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Airborne Particulate Matter and Culturable Bacteria Reduction from Spraying Slightly Acidic Electrolyzed Water in an Experimental Aviary Laying-Hen Housing Chamber

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Abstract

Compared to conventional cage laying-hen houses, aviary hen houses generally have much higher concentrations of airborne dust and bacteria due to generation of bioaerosols by the hensâ€™ access to and activities on the litter floor. Hence, reducing these airborne agents is important to safeguard the health of the animals and workers in such housing systems. Spraying slightly acidic electrolyzed water (SAEW) is a novel approach to reducing airborne culturable bacteria (CB) and particulate matter (PM) levels in hen houses. The objective of this study was to evaluate the efficacy of reducing airborne CB and PM in an experimental aviary chamber by periodic spraying of SAEW (Trt), as compared to no spraying ($Ctrl_{ns}$) or spraying of tap water ($Ctrl_w$). The hens were provided 16 h light and 8 h dark (lights on at 6:00 h and off at 22:00 h) and were given access to the litter floor from 12:00 h to 22:00 h. The Trt regimen sprayed SAEW at 14:00 h for 15 min at a dosage of 80 mL m^{-2}; the $Ctrl_{ns}$ regimen had no spraying; and the $Ctrl_w$ regimen sprayed tap water following the same procedure as with Trt. Concentrations of airborne CB and PM in six aerodynamic size ranges (0.65-1.1, 1.1-2.1, 2.1-3.3, 3.3-4.7, 4.7-7.1, and >7.1 Î¼m) were measured at 1.5 m above the floor in the center of the room during the periods of 13:45-14:00 h and 14:45-15:00 h. Compared to $Ctrl_{ns}$, spraying SAEW significantly reduced airborne CB (>2.1Â Î¼m) by up to 49% Â±10% ($p < 0.05$), while $Ctrl_w$ did not show a reduction effect. No significant difference was found between Trt and $Ctrl_w$ in reducing airborne PM, although both reduced or tended to suppress PM >7.1 Î¼m in size. The results show that spraying SAEW can inactivate airborne CB attached to PM. Thus, this is a promising technique for alleviating the adverse health impacts of bioaerosols in aviary laying-hen housing systems.

Keywords

Aviary hen housing, Animal health and welfare, Indoor air quality

Disciplines

Agriculture | Bioresource and Agricultural Engineering

Comments

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Authors

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AIRBORNE PARTICULATE MATTER AND CULTURABLE BACTERIA REDUCTION FROM SPRAYING SLIGHTLY ACIDIC ELECTROLYZED WATER IN AN EXPERIMENTAL AVIARY LAYING-HEN HOUSING CHAMBER

W. Zheng, Y. Zhao, H. Xin, R. S. Gates, B. Li, Y. Zhang, M. L. Soupir

ABSTRACT. *Compared to conventional cage laying-hen houses, aviary hen houses generally have much higher concentrations of airborne dust and bacteria due to generation of bioaerosols by the hens' access to and activities on the litter floor. Hence, reducing these airborne agents is important to safeguard the health of the animals and workers in such housing systems. Spraying slightly acidic electrolyzed water (SAEW) is a novel approach to reducing airborne culturable bacteria (CB) and particulate matter (PM) levels in hen houses. The objective of this study was to evaluate the efficacy of reducing airborne CB and PM in an experimental aviary chamber by periodic spraying of SAEW (Trt), as compared to no spraying ($Ctrl_{ns}$) or spraying of tap water ($Ctrl_w$). The hens were provided 16 h light and 8 h dark (lights on at 6:00 h and off at 22:00 h) and were given access to the litter floor from 12:00 h to 22:00 h. The Trt regimen sprayed SAEW at 14:00 h for 15 min at a dosage of 80 mL m^{-2}; the $Ctrl_{ns}$ regimen had no spraying; and the $Ctrl_w$ regimen sprayed tap water following the same procedure as with Trt. Concentrations of airborne CB and PM in six aerodynamic size ranges (0.65-1.1, 1.1-2.1, 2.1-3.3, 3.3-4.7, 4.7-7.1, and >7.1 μm) were measured at 1.5 m above the floor in the center of the room during the periods of 13:45-14:00 h and 14:45-15:00 h. Compared to $Ctrl_{ns}$, spraying SAEW significantly reduced airborne CB (>2.1 μm) by up to 49% ±10% ($p < 0.05$), while $Ctrl_w$ did not show a reduction effect. No significant difference was found between Trt and $Ctrl_w$ in reducing airborne PM, although both reduced or tended to suppress PM >7.1 μm in size. The results show that spraying SAEW can inactivate airborne CB attached to PM. Thus, this is a promising technique for alleviating the adverse health impacts of bioaerosols in aviary laying-hen housing systems.*

