The Journal of Veterinary Medical Science

FULL PAPER

Avian Pathology

# Enhancement of bactericidal effects of sodium hypochlorite in chiller water with food additive grade calcium hydroxide

**Chiharu TOYOFUKU[1)], Md. Shahin ALAM[1)], Masashi YAMADA[1)], Miyuki KOMURA[1)], Mayuko SUZUKI[1)], Hakimullah HAKIM[1)], Natthanan SANGSRIRATANAKUL[1)], Dany SHOHAM[1,2)] and Kazuaki TAKEHARA[1)]***

[1)]Laboratory of Animal Health, Department of Veterinary Medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8, Saiwai-cho, Fuchu-shi, Tokyo 183-8509, Japan
[2)]Bar-Ilan University, Begin-Sadat Center for Strategic Studies, Ramat Gan 5290002, Israel

*J. Vet. Med. Sci.*
79(6): 1019–1023, 2017
doi: 10.1292/jvms.17-0089

Received: 21 February 2017
Accepted: 28 April 2017
Published online in J-STAGE: 12 May 2017

**ABSTRACT.** An alkaline agent, namely food additive grade calcium hydroxide ($FdCa(OH)_2$) in solution at 0.17%, was evaluated for its bactericidal efficacies in chiller water with sodium hypochlorite (NaOCl) at a concentration of 200 ppm total residual chlorine. Without organic material presence, NaOCl could inactivate *Salmonella* Infantis and *Escherichia coli* within 5 sec, but in the presence of fetal bovine serum (FBS) at 0.5%, the bactericidal effects of NaOCl were diminished completely. $FdCa(OH)_2$ solution required 3 min to inactivate bacteria with or without 5% FBS. When NaOCl and $FdCa(OH)_2$ were mixed at the final concentration of 200 ppm and 0.17%, respectively, the mixed solution could inactivate bacteria at acceptable level ($10^3$ reduction of bacterial titer) within 30 sec in the presence of 0.5% FBS. The mixed solution also inhibited cross-contamination with *S.* Infantis or *E. coli* on chicken meats. It was confirmed and elucidated that $FdCa(OH)_2$ has a synergistic effect together with NaOCl for inactivating microorganisms.

**KEY WORDS:** bacterial disinfection, food additive grade calcium hydroxide, inhibition of cross contamination, sodium hypochlorite, synergistic effect

Recently, food safety has been required at higher level, and hazard analysis and critical control point (HACCP) has been introduced at farm level in Japan [17]. In order to make HACCP work appropriately, it is necessary to enhance biosecurity, such as disinfecting pathogens along the route from farm to table.

In Japan, foodborne illnesses caused by *Salmonella* spp., *Campylobacter* and *Escherichia coli* are still high, with 1,918, 2,089, and 518 patients, respectively, in 2015 [18]. In the United States of America (U.S.A.), *Campylobacter* and *Salmonella* are the top 2 bacterial foodborne human enteropathogens associated with poultry-related foodborne illness [3].

Chlorine has been a common antimicrobial utilized for prevention of bacterial carcass cross-contamination in immersion chilling systems and throughout the poultry processing plant [19, 23, 25]. Early studies demonstrated potency of chlorine as an antimicrobial, with only 0.1 mg/*l* (0.1 ppm) of free chlorine being required to inactivate 99% of *Campylobacter* strains after 5 min of contact [4]. The relatively low cost and low concentration required for efficacy promoted widespread use of chlorine in processing plants. However, the effectiveness of chlorine in immersion tanks (=chiller tanks) has been shown to diminish due to a longer residence time [1, 29], and its effectiveness is highly affected by pH changes and the amount of organic matter in the chiller tank [1, 13]. Tsai *et al.* [27] and Northcutt *et al.* [22] reported that chiller tanks at the processing plants contained around 0.35 or 0.16% suspended solids, respectively. Although chicken is chemically sanitized during processing and subsequently cleaned before packaging, some microorganisms cannot be removed completely, especially bacteria strongly attached to chicken skin [5, 14, 23, 30]. This is because these microorganisms, including *Salmonella* and *Campylobacter,* are able to become entrapped in deep skin layers, crevices, or feather follicles, thus becoming protected from chemical agents [5, 15, 29].

