Effect of Cooled and Chlorinated Chiller Water on *Campylobacter* and Coliform Counts on Broiler Carcasses during Chilling at a Middle-Size Poultry Processing Plant

Mitsuhiro KAMEYAMA1), Takehisa CHUMA2)**, Tadahiro NISHIMOTO1), Hiroyuki ONIKI1), Yasuo YANAGITANI1), Ryouichi KANETOU1), Kouichi GOTOU1), Francis SHAHADA3), Hiroyuki IWATA4) and Karoku OKAMOTO2)

1)Iwakuni Health Welfare and Center of Yamaguchi Prefecture, 6264-3 Kuga-machi, Iwakuni, Yamaguchi 742-0433, Japan
2)Department of Veterinary Public Health, Faculty of Agriculture, Kagoshima University, 1–21–24 Korimoto, Kagoshima 890–0065, Japan
3)Safety Research Team, National Institute of Animal Health, 3–1–5 Kannondai, Tsukuba, Ibaraki 305–0856, Japan
4)Department of Veterinary Medicine, Faculty of Agriculture, Yamaguchi University, 1677–1 Yoshida, Yamaguchi 753–8515 Japan

(Received 8 April 2011/Accepted 23 August 2011/Published online in J-STAGE 6 September 2011)

ABSTRACT. To evaluate the effect of cooled and chlorinated chill water for *Campylobacter* and coliforms at a middle-size processing plant which was considered to be difficult for eliminate pathogenic bacteria on carcasses, following three conditions were examined; keeping temperature at < 20, < 10 and < 10 °C, which was considered to be difficult for eliminate pathogenic bacteria on carcasses, following three conditions were examined; keeping temperature at < 20, < 10 and < 10 °C, and chlorine concentration at < 50, < 50 and 50 to 70 ppm during processing in experiment 1, 2 and 3 respectively. Fifteen prechill and 15 postchill carcasses were examined in each experiment. In lower temperature of experiment, chilling process, chlorine concentration, MPN.

NOTE

Public Health

*Campylobacter* is one of the main causative agents of human bacterial gastroenteritis worldwide. Human campylobacteriosis is occurred to food-borne transmission either directly or indirectly which is thought to be the most important route of infection. Poultry, mainly chicken and their products are known to be a major source of infection, and they are frequently contaminated with high numbers of campylobacters [3, 10, 15].

Contamination of chicken products by *Campylobacter* may occur during processing, dismantling, and consumption, although contamination frequently occurs at the processing stage. The investigation through four processing steps (hanging, post-defeatheing, post-evisceration and post-chilling) revealed that the proportion of carcasses contaminated with these microorganisms increased during evisceration step and significantly reduced during chilling [6]. It was reported that the number of *Campylobacter* on carcasses increased after evisceration in three processing plants and that scalding was the most effective process for decreasing overall microbial levels [9]. Intestinal cuts are the important source of contamination on carcasses occurred during evisceration step. The incidence of intestinal cuts was reported to be ranged from two to 34% of carcasses.


©2012 The Japanese Society of Veterinary Science
against microbiological contamination at a middle-size poultry processing plant by evaluating the effects of various water temperature of chiller water and chlorine concentration on the number of Campylobacter and coliform bacilli on carcasses before and after immersion chilling.

A middle-size poultry processing plant with the capacity of processing approximately 3,000 birds per day at a processing speed of 20 birds per min in western Japan was investigated. The plant has an inside-outside carcass washer machine equipped after evisceration line and only single immersion chiller tank with the capacity of 6.5 m³ in which washed carcasses are usually immersed for 30 to 60 min. The water flow system is not a counter-current flow, and not a replacing flow system, namely, only circulated the chill water in the tank. In addition, the tank has no over-flow system to prevent contamination from dropped water to the carcasses or various process machines. Three experiments with different conditions were conducted to evaluate cooling effects at different chiller water temperatures, and decontamination efficacy at different concentrations of sodium hypochlorite (NaClO) solution. Each experiment was conducted in different days during July to October 2009. Two thousands seven hundreds, 2,700 and 2,856 broilers which were originated from same broiler farm had processed at experiment 1, 2 and 3, respectively. Initial chiller water volume and additional timings of large amounts of crushed ices and 12% NaClO solution in chiller water in each experiment were shown in Fig. 1. Initial water temperature was measured after crush ices had added, and in experiment 2 and 3, to lower chill water temperature under 4 °C, larger amount of crush ices were added than in experiment 1 before processing started. One hundred milliliters of chiller water were collected, using sterile bottles, at the inlet and outlet sections of the chiller. Chiller water samples were collected seven times as following; before starting of the carcass chill process, and then 25, 50, 75, 100, 130 and 190 min after immersion chilling. Sampling after 25 min reflected the fact that approximately 500 postchill carcasses were processed, 130 min was the time when the final carcass was immersed into the chiller, and 190 min represented the end of whole chilling process. Thirty swabs of 25 cm² breast areas were obtained in each experiment. The sampling was conducted five times that was at 25, 50, 75, 100 and 130 min after the beginning of chilling process. After a washing stage, each time each three carcasses were removed from shackles, marked with plastic tags at the ankle, and 25 cm² right side of breast area of each carcass was swabbed. Then, the same carcasses were traced. Lastly, 25 cm² left side of breast area of these marked carcasses were sampled after exiting the chill tank. All swab samples were suspended in 0.1% peptone buffer, cooled with ice bags, and transferred to the laboratory on the same day of sampling. Chill water temperature ( °C) was measured using a digital thermometer and concentration of residual chlorine (ppm) was measured using a simple test kit (Pack Test Residual Chlorine, Kyoritsu Chemical-Check Lab. Corp., Japan) involving potassium iodide color comparison method according to the manufacturer’s guidelines at the start and end of carcass chilling at the inlet and outlet sections of the chiller, and a mean value of the two points was calculated. Two different bacterial count methods were used in this study. The most probable number (MPN) method was

