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Preventing *Salmonella* Colonization of Chickens

Electrostatic Application of Electrolyzed Oxidative Acidic Water

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Introduction

Salmonella spp. may be found in the nest box of breeder chickens, cold egg-storage rooms at the farm, on the hatchery truck, or in the hatchery environment (5). These bacteria may then be spread to fertilized hatching eggs on the shell or, in some cases, may penetrate the shell and reside just beneath the surface of the eggshell.

Research has demonstrated that contamination of raw poultry products with *Salmonella* spp. may be attributable to cross-contamination in the hatchery from *Salmonella* infected eggs or surfaces to uninfected baby chicks during the hatching process. Cox et al. (6 and 7) reported that broiler and breeder hatcheries were highly contaminated with *Salmonella* spp. Within the broiler hatchery, 71 percent of eggshell fragments, 80 percent of chick conveyor belts swabs, and 74 percent of pad samples placed under newly hatched chicks contained *Salmonella* spp. (6).

Cason et al. (4) reported that, although fertile hatching eggs were contaminated with high levels of *Salmonella typhimurium*, they were still able to hatch. The authors stated that paratyphoid salmonellae do not cause adverse health affects to the developing and hatching chick. During the hatching process, *Salmonella* spp. is readily spread throughout the hatching cabinet due to rapid air movement by circulation fans. When eggs were inoculated with a marker strain of *Salmonella* during hatching, greater than 80 percent of the chicks in the trays above and below the inoculated eggs were contaminated (4). In an earlier study, Cason et al. (3) demonstrated that salmonellae on the exterior of eggs or in eggshell membranes could be transmitted to baby chicks during pipping.

Salmonella may persist in hatchery environments for long periods of time. When chick fluff contaminated with *Salmonella* was held for 4 years at room temperature, up to 1,000,000 *Salmonella* cells per gram could be recovered from these samples (12).

Researchers have demonstrated a link between cross-contamination in the hatchery and contaminated carcasses during processing. Goren et al. (8) isolated salmonellae from three different commercial hatcheries in Europe and reported that the same serotypes found in the hatcheries could be found on processed broiler chicken carcass skin. Proper disinfection of the hatchery environment and fertile hatching eggs, therefore, is essential for reducing *Salmonella* on ready-to-cook carcasses.

Numerous studies have been conducted to evaluate sanitizing agents for disinfecting eggshell surfaces and membranes. Bailey et al. (1) reported that 2.5 percent H2O2, administered using 100 or 500 mL/h, reduced *Salmonella typhimurium* positive eggshells by 55 percent and the number of positive chicks by 53 percent. H2O2 reduced total aerobic bacterial counts (APC) in air in hatching egg incubators from 3.6 colony forming units (CFU)/L for water fogging to 0.35 CFU/L when the incubator was fogged with 3 percent H2O2 (13). Sheldon and Brake (14) demonstrated that 5 percent H2O2 reduced APC on hatching eggs from 3.98 cfu/egg for water sprayed eggs to 0.99 cfu/egg for treated eggs. Bailey et al. (1) reported that ozone at 0.2 to 0.4 ppm reduced *Salmonella typhimurium* positive eggshells by 10 percent and the number of positive chicks by 26.7 percent. In another study, ozone at 3.03 percent by weight was able to reduce APC on broiler hatching eggs by 2.57 log10 cfu (15). Brake and Sheldon (2) observed that a quaternary ammonium sanitizer at 3.0 percent reduced APC on broiler hatching eggs by 99.9 percent within 30 minutes of application. The methods used to apply sanitizers in these studies varied from gaseous exposure to fogging and dipping. Method of application may have a dramatic impact on efficacy of the sanitizers used.

Law (10) developed an electrostatic spray-charging system using air atomization which achieved a 7 fold increase in spray deposition over conventional application methods (See Figure 1).

Figure 1. Electrostatic Spray Nozzle.

