NOTE *Public Health*

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

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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, 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 2, decreasing rate (%) of coliforms was significantly higher (P<0.01) than that in experiment 1. In higher chlorination of experiment 3, no *Campylobacter* was detected from all postchill carcasses.

KEY WORDS: *Campylobacter*, chilling process, chlorine concentration, MPN.

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Campylobacter is one of the main causative agents of human bacterial gastroenteritis worldwide. Human campylobacterosis 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-defeathering, 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 evaluated in a processing plant, previously [17]. Other investigations revealed that 25% and 22% of crops were damaged during the evisceration stage [5, 8]. These observations suggested that break of intestine or ruptured crops may serve as a source of carcass contamination with food-borne pathogens.

The chilling process, which is performed after carcass washing, is usually conducted using chiller water containing chlorine to reduce bacteria. The Food Safety and Inspection Service, U.S. Department of Agriculture recommended that if chiller water use, water temperature maintained less than 40 degrees Fahrenheit (approximately 4.4°C) and used 20 to 50 ppm free available chlorine to reduce bacteria in the water and reduce carcass cross contamination [7]. Likewise, Department of Food Safety, the Ministry of Health, Labour and Welfare, Japan, have mentioned that pre-chilling and main-chilling water temperature kept less than 16°C and 5 °C, respectively, however, there was no description concerning the chlorine concentration in chiller water [14]. In Japan, there are quite a few middle-size processing plants that are not fully equipped, namely, without carcass-washing machine, a pre-chiller tank, or mechanical evisceration system. The meat products which processed at middle-size processing plants might contaminated more frequently with pathogenic bacteria like *Campylobacter* spp. than entirely equipped large-size processing plants. Moreover, the data describing the number of *Campylobacter* on carcasses in relation to variation of the chiller water temperature and chlorine concentration from the start to the end of the chilling process at middle-size processing plants are sparse. The purpose of this study was to present successful management

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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 m^3 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 cm^2 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 cm^2 right side of breast area of each carcass was swabbed. Then, the same carcasses were traced. Lastly, 25 cm^2 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

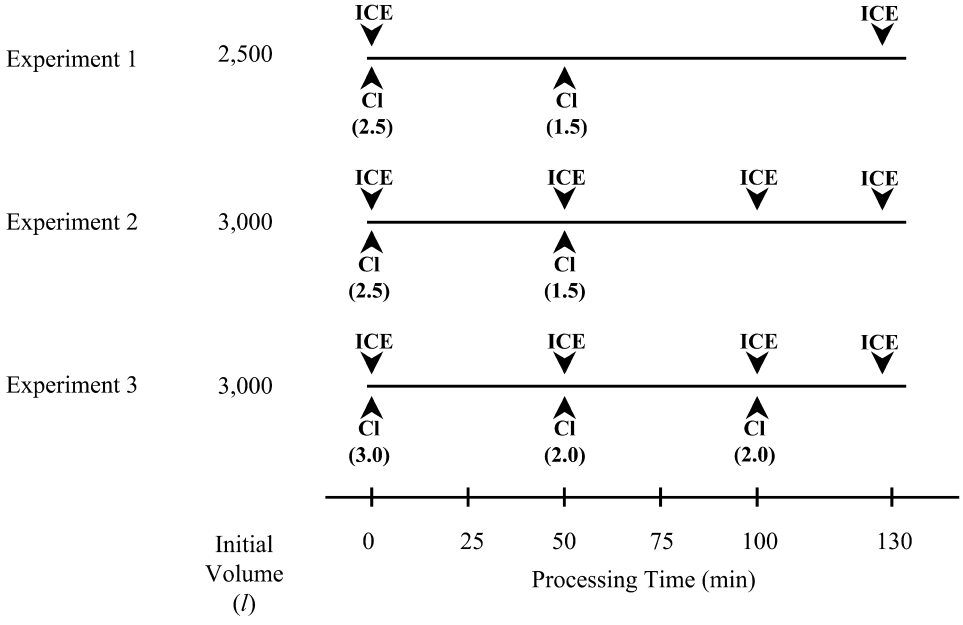

Fig. 1. Initial chiller water volume and additional timing of ices and NaClO solution during processing in each experiment. ICE, large amount of ices added in chiller water. Cl, twelve percent NaClO solution added in chiller water (volume, *l*).

