Application of Ionized Reactive Oxygen Species for Disinfection of Carcasses, Table Eggs, and Fertile Eggs

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Primary Audience: Processing Plant Managers, Hatchery Managers

SUMMARY

We evaluated the effect of ionized reactive oxygen species created using Binary Ionization Technology (BIT) for disinfection of broiler carcasses, table eggs, and treatment of fertile eggs. Previous research has indicated that BIT creates a high concentration of reactive oxygen species (ROS) that lyse bacterial cells on contact. Application of BIT to broiler carcasses that had been intentionally inoculated with 1.58×10^6 *Salmonella enterica* Enteritidis (SE) caused a 1 to 3 log reduction in recoverable SE, depending on the duration of the treatment. Additionally, after inoculation of table eggs with 6.8×10^8 cfu of SE, we recovered SE from 95% fewer eggs following enrichment and found significantly fewer (7.77 and 7.41 log reduction) colony-forming units recovered from eggs treated with BIT compared with nontreated control eggs. We also evaluated whether application of the BIT treatment had any effect on hatchability of broiler breeder eggs to determine whether use of this technology could be feasible in a hatchery environment for disinfection of eggs. There were no significant effects of BIT on the hatchability (of total set) of treated eggs as compared with nontreated control eggs; however, there was a slight numerical increase in hatchability, between 5 and 10% in 2 trials. These data suggest that application of BIT technology to carcasses and table eggs could reduce contamination with pathogens and that the application to fertile eggs may not have effects on hatchability of eggs set.

Key words: *Salmonella*, carcass, table egg, fertile egg, disinfection

2005 J. Appl. Poult. Res. 14:716–720

DESCRIPTION OF PROBLEM

In 1996, the Foodborne Diseases Active Surveillance Network (FoodNet) collected data on 9 foodborne diseases in several sites within the United States. Since the start of this program, *Campylobacter* and *Salmonella* have been the leading causes of laboratory-confirmed foodborne illness. In 1997, *Campylobacter* (3,966 cases) and *Salmonella* (2,204 cases) accounted for over 76% of the confirmed foodborne-related diseases [1]. As these pathogens are often associated with the consumption of poultry products, consumer concern over food safety has had a

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major effect on the poultry industry. Data collected by the USDA indicate that the percentage of broiler chickens contaminated with *Salmonella* in 2003 was 12.8%, the highest level since 1998 [2]. Although *Salmonella* contamination on shell and fertile eggs is difficult to quantify, it can be present at low levels. One risk assessment program has estimated using an egg production module that 1 out of 20,000 table eggs are contaminated with *Salmonella enterica* Enteritidis, or approximately 23 million eggs per year [3]. In breeder flocks, the isolation of *Salmonella* is often lower than the isolation frequency from the chicks derived from those flocks, causing confusion as to where the contamination is taking place [4]. Padron [5] showed that the exposure of hatching eggs to *Salmonella* contaminated litter can cause the chorioallantoic membrane and yolk sacs to become contaminated, and presumably the chicks will be *Salmonella*-positive at hatch.

The poultry industry has already demonstrated that poultry can be economically produced and that it is possible to continue meeting the needs of a growing global population. Therefore, technology that can reduce contamination of poultry products in a safe and effective manner is valuable to all areas of poultry production. Currently, costs are incurred by the processor through government mandates to control the spread of poultry-associated foodborne pathogens through programs such as hazard analysis and critical control points (HAACP). However, from a more global perspective, one study calculated an annual *Salmonella*-related loss of approximately $1.4 billion in lost human productivity, medical expenses, and increased animal production costs in the United States alone [6]. For these reasons, a significant priority is the identification of pathogen control strategies that offer the producer safe methods for producing clean products that can be readily accepted by consumers.

Binary Ionization Technology (BIT), developed by Intecon [7, 8], is the process of passing a cleaning and disinfecting mist through plasma (ionized gas) resulting in an effective and short disinfection and cleaning process. A potential attribute of the BIT product over previous H_2O_2 and plasma systems is that BIT works at standard atmospheric conditions and does not require a vacuum (i.e., BIT can be used in the open air without special enclosures for containment). According to Intecon, as the H_2O_2 and associated ionized products complete their reaction, the only byproducts of BIT application are presumably H_2O and molecular oxygen, thus providing an environmentally friendly solution for disinfection of eggs and poultry carcasses [7, 8].

MATERIALS AND METHODS

Salmonella Inoculum

Salmonella enterica Enteritidis (SE) PT 13A is a primary poultry isolate that can be obtained from the National Veterinary Services Laboratory in Ames, IA, and is resistant to novobiocin (25 μg/mL) and nalidixic acid (20 μg/mL). All agar media contained these antibiotics. In all experiments, SE was grown overnight in tryptic soy broth and then washed by centrifugation with sterile saline 3 times. The concentration was approximated using a spectrophotometer and determined by serial dilution and spread plating retrospectively.

