

# Spraying hatching eggs with electrolyzed oxidizing water reduces eggshell microbial load without compromising broiler production parameters

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**ABSTRACT** An experiment was conducted to determine whether spraying hatching eggs with electrolyzed oxidizing (EO) water would decrease eggshell microbial load and hence improve hatchability, chick quality, and broiler growth performance. Eggs were collected from a broiler breeder flock; half the eggs were sprayed with EO water and the other half were left untreated. Enterobacteriaceae and total aerobic bacteria present on the eggshells of eggs from both treatments were enumerated. The eggs were incubated, and the broiler chicks were grown out to 39 d. Eggshell microbial load was significantly decreased by spraying the eggs with acidic

EO water before incubation, with no effect on cuticle structure [as measured by egg weight (moisture) loss], normal embryonic development, and hatchability. Chick quality, as determined by visual assessment and BW at the time of hatch, was also not affected. However, broiler mortality during the first 2 wk of the production period was significantly reduced in the chicks that hatched from eggs sprayed with EO water compared with chicks hatching from control eggs. The ability of EO water to reduce eggshell microbial load without negatively affecting hatchability or chick quality may make it a useful product for hatching egg sanitation.

**Key words:** electrolyzed oxidizing water, hatching egg sanitation, hatchability, chick quality, eggshell microbial load

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## INTRODUCTION

Maximizing hatchability and chick quality is a crucial step in optimizing broiler production efficiency. Reducing microbial contamination of eggshells may help to decrease the incidence of bacterial infections in developing embryos and newly hatched chicks. Microorganisms can penetrate the eggshell through shell pores or cracks (Berrang et al., 1999) and can kill the developing embryo, reduce hatchability, and negatively affect the chick posthatching (Williams, 1970). Hatched chicks can also be infected through contact with contaminated eggshells and hatchery equipment (Cason et al., 1994), with infected chicks then transmitting bacteria such as *Salmonella enterica* serovar Enteritidis, pathogenic *Escherichia coli*, and *Listeria monocytogenes* to other chicks in the growing flock (Venkitanarayanan et al., 1999). The presence of these microbes can be detrimental to flock health and productivity and can threaten the food safety of broiler meat products.

Commercial sanitization of hatching eggs in the North American poultry industry often involves fog-

ging incubating eggs with formaldehyde or hydrogen peroxide (Russell, 2003). Table eggs are often rinsed with alkaline detergents and chlorine solutions to reduce microbial load on the shell (Bialka et al., 2004). However, there are disadvantages to using each of these sanitizers. Formaldehyde is a known carcinogen and poses a threat to worker health and safety (Occupational Safety and Health Administration, 1991). Alkaline detergents and chlorine solutions are not suitable for hatching eggs because they can damage the eggshell cuticle (Bialka et al., 2004), thus reducing one of the protective mechanisms of the egg against microbial invasion (Berrang et al., 1999). In addition, the sanitizing ability of chlorine is reduced in the presence of heavily soiled surfaces containing a lot of organic matter (Tsai et al., 1992).

Electrolyzed oxidizing (EO) water is a nontoxic sanitizer (Russell, 2003) that may provide an alternative to other methods currently used to disinfect eggs. Electrolyzed oxidizing water is produced through electrolysis of a weak salt water solution using an EO water generator specifically designed for the procedure (Biostel, 2004). During electrolysis, salt and water molecules dissociate into positively charged  $\text{Na}^+$  and  $\text{H}^+$  ions, which are drawn toward a negatively charged electrode. Negatively charged  $\text{Cl}^-$  and  $\text{OH}^-$  ions are drawn toward a

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positively charged electrode, resulting in 2 different solutions separated by a semipermeable membrane. The water produced on the negatively charged side of the membrane contains sodium hydroxide and has an alkaline pH between 11 and 13 (Biostel, 2004). The acidic water produced on the positively charged side of the membrane contains a combination of hydroxyl anions, chlorine, hypochlorous acid, and hydrogen peroxide and typically has a pH between 2.5 and 4.5 (Biostel, 2004).