In later studies, a 1.6- to 24-fold increase in deposition was reported (11). Herzog et al. (9) observed that insect control on cotton plants was equal to or better than conventional spray application using only one-half the amount of insecticide. Thus, the electrostatic spraying method may be an appropriate means of applying sanitizers in the hatchery environment — by distributing the sanitizer more effectively over the surface of eggs and equipment and by reducing the amount of sanitizer needed to eliminate pathogenic bacteria.

If EO water applied using ESS can significantly reduce *Salmonella* on the surface of fertile eggs, it is believed that this method may result in significant reductions in *Salmonella* contamination of raw poultry products. Although these studies collectively point to the fact that reduction of *Salmonella* in the hatchery should result in reductions on final product, this relationship has never been proven scientifically. Moreover, if a safe, nontoxic sanitizer could be proven effective in the hatchery, its use would be a welcomed replacement for formaldehyde, since formaldehyde is objectionable to both chicks and workers.

Photos of electrostatic spray nozzles and their operation in a commercial hatchery are presented in Figures 2-4.

As is demonstrated in the figures, the sanitizer is sprayed as a very fine fog and, in a short period of time, completely disappears. This fog completely covers every surface within the hatching cabinet, including eggs. Complete coverage has been demonstrated using fluorescent dye sprayed onto surfaces. After spraying, the area can be evaluated using a black light, and even the most difficult to reach spaces are completely covered.

Figure 2. Electrostatic spray nozzle in a hatchery plenum.

Figure 3. Electrostatic spraying of sanitizer in a hatching cabinet.

Figure 4. Electrostatic application of sanitizer in a hatchery plenum.

Matching the Sanitizer to the Electrostatic Spraying System

A major consideration when using electrostatic spraying is the type of sanitizer being used. Applying an electrical charge or atomization has the potential to completely eliminate the killing power of some sanitizers. When using electrostatic spraying, it is best to evaluate the sanitizer to be used in light of this limitation.

As mentioned previously, currently used sanitizers are objectionable for various reasons. Formaldehyde is difficult to work with and presents a worker safety hazard. Formaldehyde gas burns people's lungs and eyes when they are exposed to it, and many have compared the experience to that of being exposed to teargas. Glutaraldehyde is also unpleasant for workers. Hydrogen peroxide, while effective, is corrosive to equipment and is irritating to the lungs. It would seem that exposing baby chicks on day of hatch to these chemicals would be disadvantageous if other chemicals could be used that would be as effective at eliminating pathogenic bacteria.

Mixed Oxidants

A method has been developed for splitting salt water into streams of mixed oxidants. One such system (EAU Limited) is displayed in Figure 5. By mixing a 20 percent solution of salt water, and placing the water in the container inside the machine, the water is then taken up by the instrument, passed over an electrode, and various oxidizing chemicals are generated. The water comes out of the machine as two different mixed oxidant streams. One stream is acidic (pH 2.1) and the other is alkaline (pH 10.8). The acidic portion of this water has been shown to be effective for killing various pathogenic bacteria of concern to the poultry industry in an experimental setting (K. S. Venkitanarayanan et al., 1999a, and K. S. Venkitanarayanan et al., 1999b). Some of the possible mixed oxidants produced by electrolyzing the salt water are H2O2, Cl-, HOCl, O3 and ClO2. All of these compounds have been proven to be effective sanitizers, but the unique aspect of this methodology is that these compounds are produced in very low concentrations (3 to 80 ppm, depending on the system). Collectively, these chemicals exhibit bacteriocidal activity while the water remains safe enough to drink and is not harmful to equipment. The advantage of using electrolyzed water is that it costs almost nothing to produce, is safe enough to drink and breathe (when atomized), is effective against pathogenic bacteria, and will not corrode equipment.

The purpose of the studies described in this publication was to determine if electrostatic application (ESS) of a nontoxic, novel sanitizer would be effective in eliminating *Salmonella* spp. from fertile hatching eggs, and to evaluate if this reduction carries through to a significant reduction in colonization of chickens during the growout process.

