

## Article

# Assessment of Microbial Load in Fresh and Frozen Broiler Chicken Samples from Lahore

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**Abstract:** Contaminated chicken meat and its products pose significant hazards to human health. This study was designed to evaluate the quality and microbial safety of fresh and frozen broiler chicken meat samples. A total of 60 samples (30 frozen, obtained from industrially processed sources, and 30 fresh, collected from open vendors) were randomly selected from six locations in Lahore, Punjab, Pakistan. Quality parameters such as pH and color were assessed. The L\* value (brightness) was significantly higher in fresh chicken meat ( $56.64 \pm 0.693$ ) compared to frozen meat ( $44.46 \pm 1.456$ ). For a\* (red-green coordinate), mean values ranged from  $4.6 \pm 0.442$  to  $8.0 \pm 0.131$  in fresh chicken and  $7.6 \pm 0.584$  to  $13.4 \pm 0.806$  in frozen chicken. Similarly, b\* (yellow-blue coordinate) values ranged from  $5.6 \pm 0.765$  to  $7.60 \pm 0.654$  in fresh chicken and  $7.2 \pm 0.463$  to  $10.6 \pm 0.543$  in frozen samples. Microbial analysis revealed the presence of *Salmonella* spp. and *Listeria monocytogenes* in fresh samples, while frozen samples tested negative. Fecal coliforms showed optimal growth in frozen samples, but overgrowth was observed in fresh samples. *Escherichia coli* O157:H7 was absent in frozen samples but detected in fresh samples from three out of five zones. The total bacterial count in frozen chicken remained within safe limits, whereas fresh samples exceeded acceptable levels. Overall, the study concluded that frozen chicken meat is microbiologically safer and of better quality compared to fresh chicken meat.


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## 1. Introduction

Meat is a valuable and easily accessible source of high-quality animal protein for consumers. Among essential foods, it ranks as one of the most consumed due to its nutrient-rich profile, which also provides a conducive environment for microbial growth [1]. In Pakistan, broiler meat production has shown significant growth, reaching 587,000 metric tons in 2010 [2]. From 1994-1995 to 2015-2016, the annual average growth rates of meat production and exports were reported at 3% and 32%, respectively. Projections based on these trends estimate that by 2029-2030, total meat production in Pakistan could reach 6,078 thousand tons, with exports valued at \$17,477 million [3]. However, concerns regarding the microbiological quality of chicken meat persist, as contamination during processing is a significant challenge [4].

Broiler chicken meat is a crucial dietary component, rich in essential vitamins such as A, D, and E [5]. However, chicken carcasses are frequently contaminated with pathogenic microorganisms like *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7, which are associated with foodborne illnesses, including diarrhea and abdominal pain [6]. Studies indicate that *E. coli*, *Salmonella*, and *Listeria* are major contributors to meat-related illnesses, with *E. coli* accounting for up to 90% of these cases. These pathogens have also been detected in various food products, including raw and cooked

poultry, seafood, shawarma, sandwiches, ready-to-eat (RTE) salads, mayonnaise, and even on the hands of food handlers, highlighting significant food safety concerns [7].

In Pakistan, foodborne diseases such as salmonellosis, shigellosis, campylobacteriosis, cholera, gastroenteritis, and brucellosis are widely recognized. RTE foods are often contaminated with pathogens like *Campylobacter*, *Staphylococcus*, *Salmonella typhimurium*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*. Inadequate sanitation and hygiene practices exacerbate these risks, making foodborne illnesses a serious public health issue, particularly diarrhea, which disproportionately affects children and is a major health concern in the country [8].

Raw chicken is highly susceptible to contamination, harboring microorganisms on its feathers, skin, and intestinal tract. During slaughter, de-feathering, evisceration, storage, and distribution, bacterial contamination becomes inevitable. Boneless poultry products, while versatile, are also prone to microbial spoilage if proper hygiene and handling practices are not followed [9]. Without stringent controls, microbial contamination can result in foodborne illnesses, posing a significant public health risk to consumers. Proper hygiene, storage, and cooking practices are crucial in mitigating these risks [10].

The objective of the current study was to assess the microbial load in fresh and frozen broiler chicken samples collected from various sources at selected sale points in the Lahore market. This study aimed to evaluate and compare the microbiological quality of chicken meat from different outlets, highlighting potential public health risks and the need for improved hygiene and handling practices in the supply chain.