![Fig. 1. Initial chiller water volume and additional timing of ices and NaClO solution during processing in each experiment. ICE, large amount of ices added in chiller water. Cl, twelve percent NaClO solution added in chiller water (volume, l).](image-url)
applied to the samples that collected in chill water and on postchill carcasses of *Campylobacter* and in chill water of coliform bacilli which predicted a low amount of bacteria might recovered. An another method, the direct plating method, was applied to the samples that collected on prechill carcasses of *Campylobacter* and on pre- and postchill carcasses of coliform bacilli which predicted more than 1,100 cfu/25 cm² bacteria might recovered [12, 13]. The Preston *Campylobacter* selective enrichment broth (Nutrient broth No. 2 supplemented with Preston Campylobacter selective supplement, Oxoid, LTD, Hampshire, England) supplemented with 5% defibrinated horse blood and Campylobacter Blood-free Selective Agar Base supplemented with CCDA supplement (Oxoid, LTD) plate were used to grow and select *Campylobacter*, respectively. Statistical analysis was performed in an Excel spreadsheet. Both numbers of *Campylobacter* and coliform bacilli recovered from pre- and postchill carcasses were transformed log₁₀ cfu or MPN/25 cm², then means of 15 (each number of experiments) carcasses and decreasing rate (%) of *Campylobacter* and coliforms in each experiment were calculated. Differences between the data from pre- and postchill were analyzed by t test, and differences among the data from experiment 1 to 3 were analyzed by using Microsoft Excel Analyze-It statistical tests for ANOVA. Statistical significance determined at $P \leq 0.05$.

Relationships between chiller water temperature and chlorine concentration, and bacterial counts in each experiment are shown in Fig. 2. No *Campylobacter* were detected in experiment 3 except for 75 min after processing (1.96 log MPN/100 ml), and number of coliform in chill water in experiment 2 and 3 were lower than that of experiment 1. Numbers of *Campylobacter* on postchill carcasses were significantly decreased although low temperature and high chlorine conditions of experiment 1 ($P<0.01$). Moreover in experiment 3, *Campylobacter* were not recovered from all postchill carcasses (0.18 log MPN/25 cm²). Numbers of coliform on postchill carcasses were also decreased significantly in experiment 2 and 3 (Table 1). In comparison among three experiments, number of *Campylobacter* and coliforms on carcasses were significantly decreased in experiment 3 and 2, respectively ($P<0.05$ and $<0.01$, respectively).

In experiment 1, it was observed that 3.96 log CFU/25 cm² of *Campylobacter* were recovered from prechill carcasses. The number was relatively higher when compared

---

**Fig. 2.** A; Water temperature and chlorine concentration in chiller water. ▲ temperature; ●, chlorine concentration. B; Number of coliform bacilli recovery in chiller water. C; Number of *Campylobacter* recovery in chiller water. * < 30 MPN / 100 ml.
with following reports, 1.71, 2.39 and 3.04 log CFU/1,000 cm² of the organism was recovered from prechill carcasses at three processing plants in the United States, respectively [9], and 0.3 MPN/cm² of the organism obtained from prechill carcasses at a large-size processing plant which had a capacity of 19,000 birds per day in Japan [18]. The differences of capacities or equipment between plants may be one of the reason why high contamination was occurred at the plant we examined. On the other hand, Campylobacter counts were significantly reduced on postchill carcasses as opposed to the coliforms. Furthermore, there were some postchill carcasses which coliform counts increased when compared with prechill carcass coliform counts. These observations could provide the evidence that cross-contamination has occurred in chiller water. Previous reports [16, 19] have described cross-contamination occurring frequently during processing especially defeathering, evisceration and chilling stages. In experiment 2, whereby chiller water temperature ranged from 2 to 9 °C, the number of coliforms was significantly lower than those observed in the preceding experiment. This indicates that keeping the chiller water temperature low is somewhat but surely effective on the reduction of coliforms from the surface of the carcasses. The result in experiment 3 indicated that keeping higher chlorine concentration (over 50 ppm) proved to be effective on eliminating Campylobacter on postchill carcasses at the processing plant without a pre-chiller tank. It is reported that the chilling process with water containing 0.5 to 0.75 ppm of free chlorine was significantly associated with an important reduction in Campylobacter counts in broiler carcasses when compared with post-evisceration and post-chilling stages [6]. A similar study carried out by Buhr et al. [4] demonstrated that addition of chlorine at 20 ppm in the chiller water significantly decreased E. coli, coliforms, total aerobes and Campylobacter counts, but did not affect Salmonella recovery. Our findings in experiment 1 and 2 showed a good agreement to the above reports that Campylobacter were significantly reduced at low-chlorinated chiller water. Li et al. [11] demonstrated that chlorinated chiller water at 50 ppm was expected to be effective in controlling cross-contamination or in preventing the bacterial transfer from one carcass to another. However, that level of concentration was not effective on entire elimination of Campylobacter as shown in experiment 2, because the midle-size plant we investigated had no pre-chiller tank. It’s noteworthy in experiment 3 that using chlorinated chiller water at 50 to 70 ppm prevented cross-contamination of coliforms and eliminated Campylobacter counts on carcasses. Still, reducing organic debris such as feathers, bloods, and enteric contents entering into chiller water during processing should be important for keeping the efficacy of chlorine. Bashor et al. [1] revealed that washing systems consisting of one to three washers were effective in reducing Campylobacter counts, and more effective treatment was attained by washing with trisodium phosphate or acidified sodium chloride. The processing plant investigated in the present study was equipped with only one section of washing system, and used no chemicals through the washing. Therefore, establishing a washing system with more washers or applying some additive could be one of the strategies to reduce bacterial counts on carcasses at the plant.