Figure 5. Primacide mixed oxidant generator with the door closed.

The Studies

Materials and Methods

Study I

Pathogenic Bacterial Isolates

Salmonella typhimurium, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli* were obtained from the United States Department of Agriculture, Agricultural Research Service's (USDA-ARS) Poultry Microbiological Safety Unit laboratory. These isolates were originally collected from commercial broiler carcasses. Each isolate was assayed for Gram reaction, cytochrome oxidase activity and production of catalase, and was identified using either the Vitek, Biolog or Micro-ID rapid identification methods.

EO Water Preparation

A solution of EO water was prepared by electrolysis of a 20 percent saline solution made with tap water. The final pH and oxidation-reduction potential of this solution were 2.1 and 1150, respectively. Due to electrolysis of the saline solution, small concentrations of antimicrobial substances were produced including chlorine ions (8 ppm free chlorine), chlorine dioxide, ozone and hydrogen peroxide (See Figure 5). It is believed that the combination of very low concentrations of these compounds in an acidic environment is the mechanism of action for EO water.

Egg Preparation
Eggs were collected from layer chickens housed at The University of Georgia Poultry Research Center. After collection, the eggs were washed using a commercially available chlorine based sanitizer and allowed to dry. Each egg was then rinsed thoroughly three times using sterile deionized water to remove any residual sanitizer that may have remained from the washing process.

Egg Inoculation
An inoculation solution was prepared by placing 0.1 mL of an actively multiplying pure bacterial culture (incubated 24 h in brain heart infusion broth5 at 35 degrees C) into 200 mL of sterile 1 percent peptone broth. The bacterial cultures used were *Salmonella typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Escherichia coli*. Eggs were individually dipped into the inoculum and allowed to dry under a laminar flow hood for 1 h. This procedure provided time for the bacteria to attach to the surface of the egg.

Electrostatic Spraying of Eggs
Each egg was placed into a clean egg flat and positioned in an electrostatic spraying chamber. Tap water (2 repetitions) or EO water (4 repetitions) was sprayed onto the eggs using two electrostatic spray nozzles for 15 s each hour for 24 h (See Figures 6 and 7). After treatment, the eggs were allowed to dry under a laminar flow hood for 1 h. In addition, 2 eggs were dipped in each bacterial isolate, allowed to dry and stored for 24 h in an enclosed chamber with 96 percent humidity as a control.

Neutralization of Sanitizer
Each control and treated egg was cracked using a sterile blade and the contents were removed. Eggshells and membranes were placed into 25 mL of sterile 1 percent peptone broth containing 3 percent Tween 80, 0.3 percent lecithin and 0.1 percent histidine to neutralize the sanitizers. Microbiological Evaluation

One milliliter of this mixture was placed into 9 mL of sterile BHI, which acts as a growth medium for conducting impedance or conductance assays, and vortexed. One mL of this mixture was placed into a Bactometer module well in duplicate. Samples were monitored using the Bactometer Microbial Monitoring System M128. All of the bacterial isolates tested were monitored at 35 degrees C. All samples were monitored for 48 h using impedance except for E. coli, which was monitored using conductance.

Statistical Analysis
The experimental design was a 4 x 4 x 2 of replication, bacterial type and treatment (EO water and controls). All microbiological analyses were conducted in duplicate. Data were analyzed after averaging the duplicates. Results were analyzed using the General Linear Models (GLM) procedure of SAS software (SAS Institute, 1994). Treatment means were separated using Fisher's Least Significant Difference option of SAS software (SAS Institute, 1994). All values reported as significant were analyzed at the α=0.05 level.

Study II

Pathogenic Bacterial Isolates
Salmonella typhimurium was obtained from the United States Department of Agriculture, Agricultural Research Service's (USDA-ARS) Poultry Microbiological Safety Unit laboratory. These isolates were originally collected from commercial broiler carcasses. Each isolate was assayed for Gram reaction and cytochrome oxidase activity, and identified using the Vitek rapid identification method.