## 2. Materials and Methods

### 2.1 Study Design and Sampling

This study was conducted in the Food Science and Technology Laboratory (Lab No. 102) at the University Institute of Diet and Nutritional Sciences (UIDNS), The University of Lahore. The investigation focused on comparing the quality standards of frozen chicken meat versus fresh local chicken. Specifically, the study evaluated the total bacterial count (TBC), *Escherichia coli* O157, fecal coliform, *Salmonella*, and *Listeria*, along with pH and color characteristics as indicators of microbial quality and meat freshness.

A total of 60 chicken samples were collected, comprising both frozen and fresh meat. The frozen samples were sourced from supermarket chains, while the fresh samples were obtained from local markets in Lahore. For sampling, the city of Lahore was divided into six zones: Gawalmandi (Zone A), DHA-1 (Zone B), Model Town (Zone C), Bhati Gate (Zone D), Kot Abdul Malik (Zone E), and Bahria Town (Zone F). From each zone, five frozen and five fresh chicken samples were collected to ensure a comprehensive assessment of quality standards across different sale points in the city.

### 2.2 Sample Inclusion and Exclusion Criteria

Samples of industrially processed broiler chicken meat were included only if they were properly sealed and stored at  $-18^{\circ}\text{C}$ . Samples from open vendors were included if they were fresh and unpacked. These samples were immediately packed in sanitized polyethylene bags upon collection. Frozen samples with ruptured packaging or those subjected to temperature abuse were excluded. Fresh samples that were old, slaughtered earlier, or pre-packed were not included.

### 2.3 pH Analysis

The pH of chicken meat samples was measured using a digital pH meter (InoLab 720, Germany). The meter was calibrated with standard buffer solutions of pH 7.0 and 4.0 prior to use. The pH measurement was conducted by directly inserting the electrode into the meat samples following the protocol described by others [11].

### 2.4 Color Analysis

The color of chicken meat was assessed instrumentally using a tristimulus colorimeter (CIELAB SPACE, Color Tech-PCM, USA). The instrument was calibrated with standard CTn values of 151 (lightness) and 54 (darkness). Samples were analyzed using a

photocell to determine color values in the CIE Lab Cartesian coordinate system for lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ), following methods described earlier [11, 12].

### 2.5 Microbial Assay

The microbiological quality of chicken meat was assessed by determining the total bacterial count (TBC), fecal coliform (FC) count, *Escherichia coli* O157 (EC) count, *Listeria* spp. and *Salmonella* spp. Samples were plated on selective growth media, including MacConkey agar, Plate Count Agar, and XLT4 (Xylose Lysine Tergitol 4) agar, to enumerate specific pathogens [13, 14].

**Total Bacterial Count (TBC):** The microbiological status of fresh and frozen chicken meat was assessed using a 25 g sample diluted in 225 ml of BPW for a  $10^{-1}$  serial dilution. Pathogens such as Enterobacteria, *Bacillus cereus*, and Staphylococci were analyzed using standard techniques [15].

***E. coli* O157:** A 25 g sample was diluted in 225 ml of Buffered Peptone Water (BPW) to prepare a  $10^{-1}$  serial dilution. The dilution was plated on Eosin Methylene Blue (EMB) agar and incubated overnight at 37°C for 24 hours. Colonies were counted and expressed as CFU/ml [16].

**Fecal Coliform (FC):** The 25 g diluted sample was plated on MacConkey agar and incubated at 37°C for 24 hours. Colony counts were expressed as CFU/ml [16]. ***Salmonella* species:** A 25 g meat sample diluted in 225 ml of BPW to prepare a  $10^{-1}$  serial dilution. The ISO6579 protocol was followed, including serotyping and phage typing to determine contamination levels. Studies from public health laboratories were referenced for baseline contamination rates [17].

***Listeria* species:** Samples were diluted as mentioned above. Different *Listeria* species, including *L. monocytogenes*, *L. welshimeri*, *L. innocua*, and *L. seeligeri*, were detected. Studies were aligned with findings from retail chicken surveys in South Africa, indicating contamination of both fresh and frozen chicken meat [18, 19].

