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MICROBIOLOGICAL ASSESSMENT OF CHICKEN BREAST MEAT FROM UNLICENSED AND LICENSED SLAUGHTERHOUSES DURING REFRIGERATION AND FREEZING STORAGE

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ABSTRACT

This study was aimed to compare sanitary conditions by detection of hygiene statue of the chicken breast meat taken from license and unlicensed slaughterhouses. Different microbiological indicators were measured to determine some traits of the local fresh chicken breast meat during different storage periods under refrigeration and freezing temperature. All tests were made in the post-graduate laboratories of Animal Sciences Department, College of Agricultural sciences, University of Sulaimani. The microbial content of breast meat in this study showed that the TPC was increased from 3.63×10^4 to 8.56×10^4 CFU/gm meat and from 4.13×10^3 to 9.33×10^3 CFU/gm meat for both unlicensed and license slaughterhouses respectively, that stored at fridge temperature. In freezing storage, total bacterial count was fluctuated in all samples of breast meats during 90 days of storage. The coliform bacteria count in breast meat for unlicensed slaughterhouse were increased from 7.65×10^3 to 1.14×10^4 CFU/gm meat and were significant differences ($P\leq0.05$) in all storage periods at refrigeration temperature were found. At freezing temperature, coliform have found only in 0 and 15 days of storage for both unlicensed and licensed. For psychrotrophic bacteria, the count have increased after 6 days of refrigerated storage for both unlicensed and licensed slaughterhouses samples and significant differences were obtained in most storage periods, whereas the count have fluctuated after 90 days of freezing storage. In generally, the results microbial indicators were within the standard limits of permission, Coliform bacteria count revealed that the number was higher than the acceptable count (more than 10^3 CFU/gm meat) in case of unlicensed samples.

INTRODUCTION

Chicken meat have a high nutritional value, low cholesterol, cheap prices compared to red meat and contain less saturated fatty acids level which are the main reasons for arteriosclerosis, and heart diseases due to the deposition on the blood vessels (1). Also, chicken muscle contained not less than 20% protein, high levels of essential amino acids and vitamins which are necessary for human growth, in addition to being easy to prepare and to digest and suitable for all age groups (2). In most countries, two kinds of poultry slaughtering are used, one is an automated poultry slaughtering process established recently, whereby automated systems are used for scalding, plucking, eviscerating, rinsing, and packaging carcasses. Carcasses are then stored at 4°C or freezing before selling to supermarkets. The second is traditional slaughtering, which is commonly practiced in shops under poor hygienic conditions. Most of poultry slaughtering is done by traditional procedures (3). In poorer regions, poultry are often sold live or are slaughtered at the point of sale, and 30% of all the world's poultry be marketed in this way (4). Shelf life is a most important parameters affecting the quality of chicken meat after its distribution to the market. It is the result of poultry management conditions, distribution, processing and storage conditions both on the market and in consumers' households (5). Chicken meat is highly perishable and the time that leads to deterioration varies from four to about twelve days after slaughter, even when maintained in a cooling environment (6). The hypothesis is meat taken from unlicensed slaughterhouses are highly effected by the action of microorganism and therefore leads to unfit meat for human consumption, so the objective of this study are Compare between license and unlicensed broiler slaughter house in sanitary condition by detection of hygienic state of meat from those slaughter house.

MATERIAL AND METHODS

Seventy-two broiler chicken from one source, at same ages and convergent weights (3250-3700 Kg), were divided into two groups. The first group was slaughtered in unlicensed slaughterhouse and then carcasses were cut up and the breast meat were separated, also the second group was slaughtered in licensed slaughterhouse and then carcasses were cut up and the breast meat were separated. All samples were transferred inside a cork chilled box (ice box) to the laboratory. The sensory evaluation was done immediately whenever the samples reached the laboratory. Tests were made in the post-graduate laboratories of Animal Sciences Department,

College of Agricultural sciences, University of Sulaimani, Kurdistan region, Iraq. The chicken breast meat stored at refrigeration (4°C) and freezing (-18°C) temperature.