EO Water Preparation
A solution of EO water was prepared by electrolysis of a 20 percent saline solution made with tap water. The final pH and oxidation-reduction potential of this solution were 2.1 and 1150, respectively. Due to electrolysis of the saline solution, small concentrations of antimicrobial substances were produced including chlorine ions (8 ppm free chlorine), chlorine dioxide, ozone and hydrogen peroxide. It is believed that the combination of very low concentrations of these compounds in an acidic environment is the mechanism of action for EO water.

Industrial Hatchability Study
Thirty thousand eggs were placed into clean egg flats and positioned in two separate commercial hatchers (15,000 each) at a commercial primary broiler breeder facility. Tap water in one hatching cabinet and EO water in the other hatching cabinet were sprayed onto the eggs using two electrostatic spray nozzles. The timers were set to deliver EO water to the eggs for 5 minutes immediately upon placement and then 2 minutes every 6 hours for the first day in the hatching cabinet. On the second day, the nozzles delivered EO water for 2 minutes every 4 hours. On the final day of hatch, the nozzles produced EO water for 2 minutes every 2 hours.

These nozzles were designed to spray a volume of 280 mL of liquid per minute. After hatching, the percent hatchability was determined for each hatching cabinet.

Egg Preparation and Inoculation

Fertile hatching eggs were collected from broiler breeder chickens housed at The University of Georgia, Poultry Research Center and were incubated for 18 days in setters. An inoculation solution was prepared by placing 0.1 mL of an actively multiplying culture of *Salmonella typhimurium* (incubated 24 h in brain heart infusion broth at 35 degrees C) into 200 mL of sterile 1 percent peptone broth. Eggs were individually dipped into the inoculum and allowed to dry for 1 h. This procedure provided time for the bacteria to attach to the surface of the egg.

Electrostatic Spraying of Eggs

In the second portion of Study II conducted at the UGA Poultry Research Center, 40 eggs were placed into clean egg flats and positioned in two separate commercial hatchers in two separate repetitions. Tap water in one hatching cabinet and EO water in the other hatching cabinet were sprayed onto the eggs using two electrostatic spray nozzles. The timers were set to deliver EO water to the eggs for 5 minutes immediately upon placement and then 2 minutes every 6 hours for the first day in the hatching cabinet. On the second day, the nozzles delivered EO water for 2 minutes every 4 hours. On the final day of hatch, the nozzles produced EO water for 2 minutes every 2 hours. These nozzles were designed to spray a volume of 280 mL of liquid per minute. After hatching, the percent hatchability was determined for each hatching cabinet. After hatching, the percent hatchability was determined for each hatching cabinet.

Growout

After hatching, the chicks from each hatcher were transported separately to different research facilities that had been thoroughly disinfected. The chicks were reared to 4 weeks of age being fed and watered ad libitum. After 4 weeks, the birds were euthanized using CO2 gas. The lower digestive tract (including the ileal junction, ceca, rectum and cloacae) were removed from each bird, placed into a sterile plastic bag, encoded and transported to Woodsen-Tenant Laboratories for evaluation for the presence (colonization) of *Salmonella*.

Microbiological Evaluation

The lower digestive tracts of each bird were evaluated using the following method:

1. Intestines and ceca were homogenized in 250 mL of universal preenrichment broth and incubated for 24 h at 35 degrees C.
2. A 1 mL aliquot was transferred to 10 mL selenite cysteine (SC) broth and a 1 mL aliquot was transferred to 10 mL tetrathionate (TT) broth. The SC broth tubes were incubated at 35 degrees C for 8 hours and the TT broth tubes were incubated at 42 degrees C for 8 hours in a water bath.
3. 1 mL aliquots from SC and TT broth tubes were placed separately into two tubes containing M-broth and incubated at 35 degrees C for 6 hours in a water-bath.
4. Tecra ELISA visual immunoassays were used to evaluate the tubes for the presence of *Salmonella*.
5. Presumptive positives were streaked onto xylose lysine desoxycholate (XLD), Hektoen enteric (HE), bismuth sulfate (BS), and xylose lysine tergitol (XLT4) agars and incubated at 35 degrees C for 24 hours.
6. Colonies were streaked onto triple sugar iron (TSI) and lysine iron agar (LIA) slants and incubated at 35 degrees C for 24 hours.
7. Slants exhibiting typical reactions for *Salmonella* were evaluated using Poly A-I and Vi and Poly a-z for “O” and “H” antigens.

Statistical Analysis

The experimental design for the industrial hatchability study was a 1 x 2 x 15,000 of replication, treatment (tap water or EO water), and egg. The experimental design for the hatchability study at the university was a 2 x 2 x 80 of replication, treatment (water or EO water), and egg. The experimental design for the *Salmonella* recovery portion of the study was a 2 x 2 x 40 of replication, treatment, and egg. Results were analyzed using the logistic progression procedure of SAS software (SAS Institute, 1994). All values reported as significant were analyzed at the α=0.05 level.

Results and Discussion

Study I

Bacterial proliferation requires the availability of nutrients such as carbohydrates, proteins, or lipids. As bacteria break down and utilize these nutrients, they release charged byproducts such as lactic acid and acetic acid (Cady, 1974). As charged metabolites accumulate, the conductance and capacitance of the growth medium increases, and impedance decreases. A significant and dramatic shift in the electrical component of the medium occurs when bacterial populations reach a threshold of 106 to 107 cells/mL (Firstenberg-Eden, 1983). The time required for this shift to occur is called the detection time (DT). DT is dependent on the initial concentration of bacteria, the rate at which bacteria in the sample reproduce, the temperature and the test medium used (Richards et al., 1978; Silley and Forsythe, 1996). Using electrical methods, highly contaminated samples would be detected first. For example, a sample that initially contains 105 organisms would require fewer cell divisions to reach the 106 detection threshold, than a sample that initially contains only 101 bacteria. Thus, DT is inversely proportional to the initial bacterial level in the sample. If impedance or conductance detection times are significantly increased when bacterial populations are exposed to a chemical sanitizer, then the sanitizer had an inhibitory effect on the proliferation of the bacterium or group of organisms. In addition, if no detection time is recorded in 48 h, then it is assumed that the organism was deactivated or injured beyond repair by the sanitizer, as it was unable to multiply under optimal growth conditions.

In this study, significant differences in bacterial inhibition by EO water were observed between replicates. For each replicate, different concentrations of bacteria were used and the oxidation/reduction potential (ORP) of the EO water evolving from the electrostatic spray nozzle head varied within and between replicates. Thus, the differences observed between replicates may be attributed to application of high numbers of bacteria in some instances and fluctuation in ORP values. Fluctuation in ORP at the nozzle head may be attributed to the charge of the liquid coming out of the nozzle, the air speed of compressed air carrying the sanitizer, and the size of the liquid droplet coming from the nozzle. None of these variables are associated with the sanitizer but are able to be controlled by adjustments to the electrostatic spray nozzle system, especially if this system is to be used in an industrial setting.

Colony forming units (cfu) of bacteria per milliliter of inoculum exposed to EO water are presented in Table 1. Please note that, in some cases, very high concentrations of bacteria were challenged in this study to determine the effect of the sanitizer on high numbers of actively growing pathogens and indicator populations of bacteria.