Carcass Decontamination

Fresh broiler chicken carcasses were obtained from a local processing plant, maintained on wet ice prior to use, and used for each experiment within 6 h of processing. The SE was applied to the skin covering the keel of all carcasses with a pipet and spread cranial to caudal over the entire pectoralis major with a sterile bent glass rod.

In the first experiment, carcasses (n = 80; 20 per treatment) were contaminated with 25 cfu of SE in 100 μL of physiological saline and then treated with BIT spray (administered at 10,000 V) or water (controls). The control carcasses were sprayed with sterile water for 5 s, and treated groups were sprayed with BIT for 4 s, 8 s, or 3 times for 4 s with 4 s between pulses. Treatments were administered at a rate of 30 mL/min. All carcasses were rinsed in sterile bags with 100 mL of sterile saline [9]. Carcasses were held less than 2 h prior to recovery. The carcasses were massaged manually for 60 s and then the rinse water was recovered and the sample volume was doubled with 2× tetrathionate broth [10]. Samples were incubated overnight at 37°C and then streaked for isolation onto XLD agar

TABLE 1. Recovery of *Salmonella enterica* Enteritidis (SE) PT13A from broiler carcass rinses after treatment with Binary Ionization Technology (BIT) spray (experiment 1)

Treatment[1]	SE positive/total	SE recovery (%)
Control	15/20	75
8 s	12/20	60
4 s pulsed	12/20	60
4 s	14/20	70

[1]Broiler carcasses were inoculated with 25 cfu of SE/carcass on the breast skin prior to treatment.

plates [11] containing novobiocin and nalidixic acid. Samples containing characteristic *Salmonella* colonies were considered positive.

In the second experiment, each carcass (n = 40; 10 per treatment) was contaminated with 1.58×10^6 cfu of SE in 100 μL of sterile saline applied to the skin covering the pectoralis major as described above. Carcasses were treated with BIT spray (30 mL/min, 15,000 V) for 60 s, 4 intervals of 15 s with 5 to 10 s between each interval, or 3 intervals of 12 s and sprayed with air for 12 to15 s between each interval. Control carcasses were sprayed with sterile water for 60 s, also at 30 mL/min. The skin covering the pectoralis major was excised with a sterile scalpel from each carcass following treatment. Each sample was then divided into 2 equal pieces and placed in sterile sample bags with 10 mL of sterile saline where samples were stomached [12] for 30 s. One milliliter of each sample was serially diluted and then spread plated on XLD agar plates [11] containing novobiocin and nalidixic acid. The number of *Salmonella* colonies was counted for each sample, and the colony-forming units per milliliter of sample were calculated.

TABLE 2. Recovery of *Salmonella enterica* Enteritidis (SE) PT13A from breast skin of broiler carcasses after treatment with Binary Ionization Technology (BIT) spray (experiment 2)

Treatment[1]	Log_{10} cfu/mL
Control	4.30 ± 0.64^{a}
60 s	3.13 ± 1.42^{ab}
15-s intervals × 4	2.37 ± 1.01^{b}
12-s intervals × 3	1.05 ± 1.97^{c}

[a–c]Means within columns with different superscripts are significantly different ($P < 0.05$).
[1]Broiler carcasses were inoculated with 1.58×10^6 cfu of SE/carcass on the breast skin prior to treatment.

Table Egg Decontamination

Forty table eggs were obtained from the University of Arkansas Poultry Research Farm for each trial. First, melted wax was applied with a cotton swab in a small circle covering approximately one-fourth of the air cell and allowed to dry. Then a pipet was used to drop 100 μL of saline containing 6.8×10^8 cfu of SE within the wax circle on the air cell. The wax prevented the inoculum from running down the egg before drying. After incubation at room temperature (23°C) for 10 to 60 min, which allowed the SE suspension to dry on the eggshell, treatments were applied to each egg individually. Control eggs were sprayed for 5 s with sterile water and BIT-treated eggs were sprayed for 5 s with BIT treatment at 10,000 V. The total volume each egg received by spray was approximately 0.83 mL. Eggs were collected into sterile sample bags and rinsed with 10 mL of sterile saline for 30 s each [13]. One milliliter of the resulting suspension was removed from each sample and serially diluted for spread plating on XLD agar plates [11] for enumeration of SE. Then 9 mL of 2× tetrathionate broth [10] was added to each bag, and the bags were incubated overnight then streaked for isolation for detection of SE.