Breast meat samples from unlicensed and licensed slaughterhouse were stored in two types (refrigerator (4 C°) and freezer (-18 C°), each type had six replicates and each replicates composed from three chicken breast meat as follows:

- Breast of chicken meat that slaughtered in unlicensed slaughterhouse and stored at refrigeration temperature (4±1°C) for 6 days.
- Breast of chicken meat that slaughtered in unlicensed slaughterhouse and stored at freezing temperature (-18±1°C) for 90 days.
- Breast of chicken meat that slaughtered in licensed slaughterhouse and stored at refrigeration temperature (4±1°C) for 6 days.
- Breast of chicken meat that slaughtered in licensed slaughterhouse and stored at freezing temperature (-18±1°C) for 90 days.

Several tests were carried out on the breast meat samples during 4 months from September to the end of December 2015.

Microbiological Tests (Sample Preparation) (7)

Aseptically, 25 ± 0.1 gm of the sample had been weighted without thawing (for frozen samples), softened by putting in refrigerator for 18 hr at 4-5ºC, grinding and mixing with 250 ml of sterile peptone water. This became the 1:10 dilution. The foam was permitted to settle, then 10 ml of the blended 1:10 dilution was pipetted into a 90 ml dilution blank to make 1:100 dilution. The procedure had been repeated to prepare serial dilutions of 10^{-3}, 10^{-4}, etc.

Total plate bacteria count (8)

The dilutions prepared before (previous section) used, 0.1 ml from the 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} etc., dilutions were poured into each of petri dishes. Additional dilutions plates were used when expecting higher bacterial levels. The nutrient agar was allowed to harden and one series of duplicate plates were placed in a 35 ±1ºC incubator for 48 hr. A colony counter has been used to count colonies on the plates in a suitable range (30-300 colonies per plate). Average of the counts was obtained from duplicated plates, multiplied by the dilution factor and reported this number as the aerobic plate count (Standard plate count) per gram at the incubation temperature used.

Total Coliform Bacterial Counts (9)

Coliforms were determined on MacConkey agar containing bile salts (Himedia labs Pvt. Ltd) incubated at 37 °C for 48hr.

Psychrotrophic bacterial count (10)

Nutrient agar was used; 0.1ml of the series dilutions that were prepared earlier was used, then the plates were incubated at 5-7ºC for 10 days. The same procedure for counting was used as in Total Plate count.

Statistical Analysis

The statistical analysis system XLSTAT (11) program was used to analyse the data obtained study. Factorial Complete Randomized Design (CRD), was used to analysis data, The significance of differences between means of traits were determined using Duncan's multiple range tests under the probability ($P< 0.05$) (12).

RESULTS

Total plate count (TPC)

TPC is one of the most important tests performed on meat and its products either fresh or frozen. It considered as an indicator of the safety and health conditions for the consumption of meat and meat products (13; 14).

At the refrigeration storage as seen in table (1), the highest count of total bacterial was found in the last day (after 6 days) of storage for breast meat of unlicensed and licensed slaughterhouses which were (8.51×10^4 and 9.33×10^3 CFU/gm meat) respectively.

The results in the table (1) showed significant difference ($P\leq0.05$) among counts in breast meat of unlicensed and licensed slaughterhouses, in 0 day and after 6 days of storage. TPC in breast meat of unlicensed slaughterhouse after 6 days showed higher significantly ($P\leq0.05$) differences compared to other storage periods and also significant differ between day 0 of unlicensed with all storage days of licensed breast meat. On the other hands, results showed insignificant decrease in T.P.C after 2 days at refrigeration temperature and this may be due to bacteria needed to adapt with new condition.

Table 1: Total plate count (CFU/g meat) of breast meat that stored for different periods at refrigeration (4±1 ºC).