Impedance and conductance detection times (hours), and log10 cfu estimations for pure cultures of *Salmonella typhimurium* (ST), *Staphylococcus aureus* (SA), *Listeria monocytogenes* (LM), and conductance detection times (hours) for *Escherichia coli* (EC) on eggs that have been treated with tap water (2 replicates) or EO water (4 replicates) using electrostatic spraying, and control eggs that were not treated are presented in Tables 2 and 3, respectively (page 10). EO water completely eliminated all *Salmonella typhimurium* on 3 (20%), 7 (46.7%), 1 (6.7%) and 8 (53.3%) eggs of 15 tested in Reps 1, 2, 3 and 4, respectively. In all Reps, for the sanitizer to eliminate ST on an egg completely, a minimum of a 5 log10 reduction would be required. In Rep 4, when 53.3 percent of eggs were negative for ST, 6 log10 ST were killed. In addition, for eggs that remained positive, the number of ST remaining were significantly reduced by a minimum of 4 log10 when compared to control eggs.

EO water was able to eliminate *Staphylococcus aureus* on 12 (80%), 11 (73.3%), 12 (80%) and 11 (73.3%) eggs of 15 tested in Reps 1, 2, 3 and 4, respectively

Table 1. Number of bacteria (cfu/mL) inoculated onto the surface of eggs.

Bacterium	Repetition 1	Repetition 2	Repetition 3	Repetition 4
Salmonella typhimurium	>1,000,000	371,000	620,000	>1,000,000
Staphylococcus aureus	820	9,350	1,070,000	719,600
Listeria monocytogenes	225	200	15,350	62,300
Escherichia coli	70,140	67,300	47,500	1,415,000

(Table 2). In Reps 3 and 4, for the sanitizer to eliminate SA on an egg completely, a minimum of a 6 log10 and a 5 log10 reduction would be required, respectively. In addition, for eggs that remained positive, the number of SA remaining were significantly reduced by a minimum of 3 log10 when compared to control eggs.

For *Listeria monocytogenes*, EO water eliminated all bacteria on 8 (53.3%), 13 (86.7%), 12 (80%) and 14 (93.3%) eggs of 15 tested in Reps 1, 2, 3 and 4, respectively (Table 2). In Reps 3 and 4, for the EO water to eliminate LM on an egg completely, a minimum of a 4 log10 reduction would be required. In addition, for eggs that remained positive, the number of LM remaining were significantly reduced by a minimum of 1 log10 (Rep 2) or 2.2 log10 (Reps 3 and 4) when compared to control eggs, except in Rep 1.

EO water eliminated all *Escherichia coli* (E. coli) on 9 (60%), 11 (73.3%), 15 (100%) and 11 (73.3%) eggs of 15 tested in Reps 1, 2, 3 and 4, respectively (Table 2). In all Reps, for the sanitizer to eliminate EC on an egg completely, a minimum of a 4 log10 reduction would be required. In Rep 4, when 73.3 percent of eggs were negative for EC, 6 log10 EC were killed. In addition, for eggs that remained positive, the number of EC remaining were significantly ($P \leq 0.05$) reduced by a minimum of 2 log10 when compared to control eggs. In Rep 3, EO water performed especially well by eliminating all EC on all eggs, even when a concentration of 47,500 cfu/mL were used.

These data are promising in that EO water is nontoxic and can be consumed as produced. Moreover, this sanitizer is environmentally friendly and is not harmful to humans. Because *Salmonella* testing is part of the USDA.-Food Safety and Inspection Service (F.S.I.S.) Pathogen Reduction Final Rule (USDA-FSIS, 1996), and *Salmonella* is spread throughout the hatchery environment, leading to cross-contamination and eventual contamination of the product, this sanitizer should prove effective as a means of treating hatching eggs. Currently used hatchery sanitizers (formaldehyde gas and glutaraldehyde) are noxious to humans and chicks, and may pose a serious health risk. Thus, a sanitizer that does not harm chicks, is inexpensive to produce, and is effective would be a useful tool for the poultry industry.

Table 2. Impedance or conductance detection times (DT-hours) for eggs coated with *Salmonella typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Escherichia coli* populations and treated with tap water and electrolyzed oxidative acidic (EO) water applied using electrostatic spraying, or no treatment (controls).