Acidic EO water has been found to be more effective in killing *E. coli* and *Salmonella* Enteritidis on eggshells than alkaline EO water (Bialka et al., 2004). The low pH of acidic EO water, as well as a free chlorine content of approximately 50 mg/L, makes it a prospective disinfectant for hatching eggs (Fabrizio et al., 2002; Bialka et al., 2004). In addition, the oxidation-reduction potential (ORP) value of acidic EO water (approximately 1,150 mV; Biostel, 2004) is another determining factor of the sanitizing efficacy of the solution (Kim et al., 2000). The positive ORP enables acidic EO water to capture electrons from the cellular membranes of microorganisms and render these membranes unstable, thus allowing the chemical to enter and inactivate the bacterial cell (Jay, 2000). Acidic EO water has been found to completely inactivate *E. coli* O157:H7, *Salmonella* Enteritidis, and *L. monocytogenes* after 10 min of exposure (Venkitanarayanan et al., 1999).

With respect to hatching eggs, it is important that any product used to sanitize the shell does not damage the integrity of the shell cuticle, or harm the developing embryo. Research indicates that washing eggshells with acidic EO water does not affect eggshell strength but may degrade the shell cuticle (Bialka et al., 2004). Although the disinfecting properties of EO water have been established (Venkitanarayanan et al., 1999; Fabrizio et al., 2002; Bialka et al., 2004), there have been no studies conducted to determine the effects of egg sani-

tation with EO water on subsequent hatchability, chick quality, and broiler offspring production efficiency.

The objective of the present study was to establish whether acidic EO water can reduce total aerobic bacteria and coliform bacteria counts on broiler hatching eggs, and possibly improve hatchability, chick quality, and broiler production parameters. The hypothesis tested was that spraying hatching eggs with acidic EO water before incubation would decrease the numbers of total aerobic bacteria and Enterobacteriaceae on eggshells, thus improving hatchability, chick quality, broiler liveability, and growth.

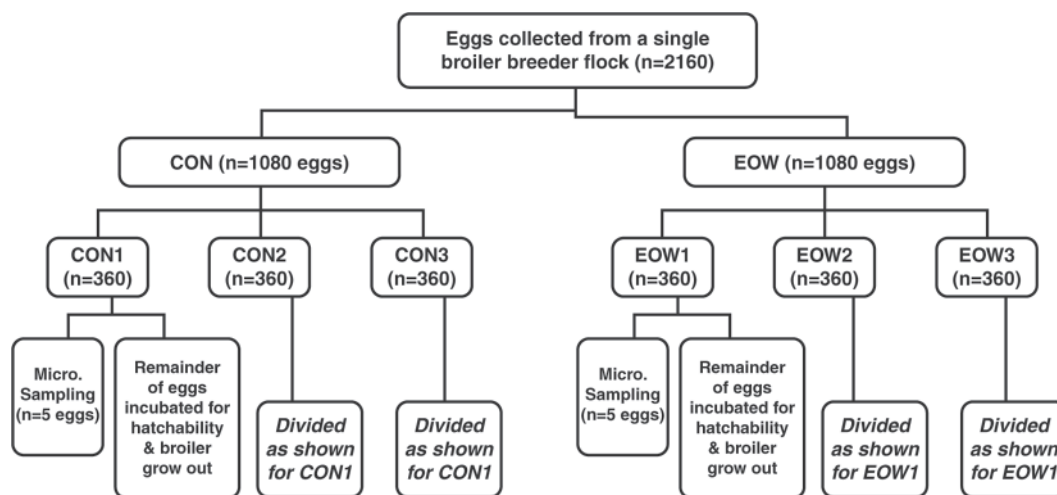
## MATERIALS AND METHODS

The experimental protocol was approved by the University of Alberta Faculty Animal Policy and Welfare Committee in accordance with the guidelines set forth by the Canadian Council on Animal Care (1993).

### Egg Collection and Allocation of Eggs to Treatment Groups

A total of 2,190 hatching eggs weighing  $68 \pm 4$  g were collected from a 55-wk-old Cobb 500 commercial breeder flock; a subset of 30 eggs weighing within a narrow weight range (68.0 to 68.5 g) was identified, labeled, and set aside for later microbiological testing. The remaining 2,160 eggs were individually weighed, numbered, and randomly divided into 2 groups of 1,080 eggs. One group was allocated to a control (CON, not sprayed with any water) and the other to a treatment group (EOW, to be sprayed with acidic EO water; Figure 1).

