Efficacy of chlorine dioxide gas in reducing *Escherichia coli* and *Salmonella* from broiler house environments

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Primary Audience: Veterinarians, Poultry farmers, Poultry scientists

SUMMARY

Escherichia coli and *Salmonella* spp. are considered to be the major pathogens associated with human transmissible infectious diseases in the air of poultry houses. Chlorine dioxide (ClO_2) is an effective biocide against a wide range of microorganisms. Accordingly, this study investigated the efficiency of gaseous ClO_2 application for disinfecting broiler houses by collecting air samples before and after fumigation using a passive method. Fumigation was performed with 125 mL or 250 mL of ClO_2 liquid (containing 2,000 ppm of ClO_2) and 3 trials were conducted for each dose. A total of 27 petri dishes were used for each trial (for each type of bacteria: *E. coli* or *Salmonella*) and placed in 3 different locations (front, middle and back) and 3 different positions (top, middle and floor) of the broiler shed. Air samples were collected at 10 min, 1 h, 3 h, 6 h, and 12 h before and after fumigation to evaluate the air quality in terms of the concentration of *E. coli* and *Salmonella.* Both levels of ClO_2 were capable of reducing the concentration of *E. coli* from broiler house air during all measuring periods except 10 min, with highest disinfection rate being observed at 6 h. With the exception of 1 h, the concentration of *Salmonella* was also reduced after fumigation with ClO_2 in all measuring period; with the highest disinfection rate occurring at 6 h. Fumigation with ClO_2 had no negative effect on birds' health condition. Taken together, these results suggest that the application of gaseous ClO_2 at the investigated levels can be an effective option for reducing bacterial load from broiler house environments.

Key words: chlorine dioxide, *Escherichia coli*, *Salmonella*, broiler house

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DESCRIPTION OF PROBLEM

Avian colibacillosis and salmonellosis are infectious diseases caused by *Escherichia coli* and a variety of *Salmonella* species, respectively. These organisms are considered the principal causes of morbidity and mortality in broiler houses, resulting in considerable economic loss to the poultry industry. These organisms are also of prime concerns because they are transmissible to humans by direct contact or via meat and eggs. The intestinal tract is believed to be the main source of pathogenic bacteria, whereas feces and dust preserve bacteria, enabling further spread [1]. Inadequate cleaning and disinfection has been reported as important risks for *E. coli*

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and *Salmonella* persistence in poultry houses [2, 3]. Therefore, adequate cleaning and disinfection of poultry house is important.

Fumigation has long been an accepted method of environmental disinfection because the production of gas or vapor allows disinfectants to easily penetrate hard-to-reach areas [4]. Fumigants commonly used to disinfect workplaces are formaldehyde, hydrogen peroxide, and chlorine dioxide [5, 6]. Chlorine dioxide (ClO_2) is a greenish-yellow gas used as a disinfectant that is recommended by the World Health Organization [7] of the United Nations. This compound is a powerful, broad-spectrum bactericide, fungicide, virucide, algicide, and sporacide, and it also destroys protozoa [8–10]. Chlorine dioxide's unique properties allow it to serve as one of the most environmentally friendly agricultural chemicals, because the ClO_2 reaction mainly forms inorganic disinfection byproducts, predominantly harmless chlorite and chloride, that leave little to no footprint following application. Additionally, ClO_2 kills microorganisms at low concentrations, even when they are inactive [11]. Furthermore, no bacteria are known to be resistant to ClO_2. Gaseous ClO_2, like aqueous ClO_2, has been shown to be an effective surface disinfectant [6, 12] Therefore, ClO_2 is now globally used as an antimicrobial and oxidizing agent for disinfection of drinking water, poultry process water [13], and fruits and vegetables [12], as well as to decontaminate animal-care facilities [14]. However, a search of the literature revealed no studies investigating fumigation of poultry barns with ClO_2. Therefore, this experiment was conducted to evaluate the effects of gaseous ClO_2 for disinfection of broiler-house air quality based on its effect on the most common bacteria isolated from broiler sheds, *E. coli* and *Salmonella*.

MATERIALS AND METHODS

This study was conducted in the experimental broiler shed of Sunchon National University, Suncheon, Republic of Korea. All the experimental procedures were approved by the Institutional Animal Care and Use committee of Sunchon National University. A total of 3 trials of ClO_2 fumigation were conducted at the end of 5a 5-wk rearing period in the presence of live birds to determine whether ClO_2 fumigation had any detrimental effect on birds. Each trial was performed at an intervals of 40 d.