In the European Union (EU), no disinfectant is allowed to be used at the processing plants, especially in chiller tanks [7]. Europe should not authorize chemical washes such as chlorine or peroxyacetic acid on poultry carcasses. In the U.S.A., United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) allows for addition of chlorine to processing waters at levels up to 50 ppm, calculated as free available chlorine in carcass wash applications and chiller make-up water [8].

*Correspondence to: Takehara, K., Laboratory of Animal Health, Department of Veterinary Medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8, Saiwai-cho, Fuchu-shi, Tokyo 183-8509, Japan. e-mail: takehara@cc.tuat.ac.jp

FSIS also requires that chlorinated water containing a minimum of 20 ppm available chlorine be applied to all surfaces of carcasses when the inner surfaces have been re-processed (due to carcass contamination), other than solely by trimming [8]. In Japan, NaOCl at 35–200 ppm as total chlorine concentration is used to disinfect poultry carcasses; however, many cross-contaminations still occur, especially during chilling process at the slaughter house, and bacteria from poultry meats are detected after processing, or at the retail markets [19, 25]. Munther *et al*. [21] also reported that while chlorine control was important for reducing *Escherichia coli* levels during chilling, it played a less significant role in the management of cross-contamination issues. Once the bacteria attach to the surface of chicken meats, it is very difficult to remove or kill these bacteria [1, 14, 30]. It is desirable to kill bacteria before they attach to poultry meats to reduce cross-contamination.

Chlorine has been shown to kill micro-organisms within very short period (within 5 sec) [10] and this short period allowed chlorine to inactivate Newcastle disease virus (NDV) in the air [12]. However, in the presence of organic matter, the efficacies were diminished [1, 10, 12].

In the present study, food additive grade calcium hydroxide ($FdCa(OH)_2$) [9] was evaluated for its inactivation efficacy toward *Salmonella* Infantis and *E. coli,* and the efficacy with NaOCl was evaluated, too. To get better efficacy, 0.17% of $Ca(OH)_2$ that is saturated concentration was used in this study [2]. And also for the application at poultry processing plants, "food additive grade" was chosen.

## MATERIALS AND METHODS

### *$FdCa(OH)_2$ powder and NaOCl*

$FdCa(OH)_2$ powder at pH 13, made of natural calcium carbonates derived from limestone through calcination process, with the average diameter of the powder size at 10 $\mu$m, was kindly provided by Fine Co., Ltd. (Tokyo, Japan) [9]. NaOCl (Turukuron®, at 12% chlorine concentration) was purchased from Toagosei Co., Ltd. (Tokyo, Japan). $FdCa(OH)_2$ powder of 170 mg was prepared in 100 m*l* of redistilled water ($dW_2$) and centrifuged at 1,750 ×g for 10 min at 4°C. The resulted supernatant was used as saturated 0.17% $FdCa(OH)_2$ [2]. NaOCl containing 200 ppm chlorine (200 ppm NaOCl) was prepared in $dW_2$. For the mixed solution (200 ppm NaOCl and 0.17% $FdCa(OH)_2$), NaOCl was diluted to 200 ppm chlorine in 0.17% $FdCa(OH)_2$ solution. Solutions containing NaOCl, namely 200 ppm NaOCl and the mixed solution, were used for the experiments immediately within 1 min after preparation.

### *Inocula preparation*

Bacterial suspensions of *E. coli* strain NBRC106373 and *S*. Infantis were prepared and enumerated as described previously [11]. In order to make rifampicin resistant bacteria, 100 $\mu l$ of log phase culture of *E. coli* was employed through its surface plating on deoxycholate hydrogen sulfide-lactose (DHL) agar containing different concentrations of rifampicin (from 0–100 $\mu$g/m*l*), followed by overnight incubation at 37°C. Colonies grown in the area containing higher concentration of rifampicin were picked up and sub-cultured on the DHL agar containing 100 $\mu$g/m*l* rifampicin. After overnight culture, around five colonies were picked up and subsequently cultured in the Luria-Bertani (LB) medium containing the same concentration of rifampicin. Log phase of bacteria from the solution culture was used for the experiments, and stocked as EC-Rif in 10% skim milk at −80°C until used. *E. coli*-Rif was enumerated on DHL agar containing 100 $\mu$g/m*l* rifampicin.

