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Effects of different slaughterhouse water chiller temperatures on the microbial quality of poultry carcasses

Seyedeh Ommolbanin Ghasemian[1*], Abbas Fardaei[1], Ehsan Gharib Mombeni[2]

1- Department of Veterinary, Behbahan Branch, Islamic Azad University, Behbahan, Iran
2- Department of Veterinary, Shoushtar Branch, Islamic Azad University, Shoushtar, Iran

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Abstract

Pathogenic bacteria are the cause of many human food poisonings during the consumption of contaminated poultry products. This study was conducted to investigate the difference in the microbial load of poultry carcasses slaughtered at different temperatures in water chillers in the slaughterhouses of Kohgiluyeh and Boyer-Ahmad provinces, Iran. One hundred twenty samples were taken randomly, under sterile conditions from chillers with temperatures of 24, 10, and 4 °C. The total count of microorganisms and bacterial isolation was done according to the national standard of Iran. The results showed that 3.3% of the samples were above the permissible limit in terms of the number of microorganisms, and all the positive samples belonged to chillers with a temperature of 24 °C. Furthermore, 28.3% of the samples were positive for contamination with *E. coli* and the highest contamination belonged to the first chiller (24 °C). In addition, 16.2% of the samples were reported to be positive for *Salmonella* spp. This study showed that the cooling steps significantly ($p < 0.05$) decreased the number of microorganisms, *E. coli* and *Salmonella* spp. *E. coli* and *Salmonella* bacteria could be isolated from the studied poultry carcasses at all stages. Although the microbial load of carcasses decreases after cooling in chillers, due to cross-contamination with some bacteria, such as *E. coli* and *Salmonella* spp., there is a need to comply with health standards to modify the slaughter process and use other kinds of chillers that are not less carcasses combination such as air chillers instead of water chillers. Besides, as the *Salmonella* spp. Sources are mainly from the intestinal should special attention be paid to the discharge of internal organs during the slaughtering process and the reduction of *Salmonella* spp. Contamination during the breeding period in broiler farms.

Introduction

Chicken meat is one of the most important sources of protein and minerals for humans, and its consumption has increased in recent decades in many countries of the world, despite having proteins with high digestibility, it is considered one of the sources of microbial contamination. It is also known to be pathogenic to humans. In many countries, one of the biggest challenges for the poultry industry is the contamination of this product

***Corresponding author:** *ghasemian1249@yahoo.com*
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with pathogenic microorganisms (1). Contamination of chicken carcasses with pathogenic microorganisms occurs from two main sources: the slaughterhouse environment (including processes, equipment, and workers' hands) and the live chicken (including digestive contents, skin contamination, etc.). The transport and distribution stages can significantly impact the generation and spread of contamination of carcasses (2). Research has shown that chilling chicken carcasses in the final stages of the slaughter process are crucial in maintaining chicken quality. The contamination of the cold-water tank can be attributed to several factors, including chicken contamination before entering the tank, the amount of alternative overflow water per carcass, and the ratio of chicken carcasses to water volume in the tank. The transmission of infections and poisoning through food has been increasing, particularly in countries with low levels of health. This has resulted in significant economic and human losses (3).

Cooling poultry carcasses in the final stages of the slaughter chain is considered one of the important factors in maintaining the quality of chicken meat. After slaughtering; biochemical, physical, chemical, and histopathological changes occur due to autolytic and bacterial activities. Temperature is one of the important and effective factors in accelerating these changes (4). The faster the poultry meat reaches the desired low temperature (the depth of the breast muscle 0.5 to 4 °C), the desired changes will be the least. Therefore, cooling the chicken carcasses is one of the important steps in the chicken slaughter chain. The effective factors in the degree of contamination of the carcasses in the cooling method using water chillers can include: the contamination load of the carcasses before cooling, the amount of running water, and the replacement for each carcass is the ratio of the number of carcasses to the amount of cooling water and the amount of free chlorine in the cooling water (2, 5). The primary method for cooling chicken carcasses in industrial slaughterhouses is immersion in water chillers. However, it is important to note that due to the potential for bacterial contamination and high microbial load of chicken carcasses before entering the cooler, as well as the high ratio of carcass to water volume and the absence of free chlorine levels in water coolers, there is a risk of increased microbial load and contamination with foodborne bacteria in the chicken carcasses after cooling (6).