Keywords. *Aviary hen housing, Animal health and welfare, Indoor air quality.*

In recent years, providing a healthy indoor environment for animals and workers has received increasing attention in egg production. Exposure to high concentrations of airborne bacteria and particulate matter (PM) can impair the health of the animals and workers (Seedorf et al., 1998; Whyte, 2002; Andersen et al., 2004; Mitchell et al., 2004). Some microorganisms can be transmitted through the air and cause animal diseases (Zhao et al., 2011a; Zhao et al., 2013a; Dee et al., 2009; Otake et al., 2010) and enter the food chain (Leach et al., 1999; Hajmeer et al., 2006). Airborne PM in livestock houses is considered a carrier of microorganisms (Gustafsson, 1999; Lee et al., 2006; Nehme et al., 2008; Nonnenmann et al., 2010; Hong et al., 2012). Reducing airborne bacteria and PM is essential to improving the air environment in animal houses. Aviary housing is an alternative egg production system that accommodates natural behaviors of the hens; however, much higher airborne PM and bacteria concentrations exist in aviary housing than in cage housing (Ellen et al., 2000; Protais et al., 2003; Hayes et al., 2012).

Spraying disinfectants (minimizing the use of therapeutic drugs) is a method to reduce airborne culturable bacteria (CB) and airborne PM levels in poultry houses (Böhm, 1998; Zheng et al., 2012a). Slightly acidic electrolyzed water (SAEW) has been considered to be an effective and environmentally friendly disinfectant in the food industry (Koide et al., 2009; Quan et al., 2010; Abdulsudi et al., 2011). Spraying SAEW in poultry houses improves the

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indoor air quality by reducing airborne bacteria (Hao et al., 2013; Zheng et al., 2013). The significant bactericidal effect of SAEW has been proven when CB were directly exposed to SAEW (Cao et al., 2009; Zhang et al., 2011; Zheng et al., 2012b). In livestock houses, airborne bacteria are normally attached to PM, which may protect the bacterial livability (Lai et al., 2009; Cambra-López et al., 2010). Spraying water has been used in livestock houses to reduce airborne PM (Takai and Pedersen, 2000; Kim et al., 2006). However, little information is found regarding reduction of airborne CB and PM by spraying SAEW in livestock houses, especially in alternative housing systems such as aviary hen houses. With increasing use of aviary hen-housing systems, investigation of the bactericidal effect of spraying SAEW on dust-attached airborne CB in such systems is thus warranted. In particular, characterizing the relationship of airborne PM and airborne CB reductions resulting from SAEW application in aviary housing systems may aid in understanding the bioaerosol-reducing behaviors of spraying SAEW and subsequently developing control techniques for improved indoor air quality.

The objective of this research was to investigate the efficacy of spraying SAEW on reduction of airborne PM and airborne CB in six aerodynamic size ranges over the range of 0.523 to 20.535 μm in an experimental aviary laying-hen chamber.

MATERIALS AND METHODS

EXPERIMENTAL AVIARY LAYING-HEN CHAMBER

The three-month experiment was conducted in a 2.2 × 2.3 × 2.4 m environmentally controlled chamber at the Livestock Environment and Animal Physiology (LEAP) Laboratory at Iowa State University in Ames, Iowa. Thirty-four 78-week-old (onset age) CV22 laying hens were kept in the environmental chamber (figs. 1 and 2), which contained a two-tier aviary system (1.8 × 1.0 × 1.75 m). The floor of the chamber was covered with litter (sawdust + dry manure, 1.8 × 1.8 m), and the thickness of the litter (1 to 2 cm) mimicked that at the commercial farm where the hens were procured. Light was scheduled to be on at 6:00 h and off at 22:00 h (16 h light:8 h dark). Hens were given access to the litter from 12:00 h to 22:00 h (10 h) of each day. Feeders, drinkers, perches, and a nest box (0.6 × 0.5 × 0.5 m) were provided in the colony cage, and the resource allowance is listed in table 1. A negative-pressure ventilation system was used that consisted of a variable-speed sidewall exhaust fan and a bi-directional ceiling diffuser. A manure collection tray was placed under the colony cage, and the collected manure was scraped off and removed every four days.