### *Computation of reduction factor*

Reduction factor (RF) was calculated using the equation below after conversion of sample titer to $\log_{10}$ CFU/m*l*:

RF=tpc–ta

In the above equation, *tpc* is bacterial titer from an untreated sample in $\log_{10}$ CFU/m*l*, whereas *ta* is the titer of recovered bacteria from treated samples. The inactivation rate was acceptable when the RF was greater than or equal to 3 [8, 11, 16, 26].

### *Evaluation of bactericidal efficacies in aqueous phase*

Four hundreds micro-liter of solutions (0.17% $FdCa(OH)_2$, 200 ppm NaOCl and the mixture) were mixed with 100 $\mu l$ of bacteria in a microtube, incubated for indicated time (0, 5, 30 sec, 1 or 3 min) at 4°C and then neutralized by adding 500 $\mu l$ of blocking solution containing 0.7 M Tris-HCl (pH 7.2) and 30% fetal bovine serum (FBS), which made pH of the tested solutions around 8.2. In the presence of FBS, 2.5$\mu l$ (0.5%), 5.0$\mu l$ (1.0%) or 25$\mu l$ (5.0%) of FBS was added, respectively, into the microtubes before adding bacteria. For the positive control, 100 $\mu l$ of *E. coli*, *E. coli*-Rif and *S.* Infantis was individually inoculated in 400 $\mu l$ of PBS then, after 3 min incubation, 500 $\mu l$ of the blocking solution was added. These experiments were performed in triplicate.

### *Evaluation of inhibition of cross-contamination*

Commercial chicken thigh meats, purchased at a retail market were cut into ten gram pieces and each piece was spiked with *E. coli*-Rif or *S.* Infantis at 1 m*l* of $10^8$ CFU/m*l* for 15 min. The spiked chicken meat piece was placed in a 250 m*l* glass beaker containing 40 m*l* of each tested solution at 4°C, namely 0.17% $FdCa(OH)_2$, 200 ppm NaOCl and the added mixture with 0.2% FBS, plus $dW_2$ containing 0.2% FBS as control; then the non-spiked chicken meat piece was placed into the same beaker immediately (within 1 sec) and incubated for 5 min with shaking at 100 rpm in Bio-Shaker BR-22UM (TAITEC Co., Saitama, Japan). The non-spiked piece was taken out from the beaker and rinsed with 40 m*l* of $dW_2$ twice, transferred into a stomacher

**Table 1.** Bactericidal efficacies of 0.17% $FdCa(OH)_2$ toward *Salmonella* Infantis and *Escherichia coli*

| Bacteria | FBS (%) | Control[b] | RF[a] | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0[c] sec | 5 sec | 30 sec | 60 sec | 180 sec |
| *Salmonella* Infantis | 0 | 8.60 ± 0.08[d] | 0.00 ± 0.00 | 0.91 ± 0.22 | 1.86 ± 0.10 | 2.11 ± 0.17 | ≥6.00 ± 0.08 |
| | 1 | | | 0.90 ± 0.20 | 1.87 ± 0.33 | 2.33 ± 0.27 | ≥6.00 ± 0.08 |
| | 5 | | | 1.19 ± 0.16 | 2.27 ± 0.39 | 2.29 ± 0.12 | ≥6.00 ± 0.08 |
| *Escherichia coli* | 0 | 8.29 ± 0.07 | 0.00 ± 0.00 | 0.55 ± 0.03 | 1.28 ± 0.04 | 2.25 ± 0.07 | ≥5.69 ± 0.07 |
| | 1 | | | 0.51 ± 0.02 | 1.22 ± 0.06 | 1.94 ± 0.15 | ≥5.69 ± 0.07 |
| | 5 | | | 0.44 ± 0.01 | 0.95 ± 0.03 | 1.65 ± 0.08 | 5.30 ± 0.16 |

a) Reduction factor (RF)=$\log_{10}$ (titer of control/m*l*) −$\log_{10}$ (titer of treated samples/m*l*). b) Titer of bacteria in the control ($\log_{10}$ CFU/m*l*). c) Contact times. d) Data represent means ± standard deviation of three different experiments.