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 few 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 cm^2 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 $\log_{10}$ cfu or MPN/25 cm^2, 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 m*l*), 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 cm^2). 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 cm^2 of *Campylobacter* were recovered from prechill carcasses. The number was relatively higher when compared

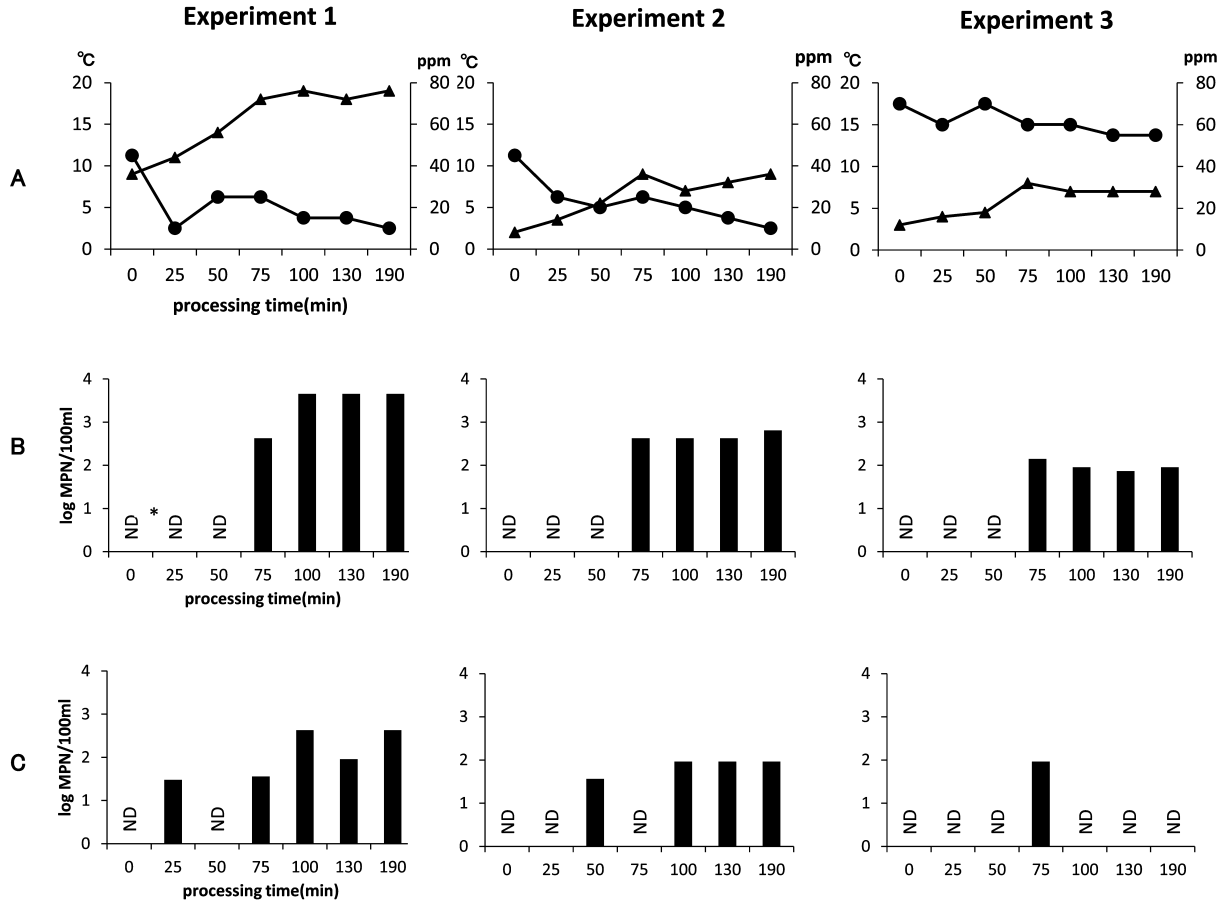

Fig. 2. A; Water temperature and chlorine concentration in chiller water, ▲ temperature; ●, chlorine concentration. B; Number of coliform bacilli recovery in chiller water. C; Number of *Campylobacter* recovery in chiller water. * < 30 MPN / 100 m*l*.

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

Experiments	Temperature range (° C)	Chlorine concentration range (ppm)	Number of bacteria in each 15 carcasses (means ± SE[a,b])							
			Compylobacter				Coliform			
			Prechill logCFU/25 cm^2	Postchill logMPN/25 cm^2	*P* value	Decreasing rate (%)[b]	Prechill logCFU/25 cm^2	Postchill log CFU/25 cm^2	*P* value	Decreasing rate (%)[b]
1	10–19	10–50	3.96 ± 0.48	1.15 ± 0.16	<0.01	70.0[1]	2.40 ± 0.49	2.31 ± 0.13	0.59	1.5[1]
2	2–9	10–50	3.18 ± 0.52	0.70 ± 0.14	<0.01	77.7[1]	3.24 ± 0.58	1.65 ± 0.20	<0.01	43.7[2]
3	2–9	50–70	2.96 ± 0.48	0.18 ± 0.00[c]	<0.01	93.9[2]	3.45 ± 0.39	1.99 ± 0.18	<0.01	42.2[2]

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.

with following reports, 1.71, 2.39 and 3.04 log CFU/1,000 cm^2 of the organism was recovered from prechill carcasses at three processing plants in the United States, respectively [9], and 0.3 MPN / cm^2 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 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 chlorite. 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 cm^2 breast skin samples were collected from postchill carcasses using a sterile scalpel and tweezers. Then, these were weighed, mixed with approximately 90 m*l* 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.