Slaughterhouses	Storage Periods (days)			
	0	2 nd	4 th	6 th
Unlicensed	3.63×10^4 ± 2691.55 *b	2.55×10^4 ± 4814.90 bc	2.56×10^4 ± 4174.71 bc	8.51×10^4 ± 9495.613 a
Licensed	4.13×10^3 ± 901.97 c	1.76×10^3 ± 303.795 c	2.83×10^3 ± 600.92 c	9.33×10^3 ± 1190.238 c

*** Number in the table represent mean and standard error. Means with different letter significantly differ ($P\leq 0.05$).**

For the freezing storage, total plate count for unlicensed slaughterhouses breast was reached to highest count after 30 days of storage and was 6.1×10^4 CFU/gm meat but for licensed, the highest count was 1.05×10^4 CFU/gm meat after 15 days of storage, in breast meat (2).

The TPC in breast meat of unlicensed slaughterhouses was increased during freezing storage until 30 day of storage and then was decreased. For licensed the increased showed only after 15 days of storage. Significant differences ($P\leq0.05$) between counts of slaughterhouses in the 0, 15th and 30th day of storage were also noticed. TPC after 30 days of unlicensed showed higher significantly differences ($P\leq0.05$) compared to other storage periods and also in day 0 and 15th of unlicensed slaughterhouse were differ significantly to other storage days of both slaughterhouses.

Table 2: Total plate count (CFU/g meat) of breast meat that storage for different periods at freezing (-18±1 ºC).

Slaughterhouses	Storage Periods (days)				
	0	15 th	30 th	60 th	90 th
Unlicensed	3.2×10^4 ± 875.00 *b	3.63×10^4 ± 1250.00 b	6.1×10^4 ± 7824.90 a	4.35×10^3 ± 367.42 c	3.76×10^3 ± 545.24 c
Licensed	4.6×10^3 ± 601.90 c	1.05×10^4 ± 426.95 c	6.5×10^3 ± 657.48 c	1.73×10^3 ± 217.46 c	1.4×10^3 ± 213.60 c

***Number in the table represent mean and standard deviation. Means with different letter significantly differ ($P \leq 0.05$).**

Coliform bacteria count

The coliform bacteria used as an indicator of the presence of pathogenic organisms be source from fecal origin, this is because Some of this bacteria is considered one of the causes of severe diseases (15).

Table 3: Coliform bacteria count (CFU/g meat) of breast meat that storage for different periods at refrigeration (4±1 ºC).

Slaughterhouses	Storage Periods (days)			
	0	2 nd	4 th	6 th
Unlicensed	7.65×10^3 ± 396.60 *a	8.34×10^3 ± 108.01 a	9.83×10^3 ± 617.79 a	1.41×10^4 ± 549.81 a
Licensed	2×10^2 ± 25.82 b	2.5×10^2 ± 7.36 b	3.3×10 ± 31.45 b	5.9×10^2 ± 42.50 b

***Number in the table represent mean and standard error. Means with different letter significantly differ ($P \leq 0.05$).**

In the refrigeration storage, as seen in table (3), the highest count of coliform bacteria found after 6 days of storage and was 1.41×10^4 CFU/gm meat in breast

meat of unlicensed slaughterhouse. The coliform bacteria count during 6 days of storage at refrigeration temperature showed significant differences ($P\leq0.05$) between counts in breast meat of slaughterhouses in all storage periods, but not among periods.

At freezing storage, coliform bacteria count in breast meat of unlicensed slaughterhouse was recorded the highest rise (9.87×10^3 CFU/gm meat) and show significant difference ($P\leq0.05$) in counts between unlicensed and licensed breast meat of slaughterhouses in day 0. Coliform bacteria count in day 0 of unlicensed breast meat was higher significantly count with other storage periods (4). After 15 days of storage, coliform bacteria were decreased until 30 days of storage, there was not any growth in both unlicensed and licensed slaughterhouses.

Table 4: Coliform bacteria count (CFU/g meat) of breast meat that storage for different periods at freezing (-18±1 ºC).