Meat and chicken carcasses are one of the main sources of contamination and spread of zoonotic pathogenic bacteria, including *Salmonella* spp. and *Escherichia coli* (7-9). *Salmonella* spp. is one of the main causes of foodborne diseases in human societies, which are of great interest today regarding severity factors. In addition to *Salmonella* spp., *E. coli* is a very important foodborne pathogen that can cause various syndromes in addition to intestinal disease and poisoning (10, 11). According to the conducted studies and published reports (12-14), the prevention of primary and cross-contamination in meat and chicken carcasses is of utmost importance, and the chilling stage is a critical point regarding contamination. Therefore, a study is needed to optimize the temperature of water chillers used for reducing microorganisms.

Materials and Methods

Region and slaughterhouses

This study was conducted in the Kohgiluyeh and Boyer-Ahmad provinces, in Iran. For this purpose, three industrial chicken slaughterhouses in the province were investigated (**Table 1**). The slaughtered chickens are transferred to the scalding tank for soaking and stuffing. This point is considered one of the most critical points in the poultry slaughterhouse. Soaking in hot water is done in two ways: mild hot water (42-52 °C) and hot water (58-62 °C). At this stage, a suitable combination of water, time, and temperature is required to perform the filling operation efficiently. Cooling is done in special cooling tanks or chillers for this purpose. Tanks usually have two parts; in the first part, the water temperature is 10-24 °C and the temperature of the second part is 0-4 °C. The

temperature of the second part is provided by adding ice powder. In the spiral part of the chillers, the chicken is pushed forward by the rotary movement of the chiller's spiral blades and rubbed against each other, and while being washed, it is cooled to 10-24 and 0-4 °C.

Sampling

One hundred twenty samples of slaughtered chickens were randomly taken from slaughterhouses in the Kohgiluyeh and Boyer-Ahmad provinces (**Table 1**). Samples were taken from the neck skin, skin and chest muscles, and thigh muscles. These were subsequently considered as one sample. Each sample was divided into three parts, one part for the detection and isolation and general counting of microorganisms, one part for the isolation and detection of *E. coli*, and the third part for the isolation and detection of *Salmonella*, and a total of 360 samples were used for microbial culture tests. Sampling was done from the first chillers with a temperature of 10-24 °C and the second with a temperature of 0-4 °C. After sampling from the first chiller, colored ribbons were tied to the feet of the chickens, and in the sampling from the second chiller, the same chickens from the first series were sampled. The samples were promptly transferred to the Food Microbiology Laboratory at Behbahan Azad University, Behbahan, Iran, using a cool box with ice.

Microbial evaluation

One hundred twenty samples were evaluated for the total count of microorganisms. After cultivation, the microorganism count was done according to standard 5272-1 of the National Food Standards of Iran (*Escherichia coli* 2496 and *Salmonella* spp. 1810-1; 15). Based on this, samples with an abundance of more than 100,000 microorganisms (10^5) counted per gram of tested food were considered positive, and lower than this number of microorganisms were considered permissible. Briefly, the culture media Lauryl Sulfate Broth, *Escherichia Coli* Broth, Tryptone Water Broth and Eosin methylene blue were used for *E. coli* and culture media Buffered Peptone Water, Rappaport-Vassiliadis soya peptone broth, Selenite Cystine Broth and XLD Agar, for Xylose-Lysine-Deoxycholate were used for *Salmonella* spp. Plate Count Agar culture media was used to evaluate the total count of microorganisms.

The samples (120) were cultured and analyzed for the detection and counting of *E. coli* strains based on standard 2496 of the national food standards of Iran. Based on this, the number of more than 50 colonies per gram of food in the culture environment is considered positive and above the permissible limit, and below this number is considered negative.

