Application of slightly acidic electrolyzed water for inactivating microbes in a layer breeding house

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ABSTRACT Lots of microorganisms exist in layer houses can cause bird diseases and worker health concerns. Spraying chemical disinfectants is an effective way to decontaminate pathogenic microorganisms in the air and on surfaces in poultry houses. Slightly acidic electrolyzed water (SAEW, pH 5.0–6.5) is an ideal, environmentally friendly broad-spectrum disinfectant to prevent and control bacterial or viral infection in layer farms. The purpose of this work was to investigate the cleaning effectiveness of SAEW for inactivating the microbes in layer houses. The effect of SAEW was evaluated by solid materials and surface disinfection in a hen house. Results indicate that SAEW with an available chlorine concentration of 250 mg/L, pH value of 6.19, and oxygen reduction potential of 974 mV inactivated 100% of bacteria and fungi in solid materials (dusts, feces, feather, and feed), which is more efficient than common chemical disinfectant such as benzalkonium chloride solution (1:1,000 vol/vol) and povidone-iodine solution (1:1,000 vol/vol). Also, it significantly reduced the microbes on the equipment or facility surfaces ($P < 0.05$), including floor, wall, feed trough, and water pipe surfaces. Moreover, SAEW effectively decreased the survival rates of *Salmonella* and *Escherichia coli* by 21 and 16 percentage points. In addition, spraying the target with tap water before disinfection plays an important role in spray disinfection.

Key words: layer house, slightly acidic electrolyzed water, microbe, disinfection

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INTRODUCTION

Airborne microorganisms in layer houses can cause bird diseases and be harmful to the health of workers. Pathogens in animal housing are usually carried by dust (Homes et al., 1996; Gustafsson, 1999), which may originate from the skin, hair, feather, feed, manure, and bedding materials (Aarnink et al., 1999). Manure can serve as reservoirs of pathogenic bacteria, such as *Clostridium perfringens*, *Escherichia coli*, and *Salmonella* spp. (McLaughlin et al., 2009). There are many microorganisms on equipment or facility surfaces, including floors, walls, ceilings, fans, and windows, in layer breeding houses in China. Reducing microorganisms is an essential process to improve the layer housing environment.

Several approaches can be used to prevent or reduce diseases in animal breeding houses, including vaccination, antibiotic injection, wiping, and cleaning. Spraying disinfectants is used extensively in modern intensive livestock production (Rodríguez Ferri et al., 2010). Specifically, prophylactic disinfection is becoming more and more important in animal husbandry (Böhm, 1998). Cleaning and disinfection following depopulation of layer houses has been shown to have limited effectiveness (Davies and Breslin, 2003); therefore, it is essential to develop new methods for decontamination of breeding houses.

Recently, a new agent named slightly acidic electrolyzed water (**SAEW**, pH 5.0–6.5), has been increasingly used for the prevention and control of microorganism because it is easy to produce continuously through electrolysis using commercial equipment (Koide et al., 2011). Len et al. (2000) indicated that SAEW contains primarily hypochlorous acid (**HOCl**), which is an effective form of the chlorine. It has the advantage of possessing antimicrobial activity, reducing corrosion of surfaces, and minimizing the potential for damage to human health and the environment (Abadias et al., 2008; Koide et al., 2009; Rahman et al., 2010). Studies often focus on detection of the disinfection efficiency of SAEW on monoculture bacteria including *E. coli*, *Staphylococcus aureus*, *Salmonella*, *Vibrio*, *Bacillus*

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spores, and *Leuconostoc* sp. (Nakayama et al., 2003; Issa-Zacharia et al., 2010a,b; Quan et al., 2010). However, little information is available on the efficacy of SAEW on decontaminating surface pathogens in animal houses, particularly in layer houses.

The objective of this study was to evaluate the inactivation efficacy of spraying SAEW to inhibit microorganisms on solid materials and on facility surfaces and the effect of SAEW on survival rates of *Salmonella* and *E. coli* in an egg-producing house. Also, the effectiveness of SAEW compared with chemical disinfectants including benzalkonium chloride solution and povidone-iodine solution was investigated.