Slaughterhouses	Storage Periods (days)				
	0	15 th	30 th	60 th	90 th
Unlicensed	9.87×10^3 ± 588.02 *a	8.33×10^2 ± 70.31 b	0.000 ± 0.000 b	0.000 ± 0.000 b	0.000 ± 0.000 b
Licensed	6.98×10^2 ± 127.92 b	2.3×10^2 ± 21.08 b	0.000 ± 0.000 b	0.000 ± 0.000 b	0.000 ± 0.000 b

***Number in the table represent mean and standard error. Means with different letter significantly differ ($P \leq 0.05$).**

Psychrotrophic bacteria count (PST)

The most dominated type of microorganisms living on meat stored at 0 °C is psychrotrophic bacteria; they are responsible for chilling meat spoilage (١٤). In the refrigeration storage, as seen in Table (5), PSP count achieved highest growth in 6th day of storage and that was 1.27×10^4 CFU/gm meat in breast meat of unlicensed slaughterhouse. Whereas, count of PST in breast meat of unlicensed slaughterhouse was increased during 6 days of storage but for licensed there was almost no differences in growth during the same storage days. Significant differences ($P\leq0.05$) between counts of slaughterhouses within the periods 2, 4, and 6 days of storage were recorded.

After 6 days, PSP in unlicensed showed higher significantly ($P\leq0.05$) differ to other storage periods for breast meat of both slaughterhouses, also day 2nd and 4th of storage for unlicensed were significantly differ with all storage periods of breast meat of licensed slaughterhouse.

Slaughterhouses	Storage Periods (days)			
	0	2 nd	4 th	6 th
Un licence	2.7×10^3 ± 217.94 *bc	3.58×10^3 ± 225.46 b	4.55×10^3 ± 640.80 b	1.27×10^4 ± 230.70 a
Licence	1.16×10^2 ± 29.01 c	1.38×10^2 ± 24.95 c	1.6×10^2 ± 37.76 c	4.15×10^2 ± 45.27 c

***Number in the table represent mean and standard error. Means with different letter significantly differ ($P \leq 0.05$).**

Table 5: Psychrotrophic bacteria count (CFU/gm meat) of breast meat that storage for different periods at refrigeration (4±1 ºC).

At the freezing storage, Psychrotrophic bacteria count in breast meat reached the highest limit after 15 days of storage (1.22×10^4 CFU/g meat) for unlicensed slaughterhouse (table 6).

Results of PST count in breast meat during 90 days of storage were fluctuated for both unlicensed and licensed slaughterhouses. In 0 and 15 days of storage has significant differences ($P\leq0.05$) between counts of slaughterhouses were found.

PST in breast meat of unlicensed slaughterhouse in 15th day showed higher significantly differ ($P\leq0.05$) with other storage periods of both slaughterhouses, also in day 0 of unlicensed slaughterhouse was significantly differ with all storage days of licensed.

Table 6: Psychrotrophic bacteria count (CFU/g meat) of breast meat that storage for different periods at freezing (-18±1 ºC).

Slaughterhouses	Storage Periods (days)				
	0	15 th	30 th	60 th	90 th
Unlicensed	2.56×10^3 ± 121.73 *b	1.4×10^4 ± 1005.87 a	3.3×10^3 ± 77.46 bc	1.68×10^3 ± 63.79 bcd	1.36×10^3 ± 190.9 bcd
Licensed	6.4×10^2 ± 54.91 cd	2.5×10^2 ± 22.36 d	5.66×10^2 ± 54.77 cd	1.26×10^2 ± 16.66 d	1.28×10^2 ± 12.08 d

***Number in the table represent mean and standard error. Means with different letter significantly differ ($P \leq 0.05$).**

DISCUSSION

Al- Dughaym and Altabari (16) considered that microbial count of 10^5 CFU/gm was satisfactory for fresh meat, while count of 10^6 CFU/gm was considered unsatisfactory. Bacterial count of 10^6 CFU/gm for chilled meat was considered satisfactory, but count of 10^7 CFU/gm was considered unsatisfactory. Furthermore, ICMSF, (17) reported that if meat is prepared under unhygienic conditions, the initial count was higher (exceeding 10^6 CFU/gm). Thus, the results of this research for both unlicensed and licensed meat samples were acceptable for sanitary condition.