SPRAYING SYSTEM

A spray head with a 0.5 mm diameter nozzle (Pilot Mini, Walther Pilot NA, Chesterfield, Mich.) connected to an air compressor (model 204100, Campbell Hausfeld, Harrison, Ohio) at an air pressure of 140 kPa was used for

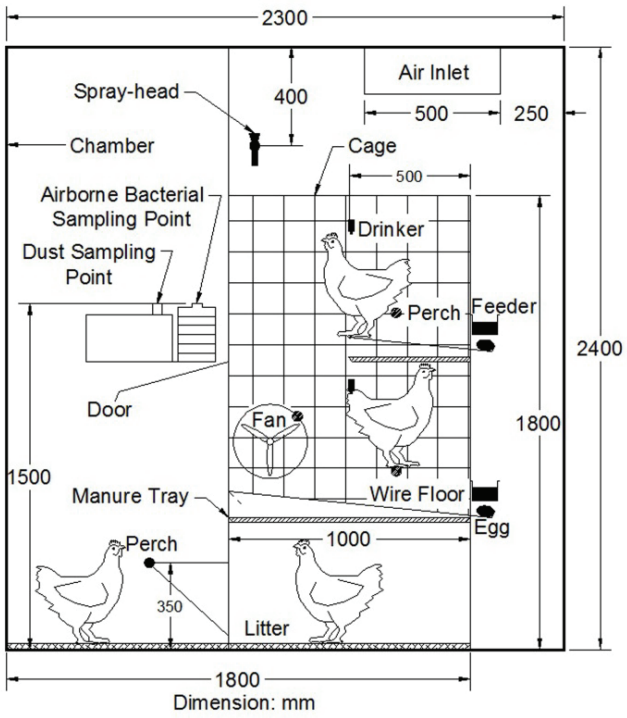

Figure 1. Cross-sectional view of the aviary laying-hen chamber.

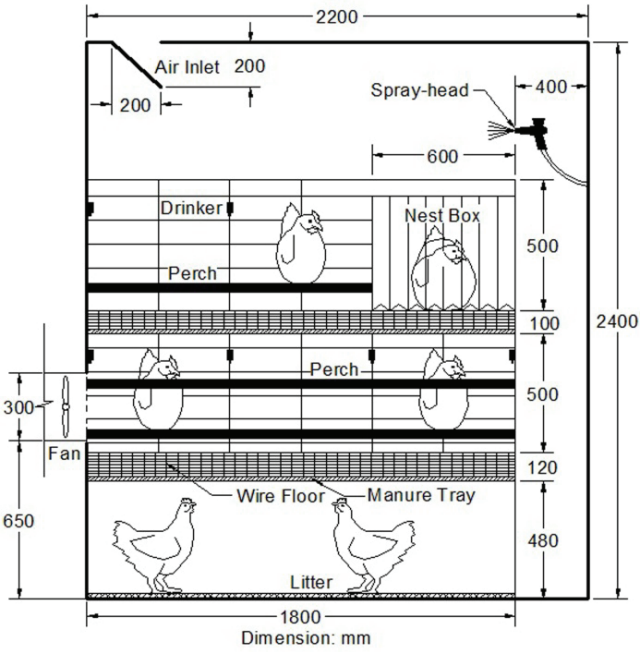

Figure 2. Longitudinal view of the aviary laying-hen chamber.

Table 1. Resource allowance in the aviary laying-hen chamber.

Wire floor area	794 cm^2 $bird^{-1}$
Litter floor area	953 cm^2 $bird^{-1}$
Nest space	88 cm^2 $bird^{-1}$
Perch space[a]	14 cm $bird^{-1}$
Drinker	5.7 birds $drinker^{-1}$
Feed through space	10 cm $bird^{-1}$

[a] Perches in the cage colony.

spraying SAEW or tap water in the experimental aviary laying-hen chamber. The size distribution of the sprayed aerosols was determined using particle image velocimetry (PIV) technology that took images at 5×10^{-7} s intervals with a high-resolution CCD camera (PCO 1600, PCO-Tech, Inc., Romulus, Mich.). The aerosols were found to have an 80 μm median particle diameter and average velocity of 60.5 m s^{-1} near the nozzle (Zhao et al., 2013b). Spraying of SAEW or tap water was activated at 14:00 h for approximately 15 min at a dose of 80 mL m^{-2} floor area.