Each of the 2 treatment groups was further divided into 3 groups of 360 eggs (CON1, CON2, CON3 and



**Figure 1.** Flowchart showing the allocation of eggs to different treatment groups. All eggs were collected from a single commercial broiler breeder flock and were then divided into 2 treatment groups (EOW = sprayed with acidic electrolyzed oxidizing water and CON = not sprayed). The eggs within each treatment were then further subdivided into 3 replicates in time (EOW1, EOW2, EOW3, CON1, CON2, CON3) for the purpose of microbiological sampling. Five eggs from each replicate group were removed for microbiological sampling, and the remaining eggs were used in the incubation, hatching, and broiler performance portion of the study.

EOW1, EOW2, EOW3) to provide 3 replicates in time for the microbiological testing (Figure 1). Five eggs from each of the replicate groups were randomly removed, discarded, and replaced with one of the 30 eggs previously identified to be within the weight range of 68.0 to 68.5 g. This procedure was conducted to minimize the surface area variability between the eggs that were being sampled for the presence of bacteria.

### ***EO Water Properties and Application***

The acidic EO water being tested had a pH of 5.54 and an ORP of 1,140 mV (Profound Technologies Inc., Olds, Alberta, Canada). The EO water was obtained from a commercial farm 24.5 h before use and was stored in plastic jugs in a refrigerator at 2°C and 21% RH. The temperature of the room where the eggs were sprayed was 24°C and the RH was 20%.

The 3 replicate groups of eggs per each EOW treatment were sprayed at 30-min intervals; the EOW1 subgroup at time 0, the EOW2 subgroup at time 0 + 30 min, and the EOW3 subgroup at time 0 + 60 min. Using a hand-operated manual spray bottle, the top surface of the EOW eggs in each 42-egg tray was sprayed 20 times (each flat of eggs was sprayed with approximately 171 mL of EO water). The 5 eggs from each replicate group that were designated for microbiological testing were allowed to air dry in the trays for 90 min before the microbiological sampling commenced. The 5 eggs from each replicate group that were designated for microbiological testing were allowed to air dry in the trays for 90 min before the microbiological sampling commenced.

### ***Microbiological Sampling Procedure***

Using aseptic techniques, each egg (total of 15 EOW and 15 CON eggs) was placed into a 700-mL Nasco Whirl-Pak bag (Nasco, Modesto, CA) containing 10 mL of sterile 0.1% peptone water (Difco, Becton, Dickinson and Company, Sparks, MD). The bag was sealed and then raised above the shoulder of the person holding the bag and then swung down to the person's hip, in a 180° arc. This motion was repeated 20 times for each egg to rinse the eggshell with the peptone wash. The egg was then removed and discarded, and the bag was resealed and stored at 4°C overnight.

Enterobacteriaceae and total aerobic bacteria were enumerated by the following methods. Ten-fold serial dilutions were prepared from each eggshell sample using sterile 0.1% peptone water. Pour plates using violet red bile agar (Difco) containing 1% glucose (Fisher Scientific, Edmonton, Alberta, Canada) were prepared for each dilution. The plates were incubated at 38°C for 18 to 24 h before enumerating total Enterobacteriaceae. Total aerobic bacterial counts were obtained by plating 0.1 mL of each dilution on plate count agar (Difco) and incubating at 23°C for 48 h. All microbiological data

were converted to log colony-forming units per square centimeter.

### ***Incubation and Hatching***

After spraying the EOW egg treatment groups, both EOW and CON eggs were stored in the same egg cooler overnight at a dry bulb temperature of 17°C and a wet bulb temperature of 13.2°C. Thus, the total time from laying to incubation was 1 d. Before incubation, the eggs were candled and any cracked or odd-shaped eggs removed and discarded. The total number of eggs placed in the incubator for the CON and EOW groups was 1,026 and 1,044, respectively. The groups of 18 eggs served as the experimental unit for the hatchability portion of the research. All the eggs were incubated in the same 5,000-egg capacity single-stage incubator (Jamesway Incubator Company Inc., Cambridge, Ontario, Canada) at a dry bulb temperature of 37.5°C and a wet bulb temperature of 29.4°C. The positions of the groups of 18 eggs were randomized within the incubator.