MATERIALS AND METHODS

Housing

Experiments were conducted in a layer house with dimensions of 100 m × 12 m × 4 m (length × width × height) located in the suburban district of Beijing, northern China. The study was carried out in the winter of 2011. This facility housed about 16,000 breeding hens (351 d old) confined in 4 rows of 3-tier stair-step cages, which provided an average space of 450 cm^2 per hen. The house was ventilated with an automatic negative-pressure ventilation system with 5 fans located in the end wall. Feed and water were supplied by an automatic feeding trough and drinking systems nipples. In the study period, the layers were kept in the cages and the researchers and animal workers applied strict hygiene to prevent cross-infections.

Disinfectants

Slightly acidic electrolyzed waters containing different available chlorine concentrations (**ACC**) were generated by electrolyzing 0.1% NaCl solution using a continuous SAEW generator (CWD-A, Shenyang Dongyu Xinbor Technology Company Ltd., Shenyang, China), which was set at 45 V and 30 A (Figure 1). The electrodes were two 30 × 15 cm^2 plates of Ti/Pt-IrO_2, and the distance between anode and cathode was set at 1 cm. Slightly acidic electrolyzed water with a pH of 5.29 to 6.30, an oxidation-reduction potential (**ORP**) of 974 to 987 mV, and an ACC of 30 to 250 mg/L was produced in the SAEW generator with different electrolyzing time. All chemicals used were of analytical grade.

The physicochemical properties of SAEW were measured immediately before use. The pH and ORP values were measured using a dual-scale pH/ORP meter (CON60, Trans-Wiggens, Singapore) with a pH electrode (PE02) or an ORP electrode (ORP06). The range of this sensor was pH 0.00 to 14.00 and ORP −999 to +999 mV, respectively. The ACC was determined using a digital chlorine test kit (RC-2Z, Kasahara Chemical Instruments Corp., Saitama, Japan). The resolution is 1 mg/L. The detection limit is 0 to 320 mg/L. All electrolyzed water treatment solutions were used within 1 h after preparation.

Benzalkonium chloride solution (Bayer Animal Health Co. Ltd., Sichuan, China) and povidone-iodine solution (Beijing Realm-Zone Animal Health Co. Ltd., Beijing, China) were purchased from a commercial supplier. For each disinfectant, the product names, classes, effective components, and recommended and practical use concentrations are shown in Table 1. Product dilutions were done with tap water.

Disinfection Efficiency of SAEW on Dust, Feces, Feathers, and Feed

Solid samples (5 of each) such as feces, feed, feathers, and dust were collected inside the layer house on 3 d of disinfection, 1 d a week for 3 consecutive weeks. The samples were gathered together with sterile moist gauze swabs and transferred using sterile forceps and spoon. Dust was collected from windows, beams, water pipes, cages, and floor. Feed was collected from different locations in the feed trough. Feces were collected from different locations on the manure board, and naturally shed feathers were sampled from different cages. Samples were stored at ambient temperature for transport to the laboratory and processed on the day of collec-