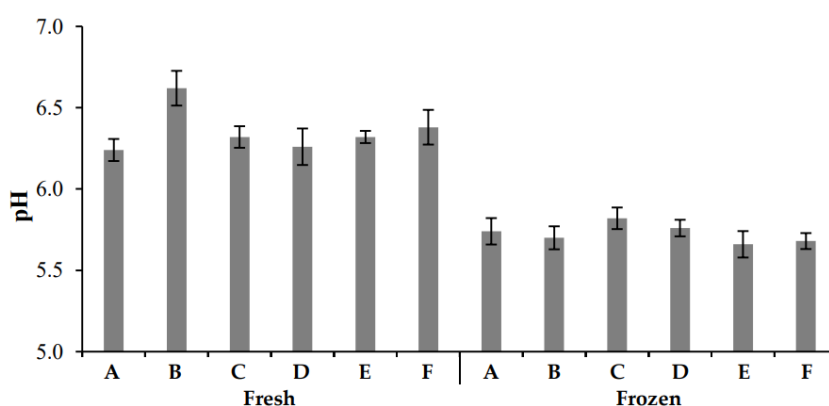
### 2.6. Statistical Analyses

Data were analyzed using the GLM Procedure of SAS (SAS for Academics) under analysis of variance (ANOVA). The sample collection points defined as zones and the frozen vs fresh meat were treated as a fixed effect. Results were considered statistically significant at  $p \leq 0.05$ .

## 3. Results and Discussion

### 3.1 pH of Meat

Figure 1 presents the pH levels in fresh and frozen chicken meat. Results indicated significant differences between fresh and frozen chicken samples as well as between different zones. The highest pH value (5.82) in fresh chicken was recorded in Zone C, followed by Zones D, A, B, F, and E. In frozen chicken, the highest pH value (6.62) was observed in Zone B, followed by Zones F, E, C, D, and A. The means of pH within the fresh and frozen groups were significantly different, while there were no significant differences within each group.

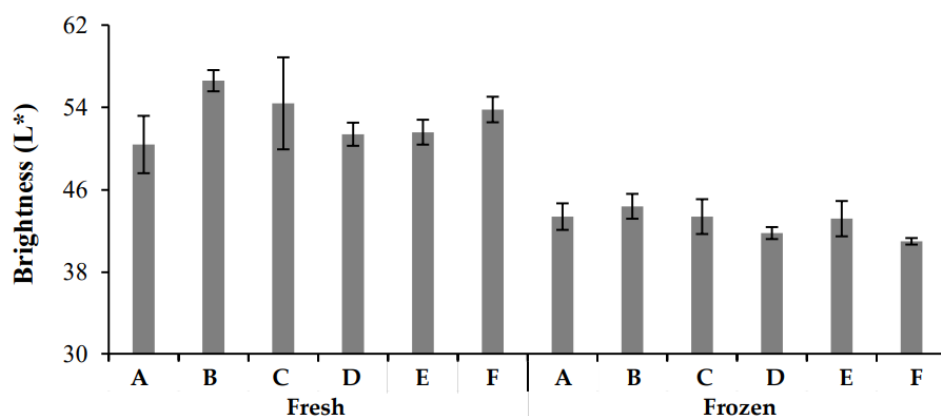


**Figure 1.** Comparison of pH in fresh and frozen chicken meat from various locations (A–F) in Lahore district. Error bars represent standard error of means (SE).

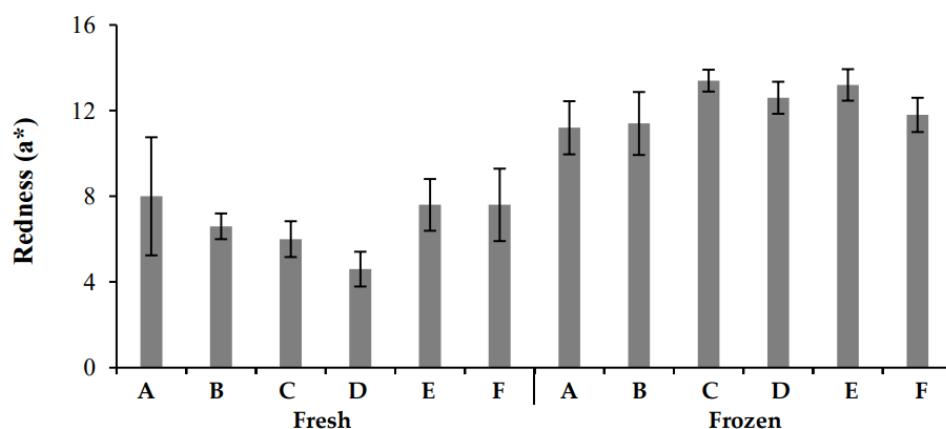
The pH of meat plays a crucial role in its water-holding capacity. Meat with lower pH has reduced water retention compared to meat samples with normal or high pH, which can lead to higher cooking losses and protein denaturation [20, 21].