All eggs were candled at 7 d of incubation. Eggs not containing viable embryos were removed and broken open to determine fertility status and if fertile the approximate day of embryonic death was noted. At 18 d of incubation, all remaining eggs were individually weighed to calculate moisture loss, and eggs from each treatment were transferred into 2 separate 5,000-egg capacity hatchers (Jamesway Incubator Company Inc.); the EOW and CON eggs were separated to reduce microbial cross-contamination between treatments during the hatching process. Both hatchers were maintained at a dry bulb temperature of 35.2°C and a wet bulb temperature of 29.4°C. From the point of collection of the eggs throughout the egg transfer process, clean and sanitized rubber gloves were worn whenever the eggs were handled.

After 21.5 d of incubation, all hatched chicks were removed from each hatcher, counted, and weighed. Chick quality was visually assessed, and chicks deemed not to be saleable according to commercial hatchery standards (chicks with unhealed navels, red hocks, or obvious abnormalities) were killed via cervical dislocation. All unhatched eggs were broken open to determine fertility status and if fertile the day of embryonic death was estimated.

### ***Broiler Production***

A total of 800 saleable chicks from each of the EOW and CON treatments were grown out separately in 1 of 4 floor pens per treatment. The chicks allocated to each pen ( $n = 200$ ) were group-weighted before placing the birds on fresh straw litter at a stocking density of 0.06 m<sup>2</sup>/bird. Mortality and feed consumption were recorded throughout the grow-out period, and the birds were group-weighted on d 39 before being shipped to a commercial processing plant for slaughter.

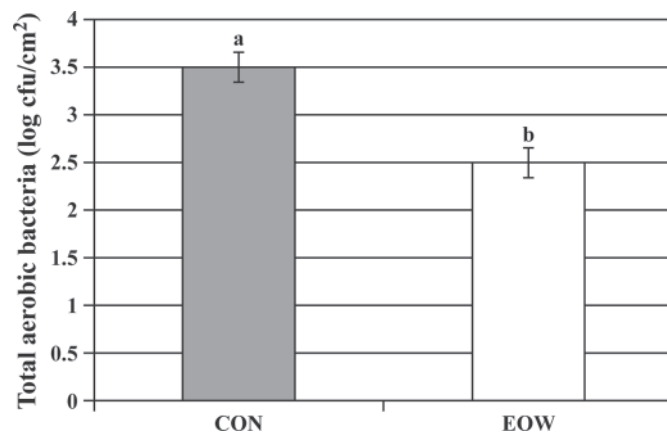
## Statistical Analysis

Microbiological data were analyzed using the GLM procedure of SAS (SAS Institute, 1999). Fertility, hatchability, and embryonic mortality data were analyzed using the CATMOD procedure of SAS. All egg weight, broiler BW, feed conversion ratio, and mortality data were analyzed using the mixed procedure of SAS. In all cases, probability was assessed at  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

### Microbiological Counts

There was no evidence of Enterobacteriaceae on the eggs from either treatment. This indicates that the eggshells were not initially contaminated with bacteria such as *E. coli* and *Salmonella* species. The CON eggs had a mean total aerobic bacteria count of 3.5 log cfu/cm<sup>2</sup>. These values are in the same range as those previously reported for clean, unwashed eggs (Kuo et al., 1996; McKee et al., 1998). The EOW eggs (2.5 log cfu/cm<sup>2</sup>) had significantly lower total aerobic bacteria counts than CON eggs (Figure 2). These results support the hypothesis that spraying eggs with EO water would reduce total aerobic bacteria counts on the eggshell. Such findings indicate that acidic EO water can be used to effectively disinfect eggshells. Bialka et al. (2004), who performed a study using specifically characterized *Salmonella* Enteritidis and *E. coli* strains, found that washing eggs in acidic EO water resulted in the reduction of these counts. The above result supports our finding of decreased bacterial loads on eggshells sprayed with EO water. In addition, Russell (2003) found that electrostatic spraying of acidic EO water on eggshell surfaces resulted in the complete elimination of *E. coli*, *L. monocytogenes*, *Salmonella enterica* serovar Typhimurium, and *Staphylococcus aureus*.