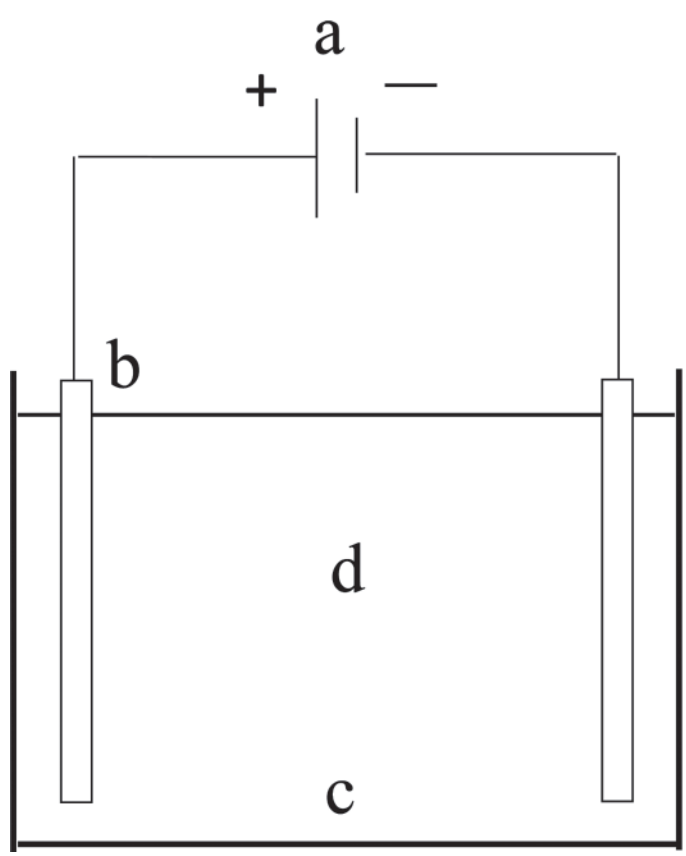

Figure 1. Schematic diagram of the slightly acidic electrolyzed water generator. a) DC power, b) electrodes, c) electrolytic cell, and d) slightly acidic electrolyzed water.

Table 1. Main characteristics of the slightly acidic electrolyzed water (SAEW), benzalkonium chloride solution, and povidone iodine solution

Disinfectant	Class of disinfectant	Effective components	Recommended use concentration	Practical use concentration
SAEW	Oxidizing agent nolvasant	Hypochlorous acid (HClO), hypochlorite (ClO^-)		30 to 250 mg/L
Benzalkonium chloride	Quaternary nolvasant	Benzalkonium chloride (20%)	1:1,000 to 1:10,000 (vol/vol)	1:1,000 (vol/vol) 1:2,000 (vol/vol)
Povidone iodine	Iodine	Povidone iodine (5%)	1:1,000 (vol/vol)	1:1,000 (vol/vol) 1:2,000 (vol/vol)

tion. Each of the dust (100 mg), feces (100 mg), feather (3 pieces), and feed (100 mg) samples was mixed into 5 mL of peptone water (Beijing Land Bridge Technology Co. Ltd., Beijing, China) and used as suspensions. All suspensions were processed as follows:

- Treatment 1: Each suspension (1 mL) was mixed with 9 mL of SAEW with an ACC of 30 mg/L for 5 min.
- Treatment 2: Each suspension (1 mL) was mixed with 9 mL of SAEW with an ACC of 250 mg/L for 5 min.
- Treatment 3: Each suspension (1 mL) was mixed with 9 mL of benzalkonium chloride solution with a dilution of 1:1,000 (vol/vol) for 5 min.
- Treatment 4: Each suspension (1 mL) was mixed with 9 mL of povidone-iodine solution with a dilution of 1:1,000 (vol/vol) for 5 min.
- Control: Each suspension (1 mL) was mixed with 9 mL of distilled tap water for 5 min.

Each suspension was serially diluted (1:10, vol/vol) in 0.1% peptone water, and each dilution (0.1 mL) was surface plated in triplicate onto plate count agar (Beijing Land Bridge Technology Co. Ltd., Beijing, China) plates to determine total bacterial counts, and onto Sabouraund dextrose agar (Beijing Land Bridge Technology Co. Ltd.) to determine fungal counts. The bacterial and fungal plates were cultured at 37°C and 28°C for 48 h, respectively (Şahin et al., 2005; Broekaert et al., 2011).