### 3.2 Color Analysis

In this study, chicken meat color was evaluated using a colorimeter, which measured the L\* (brightness), a\* (redness), and b\* (yellowness) values. The results (Figures 2–4) revealed significant differences in color parameters for both fresh and frozen chicken samples. Fresh chicken samples exhibited higher L\* (brightness) values, ranging from  $56.64 \pm 0.693$  to  $50.42 \pm 0.871$ , compared to frozen chicken, which showed lower L\* values ( $44.46 \pm 1.456$  to  $41.26 \pm 1.603$ ). For a\*, fresh chicken samples ranged from  $4.6 \pm 0.442$  to  $8.0 \pm 0.131$ , while frozen chicken samples ranged from  $7.6 \pm 0.584$  to  $13.4 \pm 0.806$ . The greatest a\* value was recorded in Zone A of fresh chicken ( $12.25 \pm 0.662$ ), while the highest value in frozen chicken was found in Zone B ( $15.52 \pm 0.432$ ). Regarding b\*, fresh chicken samples ranged from  $5.6 \pm 0.765$  to  $7.60 \pm 0.654$ , while frozen chicken samples ranged from  $7.2 \pm 0.463$  to  $10.6 \pm 0.543$ . The highest b\* value was observed in Zone A for fresh chicken ( $12.24 \pm 0.512$ ) and in Zone B for frozen chicken ( $13.22 \pm 0.234$ ). These results indicate significant differences in color characteristics, with fresh chicken showing higher brightness (L\*), while frozen chicken exhibited more redness (a\*) and yellowness (b\*).



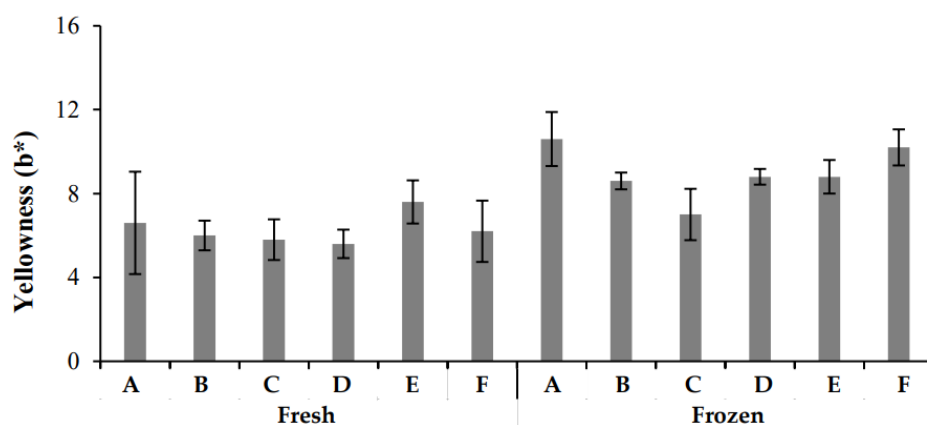
**Figure 2.** Comparison of brightness (L\*) in fresh and frozen chicken meat from various locations (A–F) in Lahore district. Error bars represent standard error of means (SE).



**Figure 3.** Comparison of redness (a\*) in fresh and frozen chicken meat from various locations (A–F) in Lahore district. Error bars represent standard error of means (SE).

Color variations in meat are primarily due to myoglobin oxidation and protein denaturation, both of which are influenced by pH. As pH decreases, meat color becomes

paler, whereas higher pH results in a more red color. The pinkish-red color of meat is due to myoglobin oxidation, which then turns into oxymyoglobin and eventually into metmyoglobin, giving the meat a brownish hue [22]. The oxidation of myoglobin is reversible.