PREPARATION OF SAEW

A cylindrical plastic electrolyzing container (32 cm H × 19 cm dia.) was used to produce SAEW in this study (Zhao et al., 2013b). Three metal electrode plates (two anode plates and one cathode plate, 15 cm L × 12.5 cm W) were installed in the container, spaced at 1 cm between each anode plate and the cathode plate. The SAEW with an available chlorine concentration (ACC) of 80 mg L^{-1} and a pH of 6.0 was generated by electrolyzing 5 L of NaCl and HCl solution (0.1% NaCl solution with a pH of 2.7) at 8 VDC for 15 min.

AIR TEMPERATURE AND RH MEASUREMENT

During the experiment, the room ventilation rate was maintained at about 3.0 m^3 h^{-1} per bird (equivalent to 9 to 10 air changes per hour for the aviary chamber). Two HOBO Temp/RH sensors (H08-032-08, Onset Computer Corp., Mass.) were used to measure the air temperature and relative humidity (RH). The Temp/RH sensors were checked and calibrated, as necessary, using a precision mercury thermometer and a motorized psychrometer before the experiment and every week during the experiment.

AIRBORNE CB SAMPLING AND ANALYSIS

A bioaerosol impactor (Six-Stage Viable Andersen Cascade Impactor, Thermo Fisher Scientific, Inc., Franklin, Mass.) was used for airborne CB sampling. The impactor collects airborne microorganisms using an agar Petri dish in each of its six stages, which differentiates the collected microorganisms according to their aerodynamic sizes. From the first to sixth stages of the impactor, airborne microorganisms in the sizes of >7.1, 4.7-7.1, 3.3-4.7, 2.1-3.3, 1.1-2.1, and 0.65-1.1 μm were collected. The impactor was operated at an airflow rate of 28.3 L min^{-1} and calibrated using a rotameter (RMC-123-SSV Rate-Master Flowmeter, Dwyer Instruments, Michigan City, Ind.) every sampling day. Each Petri dish was filled with 27 mL of sterilized nutrient agar (Trypticase Soy-Yeast Extract Agar, Fisher Scientific, Pittsburgh, Pa.). Each sampling took 15 min.

After sampling, each Petri dish with airborne CB collected on the medium was immediately rinsed three times with 2 mL of sterilized 0.9% physiological saline solution using a sterilized spreader in a biosafety cabinet, following the method described by Zhao et al. (2011b). The rinsate liquid received 20 μL of Tween 85 (Fisher Scientific, Pittsburgh, Pa.) to deagglomerate coagulated microorganisms (Krometis et al., 2009), followed by 30 s vortex mixing at a speed of 3000 rpm. The volume of the rinsate was recorded. The liquid sample was then serially diluted (1:10) in physiological saline solution, and 0.5 mL of the original and the diluted samples were plated in duplicate on TSA agar. The Petri dishes and the glass Petri dish used in the impactor were then incubated at 37°C for 24 h. After incubation, colonies in the Petri dishes with 30 to 300 colonies were enumerated.

AIRBORNE PM MEASUREMENT

The count concentration of PM was determined at 5 min intervals using an Aerodynamic Particle Sizer (APS) spectrometer (model 3321, TSI, Inc., Shoreview, Minn.). This instrument measured the particle count concentration in 51 channels (consecutive size ranges) over the aerodynamic size range from 0.523 to 20.535 μm, regardless of the particles' physical size, shape, density, or composition. The mass concentrations of PM in different size ranges were also given by the APS, assuming that all the particles were solid, spherical, and had a constant PM density of 1.0 g cm^{-3} (Lai et al., 2012). The APS was calibrated by a specialist from the manufacturer before the experiment. Due to the instrument's limit, PM >20 μm was not measured in this experiment.

EXPERIMENTAL DESIGN

As shown in table 2, no spraying ($Ctrl_{ns}$), spraying tap water ($Ctrl_w$), or spraying SAEW with an ACC of 80 mg L^{-1} (Trt) was performed every other day, four days in total per group, with $Ctrl_w$ and Trt randomly assigned. Airborne PM and airborne CB concentrations in the aviary laying-hen chamber were measured at 1.5 m above the litter floor in the center of the room (figs. 1 and 2). The airborne PM and airborne CB were simultaneously sampled during 13:45-14:00 h (before spraying) and 14:45-15:00 h (after spraying). Spraying occurred at 14:00 h for 15 min in the amount of 80 mL m^{-2} (or 400 mL for the chamber).