All media and plates are incubated in a 37°C incubator (Memmert) for the recommended period, as outlined in general microbiology references.

Statistical analysis

The SPSS version 27 software was used for statistical analysis of data. The average value of the microbial count and the count of each obtained bacteria was expressed as a logarithm based on 10. The significance limit was defined as less than $p < 0.05$. The Chi-square test (Chi-square 2) was used for data analysis. A Paired Sample-Test was used to compare the average microbial counts at temperatures of 4, 10, and 24 °C. In addition, all experiments were performed in three replicates.

Results

Out of 120 tested samples, 4 cases (3.3%) counted the number of microorganisms per gram of food was higher than the limit, and 356 cases (96.7%) were lower than the limit. Frequency distribution of samples with microorganism counts higher than the permissible limit in terms of the temperatures of the studied chillers, all four reported cases were reported in chillers with a temperature of 24 °C (100% at a temperature of 24 °C). Frequency distribution of the studied cities regarding the most contamination with counted microorganisms and above the permissible limit, all 4 cases belong to Boyer Ahmad city. Frequency distribution of the overall count of microorganisms, in terms of the studied slaughterhouses, the number of 4 cases is

higher than the limit of isolated microorganisms belonging to slaughterhouse number 1 (Table 1).

Out of 120 cultured samples, 34 samples (28.3%) with more than 50 CFU/g in the culture media tested positive. Based on this, out of 34 positive samples, 20 samples (58.8% of positive samples) were positive at 24 °C and 6 samples (17.6% of positive samples) at 10°C and 8 samples were reported as positive at 4 °C. Statistically, a significant decrease in *E. coli* contamination was observed at temperatures of 10 and 4 °C ($p < 0.05$; Table 3).

Frequency distribution of *E. coli* contamination of chicken samples slaughtered in chillers with temperatures of 24, 10, and 4 °C in terms of the slaughterhouse studied, out of the 34 reported positive cases, 18 cases (53%) belong to slaughterhouse number 1, 12 cases (35.2%) belonged to slaughterhouse number 2 and 4 cases (11.8%) belonged to slaughterhouse number 3 (Table 1).

Out of 120 chicken samples taken, 20 samples (16.2%) were positive for *Salmonella* spp, and 100 samples (83.8%) were found to be *Salmonella* negative. Out of 20 *Salmonella* positive samples, 10 samples (50% of positive samples) belonged to chillers with a temperature of 24 °C and 10 samples (50% of positive samples) belonged to chillers with a temperature of 4 °C. None of the 10 °C chillers were positive for *Salmonella*. Out of 20 *Salmonella*-positive chicken samples from the studied slaughterhouses at 24 °C temperature, 3 samples (7.5% of positive samples) were positive in both 24 °C and 4 °C chillers. Statistically, a significant decrease in *Salmonella* contamination was observed at a temperature of 10 °C ($p < 0.05$), but no significant difference was observed at a temperature of 4 °C (Table 3). Distribution of *Salmonella* infection frequency of chicken samples slaughtered in chillers with temperatures of 24, 10, and 4 °C according to the studied city, out of the 20 *Salmonella*-positive cases reported, 12 cases (60%) belong to the slaughterhouses of Boyer Ahmad County and 8 the cases (40%) belonged to the studied slaughterhouse in Gachsaran county. Distribution of the frequency of *Salmonella* contamination of chicken samples slaughtered in chillers with temperatures of 24, 10, and 4 °C in terms of the slaughterhouse studied, out of the 20 reported positive cases, 8 cases (40%) belong to slaughterhouse number 1, 4 cases (20%) belonged to slaughterhouse number 2 and 8 cases (40%) belonged to slaughterhouse number 3 (Table 1).