Disinfection Efficiency of SAEW on the Equipment or Facility Surfaces

The fecal matter was removed and the bird house was cleaned with tap water before the experiments. The house equipment was sprayed with SAEW having an ACC of 250 mg/L, pH value of 6.19, and ORP of 974 mV. The SAEW spray was conducted artificially with a high-pressure sprayer at a spray rate of 120 mL/m^2. Surface samples from floor, wall, feed trough, water pipes, and cage floor were taken with sterile swabs before cleaning, after cleaning but before disinfection, and after spraying disinfection for 5 min. Sterile swabs soaked in buffered peptone water (**BPW**) were used to collect the microbes on the surfaces (5 × 5 cm^2) of floor, wall, water pipes, and feed trough. The cage surface samples were obtained from 10 adjacent grids per swab and each grid had a dimension of 3 × 3 cm. Swab samples were soaked in BPW (5 mL) and sent to the laboratory under ambient conditions on the day of collection and processed immediately. The swab suspensions were serially diluted (1:10) in 0.1% peptone water, and 0.1 mL of each dilution was plated in duplicate onto plate count agar plates for total bacteria determination. The plates were incubated at 37°C for 48 h. The dilution selected for counting was that producing 30 to 300 cfu/mL.

Effectiveness of SAEW Against Salmonella and E. coli

The existence of *Salmonella* in the hen house was confirmed following the ISO-6579:2002 method. A total of 289 samples were selected before disinfection, and the same group of 289 samples was collected after spraying disinfection with SAEW. Each sample group consisted of the following number of swabs: floor (25), wall (13), water pipes (30), feed troughs (25), egg surface (26), cages (25), vehicles (8), workers' hands (8), feces (37), feed (40), feathers (15), floor sweepings (6), beams (9), windows (11), dust on water pipes (5), and dust around cages (6). Samples from workers' hands were collected with sterile moist gauze swabs, which were wiped back and forth on the whole surface of hands. One hand of each worker was sampled before disinfection, and another hand was sampled after disinfection. All samples were taken directly into 10 mL of buffered peptone water and preenriched at 37°C for 18 h, followed by selective enrichment on modified semisolid Rappaport-Vassiliadis broth (Aoboxing Bio-Tech Co., Ltd., Beijing, China) for 48 h at 41.5°C. Subcultures were streaked onto Xylose Lysine Desoxycholate or Brilliant Green agar (Aoboxing Bio-Tech Co. Ltd.) and incubated at 37°C for 24 h. Meanwhile, after the first stage of preenrichment in BPW for 18 h at 37°C, 1 mL of each suspension was enriched in selective cultivation of MacConkey broth and subcultivated onto MacConkey agar (Aoboxing Bio-Tech Co. Ltd.) for 48 h at 37°C.

Statistical Analyses

Statistical analyses of the number of bacteria were performed after logarithmic transformation. Data were analyzed using the ANOVA (SAS Institute Inc. Cary, NC). Results were reported as means and SE. A significance level of less than 0.05 was performed using Student's *t*-test.

RESULTS

Microbial Inactivation Efficiency of SAEW on Dust, Feces, Feathers, and Feed in a Layer House

The disinfection efficiency comparison of 3 disinfectants on dust, feces, feathers, and feed in a layer house is shown in Table 2. As the data presented in this table, the inactivation efficiency of SAEW on all materials increased with increasing ACC. The SAEW with ACC of 30 and 250 mg/L significantly reduced bacteria and fungus in dust, feces, feathers and feed ($P < 0.05$). The detection of bacteria on feces, feathers, and feed (initial populations of 9.02, 4.75, and 7.64 $\log_{10}$ cfu/mL) and fungi on dusts, feces, and feed (initial populations of 7.75, 7.07, and 5.47 $\log_{10}$ cfu/mL) was below detectable levels by SAEW treatment at an ACC of 250 mg/L for 5 min. The initial population of fungus (2.47 $\log_{10}$ cfu/mL) on feathers was inactivated to below detectable levels by SAEW at an ACC of 30 mg/L.

Furthermore, there were significant differences ($P < 0.05$) on the inactivation of microbes among SAEW (250 mg/L), benzalkonium chloride and povidone-iodine solutions. Treatment of 4 solid materials (dust, feces, feathers and feed) with benzalkonium chloride and povidone-iodine solutions only partially decreased the bacterial populations (benzalkonium chloride solution resulted in a reduction of 2.51, 3.98, 0.99, and 2.93 $\log_{10}$ cfu/mL, while the bacterial populations were reduced about 1.78, 4.55, 1.28, and 2.95 $\log_{10}$ cfu/mL by povidone-iodine solution).