DATA ANALYSIS

The actual stage kernel functions of the Anderson impactor are sigmoidal in nature (Vaughan, 1988). However, for simplicity, each stage was assumed to have an ideal cut-off in order to determine airborne CB concentration for each stage. The airborne CB concentration in each range was calculated using equation 1, and the airborne CB concentrations calculated from the duplicate counting were averaged:

Table 2. Operations in the experiment evaluating the efficacy of spraying slightly acidic electrolyzed water (SAEW) on reduction of airborne PM and airborne culturable bacteria in the aviary hen housing chamber.

	Time of Day			
Spray Regimen	13:45 h-14:00 h	14:00 h-14:15 h	14:45 h-15:00 h	Replications
No spraying ($Ctrl_{ns}$)	Sampling	No spraying	Sampling	4
Spraying water ($Ctrl_w$)	Sampling	Spraying water	Sampling	4
Spraying SAEW (Trt)	Sampling	Spraying SAEW	Sampling	4

$$C = \frac{\frac{N_1 V_1}{V_2} \times 10^a + N_2}{Q \times t} \quad (1)$$

where

C = airborne CB concentration at one of the six size ranges (colony-forming unit, CFU m^{-3})

N_1 = number of colonies in a Petri dish with 30 to 300 colonies where 10^{-a} liquid sample is cultured (CFU)

V_1 = total volume of 10^0 liquid sample (mL)

a = dilution factor of the rinsing-off liquid

V_2 = volume of 10^{-a} liquid sample cultured and plated on TSA agar (0.5 mL in this study)

N_2 = number of colonies in the Petri dish used in the impactor (CFU)

Q = airflow rate through the impactor with the Petri dishes (28.3 L min^{-1} = 0.0283 m^{-3} min^{-1})

t = sampling duration (15 min).

Airborne PM concentrations in similar size ranges to those for the airborne CB (i.e., 0.65-1.1, 1.1-2.1, 2.1-3.3, 3.3-4.7, 4.7-7.1, and 7.1-20 μm) were calculated by utilizing the count and mass within the APS size channels, assuming a linear distribution between size channels that spanned the cut-off diameters between successive impactor stages. As examples, PM count and mass concentrations in the range of 0.65-1.1 μm were calculated using equations 2 and 3, respectively. PM count and mass concentrations in ranges of 1.1-2.1, 2.1-3.3, 3.3-4.7, 4.7-7.1, and 7.1-20 μm were calculated similarly:

$$\begin{aligned} N_{0.65-1.1} = {} & N_{0.649-1.075} \\ & - \frac{DL_1 - 0.649}{MD_{0.649-0.698} - MD_{0.604-0.649}} \\ & \times \left(N_{0.649-0.698} - N_{0.604-0.649}\right) \\ & + \frac{DL_2 - 1.075}{MD_{1.075-1.555} - MD_{1.000-1.075}} \\ & \times \left(N_{1.075-1.555} - N_{1.000-1.075}\right) \end{aligned} \quad (2)$$

$$\begin{aligned} M_{0.65-1.1} = {} & M_{0.649-1.075} \\ & - \frac{DL_1 - 0.649}{MD_{0.649-0.698} - MD_{0.604-0.649}} \\ & \times \left(M_{0.649-0.698} - M_{0.604-0.649}\right) \\ & + \frac{DL_2 - 1.075}{MD_{1.075-1.555} - MD_{1.000-1.075}} \\ & \times \left(M_{0.649-0.698} - M_{0.604-0.649}\right) \end{aligned} \quad (3)$$

where

$N_{0.65\text{-}1.1}$, $N_{0.649\text{-}1.075}$, $N_{0.649\text{-}0.698}$, $N_{0.604\text{-}0.649}$, $N_{1.075\text{-}1.555}$, and $N_{1.000\text{-}1.075}$ = PM count concentrations in the ranges of 0.65-1.1, 0.649-1.075, 0.649-0.698, 0.604-0.649, 1.075-1.555, and 1.000-1.075 μm, respectively (particles m^{-3})