		Repetition 1			Repetition 2			Repetition 3			Repetition 4		
Sanitizer Used	**Bacterium**	**Pos[1]**	**Avg DT[2]**	**Control DT[3]**	**Pos**	**Avg DT**	**Control DT**	**Pos**	**Avg DT**	**Control DT**	**Pos**	**Avg DT**	**Control DT**
Tap water	*Salmonella typhimurium*	10/10	6.44	—[4]	10/10	6.34	—	—	—	—	—	—	—
EO water	*Salmonella typhimurium*	12/15	7.2[a]	2.35[b]	8/15	14.6[a]	5.07[b]	14/15	9.4[a]	7.1[b]	7/15	8.2[a]	5.2[b]
EO water	*Staphylococcus aureus*	3/15	11.2[a]	3.9[b]	4/15	8.2[a]	4.2[b]	3/15	13.8[a]	3.6[b]	4/15	10.7[a]	5.6[b]
EO water	*Listeria monocytogenes*	7/15	8.2	8.5	2/15	9.6[a]	7.6[b]	3/15	10.7[a]	6.5[b]	1/15	19.4[a]	5.4[b]
EO water	*Escherichia coli*	6/15	6.05[a]	1.05[b]	4/15	16.0[a]	5.35[b]	0/15	4.80[a]	8.7[b]	4/15	7.1[a]	5.0[b]

[a-b] Numbers with no common superscripts differ significantly ($P \leq 0.05$).

[1] Pos is the number of eggs that were positive for growth and produced a detection time out of the number evaluated. (DT = time required for bacterial populations to reach 106 cfu/mL; no detection time indicates no bacteria survived.)

[2] Avg DT is the average time required for bacteria that survived treatment to multiply to 106 (longer DT = fewer bacteria).

[3] Control DT is the average time required for control eggs dipped in each bacterium that were not exposed to sanitizer to multiply to 106.

[4] — Samples were not evaluated.

Table 3. Log10 cfu/mL estimations1 from impedance or conductance detection times (DT-hours) for eggs coated with *Salmonella typhimurium*, *Staphylococcus aureus*, *Listeria Monocytogenes* and *Escherichia coli* populations and treated with tap water and electrolyzed oxidative acidic (EO) water applied using electrostatic spraying, or no treatment (controls).

		Repetition 1			Repetition 2			Repetition 3			Repetition 4		
Sanitizer Used	**Bacterium**	**Pos[2]**	**Avg log[10]**	**Control log[10]**	**Pos**	**Avg log[10]**	**Control log[10]**	**Pos**	**Avg log[10]**	**Control log[10]**	**Pos**	**Avg log[10]**	**Control log[10]**
Tap water	*Salmonella typhimurium*	10/10	4.6	—	10/10	4.6	—	—	—	—	—	—	—
EO water	*Salmonella typhimurium*	12/15	4.0	7.5	8/15	>0.1	5.5	14/15	2.4	4.1	7/15	3.3	5.4
EO water	*Staphylococcus aureus*	3/15	>0.1	5.9	4/15	2.2	5.6	3/15	>0.1	6.1	4/15	0.1	4.4
EO water	*Listeria monocytogenes*	7/15	5.0	4.9	2/15	4.4	5.3	3/15	3.8	5.9	1/15	>0.1	6.4
EO water	*Escherichia coli*	6/15	4.0	8.8	4/15	>0.1	4.7	0/15	0	1.4	4/15	3.0	5.0

[1] Impedance or conductance detection times (hours) were subjected to analyses using line equations from established calibration curves for each bacterial species and log10 estimations were generated.

[2] Pos is the number of eggs that were positive for growth and produced a detection time out of the number evaluated. (DT = time required for bacterial populations to reach 106 cfu/mL; no detection time indicates no bacteria survived.)