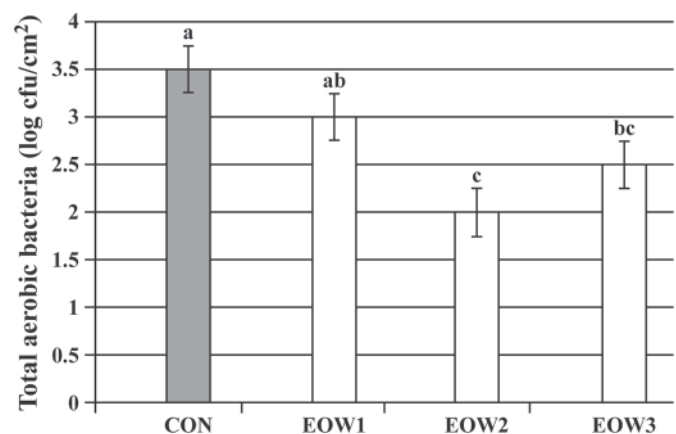
**Figure 2.** Effect of spraying eggshells with acidic electrolyzed oxidizing (EO) water on mean log total aerobic bacteria counts. Control (CON) eggs ( $n = 15$ ) were not sprayed with acidic EO water before incubation. The EO water-treated (EOW) eggs ( $n = 15$ ) were sprayed with acidic EO water before incubation. Least squares means that do not share a common letter differ significantly ( $P \leq 0.05$ ).

Significant differences in the reduction of total aerobic bacteria were observed between EOW1 (3.0 log cfu/cm<sup>2</sup>) and EOW2 (2.0 log cfu/cm<sup>2</sup>) replicates, neither of which differed from the EOW3 (2.5 log cfu/cm<sup>2</sup>) group (Figure 3). However, these groups differed by only 1 log reduction. These differences between replicates could likely be reduced or eliminated through the use of a commercial spraying cabinet that would apply the EO water with more precision.

### Egg Weight

Egg set weights (EOW 67.9 g; CON 68.0 g) and egg transfer weights (EOW 59.8 g; CON 60.0 g) were not significantly different between treatment groups (Table 1). No significant differences were found in percentage of egg weight loss from setting to transfer between treatments (EOW 11.8%; CON 11.8%). Because it is known that cuticle removal increases water loss (Peebles et al., 1998), these results suggest that spraying eggshells with acidic EO water does not appear to affect the integrity of the shell cuticle; if the cuticle had been partially damaged or removed by the EOW treatment, it would have likely resulted in an increase in egg weight loss, due to an increase in water loss through the pores of the shell (Peebles et al., 1998). However, measuring egg moisture loss, as was done in this study, is an indirect approach to assessing the integrity of the eggshell cuticle. We recommend that in future studies, direct measures of cuticle integrity, such as those described by Messens et al. (2007), be used.

These findings are not consistent with the results of a previous study, which showed that application of acidic EO water to eggshells resulted in degradation and partial removal of the cuticle (Bialka et al., 2004). This



**Figure 3.** Effect of spraying eggshells with acidic electrolyzed (EO) water on mean log total aerobic bacteria counts between replication groups. Control (CON) eggs ( $n = 15$ ) were not sprayed with acidic EO water before incubation. The EO water-treated (EOW) eggs were sprayed with acidic EO water before incubation. Replication group EOW2 was treated 30 min after group EOW1, and group EOW3 was treated 30 min after group EOW2. Each replication group (EOW1, EOW2, EOW3) was comprised of 5 eggs. Least squares means in columns that do not share a common superscript differ significantly ( $P \leq 0.05$ ).

**Table 1.** Set weight, transfer weight, and percentage of weight loss of control eggs (CON) and eggs sprayed with acidic electrolyzed oxidizing water (EOW) before incubation

Treatment	n <sup>1</sup>	Set weight (g)	Transfer weight (g)	Weight loss <sup>2</sup> (%)
CON	57	68.0 <sup>a</sup>	60.0 <sup>a</sup>	11.8 <sup>a</sup>
EOW	58	67.9 <sup>a</sup>	59.8 <sup>a</sup>	11.8 <sup>a</sup>
SEM		0.1	0.1	0.1

<sup>a,b</sup>Means within a column with different superscripts differ significantly ( $P < 0.05$ ).

<sup>1</sup>Number of experimental units. Experimental unit = 18 eggs.

<sup>2</sup>Egg weight loss = [(egg set weight – egg transfer weight)/egg set weight] × 100.

may be in part due to differences in the way the EO water was applied to the eggs. In the present study, eggs were sprayed with EO water, whereas in the study by Bialka et al. (2004), eggs were completely immersed in EO water. The differences observed may also be due to the age of the parent flock from which the eggs were collected. Cuticle thickness is known to vary with flock age (Ruiz and Lunam, 2000). The study by Bialka et al. (2004) used eggs from 22-wk-old hens, whereas the current study used eggs from 55-wk-old hens. Because cuticle thickness was not directly measured in this study, it is unknown whether the eggs had greater, average, or less-than-average cuticle thickness. In the current study, if cuticle thickness was less than average, any further removal of cuticle by the EO water treatment would likely have had a nominal effect on egg weight loss measured to 1 decimal place.