Bacteria Inactivation Efficiency of SAEW on Equipment or Facility Surfaces in Layer Houses

The cleaning efficiency of SAEW on the floor, walls, feed troughs, water pipes, and cage floors is illustrated in Table 3. It shows that SAEW significantly reduced the population of organisms on the floor, wall, feed trough, and water pipes ($P < 0.05$) after spraying a layer breeding house for 5 min. The microbes on the floor were inactivated at a count of 0.79 $\log_{10}$ cfu/cm^2. Moreover, cleaning with tap water had a significant effect on reducing the microbes on floor and wall surfaces ($P < 0.05$); the reductions were 0.37 and 0.88 $\log_{10}$ cfu/cm^2, respectively. No significant difference ($P > 0.05$) was found in tests of cage floor during the whole cleaning and disinfection process.

Effect of SAEW on Salmonella and E. coli Survival in Layer Houses

Prevalence of *Salmonella* and *E. coli* obtained from various sample sites in the layer breeding house before and after disinfection is measured in Table 4. The data illustrate that *Salmonella* and *E. coli* can be isolated from almost all of the surfaces and solid materials, and that SAEW can decrease their prevalence. There was an overall *Salmonella* total isolation rate of 60% positive before disinfection, which was double that of *E. coli*. However, the isolation rates of *Salmonella* and *E. coli* showed a decrease of 19 and 16 percentage points after spraying with SAEW, respectively. Specifically, feed samples from 40 sites in the breeding house were identified as *Salmonella*-positive and *E. coli*-negative before disinfection, and spraying SAEW reduced *Salmonella* isolation by 72% of the feed samples. A *Sal-*

Table 2. Mean populations of microorganisms in dust, feces, feathers, and feed after disinfection with slightly acidic electrolyzed water (SAEW), benzalkonium chloride solution, and povidone iodine solution[1]

	Survival population ($\log_{10}$ cfu/mL)/% of reduction				
Item	Tap water (control)	SAEW (30 mg/L)	SAEW (250 mg/L)	Benzalkonium chloride (1:1,000, vol/vol)	Povidone iodine (1:1,000, vol/vol)
Bacteria					
Dust	9.55 ± 0.45^a	8.00 ± 0.10^b/97	6.77 ± 0.10^c/100	7.04 ± 0.07^c/100	7.77 ± 0.03^b/98
Feces	9.02 ± 0.12^a	8.74 ± 0.09^b/48	ND^{2e}/100	5.04 ± 0.05^c/100	4.47 ± 0.06^d/100
Feathers	4.75 ± 0.06^a	3.60 ± 0.09^c/93	ND^e/100	3.76 ± 0.08^b/90	3.47 ± 0.07^d/95
Feed	7.64 ± 0.08^a	6.25 ± 0.05^b/96	ND^d/100	4.72 ± 0.05^c/100	4.70 ± 0.04^c/100
Fungi					
Dust	7.75 ± 0.11^a	4.44 ± 0.09^b/100	ND^c/100	ND^c/100	ND^c/100
Feces	7.07 ± 0.06^a	5.63 ± 0.08^b/96	ND^d/100	3.46 ± 0.06^c/100	ND^d/100
Feathers	2.47 ± 0.07	ND/100	ND/100	ND/100	ND/100
Feed	5.47 ± 0.06^a	5.46 ± 0.05^a/2	ND^b/100	ND^b/100	ND^b/100

[a–e]Means in the same row followed by different superscripts are significantly different as determined by Student's *t* test ($P < 0.05$).

[1]Data reported as means ± SD. The treatment duration was 5 min.

[2]ND = negative by enrichment and no detectable survivors by a direct plating procedure.