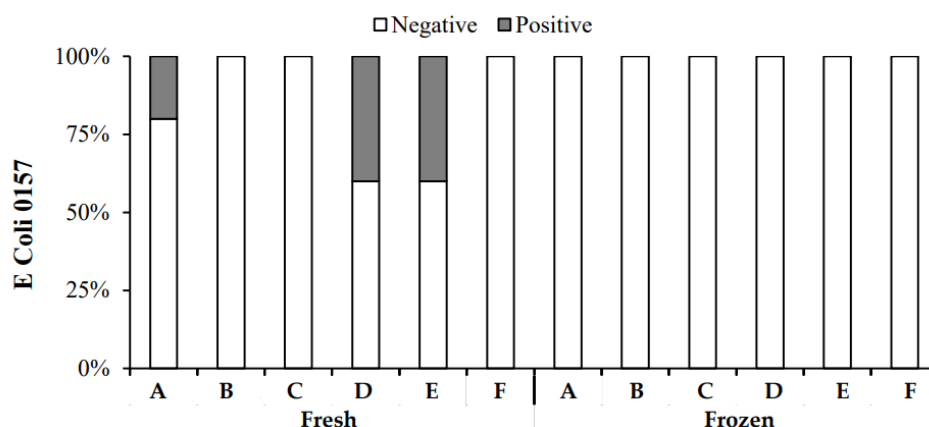


**Figure 4.** Comparison of yellowness ( $b^*$ ) in fresh and frozen chicken meat from various locations (A–F) in Lahore district. Error bars represent standard error of means (SE).

### 3.3. Microbial Assay

#### 3.3.1 E. coli Count

In this study, both fresh and frozen chicken meat samples from various zones in Lahore were tested for *E. coli* O157:H7. The results showed that no *E. coli* O157:H7 was detected in any of the frozen chicken samples. In contrast, fresh chicken samples from Zones A, D, and E were found to be positive for *E. coli* O157:H7, although other fresh samples were negative. The results (Figure 5) showed that *E. coli* O157:H7 was not detected in frozen chicken and was only present in certain fresh chicken samples.



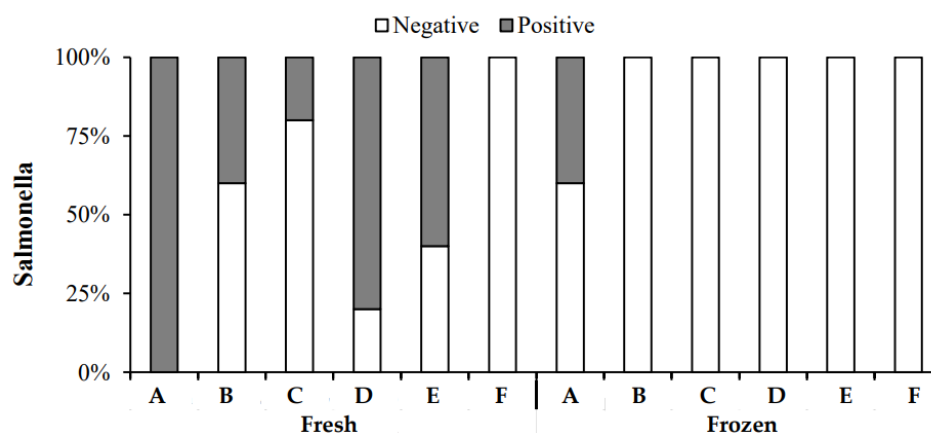
**Figure 5.** Comparison of *E. coli* 0157 presence in fresh and frozen chicken meat from various locations (A–F) in Lahore district. Error bars represent standard error of means (SE).

*E. coli* is a key indicator of meat quality and safety [23, 24]. *E. coli* O157:H7 is a particularly concerning strain, often associated with meat and linked to several outbreaks globally. For instance, an outbreak in the USA in 2002 was traced to contaminated beef, resulting in the hospitalization of several individuals [25, 26].