$MD_{0.649\text{-}0.698}$, $MD_{0.604\text{-}0.649}$, $MD_{1.075\text{-}1.555}$, and $MD_{1.000\text{-}1.075}$ = PM midpoint diameters in the ranges of 0.649-0.698, 0.604-0.649, 1.075-1.555, and 1.000-1.075 μm, respectively (μm)

DL_1 and DL_2 = lower and upper diameter boundaries of the size range for CB (i.e., 0.65 μm and 1.1 μm)

$M_{0.65\text{-}1.1}$, $M_{0.649\text{-}1.075}$, $M_{0.649\text{-}0.698}$, $M_{0.604\text{-}0.649}$, $M_{1.075\text{-}1.555}$, and $M_{1.000\text{-}1.075}$ = PM mass concentrations in the ranges of 0.65-1.1, 0.649-1.075, 0.649-0.698, 0.604-0.649, 1.075-1.555, and 1.000-1.075 μm, respectively (mg m^{-3}).

For each treatment group (Trt, $Ctrl_w$ and $Ctrl_{ns}$), PM mass concentration (0.65 to 20 μm) and airborne CB concentration (0.65 to 7.1 μm, and >7.1 μm) in each size range during each sampling period (13:45-14:00 h and 14:45-15:00 h) were calculated. The airborne PM and airborne CB concentrations during 13:45-14:00 h (before spraying) were different from day to day. To assess the effects of treatment on the changes of airborne PM and airborne CB concentrations (from 13:45-14:00 h to 14:45-15:00 h), it was necessary to present the airborne PM or airborne CB concentration ratio of after-spraying (14:45-15:00 h) to before-spraying (13:45-14:00 h) using equation 4, and the ratios for each of the four different sampling days in each group were averaged (n = 4):

$$R_{(i)} = \frac{C_{after\,(i)}}{C_{before_{(i)}}} \quad (4)$$

where

$R_{(i)}$ = airborne CB or PM concentration ratio of after-spraying to before-spraying in the ith size range

$C_{after(i)}$ = airborne CB or PM concentration after spraying in the ith size range (CFU m^{-3} or mg m^{-3})

$C_{before(i)}$ = airborne CB or PM concentration before spraying in the ith size range (CFU m^{-3} or mg m^{-3}).

The PM mass-based airborne CB concentration for the entire size range was calculated using equation 5. For each day, the PM mass-based airborne CB concentrations at 13:45-14:00 h (before spraying) and 14:45-15:00 h (after spraying) for the entire size range were computed. PM of 0.65 to 20 μm was taken as PM >0.65 μm in this study, realizing that PM >20 μm can be hardly suspended in the air:

$$C = \frac{C_{CB}}{C_{PM}} \quad (5)$$

where

C = PM mass-based airborne CB concentration in the entire size range (CFU mg^{-1})

C_{CB} = airborne CB concentration in the entire size range (CFU m^{-3})

C_{PM} = airborne PM concentration in the entire size range (mg m^{-3}).

STATISTICAL ANALYSIS

A one-sided t-test was used to evaluate whether ratios of after-spraying to before-spraying airborne CB or airborne PM concentration were significantly different from unity for all size ranges and for the overall size range. A one-way analysis of variance (ANOVA) with main effects of Trt,

Table 3. Airborne culturable bacteria (CB) concentration ratios of after-spraying to before-spraying in different size ranges in response to different experimental regimens of control (no spray), spraying water, or spraying SAEW.[a]

Spraying Regimen[b]	Size Range (μm)						
	0.65-1.1	1.1-2.1	2.1-3.3	3.3-4.7	4.7-7.1	>7.1	>0.65
No spraying ($Ctrl_{ns}$)	1.46 ±0.19	1.61 ±0.84	1.80 ±0.15 A	1.60 ±0.10 A	1.78 ±0.53	2.22 ±0.60 A	2.12 ±0.27 A
Spraying water ($Ctrl_w$)	1.73 ±0.16	1.08 ±0.35	1.40 ±0.75 AB	1.45 ±0.85 AB	1.66 ±0.83	2.09 ±0.11 A	1.97 ±0.11 A
Spraying SAEW (Trt)	1.14 ±0.63	1.55 ±0.87	1.10 ±0.41 B	0.65 ±0.38 B	1.00 ±0.42	0.94 ±0.38 B	0.92 ±0.17 B

[a] Airborne CB concentration ratios were calculated using equation 4. Values are means ± standard deviations (n = 4). Values in the same column followed by different letters are significantly different ($p < 0.05$).