Study II

Results for hatchability of commercial broiler breeder chicks from hatching eggs treated electrostatically with tap water or EO water during hatch are presented in Table 4. Although treatment with EO water seemed to lower hatchability slightly when compared to tap water treated fertile eggs, the hatchery manager indicated that this was expected because he used older fertile eggs for the EO water treated hatching cabinet. This effect was corroborated in the later hatchability study at the University of Georgia.

Table 4. Results for hatchability of commercial broiler-breeder chicks from hatching eggs treated electrostatically with tap water or EO water during hatch under commercial conditions.

	Normal Hatch*	Tap Water Treated	EO Water Treated
Hatchability	85%	82%	79%
n		15,000	15,000

* Fertile eggs used in this study for the EO water treatment were older and expected hatchability was lower than the normally expected hatch.

Results for hatchability of chicks from fertile hatching eggs obtained at the University of Georgia and treated electrostatically with tap water or EO water during hatch are presented in Table 5. No differences were observed in hatchability between EO treated or tap water treated eggs at 93 percent each. These data are very consistent with expected hatch percentages from the incubators at the UGA Poultry Research Center. Thus, hatchability does not seem to be a significant factor when considering the use of EO water for sanitizing hatching eggs during the hatching process.

Table 5. Results for hatchability of chicks from hatching eggs treated electrostatically with tap water or EO water during hatch under research conditions at the University of Georgia Poultry Research Center.

	Normal Hatch	Tap Water Treated	EO Water Treated
Hatchability	92%	93%	93%
n		160	160

We observed in these studies that electrostatic application of EO water completely removed the dust, fluff and dander from the air, upper surfaces of the hatching cabinet, and the eggs. It is believed that, by charging the EO water using electrostatic spraying, the dust and dander were also charged and fell to the floor, away from the eggs and chicks.

Results for *Salmonella typhimurium* prevalence in the lower intestines of broiler chickens from hatching eggs treated electrostatically with tap water or EO water during hatch are presented in Table 6. These results are extremely encouraging in that 65 to 95 percent (Replicate 1 and 2, respectively) of the chickens were colonized when only tap water was used

to treat the fertile hatching eggs, indicating that our method for inducing colonization was appropriate; however, for electrostatically treated eggs using EO water, *Salmonella* was only able to colonize 1 chicken out of 40 tested over two repetitions under actual growout conditions.

Table 6. Results for *Salmonella typhimurium* prevalence (%) in the lower intestines, ceca or cloacae of broiler chickens from hatching eggs treated electrostatically with tap water or EO water during hatch.

Treatment	Repetition 1	Repetition 2
Tap water control	65%	95%
EO water control	0%	5%
n	20	20

This research has tremendous industrial application because many of the companies that are experiencing failures due to high *Salmonella* prevalence at the poultry plant are receiving flocks of birds that are 80-100 percent positive for *Salmonella* as they enter the plant. It would seem logical to suppose that if the number of chickens in field that are colonized with *Salmonella* could be reduced to the levels observed in this study, the industry would be able to meet the *Salmonella* performance standard required by the USDA-FSIS. This research describes a method that should have tremendous value to the poultry industry for reducing *Salmonella* in flocks arriving to the processing plant, which, according to our research, will translate directly into lower numbers of processed carcasses that are positive for *Salmonella*. Moreover, the electrostatic spraying system is not expensive to incorporate into a commercial hatchery. Additionally the EO water is very economical to produce and is so non-toxic as to be potable. This water does not degrade equipment and does not present an environmental hazard when discharged.

Summary

Electrolyzed oxidative water applied using electrostatic spraying is an effective means of eliminating pathogenic and indicator populations of bacteria from hatching eggs. Using this method in a pilot scale hatchery, the percentage of chickens that were colonized with *Salmonella* was dramatically reduced. These studies demonstrate that the use of EO water in combination with electrostatic spraying may provide a practical and inexpensive way for the industry to significantly lower the number of birds that arrive at the processing plant contaminated with *Salmonella*.

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