Haleem *et al.* (19) reported that T.P.C in breast meat was decreased in the first 48h at freezing storage. In poultry meat which stored at -18 °C showed depression in total plate count. Kumar *et al.* (20) reported fluctuation of TPC in breast chicken meat during freezing storage (in 90 days' storage), which support the present results for TPC indicator at freezing storage.

The decreased in total bacteria count in samples during frozen storage due to death of vegetative form of the microorganisms in meat by thermal shock, ice formation, dehydration and high solute concentration (21).

The present results agreed with Yammamoto and Harris, (21) and Gill (22), whose stated that, the number of viable bacteria tends to decline with prolonged frozen storage, during frozen storage some viable bacteria are killed, while others may only damage and can recover when thawing.

Kumar *et al.* (19) reported a comparison between breast meat from market/road side and scientifically slaughtered chicken. They showed the scientifically slaughtered chicken had significantly lower ($P \leq 0.05$) total plate count due to hygienic processing of scientifically slaughtered chicken

Although, the counts for all breast meat samples of the present study were within the standard levels in Iraqi quality regulations IQS 2270/4 and 3725/4 which determine the TPC for frozen chicken between 10^5- 10^7 CFU/gm meat (23,24). The results of our study showed that all frozen breast meat samples were acceptable for sanitary condition because all results were less than 10^5- 10^7 CFU/gm meat.

Some of coliform considered as psychrotophic bacteria and that fall into the indicator organism's categories (such as Enterobacter) and can multiply on refrigerated raw poultry carcasses and products (25, 26) reported that count of coliform bacteria in chicken meat in traditional shops (unlicensed) was more than those from supermarket (licensed).

(27) reported that no coliform bacteria in frozen chicken meat it collected from various Baghdad markets, and growth may be prevented as a result of exposure to environmental stress. Freezing process led to the formation of ice crystals that have prevented the emergence of these bacteria. This percentage increases with time of freezing storage, and freeze at low temperatures (-18 ± 2 °C), it will stop the growth of microorganisms and reduces the numbers and create unfavorable conditions for the growth of the rest of them (28).

Under frozen storage, decreased in coliform count were recorded by Abu-Ruwaida *et al.* (29) during storage of broiler chicken at -18 ºC. (30) revealed that the coliform count in the locally processed samples of breast meat were higher than those imported. Similar, comparison between breast meat from market/road side and scientifically slaughtered chicken, had significantly lower ($P<0.05$) coliform load due to hygienic processing of scientifically slaughtered chicken (19).

Since there was no specific coliform criteria in the Iraqi quality regulations specified for frozen chicken meat, so the comparison was made with limits given by HPFB (31) which determined coliform limit at 10-10^3 CFU/gm meat. Results of our samples from licensed slaughterhouse were within acceptable limits of HPFB (28), but coliform bacteria count showed contamination in day zero and in all storage periods in unlicensed breast meat samples, at refrigeration temperature.

Results of Gallas *et al*. (32) showed the psychrotrophic count in chicken breasts stored under refrigerator condition at 0, 3, 9, 14 days were 3.1, 2.7, 4.4, 6.5 $\log_{10}$ CFU/gm meat respectively, and Malav *et al*. (٣٣) found that the psychrotrophic count in chicken breast meat increase during refrigeration (4±1 °C) storage. (14) had mentioned increasing the Psychrotrophic count within long storage at low temperature. The growth yield and microbial activity of psychrotophic microorganisms are higher at low temperatures compared to temperatures close to the maximum temperature of growth; this has been usually explained as successful microbial adaptation to the natural cold environment (34).