Table 3. Mean populations of microorganisms on the facility surface before and after spraying disinfection with slightly acidic electrolyzed water[1]

Treatment site	Survival population ($\log_{10}$ cfu/cm^2)		
	Before cleaning	After cleaning	After disinfection
Floor	5.01 ± 0.11^a	4.64 ± 0.13^b	4.22 ± 0.19^c
Walls	4.01 ± 0.04^a	3.13 ± 0.08^b	2.95 ± 0.17^b
Feed troughs	3.54 ± 0.10^a	3.39 ± 0.11^{ab}	3.16 ± 0.16^b
Water pipes	5.64 ± 0.15^a	5.52 ± 0.11^a	4.39 ± 0.02^b
Cage floor	3.93 ± 0.16^a	3.87 ± 0.17^a	3.84 ± 0.19^a

[a–c]Means in the same row followed by different superscripts are significantly different as determined by Student's *t*-test ($P < 0.05$).

[1]Data reported as means ± SD. Slightly acidic electrolyzed water: available chlorine concentration of 250 mg/L, pH value of 6.19, oxygen reduction potential of 974 mV, and treatment time of 5 min.

monella isolation rate of 95% from feces samples was detected, whereas one-half of the samples were identified as *E. coli*-positive. After spraying SAEW, the *Salmonella* and *E. coli* in feces declined as by 25 and 48 percentage points, respectively. Eighty-eight percent of the workers' hands tested positive for *Salmonella* and 13% were positive for *E. coli*. There was a general reduction of 38 percentage points for *Salmonella* after washing hands in SAEW, but no reduction for *E. coli*.

DISCUSSION

Generally, we used BPW or NaCl solution to treat the samples and calculate the bacteria concentration before treatment. We found there was no significant difference on the bacteria concentration when we used distilled tap water to treat the samples (not shown in the paper). In this study, the bacteria concentration before treatment was evaluated by soaking the samples in distilled tap water. The control group shows the initial populations of bacteria and fungi. The data (Table 2) indicate that the populations of bacteria are always more than fungi irrespective of the sample (dust, feces, feathers, or feed). Treatment of SAEW with an ACC of 250 mg/L for 5 min can inactivate the bacteria (initial populations of 9.02, 4.75, and 7.64 $\log_{10}$ cfu/mL) and fungi (initial populations of 7.07, 2.47, and 5.47 $\log_{10}$ cfu/mL reduction) in feces, feathers, and feed below detectable levels. However, only 2.78 $\log_{10}$ cfu/mL bacteria in dust were decreased after treatment with the same SAEW in the same conditions. Organic matter in dust may play an important role in disinfection. But no reports have been found that dust carry more organic matter than feces and feed. The SAEW (ACC of 250 mg/L) significantly inhibited the bacterium and fungus in all materials compared with benzalkonium chloride and povidone-iodine solutions ($P < 0.05$) in this study. Therefore, SAEW (ACC of 250 mg/L) can be a potential disinfectant in layer houses.

Table 4. Detection of *Salmonella* and *Escherichia coli* isolated from various sampling sites in layer houses before and after disinfection with slightly acidic electrolyzed water[1]

Sampling sites	Number of samples	Positive number for *Salmonella*		Positive number for *E. coli*	
		Before (%)	After (%)	Before (%)	After (%)
Floor	25	16 (64[2])	12 (48)	8 (32)	8 (32)
Walls	13	29 (15)	0 (0)	2 (15)	0 (0)
Water pipes	30	19 (63)	8 (27)	8 (27)	5 (17)
Feed troughs	25	5 (20)	3 (12)	6 (24)	4 (16)
Egg surfaces	26	10 (39)	16 (62)	2 (8)	0 (0)
Cage floors	25	15 (60)	20 (80)	10 (40)	13 (52)
Vehicles	8	2 (25)	2 (25)	1 (13)	0 (0)
Hands of workers	8	7 (88)	4 (50)	1 (13)	1 (13)
Feces	37	35 (95)	26 (70)	19 (51)	1 (3)
Feed	40	40 (100)	11 (28)	0 (0)	0 (0)
Feathers	15	5 (33)	4 (27)	4 (27)	0 (0)
Floor sweepings	6	2 (33)	2 (33)	3 (50)	0 (0)
Beams	9	4 (44)	3 (33)	9 (100)	1 (11)
Windows	11	6 (55)	5 (46)	1 (9)	0 (0)
Dust on water pipe	5	4 (80)	2 (40)	0 (0)	0 (0)
Dust around cage	6	1 (17)	1 (17)	4 (67)	0 (0)
Total	289	173 (60)	119 (41)	78 (27)	33 (11)

[1]Slightly acidic electrolyzed water: available chlorine concentration of 250 mg/L, pH value of 6.19, oxygen reduction potential of 974 mV, and treatment time of 5 min.