Sample Collection before Fumigation

Microbial samples were collected from the house environment by using settling plates. Specifically, standard petri dishes containing culture media were opened and exposed to the air for a given time, then incubated to allow visible colonies to develop. These colonies were then enumerated. Air samples were collected and analyzed before fumigation to determine the background levels (expressed as control) of *E. coli* and *Salmonella*.

E. coli was cultured using Difco™ MacConkey Sorbitol agar and *Salmonella* was evaluated using BBL™ Salmonella Shigella agar. In each trial a total of 27 petri dishes were used for each bacterial type. The petri dishes were set at 3 different locations (front, middle and back) and 3 different positions (top, middle and floor) of the broiler shed (around 80 m^3) away from walls or any major obstacles. The agar dishes were opened on the benches and left open for 10 min, 1 h, 3 h, 6 h, and 12 h. After the sampling time was completed, the agar plates were covered with the lids, sealed with parafilm and incubated for 24 h at 37°C. Visible microbial colonies were counted immediately after removal from the incubator based on the color of colonies (*E. coli*, rose to pink colonies; *S.* Typhimurium, colorless with black centers).

Fumigation and Sample Collection

Fumigation was carried out for 12 h (9:00 am to 9:00 pm) through generation of ClO_2 gas by using a ClO_2 based liquid disinfectant (Mind of Farmer, Chemopia, Daejon, Korea) containing 0.2% ClO_2 (2,000 ppm) and a smoke producing liquid (TECON SMK, Gyeonggi-do, Pocheon, Korea) at a 1:1.2 ratio (the ratio was recommended by the manufacturer). The doses of ClO_2 used for fumigation in the broiler shed were 125 mL and 250 mL. Same shed were used for the dose levels by giving an interval of 5 d. Three trials were conducted for each dose. The mixture of liquids was poured in an aluminum foil plate

Table 1. Effects of fumigation with chlorine dioxide (ClO_2) on the concentration and disinfection rate of *Escherichia coli* in broiler house.[1]

	Level of ClO_2 (mL)							
	Concentration (No. of colony)			Disinfection rate (%)				
Time of disinfection	Control	125	250	Control	125	250	SEM (Conc.)	*P* value (Conc.)
10 min	7.11[a]	5.44[a,b]	2.89[b]	–	23.44	59.38	1.30	0.093
1 h	17.56[a]	7.33[b]	5.22[b]	–	58.23	70.25	2.20	0.0008
3 h	8.67[a]	4.11[b]	3.44[b]	–	52.56	60.26	0.96	0.002
6 h	19.56[a]	6.89[b]	4.44[b]	–	64.77	77.27	1.16	<0.0001
12 h	26.44[a]	11.89[b]	8.78[b]	–	55.04	66.81	2.16	<0.0001

[a,b]Within the same row, entries annotated with different superscripts exhibit a statistical difference according to Student's *t*-test ($P < 0.05$).

[1]Each entry represent the mean of 3 replicate trials (9 locations and 27 petri dishes per trial).

and fumes were produced by boiling the liquid through an electric heating plate. The temperature (around 27 to 28°C) and humidity (around 57 to 59%) of the room during fumigation was recorded using a digital humidity temperature meter [15].

To determine the effects of ClO_2 fumigation on the microbial load of the house environment, we enumerated *E. coli* and *Salmonella* in the same locations using the same method as before fumigation, with the lids of the petri dishes being opened and left open for 10 min, 1 h, 3 h, 6 h, and 12 h from the time at which fume production began.

Safety Evaluation

After completion of the fumigation process, the health conditions of the birds (aged 40 d with a stocking density of 1 sq. ft. per bird), were scored according to the method described by Arnould et al. [16] based on wounds, plumage condition, body cleanliness, breast blisters, feet conditions, broken bones including keel bone damages. Ten birds were subsequently selected at random and sacrificed, after which their skin and respiratory tracts were evaluated for lesions.

Statistical Analysis

The experiment was completed as 3 replicates, and the mean values of bacterial colonies were calculated and reported with a 95% confidence interval. Differences in the concentration of *E. coli* and *Salmonella* before and after fumigation were identified by Student's *t*-tests [17], with $P < 0.05$ used to indicate significance.