**Table 2.** Bactericidal efficacies of 0.17% $FdCa(OH)_2$, 200 ppm NaOCl or the mixture solution toward *Salmonella* Infantis and *Escherichia coli*

| Tested solution | Bacteria | FBS (%) | Control[b] | RF[a] | |
|---|---|---|---|---|---|
| | | | | 5[c] sec | 30 sec |
| 0.17% $FdCa(OH)_2$ | *Salmonella* Infantis | 0.5 | 8.01 ± 0.27[d] | 0.72 ± 0.14 | 0.98 ± 0.10 |
| 200 ppm NaOCl | | 0 | | 5.41 ± 0.27 | 5.41 ± 0.27 |
| Mixture | | 0.5 | | 0.80 ± 0.37 | 1.14 ± 0.52 |
| | | 0 | | 5.41 ± 0.27 | 5.41 ± 0.27 |
| | | 0.5 | | 1.31 ± 0.25 | 5.41 ± 0.27 |
| | | 1.0 | | 0.60 ± 0.07 | 1.04 ± 0.06 |
| 0.17% $FdCa(OH)_2$ | *Escherichia coli* | 0.5 | 8.13 ± 0.15 | 0.49 ± 0.07 | 1.19 ± 0.05 |
| 200 ppm NaOCl | | 0 | | 5.53 ± 0.15 | 5.53 ± 0.15 |
| Mixture | | 0.5 | | 0.36 ± 0.07 | 0.47 ± 0.19 |
| | | 0 | | 3.17 ± 0.82 | 5.53 ± 0.15 |
| | | 0.5 | | 0.68 ± 0.01 | 5.53 ± 0.15 |
| | | 1.0 | | 0.32 ± 0.23 | 1.29 ± 0.08 |

a) Reduction factor (RF)=$\log_{10}$ (titer of control/m*l*) −$\log_{10}$ (titer of treated samples/m*l*). b) Titer of bacteria in the control ($\log_{10}$ CFU/m*l*). c) Contact times. d) Data represent means ± standard deviation of three different experiments.

bag (Organo Co., Ltd., Tokyo, Japan), then homogenized in 20 m*l* of PBS using MINIMIX®100CC® (Interscience Ci., Ltd., Saint Nom, France). The bacteria in the homogenates, transferred to the non-spiked piece, were enumerated as described above. In the preliminary experiments, it was demonstrated that 200 ppm NaOCl could not inhibit cross contamination in 0.5% FBS, 0.2% FBS was used for this experiments.

## RESULTS

### *Evaluation of bactericidal efficacies of 0.17% $FdCa(OH)_2$*

Table 1 shows the capacity of 0.17% $FdCa(OH)_2$ to inactivate bacteria in liquid. When the blocking solution was added to the solution before adding bacteria (0 sec), no reduction was observed. Regardless of the presence or absence of FBS, 0.17% $FdCa(OH)_2$ inactivated *S.* Infantis from $10^{8.60}$ PFU/m*l* to below the detection limit (≤$10^{2.6}$ CFU/m*l*) (RF≥6.00), as well as *E. coli* from $10^{8.29}$ PFU/m*l* to below the detection limit (≤$10^{2.6}$ CFU/m*l*) (RF≥5.69), except presence of 5% FBS (RF=5.3). However, it took more than 1 min for 0.17% $FdCa(OH)_2$ to inactivate bacteria.