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

1. Bashor, M. P., Curtis, P. A., Keener, K. M., Sheldon, B. W., Kathariou, S. and Osborne, J. A. 2004. Effects of carcass washers on *Campylobacter* contamination in large broiler processing plants. *Poult. Sci.* **83**: 1232–1239.
2. Berrang, M. E. and Dickens, J. A. 2000. Presence and level of *Campylobacter* spp. on broiler carcasses throughout the processing plant. *J. Appl. Poultry Res.* **9**: 43–47.
3. Bolton, F. J. 2007. *Campylobacter* infection: Food-borne sources and isolation methods. *Jpn. J. Food. Microbiol.* **24**: 151–156.
4. Buhr, R. J., Bourassa, D. V., Northcutt, J. K., Hinton, A., Ingram, K. D. and Cason, J. A. 2005. Bacteria recovery from genetically feathered and featherless broiler carcasses after immersion chilling. *Poult. Sci.* **84**: 1499–1504.
5. Buhr, R. J. and Dickens, J. A. 2001. Crop extraction load and efficiency of crop removal during manual evisceration of broilers: 1. Evaluation of stunning voltage and method of bleeding. *J. Appl. Poultry Res.* **10**: 71–78.
6. Figueroa, G., Troncoso, M., López, C., Rivas, P. and Toro, M. 2009. Occurrence and enumeration of *Campylobacter* spp. during the processing of Chilean broilers. *BMC Microbiol.* **9**: 94.
7. Food Safety and Inspection Service, U. S. Department of Agriculture. 2010. Compliance Guideline for Controlling *Salmonella* and *Campylobacter* in Poultry, third edition. Washington, D.C., U.S.A.
8. Hargis, B. M., Caldwell, D. J., Brewer, R. L., Corrier, D. E. and DeLoach, J. R. 1995. Evaluation of the chicken crop as a source of *Salmonella* contamination for broiler carcasses. *Poult. Sci.* **74**: 1548–1552.
9. Izat, A. L., Gardner, F. A., Denton, J. H. and Golan, F. A.1988. Incidence and level of *Campylobacter jejuni* in broiler processing. *Poult. Sci.* **67**: 1568–1572.
10. Jorgensen, F., Bailey, R., Williams, S., Henderson, P., Wareing, D. R., Bolton, F. J., Frost, J. A., Ward, L. and Humphrey, T. J. 2002. Prevalence and numbers of *Salmonella* and *Campylobacter* spp. on raw whole chickens in relation to sampling methods. *Int. J. Food Microbiol.* **76**: 151–164.
11. Li, Y., Yang, H. and Swem, B. L. 2002. Effect of high temperature inside-outside spray on survival of *Campylobacter jejuni* attached to prechill chicken carcasses. *Poult. Sci.* **81**: 1371–1377.
12. Ministry of Health, Labor and Welfare. 2004. *Campylobacter*. pp. 225–235. *In*: Food Sanitation Inspection Guidelines, Microbiology volume, Japan Food Hygiene Association, Tokyo (in Japanese).
13. Ministry of Health, Labor and Welfare. 2004. Indicator bacteria of contamination, fecal indicator bacteria and *Escherichia coli*. pp. 129–144. *In*: Food Sanitation Inspection Guidelines, Microbiology volume, Japan Food Hygiene Association, Tokyo (in Japanese).
14. Ministry of Health, Labour and Welfare, Department of Food Safety. 1992 (amendment in 2005). Guideline of Sanitary Management based on HACCP System in Poultry Processing Plants. Tokyo (in Japanese).
15. Moore, J, E., Corcoran, D., Dooley, J, S, G., Fanning, S., Lucey, B., Matsuda, M., Mcdwell, D, A., Megraud, F., Millar, B, C., O'mahony, R., O'riordan, L., O'rourke, M., Rao, J, R., Rooney, P, J., Sails, A. and White, P. 2005. *Campylobacter*. *Vet. Res.* **36**: 351–382.
16. Oosterom, J., Notermans, S., Karman, H. and Engels, G. B. 1983. Origin and prevalence of *Campylobacter jejuni* in poultry processing. *J. Food Prot.* **46**: 339–344.
17. Russel, S. M. 2003. The effect of airsacculitis on bird weights, uniformity, fecal contamination, processing errors, and population of *Campylobacter* spp. and *Escherichia coli*. *Poult. Sci.* **82**: 1326–1331.
18. Sato, H., Watanabe, S. and Goto, K. 2001. Evaluation of Microbiological Sampling Methods on Poultry Carcasses and a Study of Bacterial Contamination in Broiler Carcasses. *J. Jpn. Vet. Med. Assoc.* **54**: 857–861 (in Japanese).
19. Takahashi, R., Shahada, F., Chuma, T. and Okamoto, K. 2006. Analysis of *Campylobacter* spp. contamination in broiler from the farm to the final meat cuts by using restriction fragment length polymorphism of the polymerase chain reaction products. *Int. J. Food Microbiol.* **110**: 240–245.
20. Yang, H., Li, Y. and Johnson, M. G. 2001. Survival and death of *Salmonella* Typhimurium and *Campylobacter jejuni* in processing water and on skin during poultry scalding and chilling. *J. Food Prot.* **64**: 770–776.