Fertile Egg Treatment

Broiler breeder eggs (140 and 542 eggs for trials 1 and 2, respectively) were obtained from a primary breeder hatchery prior to incubation for both trials and were treated upon arrival at our laboratory. Approximately half of the total eggs were treated with BIT for 5 s at 15,000 V prior to incubation. Control eggs received no treatment before incubation, because the objective of the experiment was to determine if application of BIT would have an effect on hatchability of eggs set. The eggs were incubated at 99°F with relative humidity of 60 to 5% and turned 5 times daily for 18 d and then transferred to a hatching cabinet (99.5°F) until day of hatch [14]. All eggs were candled at approximately 11 d of incubation and eggs not containing viable embryos were discarded. The remaining viable embryos were again candled at 18 d of incubation. At 22 d, hatched chicks were enumerated.

TABLE 3. Recovery of *Salmonella enterica* Enteritidis (SE) PT13A from egg shells after treatment with Binary Ionization Technology (BIT) spray

	Treatment[1]	Log_{10} cfu/mL	Positive/total (%)
Trial 1	Control	8.04 ± 0.13[a]	20/20 (100)[a]
	BIT	0.27 ± 0.27[b]	1/20 (5)[b]
Trial 2	Control	7.61 ± 0.07[a]	20/20 (100)[a]
	BIT	0.20 ± 0.20[b]	1/20 (5)[b]

[a,b]Means within columns with different superscripts are significantly different ($P < 0.05$).
[1]All eggs were inoculated with 6.8×10^8 cfu of SE/egg and allowed to dry prior to treatment. Control eggs were sprayed with water (30 mL/min) for 5 s each, and BIT-treated eggs were sprayed with BIT (30 mL/min) for 5 s each.

TABLE 4. Evaluation of Binary Ionization Technology (BIT) application on the hatchability of eggs set

	Treatment[1]	Total hatched/ total set	Hatch of set (%)[2]
Trial 1	Control	35/70	50
	BIT	42/70	60
Trial 2	Control	169/268	61.9
	BIT	182/274	66.4

[1]Control eggs received no treatment and BIT-treated eggs were sprayed for 5 s at a rate of 30 mL/min.
[2]All eggs were candled (examined) at approximately 11 d of incubation and nonviable embryos were discarded. The remaining viable embryos were continued to 18 d of incubation when the embryos were again candled and viable embryos enumerated and transferred to a hatching cabinet. At 22 d, hatched chicks were enumerated and compared with the total eggs set.

In these experiments, incidence data and hatchability data were analyzed using the chi-squared test of independence [15]. Numerical data from experiments 2 and 3 were analyzed by SAS software using the ANOVA test and further separated using the GLM procedure [16].

RESULTS AND DISCUSSION

In the first carcass experiment, spray application of BIT did not significantly alter our ability to recover SE from chicken carcasses following enrichment (Table 1). However, in the second experiment, a 1.93 (15-s intervals × 4) and 3.25 (12-s intervals × 3) log_{10} reduction of SE was observed on the BIT treated carcasses when compared with the control water-treated carcasses (Table 2). Although treatment times were longer in the second trial, the BIT application was also performed at a higher voltage (15,000 V compared with 10,000 V previously).

When BIT was used to disinfect table eggs that had been intentionally inoculated with SE, there was a 7.77 and 7.41 log_{10} reduction of SE (trials 1 and 2, respectively) from the BIT-treated eggs when compared with control water-treated eggs (Table 3). Further, when the egg rinse was enriched for detection of SE, a significant 95% reduction of SE was realized in the treated groups (Table 3). Additionally, application of BIT to the surface of fertile eggs did not have a significant impact on hatch of eggs set (Table 4). The hatchability obtained was low, but the source flock (primary breeders) was problematic for hatchability and, therefore, a low hatch rate was expected. A primary breeder flock was chosen as the egg source in part because it is critical for disinfection. Further research should be done to evaluate the apparent numerical improvement in hatchability observed in the fertile eggs treated with BIT (10 and 4.5%). Small improvements in hatchability could prove to be economically important.

CONCLUSIONS AND APPLICATIONS

1. Application of BIT to carcasses in experiment 1 was not effective in reducing SE recovery following enrichment, possibly due to short application time or lower voltage used for application.
2. Application of BIT to carcasses that had been contaminated with SE significantly reduced SE recovery by 1.93 and 3.25 log cfu/mL when applied 15 s for 4 times or 12 s for 3 times.
3. Recovery of SE was reduced by 95% on contaminated table eggs in 2 experiments. In these experiments significant reductions of 7.77 and 7.41 log cfu/mL were also observed.
4. Application of BIT did not have a significant effect on hatch of eggs set.

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