### *Enhancement of bactericidal efficacies of NaOCl with 0.17% $FdCa(OH)_2$*

Table 2 summarizes the results of the mixture of NaOCl and $FdCa(OH)_2$. In the absence of organic materials, namely without FBS, 200 ppm NaOCl inactivated *S.* Infantis within 5 sec from $10^{8.01}$ PFU/m*l* to below the detection limit (≤$10^{2.6}$ CFU/m*l*) (RF≥5.41), as well as *E. coli* from $10^{8.13}$ PFU/m*l* to below the detection limit (≤$10^{2.6}$ CFU/m*l*) (RF≥5.53). With 0.5% FBS, 200 ppm NaOCl could not inactivate bacteria at all within 30 sec. As shown in Table 1, 0.17% $FdCa(OH)_2$ could not inactivate bacteria within 30 sec. However, when these 2 materials were mixed, the mixture could inactivate bacteria within 30 sec in the presence of 0.5% FBS. The mixture could not inactivate bacteria in 1.0% FBS.

### *Evaluation of inhibition of cross-contamination*

When bacteria spiked chicken meats were pooled with non-spiked chicken meats for 5 min in $dW_2$ containing 0.2% FBS at

**Table 3.** Bacterial cross-contamination of the non-spiked chicken meats

| Tested solution with 0.2% FBS | | 200 ppm NaOCl | 0.17% $FdCa(OH)_2$ | Mixture | $dW_2$ |
|---|---|---|---|---|---|
| *Salmonella* Infantis[a)] | Homogenate | 4.74 ± 0.15[c)] | 3.51 ± 0.58 | 1.76 ± 0.07 | 4.74 ± 0.07 |
| | RF[b)] | 0.00 ± 0.07 | 1.24 ± 0.52 | 2.98 ± 0.07 | - |
| *E. coli*-Rif [a)] | Homogenate | 5.20 ± 0.18 | 2.90 ± 0.21 | 2.51 ± 0.40 | 5.21 ± 0.01 |
| | RF[b)] | 0.01 ± 0.08 | 2.32 ± 0.20 | 2.71 ± 0.30 | - |

a) Titer of SI in the inoculum was 8.62 ± 0.07 and that of Ec-Rif was 8.80 ± 0.04. b) Reduction factor (RF)=$\log_{10}$ (titer in $dW_2$/m*l*)−$\log_{10}$ (bacterial titer in treated/m*l*). c) Data represent means ± standard deviation of three different experiments.

4°C, the spiked bacteria were detected in the non-spiked meats at $10^{4.74}$ PFU/m*l* for *S.* Infantis and $10^{5.21}$ PFU/m*l* for *E. coli*-Rif as shown in Table 3. This demonstrated that the cross-contamination of bacteria from contaminated meats to non-contaminated meats occurred in $dW_2$. When the meats were dipped in 200 ppm NaOCl containing 0.2% FBS, bacteria were detected in the non-spiked meats in the same order as $dW_2$, namely $10^{4.74}$ PFU/m*l* for *S.* Infantis and $10^{5.20}$ PFU/m*l* for *E. coli*-Rif (Table 3). With 0.17% $FdCa(OH)_2$ containing 0.2% FBS, bacteria detected in the non-spiked meats were $10^{3.51}$ PFU/m*l* for *S.* Infantis and $10^{2.90}$ PFU/m*l* for *E. coli*, respectively. In the mixed solution containing 0.2% FBS, bacteria detected in the non-spiked meats were $10^{1.76}$ PFU/m*l* for *S.* Infantis and $10^{2.51}$ PFU/m*l* for *E. coli*, and the RF was 2.98 and 2.71, respectively.

## DISCUSSION

Cross-contamination occurs at the processing plants of poultry especially in the chiller tank [19, 25], and it was also demonstrated in the present study as shown in Table 3 even with 200 ppm NaOCl. Poultry meats will be contaminated with bacteria through attachment of feces during the processing course, and the contaminated meats will release these bacteria into the environment, and non-contaminated meats will thereby be contaminated [19, 25]. Once bacteria attached to the meats, it was difficult to kill the bacteria on meats [14, 30]. It is desirable to kill bacteria immediately before attachment to the other meats.

To recover bacteria in meats, the homogenized method was used in the present study because the wipe or rinse methods could not recover bacteria in the deep layer of skin or meats [5, 29].