### Fertility, Hatchability, and Embryonic Mortality

Fertility differed significantly between EOW (93.9%) and CON (91.8%) eggs (Table 2). This difference was not an effect of the treatment because fertility status of the eggs was determined before the treatments were imposed.

No differences in hatchability (EOW 78.4%; CON 77.6%) or hatchability of fertile eggs (EOW 83.3%;

CON 84.5%) were found between treatments, indicating that spraying eggs with acidic EO water does not negatively affect the hatchability of broiler eggs.

No significant reductions in embryonic mortality at any point during the incubation process were found between EOW and CON eggs at any particular stage of embryonic development (Table 2). The embryonic mortality observed was not due to any 1 particular cause and fell within expected parameters, based on previous reports (Fasenko et al., 2000; Tona et al., 2001). Although spraying hatching eggs with acidic EO water did not improve hatchability in the eggs tested in this research trial, it was important to establish that acidic EO water did not negatively affect embryo health or development.

### Chick Quality

There were no differences in the percentages of dead (EOW 0.2%; CON 0.6%) or culled (EOW 3.7%; CON 3.3%) chicks at the time of hatch between CON and EOW eggs (Table 2). In addition, chick weights did not differ between treatment groups (EOW 48.0 g; CON 47.9 g; Table 3). Because chick weight is 1 indicator of chick quality (Tona et al., 2004), these results do not support the hypothesis that spraying hatching eggs with acidic EO water would improve hatched chick quality at the time the hatch was pulled. However, as with

**Table 2.** Percentage of fertility, hatchability, hatchability of fertile eggs, embryonic mortality, and dead and cull chicks produced from control eggs (CON) and eggs sprayed with acidic electrolyzed oxidizing water (EOW) before incubation

Treatment	n <sup>1</sup>	Fertility <sup>2</sup> (%)	Hatchability <sup>3</sup> (%)	Hatch of fertile <sup>4</sup> (%)	Early mortality <sup>5</sup> (%)	Mid mortality <sup>6</sup> (%)	Late mortality <sup>7</sup> (%)	Total mortality <sup>8</sup> (%)	Dead chicks <sup>9</sup> (%)	Cull chicks <sup>10</sup> (%)
CON	57	91.8 <sup>b</sup>	77.6 <sup>a</sup>	84.5 <sup>a</sup>	4.6 <sup>a</sup>	1.2 <sup>a</sup>	5.6 <sup>a</sup>	11.3 <sup>a</sup>	0.6 <sup>a</sup>	3.3 <sup>a</sup>
EOW	58	93.9 <sup>a</sup>	78.4 <sup>a</sup>	83.3 <sup>a</sup>	5.4 <sup>a</sup>	1.0 <sup>a</sup>	5.4 <sup>a</sup>	11.9 <sup>a</sup>	0.2 <sup>a</sup>	3.7 <sup>a</sup>
SEM		0.8	1.7	0.6	0.8	0.4	0.9	1.2	0.2	0.7

<sup>a,b</sup>Means within a column with different superscripts differ significantly ( $P < 0.05$ ).

<sup>1</sup>Number of experimental units. One experimental unit = 18 eggs.

<sup>2</sup>Fertility = (number of fertile eggs/number of eggs set) × 100.

<sup>3</sup>Hatchability = (number of eggs hatched/number of eggs set) × 100.

<sup>4</sup>Hatch of fertile = (number of eggs hatched/number of fertile eggs set) × 100.

<sup>5</sup>Early dead = (number of dead embryos between 1 to 7 d of incubation/number of fertile eggs set) × 100.

<sup>6</sup>Mid dead = (number of dead embryos between 8 to 14 d of incubation/number of fertile eggs set) × 100.

<sup>7</sup>Late dead = (number of dead embryos between 15 d of incubation to external pipping/number of fertile eggs set) × 100.

<sup>8</sup>Total embryonic mortality = (number of dead embryos between 1 d of incubation to external pipping/number of fertile eggs set) × 100.

