standard all of the examined thigh and breast samples exceeded the upper allowable limits (10^4 / for APC).

Concerning the total *Staphylococcus* count, the mean value of examined thigh samples was $8.9 \times 10^3 \pm 3.4 \times 10^2$ CFU/g, while in breast samples was $2.7 \times 10^3 \pm 1.7 \times 10^2$ CFU/g.

Coagulase positive *Staphylococcus* was detected in 38.7% and 51.5% of the positive thigh and breast samples, respectively as recorded in Table (2). Several investigators revealed that large numbers (usually greater than 1 million per gram) of coagulase positive *Staphylococcus aureus* must contaminate the foods for producing sufficient enterotoxins to cause food poisoning (Dack *et al.*, 1960 and Gilbert *et al.*, 1972).

Contamination of poultry carcasses with *Staphylococcus aureus* usually occurs through handling by human, if such products are left without refrigeration for several hours or cooled slowly in refrigerator, growth of *Staphylococcus aureus* and enterotoxin formation may occur. Growth of *Staphylococcus aureus* in the product is favoured by lack of competitive bacteria, which are destroyed by heat (Bryan, 1980). *Salmonellae* are the major causes of foodborne diseases in many countries (Bernardo and Machodo, 1989). *Salmonella typhimurium* and *Salmonella enteritidis* reach to food directly or indirectly at a time of slaughtering and causes cross contamination.

Results reported in Table (3) revealed that *Salmonella typhimurium* O: 1,4 (s), 12 H: 1,1,2 and *Salmonella enteritidis* O:1,9,12 H: gm, (1,7) were detected from 2(5%) of thigh samples, while *Salmonella Kentucky* O, 8, 20H:1,2,6 *Salmonella tshiongue* and O: 6,8. H: ehenz 1,5 were isolated from 2 (5%) of breast samples.

Salmonellae that cause most of foodborne disease grow well in cooked poultry meat (Pivnick *et al.*, 1969). Handling of the unclean utensils or work surfaces that previously contact with raw poultry

frequently contaminate cooked poultry during boning, slicing, chopping and grinding.

In the present study the incidence of the isolated *E. coli* from thigh and breast muscles samples was 32.5% and 10%, respectively, as recorded in Table (4). Nearly similar results of examined thigh were recorded by Belal (1996) and Hafiz (1986), while for the examined breast, nearly similar results were recorded by Tamura *et al.*, (1971) and Hefnawy and Mostafa (1990).

The results achieved in Table (5) revealed that 17 *E. coli* strains were isolated from 13 (32.5%) of thigh samples and were serotyped, while 4 (10%) of *E. coli* strains were isolated from breast muscle samples and were also serotyped. These isolated serovars were belonged to EPEC, ETEC and EIEC as previously reported by Levine (1987) who added that *E. coli* O₁₁₉ and O₁₂₆ serovars caused epidemic children enteritis. The public health significant of the isolated Enteropathogenic *E. coli* serovars had been recorded to elaborate enterotoxin and implicated in cases of gastroenteritis, epidemic children diarrhoea and sporadic diarrhoea in children as well as in case of food poisoning (Bryan, 1982). Moreover, presence of *E. coli* in poultry meat increased the chance of possibility of other enteric pathogens that collectively constitute public health hazard to consumers.

The results recovered in Table (6) declared that the enteric bacteria could be isolated and identified in different percentages from thigh and breast chicken samples as *Citrobacter freundii* (20,15%), *Citrobacter diversus* (15,5%), *Enterobacter aerogenes* (15,35%), *Enterobacter cloacae* (7.5,2.5%), *Enterobacter hafnia* (5,6%), *Enterobacter agglomerance* (5,6%), *klebsiella ozaenae*(10,20%) *klebsiella pneumoniae* (17.5, 5%), *Proteus mirabilis* (15, 12.5%), *Proteus morganii* (10, 27.5%), *Proteus rettgeri* (7.5,0%), *Proteus vulgaris* (30,17.5%) *Serratia liquefaciens* (7.5, 2.5%), *Serratia marcescens* (5, 5%) and *Serratia rabidaea* (5,0%). The high percentages of enteric bacteria may be indicated for high contamination during evisceration, handling and cross contamination may occurred.

It is evident from Table (7) that the mean values of pH in the examined chicken thigh and breast samples were 6.37 ± 0.05 and 5.90 ± 0.03 , respectively. The higher pH value of the examined samples may be attributed to increase blood supply, fat deposit and increase the chance of possibility of contamination during evisceration. Hudson and Roberts (1984) reported that meat of high pH become bacteriologically spoiled more rapidly than meat of normal pH.

Nowadays, antibiotics are used in veterinary field and poultry farms in a large scale for prophylaxis and treatment of different infectious diseases as well as feed additives as a growth promoters. The greatest danger to human health deriving from the ingestion of food stuffs of animal origin containing antibiotic residues are allergic phenomena, sensitization, antibiotic resistance as well as carcinogenic, tertatogenic, liver damage and renal failure. The results obtained in Table (8) indicated that microbiological inhibition test of the examined chicken (thigh and breast) samples for antibiotic residue were (42.5%, 12.5%), respectively. The high incidence of antibiotic residues in thigh muscles were attributed to the thigh is the most site of intramuscular injection of antibiotic which may persisted for long periods (Pakkala *et al.*, 1976).

On the other hand, results presented in Table (8) revealed that the incidence of antimicrobial residues of liver, kidney, heart, spleen and gizzard was 85, 80, 60, 75 and 45%, respectively. the high incidence of antibiotic residues in the liver may be due to metabolism and detoxication of drug by its microsomal enzymes, also the high concentration of antibiotic residues in kidney is due to the filtration and clearance of the blood from drugs which are carried out by such organ. Presence of bacteriologic lysozyme in the kidney may be responsible for the appearance of false positive results (El-mossalami *et al.*, 1985). The highly recommended results from the study revealed that all poultry holder, allendance and owners must strictly follow the withdrawal period of all drugs before slaughtering time to become sure that the antibiotic residues completely disappeared from tissues and organs.

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