To confirm the effect of chlorine on Campylobacter on postchill carcasses, an additional experiment was conducted. In the same condition of the experiment 3, 15 of 25 cm² breast skin samples were collected from postchill carcasses using a sterile scalpel and tweezers. Then, these were weighed, mixed with approximately 90 ml of 0.1% peptone buffer, shook with stomacher and estimated Campylobacter population by the MPN method [12]. Campylobacter was recovered from all 15 samples, with ranged from 0.3 to 150 MPN/g (means of 2.56 MPN/g), although this organism couldn’t detect from swab samples in the same condition of experiment 3. This indicated that high chlorine concentration in chill water was effective to reduce Campylobacter on postchill carcasses but couldn’t eliminate completely the organism. Our result also indicated that chlorination of chiller water did not effectively reduce Campylobacter attached to broiler skins in the same way as the result described previously [20]. It was reported that 3.8 log cfu/g of the organism was found in the breast skin (excluding feathers) before carcasses enter the scalding tank [2]. A few amount of Campylobacter in the skin as low level as the amount we detected in the present study might pose a risk of food poisoning. During dismantling, a cross-contamination could occur due to the presence of a small number of Campylobacter on the surface of the carcasses.

Table 1. Number of Campylobacter and coliform recovery from pre and postchill carcasses

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Temperature range (°C)</th>
<th>Chlorine concentration range (ppm)</th>
<th>Campylobacter</th>
<th>Coliform</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prechill log CFU/25 cm²</td>
<td>Postchill log CFU/25 cm²</td>
<td>Decreasing rate (%)</td>
<td>Prechill log CFU/25 cm²</td>
</tr>
<tr>
<td>1</td>
<td>10–19</td>
<td>10–50</td>
<td>3.96 ± 0.48</td>
<td>1.15 ± 0.16</td>
</tr>
<tr>
<td>2</td>
<td>2–9</td>
<td>10–50</td>
<td>3.18 ± 0.52</td>
<td>0.70 ± 0.14</td>
</tr>
<tr>
<td>3</td>
<td>2–9</td>
<td>50–70</td>
<td>2.96 ± 0.48</td>
<td>0.18 ± 0.00</td>
</tr>
</tbody>
</table>

a) Standard error.

b) Within columns, means lacking a common superscript differ significantly (P<0.05, at Campylobacter and P<0.01, at coliform).

c) Campylobacter were undetectable from all 15 carcasses.

Campylobacter as shown in experiment 2, because the middle-size plant we investigated had no pre-chiller tank. It's noteworthy in experiment 3 that using chlorinated chiller water at 50 to 70 ppm prevented cross-contamination of coliforms and eliminated Campylobacter counts on carcasses. Still, reducing organic debris such as feathers, bloods, and enteric contents entering into chiller water during processing should be important for keeping the efficacy of chlorine. Bashor et al. [1] revealed that washing systems consisting of one to three washers were effective in reducing Campylobacter counts, and more effective treatment was attained by washing with trisodium phosphate or acidified sodium chloride. The processing plant investigated in the present study was equipped with only one section of washing system, and used no chemicals through the washing. Therefore, establishing a washing system with more washers or applying some additive could be one of the strategies to reduce bacterial counts on carcasses at the plant.
Although the middle-capacity of processing plants such as we examined are generally considered to be more difficult to eliminate the contamination of bacteria like Campylobacter than fully equipped processing plants, our result revealed that keeping low chiller water temperature and higher chlorine concentration in immersion chillers could reduce the numbers of Campylobacter and coliforms considerably on postchill carcasses.

REFERENCES