RESULTS AND DISCUSSION

Chlorine dioxide is widely used as an antimicrobial and sanitizing agent for treatment of drinking water, fruits and vegetables, poultry process water, swimming pools, and mouthwash preparations. Gaseous ClO_2, like aqueous ClO_2, has been shown to be an effective surface disinfectant. Several studies have revealed the efficacy of gaseous ClO_2 as a sanitizer for reducing *E. coli*, *Salmonella,* yeast, and mold from fruit surfaces, food processing plants, and libraries [12, 18]. However, to the best of our knowledge, this is the first study to investigate the use of ClO_2 to disinfect animal facilities. In our study, we applied 2 levels of ClO_2 to disinfect broiler houses in terms of the concentration of *E. coli* and *Salmonella* in the house air (Tables 1 and 2, respectively). According to Table 1, the ability of *E. coli* to grow in the agar media increased with increasing time period prior to fumigation (with the exception of 3 h). However, regardless of the time factor, both levels of ClO_2 significantly reduced the number of *E. coli* ($P < 0.05$), except at 10 min, when only a tendency for reduction was noted in response to fumigation with 250 mL ClO_2 ($P < 0.10$). The highest disinfection rate against *E. coli* was recorded at 6 h in response to fumigation with 250 mL ClO_2. The concentrations of *Salmonella* in the broiler house atmosphere were also lower at all measuring periods (except 1 h) as a result of fumigation

Table 2. Effects of fumigation with chlorine dioxide (ClO_2) on the concentration and disinfection rate of *Salmonella* spp. in broiler houses.[1]

	Level of ClO_2 (mL)							
	Concentration (No. of colonies)			Disinfection rate (%)				
Time of disinfection	Control	125	250	Control	125	250	SEM (Conc.)	*P* value (Conc.)
10 min	2.56[a]	1.11[b]	1.00[b]	–	56.52	60.87	0.34	0.006
1 h	3.44	2.56	1.56	–	25.81	54.84	0.67	0.17
3 h	3.67[a]	1.78[b]	1.22[b]	–	51.52	66.67	0.47	0.004
6 h	6.44[a]	2.78[b]	1.67[b]	–	56.90	74.14	0.77	0.001
12 h	15.67[a]	8.67[b]	4.44[b]	–	44.68	71.63	1.59	0.0005

[a,b]Within the same row, entries annotated with different superscripts exhibit a statistical difference according to Student's *t*-test ($P < 0.05$).

[1]Each entry represent the mean of 3 replicate trials (9 locations and 27 petri dishes per trial).

Table 3. Effects of fumigation with chlorine dioxide (ClO_2) on bird health conditions and gross lesion in skin and respiratory tract.[1]

		Gross lesion/ no. of birds tested	
Level of ClO_2 (mL) for fumigation	Sick birds/no. of birds tested	Skin	Respiratory tract
Control (No fumigation)	0/60	2/30	0/30
125 mL	0/60	2/30	0/30
250 mL	0/60	1/30	0/30

[1]Each entry represent the result of 3 replicate trials.

with 125 and 250 mL ClO_2 ($P < 0.05$), with highest disinfection rate being observed at 6 h. As a disinfectant, ClO_2 works through oxidation [19] and disrupts the cell wall, resulting in death of the microorganism by breaking up the cell [20]. Chlorine dioxide also penetrates bacterial cell walls and alters the protein involved in the structure of microorganisms, resulting in rapid destruction of the bacteria. This reaction is not dependent on reaction time or concentration. Hsu et al. [6] reported that regular application of ClO_2 in a student health center yielded high disinfection efficiency against bacteria and fungi. The efficacy of ClO_2 gas as a potential fumigant for the inactivation of fungal colonies was also reported by Wilson et al. [21].

None of the birds became sick or showed any gross lesions in the respiratory tract after fumigation (Table 3). A few birds did show some skin lesions that may have occurred due to overweight or the group rearing system. Lin et al. [22] reported that skin application or oral ingestion of ClO_2 at up to 500 ppm had no toxic effect on 5-week-old Leghorn chicken, indicating that ClO_2 is a safe disinfectant that can be used to treat infections of the upper respiratory tract, oral cavity, or superficial infections.

CONCLUSIONS AND APPLICATIONS

1. This study showed that ClO_2 gas can be effective as a fumigant for controlling the *E. coli* and *Salmonella* in the broiler house environment without any toxic effect.
2. Fumigation of a broiler shed with 125 and 250 mL ClO_2 had no negative effect on birds' health.
3. Overall, ClO_2 has the potential for use as a fumigant for broiler house disinfection; however, further investigations using different doses are warranted.

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