As shown in Table 1, the saturated concentration, 0.17%, $FdCa(OH)_2$ can kill bacteria even in the presence of 5% FBS, although it takes more than 1 min to inactivate them even without FBS. So the concentration 0.17% was used for the further experiments. In Japan, 200 ppm NaOCl is used as disinfectant in the chiller tank at processing plants, but many reports demonstrated that it is difficult to inactivate bacteria in the chiller tanks because of organic material contamination in the tanks [1, 19, 25]. In the present study, as shown in Table 2, 200 ppm NaOCl could inactivate bacteria within 5 sec. In the preliminary experiment, 200 ppm NaOCl could inactivate bacteria in the presence of 0.15% FBS within 5 sec (data not shown). However in the presence of 0.5% FBS that mimics the chiller tank containing about 0.35% suspended solids [22, 27], the inactivation activity of 200 ppm NaOCl was diminished (Table 2). The mixed solution containing 0.17% $FdCa(OH)_2$ and 200 ppm NaOCl could inactivate bacteria within 30 sec to the undetectable level even in the presence of 0.5% FBS. However, with 1.0% FBS, the mixture could not inactivate bacteria within 30 sec (Table 2). These data demonstrated a synergistic effect of NaOCl and $Ca(OH)_2$ for inactivation of bacteria. The mechanism of the synergistic effect seems to be that high pH and calcium made the bacterial cell membrane damaged [20] and $OCl^-$ ion could pass the damaged membrane and denature proteins in cytosol of bacteria. Normally, in 200 ppm NaOCl solution around pH 8.0–9.0, HOCl molecule composes about 10% while hypochlorite ion ($OCl^-$) composes about 90%, and the molecule only can pass through the cell membrane [28]. However, under high pH condition with $Ca(OH)_2$, all of the latter becomes $OCl^-$ ion and can enter the bacterial cells through the damaged membrane. In the present study, it was shown that it took 5 to 10 sec to make the damage of the bacterial cell membrane. As shown in Table 2, in the 0% FBS condition, 200 ppm NaOCl could inactivate *E. coli* under detectable level within 5 sec, while, the mixture inactivated partially within 5 sec. The reason seemed to be that in high pH all became $OCl^-$ but could not enter bacterial cytosol, because little damage of the cell membrane was created. At 10 sec post-incubation, *E. coli* was inactivated to the undetectable level in the mixture in the 0% FBS condition (data not shown).

In the chiller tank, cross-contamination occurs easily as shown in Table 3, and implementation of logistic slaughter (where *Campylobacter*-negative flocks are slaughtered first) was suggested [25]; however, there is conflict regarding logistic slaughter with respect to *Salmonella* and with respect to *Campylobacter* status [6, 24]. Inhibition of cross-contamination through immediate bacterial inactivation seems to be applicable by adding $FdCa(OH)_2$ in chiller tanks containing NaOCl, as shown in Table 3. In chiller tanks, organic materials may be released from chicken meats so the organic material contamination will be more than 0.2% FBS [22, 27]. In the present experiments for "*Evaluation of inhibition of cross-contamination*", 0.2% FBS, but not 0.5%, was used to mimic field conditions, because the experiments contained chicken meats. It is also important to clean the processing plant environment with materials such as $FdCa(OH)_2$, that can have effect even in the presence of organic materials.

ACKNOWLEDGMENT. This study was supported in part by Regulatory Science, Ministry of Agriculture, Forestry and Fisheries, Japan (MAFF) 2016.