#### 3.3.2 Salmonella

In this study, the presence of *Salmonella* in fresh and frozen chicken samples was assessed using traditional methods. Figure 6 presents the results, which showed that *Salmonella* was detected in some fresh chicken samples. Specifically, all samples from Zone A of fresh chicken were positive for *Salmonella*, and some samples from Zones B, D, and

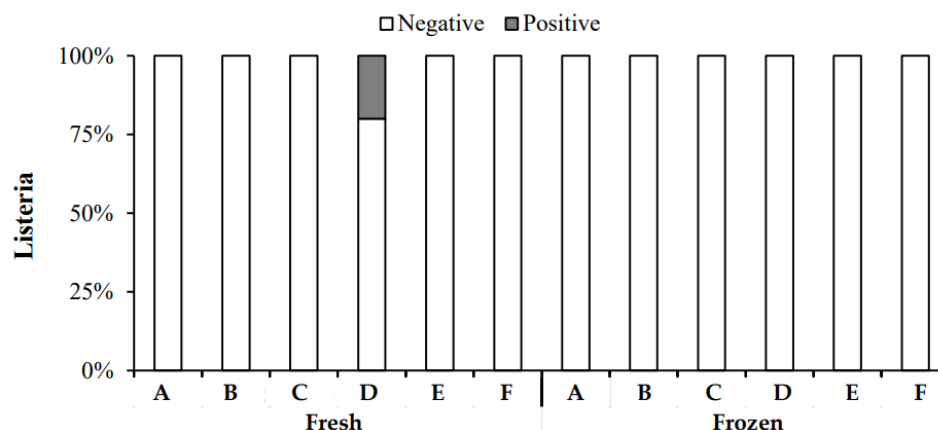
E also tested positive. However, all frozen chicken samples from the six zones tested negative for *Salmonella*, except for a few positive samples in Zone A. *Salmonella* is one of the most common foodborne pathogens, with many outbreaks linked to meat products [27].



**Figure 6.** Comparison of salmonella detection in fresh and frozen chicken meat from various locations (A–F) in Lahore district. Error bars represent standard error of means (SE).

### 3.3.3 *Listeria*

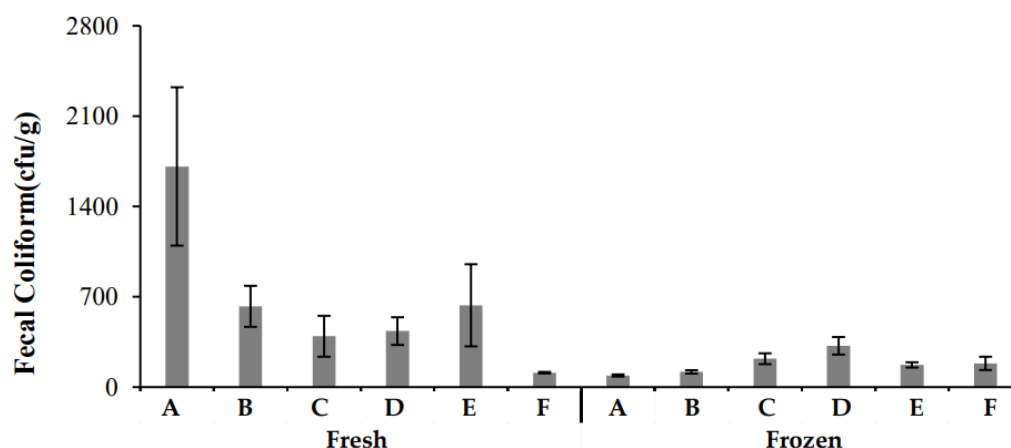
In this study, *Listeria* was tested in both fresh and frozen chicken samples. The results showed that *Listeria* was not detected in most samples, with only one fresh sample from Zone D testing positive (Figure 7). Overall, the prevalence of *Listeria* in both fresh and frozen chicken samples was low. *Listeria monocytogenes* is a significant pathogen in meat products due to its ability to grow under refrigerated conditions, making it a concern for both fresh and frozen meat [28].



**Figure 7.** Comparison of listeria detection in fresh and frozen chicken meat from various locations (A–F) in Lahore district. Error bars represent standard error of means (SE).