Hedrick *et al*. (20) showed a significant decreased in the counts of PST bacteria from samples stored at freezing condition due to the death of these bacteria in meat by thermal shock, ice formation, dehydration (reduced water activity) and high solute concentration.

The other reason for the higher count could be due to the high initial psychrotrophic bacteria count of the products before freezing which can occur during storage, distribution, handling of the product and temperature abuse or retail display (35).

The same tables showed that the psychrotrophic bacterial count of all breast meat samples were close than total plate count (mesophilic bacteria), this may approve that the increase of count might be due to the contamination of freezers or the storage area with that kind of microorganisms specially *Pseudomonas* spp. (14).

Even ICMSF, (35) confirmed that frozen poultry typically does not undergo microbial spoilage, but they also determined that storage temperature should be controlled to prevent fluctuation which directly have an effect on microbial growth.

CONCLUSION

Initial bacterial count on chicken breast have a direct effect on the shelf-life of fresh product. Chicken breast meat for unlicensed and licensed slaughterhouses were within Iraqi and international standard limits, but the unlicensed were carried more microbial than licensed that lead to exposing them to damage it faster. Coliform bacteria count showed contamination in day zero and in all storage periods in unlicensed breast meat samples, at refrigeration temperature.

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التقييم الميكروبيولوجى للحوم صدر الدجاج من المجازر غير المرخصة والمرخصة أثناء التخزين بالتبريد والتجميد

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الخلاصه

هدفت هذه الدراسة إلى التقييم المايكروبايولوجي للحم صدر الدجاج المأخوذ من المجازر المرخصة وغير المرخصة. تم قياس مؤشرات ميكروبيولوجية مختلفة لتحديد بعض سمات لحوم صدر الدجاج الطازجة المحلية خلال فترات التخزين المختلفة تحت درجة حرارة التبريد والتجميد. أجريت جميع الاختبارات في مختبر الدراسات العليا في قسم علوم الحيوان، كلية العلوم الزراعية، جامعة السليمانية. أظهر المحتوى الميكروبي للحم الصدر في هذه الدراسة أن العد الكلي الميكروبي قد زاد في اللحم ٣.٦٣ × ١٠^٤ إلى ٨.٥٦ × ١٠^٤ وحدة تكوين المستعمرة/ غم ومن ٤.١٣ × ١٠^٣ إلى ٩.٣٣ × ١٠^٣ وحدة تكوين المستعمرة/ غم لحم لكل من المجازر المرخصة وغير المرخصة على التوالي ، في درجة حرارة الثلاجة. في التخزين بالتجميد، تذبذب إجمالي العد البكتيري في جميع عينات الصدر خلال ٩٠ يومًا من التخزين. ازداد عد بكتريا القولون في لحم الصدر من المجازرغير المرخصة من ٧.٦٥ × ١٠^٣ إلى ١.١٤ × ١٠^٤ وحدة تكوين المستعمرة/ غم لحم. وكانت هناك فروقات معنوية ($P\leq0.05$) في جميع فترات التخزين عند درجة حرارة التبريد. عند درجة حرارة التجميد ، لم يتم كشف بكتريا القولون سوى بعد ١٥ يوم من التخزين لكل من غير المرخص والمرخص. بالنسبة للبكتيريا المحبة للبرودة ، ازداد العد بعد ٦ أيام من التخزين المبرد لكل من عينات الصدر من المجازر غير المرخصة والمرخصة وكانت هناك فروق معنوية في معظم فترات التخزين ، في حين تذبذب العد بعد ٩٠ يومًا من التخزين بالتجميدد. بشكل عام ، كانت نتائج المؤشرات الميكروبية ضمن الحدود القياسية المسموحة ، ام البكتيريا القولونية فأن العد كان أعلى من الحد المقبول (أكثر من ١٠^٣ وحدة تكوين المستعمرة/ غم لحم) في حالة عينات اللحوم غير المرخصة.

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