## REFERENCES

1. Bartenfeld, L. N., Fletcher, D. L., Northcutt, J. K., Bourassa, D. V., Cox, N. A. and Buhr, R. J. 2014. The effect of high-level chlorine carcass drench on the recovery of *Salmonella* and enumeration of bacteria from broiler carcasses. *Poult. Sci.* **93**: 2893–2899. [Medline] [CrossRef]
2. Bassett, H. 1954. Solubility of calcium hydroxide in water. pp. 630–632. *In*: Solubilities of Inorganic and Metal Organic Compounds, 4th ed. (Linke, W. F. ed.), American Chemical Society, Washington D.C.
3. Batz, M. B., Hoffman, S. and Morris, J. G. Ranking the risks: the 10 pathogen-food combinations with the greatest burden on public health [internet]. Emerging Pathogens Institute; 2011. Available from http://www.epi.ufl. edu/sites/www.epi.ufl.edu/files/RankingTheRisksREPORT.pdf [accessed January 11, 2017].
4. Blaser, M. J., Smith, P. F., Wang, W. L. and Hoff, J. C. 1986. Inactivation of *Campylobacter jejuni* by chlorine and monochloramine. *Appl. Environ. Microbiol.* **51**: 307–311. [Medline]
5. Chantarapanont, W., Berrang, M. E. and Frank, J. F. 2004. Direct microscopic observation of viability of *Campylobacter jejuni* on chicken skin treated with selected chemical sanitizing agents. *J. Food Prot.* **67**: 1146–1152. [Medline] [CrossRef]
6. Elvers, K. T., Morris, V. K., Newell, D. G. and Allen, V. M. 2011. Molecular tracking, through processing, of *Campylobacter* strains colonizing broiler flocks. *Appl. Environ. Microbiol.* **77**: 5722–5729. [Medline] [CrossRef]
7. European Union. Laying down specific hygiene rules for food of animal origin. 2004. Regulation (EC) No 853/2004 of the European parliament and of the council of 29 April 2004.
8. Food Safety and Inspection Service, USDA. 2017. Safe and suitable ingredients used in the production of meat, poultry, and egg products. FSIS Directive 7120.1, Rev 39.
9. Hakim, H., Toyofuku, C., Ota, M., Suzuki, M., Komura, M., Yamada, M., Alam, M. S., Sangsriratanakul, N., Shoham, D. and Takehara, K. 2017. Accuracy of the evaluation method for alkaline agents' bactericidal efficacies in solid, and the required time of bacterial inactivation. *J. Vet. Med. Sci.* **79**: 244–247. [Medline] [CrossRef]
10. Hakim, H., Thammakarn, C., Suguro, A., Ishida, Y., Kawamura, A., Tamura, M., Satoh, K., Tsujimura, M., Hasegawa, T. and Takehara, K. 2015. Evaluation of sprayed hypochlorous acid solutions for their virucidal activity against avian influenza virus through *in vitro* experiments. *J. Vet. Med. Sci.* **77**: 211–215. [Medline] [CrossRef]
11. Hakim, H., Alam, M. S., Sangsriratanakul, N., Nakajima, K., Kitazawa, M., Ota, M., Toyofuku, C., Yamada, M., Thammakarn, C., Shoham, D. and Takehara, K. 2016. Inactivation of bacteria on surfaces by sprayed slightly acidic hypochlorous acid water: *in vitro* experiments. *J. Vet. Med. Sci.* **78**: 1123–1128. [Medline] [CrossRef]
12. Hakim, H., Thammakarn, C., Suguro, A., Ishida, Y., Nakajima, K., Kitazawa, M. and Takehara, K. 2015. Aerosol disinfection capacity of slightly acidic hypocholorous acid water towards Newcastle disease virus in the air: an *in vivo* experiment. *Avian Dis.* **59**: 486–491. [Medline] [CrossRef]
13. James, W. O., Brewer, R. L., Prucha, J. C., Williams, W. O. Jr. and Parham, D. R. 1992. Effects of chlorination of chill water on the bacteriologic profile of raw chicken carcasses and giblets. *J. Am. Vet. Med. Assoc.* **200**: 60–63. [Medline]
14. Ko, J., Ma, Y. and Song, K. B. 2005. Effect of chlorine dioxide treatment on microbial growth and qualities of chicken breast. *J. Food Sci. Nutr.* **10**: 122–129.