### 3.3.4 *Fecal Coliform*

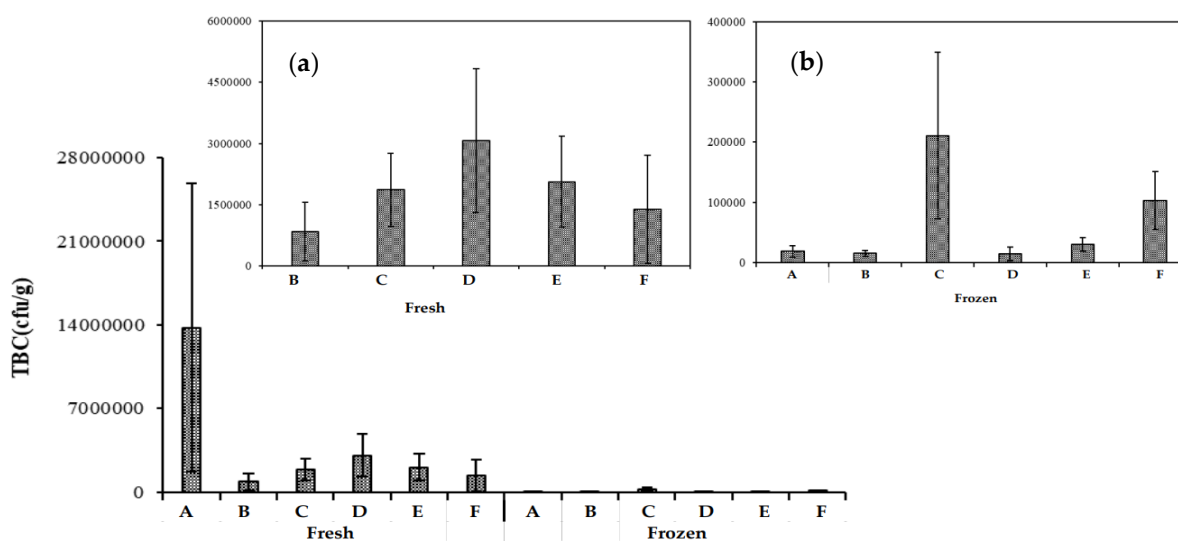
The results of fecal coliform testing (Figure 8) indicated that fresh chicken samples generally had higher fecal coliform counts compared to frozen samples. In particular, samples from Zone A showed the highest contamination levels, followed by Zones B, C, D, E, and F in descending order. Fecal coliforms, particularly those originating from improper sanitation practices, are a key indicator of meat contamination. High fecal coliform counts are associated with poor hygienic conditions during processing and handling [23, 29].



**Figure 8.** Comparison of fecal coliform count in fresh and frozen chicken meat from various locations (A–F) in Lahore district. Error bars represent standard error of means (SE).

### 3.3.5 Total Bacterial Count

Total bacterial count (TBC) is a standard method for assessing the microbiological quality of meat [30]. Chicken meat, both fresh and frozen, is prone to bacterial contamination, which can lead to foodborne illnesses [31]. The TBC results for fresh and frozen chicken samples from different zones are presented in Figure 9. The mean TBC values for frozen chicken ranged from  $1.38 \times 10^7$  to  $1.39 \times 10^6$ , while the mean TBC for fresh chicken ranged from  $1.83 \times 10^4$  to  $2.11 \times 10^5$ . These findings indicate that frozen chicken meat had higher bacterial loads compared to fresh meat, in line with other studies [32, 33].



**Figure 9.** Comparison of total bacterial count (TBC) in fresh and frozen chicken meat from various locations (A–F) in Lahore district. Panels (a) and (b) present the same TBC values on a smaller scale, separately for fresh and frozen chicken meat. Error bars represent standard error of means (SE).

## 4. Conclusion

This study concluded that frozen chicken meat is microbiologically safer and of better quality compared to fresh chicken meat. Frozen samples demonstrated lower microbial contamination, including the absence of *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7, while fresh samples exhibited overgrowth of fecal coliforms and higher total bacterial counts. Additionally, quality parameters such as color and pH indicated better preservation in frozen chicken. These findings suggest that frozen chicken meat poses fewer health risks than fresh chicken meat in the studied market conditions.



**Author Contributions:** Conceptualization, H.M.Z. and M.A.; methodology, H.M.Z., M.A. and A.A.; formal analysis, H.M.Z. and A.A.; resources, M.A., N.G. and A.A.; data curation, H.M.Z.; writing—original draft preparation, H.M.Z., M.A. and N.G.; writing—review and editing, N.G. and M.A. All authors have read and agreed to the published version of the manuscript.

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