Table 1. Frequency distribution of samples taken from slaughterhouses in the province

Slaughterhouse	County	NS for the total count	*E. coli* NS	*Salmonella* spp. NS	Total NS	Percent (%)
1	Boyer-Ahmad	48	48	48	144	40
2	Boyer-Ahmad	48	48	48	144	40
3	Gachsaran	24	24	24	72	20
Total	3	120	120	120	360	100

NS: Number of samples

Table 2. Frequency distribution of microbial culture samples in terms of *E. coli* and *Salmonella* spp.

Bacteria	Number of samples	Positive (%)	Negative (%)
E. coli	120	34 (28.3)	86 (71.7)
***Salmonella* spp.**	120	20 (16.2)	100 (83.8)

Table 3. The effect of temperature of chillers on contamination cases

Bacteria	Number of positive samples	Temperature (°C)		
		4	10	24
E. coli	34	**8**	**6**	20
***Salmonella* spp.**	20	10	**0**	10

The underlined line indicates a statistically significant difference ($p < 0.05$).

Discussion

The results of the study showed that out of 120 studied samples, in terms of the total count of microorganisms, there was 3.3% of contaminated exceeded the permissible limit, and 100% of the contaminated samples belonged to chillers with a temperature of 24 °C, which indicates the good health status of the chillers. In terms of the amount of contamination with different microorganisms, compared to the studies conducted in Iran and other parts of the world, there is a lower amount of contamination in terms of the overall count of microorganisms in the studied chillers. Furthermore, the low level of contamination in chillers at 4 and 10 °C indicates a good health condition and sufficient watering in the chillers in a cascade manner and shows that the amount of sufficient water and compliance with health standards can reduce the microbial load of water chillers.

Allen et al. reported that water chillers significantly impact carcass microbial quality through temperature and chlorine content (16, 17). Karimi and Akhoundzadeh studied the role of water chillers in the status of *Listeria monocytogenes* contamination of chicken carcasses in the industrial slaughterhouse of West Azarbaijan province (Iran). Their results showed that out of 180 chicken samples tested before entering the chillers, three were found to be *Listeria monocytogenes* positive, while 12 carcasses were evaluated positive for *Listeria monocytogenes* after entering the water chiller (18).

Mugabe et al. (2024) in the total count of bacteria, *Enterobacteriaceae*, *Listeria monocytogenesis*, *E. coli*, *Salmonella*, and *Staphylococcus aureus* in chicken carcasses at different stages of slaughtering. The results showed that the scalding stage and filling of the water chiller, there is the most important stage in the contamination of chicken carcasses in the slaughterhouse (19). Naghizadeh and Radmehr (2004) studied the microbial contamination of water used in different stages of poultry slaughtering in industrial slaughterhouses of Mazandaran province (North of Iran). They concluded that there is no significant difference between the microbial contamination of inlet water and washing water, while this difference between the amount of microbial contamination of the inlet water and the washing water of the chillers (20). Water chillers can be contaminated from earlier stages or because of the existing water. Studies have shown that *Salmonella* spp., *Campylobacter*, and *E. coli* can survive chiller steps and spread contamination. Therefore, chiller temperature and chlorination are very important (21).

Conclusion

This study showed that although the total count of microorganisms is acceptable in the water chillers of slaughterhouses in the province, the level of contamination with *Salmonella* and *Escherichia coli* in the chillers is very high, and considering that in the chillers with low temperatures and at the end of the slaughter line, the level of contamination decreases significantly, However, water chillers are considered one of the critical points of the slaughter

line due to the contact of the carcasses and the contamination of the water in the chillers by contaminated carcasses. The temperature of water used in chillers is one of the main factors affecting the microbial quality of carcasses. The results demonstrated that the samples subjected to temperatures of 4 and 10 °C exhibited the lowest levels of microorganisms. Furthermore, the results indicated that temperature is an effective factor in bacterial contamination. It can be hypothesized that adding a food-safe detergent to chiller water may prove beneficial. In some areas of the world and Iran where chlorine is used as water disinfection in chillers within the permissible limit, the level of contamination shows a reduction.

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Ethical approval

Our research protocol was approved by the Animal Research Ethics Committee of the University of the Behbahan, and the research was conducted by their guidelines and standards.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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