<sup>9</sup>Dead chicks = (number of dead chicks at hatching/number of fertile eggs set) × 100.

<sup>10</sup>Cull chicks = (number of chicks culled/number of fertile eggs set) × 100.

**Table 3.** Average BW at hatching, 39 d, and average feed conversion ratio of broilers hatched from control eggs (CON) and eggs sprayed with acidic electrolyzed oxidizing water (EOW)

Treatment	n <sup>1</sup>	Average BW at hatching (g)	Average BW at 39 d (kg)	Feed conversion ratio (kg of feed/kg of gain)
CON	4	47.9 <sup>a</sup>	2.5 <sup>a</sup>	1.6 <sup>a</sup>
EOW	4	48.0 <sup>a</sup>	2.5 <sup>a</sup>	1.6 <sup>a</sup>
SEM		0.2	0.0	0.0

<sup>a</sup>Means within a column with different superscripts differ significantly ( $P < 0.05$ ).

<sup>1</sup>Number of experimental units. Experimental unit = 1 pen containing 200 broilers.

hatchability, the results showed that there was no negative effect of spraying acidic EOW on chick quality.

### Broiler Production Parameters

There were no differences in final BW before shipping at 39 d between the CON (2.5 kg) and EOW (2.5 kg) broilers (Table 3). There were also no differences in feed conversion ratio between EOW and CON broilers over the entire 39-d production period (Table 3).

For the first 14 d of broiler grow-out, the birds that hatched from the EOW eggs had lower cumulative (0 to 14 d) mortality (0.4%) compared with the birds that hatched from the CON group (1.9%; Table 4). Although chick quality at hatching was not different according to chick weight or number of culled or dead chicks, chicks hatching from the EOW-treated eggs may have had a lower bacterial load than chicks from the CON group. This may have resulted in lower early broiler mortality. This can only be put forth as a hypothesis because the level of bacterial infection in the hatched broilers was not measured in the current study. From 21 d to shipping, there were no significant differences in cumulative mortality between the 2 treatment groups due to variability between replicate pens.

### Conclusions and Future Research

The results of this study, showing no difference in egg weight loss during incubation, provide indirect evidence that the structural integrity of the cuticle does not appear to be affected by spraying hatching eggs with EO water. Actual cuticle amount should be directly measured in future studies to definitively establish that EOW treatment does not remove the cuticle.

Although spraying hatching eggs with acidic EO water did not negatively affect embryonic development or

survival, or broiler growth, total aerobic bacteria counts were significantly lower on eggs that were sprayed with acidic EO water compared with eggs left untreated. This result may have contributed to the reduction in broiler mortality during the first 2 wk of production, by reducing the bacteria present in the young broilers. Again, definitive research showing microbial reduction inside the chick due to treatment of eggs with EOW needs to be conducted to prove this hypothesis.

Based on the results of this study, acidic EO water may provide an effective, safe, nontoxic, and relatively inexpensive hatching egg sanitizer. Further studies should be conducted to examine if particular eggs that are more susceptible to bacterial contamination (i.e., eggs with poor shell quality) would especially benefit from being sanitized with EO water. Also, comparative studies determining the efficacy and cost-effectiveness of EO water compared with that of other sanitizing methods currently employed by hatcheries would be useful.

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**Table 4.** Average cumulative mortality in broilers hatched from control eggs (CON) and eggs sprayed with acidic electrolyzed oxidizing water (EOW)

Treatment	n <sup>1</sup>	d 7 (%)	d 14 (%)	d 21 (%)	d 28 (%)	d 35 (%)	d 39 (%)
CON	4	1.5 <sup>a</sup>	1.9 <sup>a</sup>	3.0 <sup>a</sup>	4.3 <sup>a</sup>	6.0 <sup>a</sup>	7.3 <sup>a</sup>
EOW	4	0.1 <sup>b</sup>	0.4 <sup>b</sup>	1.6 <sup>a</sup>	2.8 <sup>a</sup>	4.0 <sup>a</sup>	5.1 <sup>a</sup>
SEM		0.3	0.3	0.4	0.6	0.6	0.7

<sup>a,b</sup>Means within a column with different superscripts differ significantly ( $P < 0.05$ ).

<sup>1</sup>Number of experimental units. Experimental unit = 1 pen containing 200 broilers at the time the chicks were placed.

Canada) in conducting the microbiological analysis is also gratefully acknowledged.

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