15. Lee, N. Y., Park, S. Y., Kang, I. S. and Ha, S. D. 2014. The evaluation of combined chemical and physical treatments on the reduction of resident microorganisms and Salmonella Typhimurium attached to chicken skin. *Poult. Sci.* **93**: 208–215. [Medline] [CrossRef]
16. Lombardi, M. E., Ladman, B. S., Alphin, R. L. and Benson, E. R. 2008. Inactivation of avian influenza virus using common detergents and chemicals. *Avian Dis.* **52**: 118–123. [Medline] [CrossRef]
17. Ministry of Agriculture Forestry and Fisheries "The improvement of the breeding hygiene management standard in the stage of production of the domestic animal", http://www.maff.go.jp/j/syouan/douei/katiku_yobo/k_haccp/index.html [accessed February 20, 2017].
18. Ministry of Health Labour and Welfare. 2015. Food poisoning statistics (data for foodborne disease outbreaks) in 2015 in Japan. http://www.mhlw.go.jp/file/05-Shingikai-11121000-Iyakushokuhinkyoku-Soumuka/0000116566.pdf [accessed February 20, 2017].
19. Misawa, N. 2012. Strategies for post-harvest control of *Campylobacter*. *J. Jpn. Vet. Med. Assoc.* **65**: 617–623.
20. Mohammadi, Z., Shalavi, S. and Yazdizadeh, M. 2012. Antimicrobial activity of calcium hydroxide in endodontics: a review. *Chonnam Med. J.* **48**: 133–140. [Medline] [CrossRef]
21. Munther, D., Sun, X., Xiao, Y., Tang, S., Shimozako, H., Wu, J., Smith, B. A. and Fazil, A. 2016. Modeling cross-contamination during poultry processing: Dynamics in the chiller tank. *Food Contr.* **59**: 271–281. [CrossRef]
22. Northcutt, J. K. and Jones, D. R. 2004. A survey of water use and common industry practices in commercial broiler processing facilities. *J. Appl. Poult. Res.* **13**: 48–54. [CrossRef]
23. Northcutt, J. K., Smith, D., Huezo, R. I. and Ingram, K. D. 2008. Microbiology of broiler carcasses and chemistry of chiller water as affected by water reuse. *Poult. Sci.* **87**: 1458–1463. [Medline] [CrossRef]
24. Rasschaert, G., Houf, K., Godard, C., Wildemauwe, C., Pastuszczak-Frak, M. and De Zutter, L. 2008. Contamination of carcasses with *Salmonella* during poultry slaughter. *J. Food Prot.* **71**: 146–152. [Medline] [CrossRef]
25. Sasaki, Y., Maruyama, N., Zou, B., Haruna, M., Kusukawa, M., Murakami, M., Asai, T., Tsujiyama, Y. and Yamada, Y. 2013. Campylobacter cross-contamination of chicken products at an abattoir. *Zoonoses Public Health* **60**: 134–140. [Medline] [CrossRef]
26. Thammakarn, C., Tsujimura, M., Satoh, K., Hasegawa, T., Tamura, M., Kawamura, A., Ishida, Y., Suguro, A., Hakim, H., Ruenphet, S. and Takehara, K. 2015. Efficacy of scallop shell powders and slaked lime for inactivating avian influenza virus under harsh conditions. *Arch. Virol.* **160**: 2577–2581. [Medline] [CrossRef]
27. Tsai, L. S., Schade, J. E. and Molyneux, B. T. 1992. Chlorination of poultry chiller water: chlorine demand and disinfection efficiency. *Poult. Sci.* **71**: 188–196. [CrossRef]
28. White, G. C. 2010. Chemistry of aqueous chlorine. pp. 69–173. *In*: White's Handbook of Chlorination and Alternative Disinfectants, 5th ed. (Black and Veatch), John Waley and Sons, Inc., Hoboken.
29. Yang, H., Li, Y. and Johnson, M. G. 2001. Survival and death of *Salmonella* typhimurium and *Campylobacter jejuni* in processing water and on chicken skin during poultry scalding and chilling. *J. Food Prot.* **64**: 770–776. [Medline] [CrossRef]
30. Zhang, L., Jeong, J. Y., Janardhanan, K. K., Ryser, E. T. and Kang, I. 2011. Microbiological quality of water immersion-chilled and air-chilled broilers. *J. Food Prot.* **74**: 1531–1535. [Medline] [CrossRef]