[2]Percent positive in parentheses.

It was not possible to check the fungicidal efficiency of SAEW on surfaces of floor, walls, feed troughs, water pipes, and cage floor because the fungal counts on the sample plates were too small (<30). It has been shown that SAEW with an ACC of 250 mg/L can effectively remove the bacteria on surfaces including the floor, walls, feed troughs, and water pipes ($P < 0.05$; Table 3). However, there are many technical difficulties with achieving this antimicrobial aim on all surfaces or materials, such as on the cage floor. This may be due to the poor disinfectant reception of the floor, which was designed as a web structure. Additionally, the study proved that cleaning before disinfection is an important process. This is in agreement with the report of Böhm (1998), who indicated that cleansing and disinfection are working steps in animal husbandry that could not be separated from each other without losing efficiency.

Carrique-Mas and Davies (2008) reported that *Salmonella* survive desiccation better than other *Enterobacteriaceae*. Many typing methods have been used to trace the outbreaks to the contaminated source and to elucidate the epidemiology of its infection (Helmuth and Schroeter, 1994). The ISO-6579:2002 standard that was used in this study is applicable to products intended for human consumption or feeding of animals. As indicated in the present study, no site or substance in the breeding house was free of detectable *E. coli*, except in feed and dust on the water pipe. *Salmonella* was detected in all of the test targets, specifically in feed (Table 4). It is likely that the organic matter in feed dust supplies a protective nutritive matrix to enhance the survival of *Salmonella* (Davies and Wray, 1996). Furthermore, *E. coli*, another significant pathogen in layer farms, was mainly inactivated in dust around cage and feces, as the study shows.

The objective of cleaning and disinfection with SAEW in layer houses was to reduce contamination of the building and equipment such as pathogenic microorganisms and organic matter that can harbor such organisms that cause problems following repopulation. This study indicates that spraying SAEW in layer houses can reduce *Salmonella* detection rate to 41%, and decrease *E. coli* detection rate to 11%. However, SAEW was ineffective on *Salmonella* or *E. coli* presence on cage floors. This may be because of the movement of birds. Further research needs to be done.

In the present study, SAEW (pH 5.0–6.5) with an ACC of 250 mg/L can significantly reduce the bacteria and fungi in layer houses ($P < 0.05$). Zheng et al. (2012) reported SAEW can significantly reduce the dust levels in layer breeding houses. Electrolyzed oxidizing water may contain Cl_2, HOCl, and OCl^-, all of which contribute to available chlorine or free chlorine. The relative amount of each chlorine compound (Cl_2, HOCl, or ClO^-) is dependent on the pH value and temperature (Koide et al., 2009). Accordingly, the chlorine type in SAEW at a pH value of 5.0 to 6.5 is hypochlorous acid (HOCl, approximately 95%) with strong antimicrobial activity. The SAEW produced by electrolysis of dilute hydrochloric acid, NaCl solution, or both in a nonmembrane electrolytic system is more effective, convenient, and less expensive than other electrolyzed water systems (Cao et al., 2009).

Slightly acidic electrolyzed water with a near-neutral pH value (5.0–6.5) is an effective way to reduce *Salmonella* and *E. coli* isolation rate in layer houses. It can significantly inactivate bacteria and fungi on facility surfaces or solid materials ($P < 0.05$). The disinfection activity of SAEW increased with increasing the ACC. The SAEW at an ACC of 250 mg/L had a higher inactivation effect on microorganisms in layer farms than benzalkonium chloride solution (1:1,000) and povidone-iodine solution (1:1,000). In conclusion, the present study shows a wide range of effectiveness of cleaning and disinfection with SAEW, suggesting that spraying SAEW can be a new method to significantly depopulate pathogens in layer houses.

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