[b] Spraying was performed at 14:00-14:15 h.

$Ctrl_w$, and $Ctrl_{ns}$ was performed using SAS (ver. 9.2, SAS Institute, Inc., Cary, N.C.), with Duncan's test used to test for differences between mean concentration ratios (eq. 4). The mean ratios included airborne CB concentration ratio and mean PM mass concentration ratio (after-spraying/before-spraying). A two-way ANOVA was performed on mean PM mass-based airborne CB concentrations with main effects of the treatments (Trt, $Ctrl_w$, and $Ctrl_{ns}$) and time intervals [13:45-14:00 h (before spraying) and 14:45-15:00 h (after spraying)] (eq. 5). For each treatment, the difference in mean PM mass-based airborne CB concentrations at 13:45-14:00 h (before spraying) and 14:45-15:00 h (after spraying) was tested using Tukey's test. All effects were tested at the 5% significance level.

RESULTS AND DISCUSSION

THERMAL ENVIRONMENT

Indoor air temperature ranged from 18.6°C to 25.9°C (averaging 21.1°C), and indoor RH ranged from 21% to 73% (averaging 39%) throughout the experiment. Average air temperature and RH on the days of spraying (Trt, $Ctrl_w$) are plotted in figure 3. Spraying caused a slight (~0.5°C) air temperature drop and about 10% RH rise, a result of sensible and latent heat shift of the air due to evaporation of the sprayed SAEW or tap water.

CONCENTRATION OF AIRBORNE CB

The airborne CB concentration ratios of after-spraying (14:45-15:00 h) to before-spraying (13:45-14:00 h) in different size ranges and for the entire size range (eq. 5) are given in table 3. According to the t-test, the mean airborne CB concentration ratio for Trt over the entire size range (>0.65 μm) was not different from unity ($p = 0.50$), indicating no significant change in airborne CB concentrations. However, for both $Ctrl_{ns}$ and $Ctrl_w$, the mean airborne CB concentration ratios exceeded unity for the entire size range ($p < 0.05$), indicating temporal increase of airborne CB concentrations.

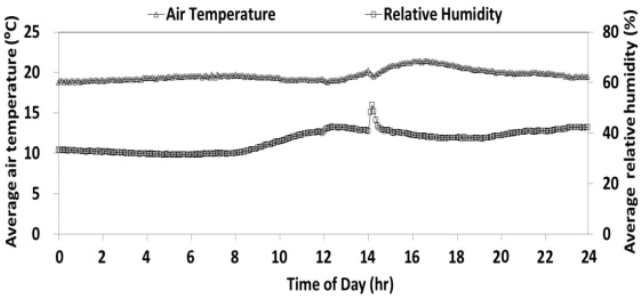

Figure 3. Average indoor air temperature and RH on spraying days. Spraying was done at 14:00 h for 15 min at a dosage of 80 mL m^{-2}.

According to the one-way ANOVA, the overall airborne CB concentration ratio (size >0.65 μm) and the largest size range (size >7.1 μm) were lower for Trt than for either $Ctrl_{ns}$ or $Ctrl_w$ ($p < 0.05$). There was no difference between $Ctrl_w$ and $Ctrl_{ns}$ for these same size ranges ($p = 0.66$ and $p = 0.71$, respectively). Hence, compared to $Ctrl_{ns}$ (no spraying) or $Ctrl_w$ (spraying tap water), spraying SAEW showed an overall reduction in the airborne CB concentrations. However, no difference was detected among Trt, $Ctrl_{ns}$, and $Ctrl_w$ for the size ranges of 0.65-1.1 μm and 1.1-2.1 μm.

SAEW improves the bactericidal activity by maximizing the use of hypochlorous acid, which is primarily responsible for inactivation of airborne CB (Zheng et al., 2012b). Higher ACC caused better inactivation efficiency of bacterial aerosols when spraying SAEW (Chuang et al., 2013). Airborne CB directly exposed to sprayed SAEW could be killed or inactivated, resulting in an overall reduction in airborne CB concentration. The lack of airborne CB reduction in the size range of 0.65-1.1 μm and 1.1-2.1 μm could be explained by the low percentage of these fine bioaerosols that were caught by the sprayed SAEW. SAEW spray could be an effective means for reducing airborne CB in laying-hen houses. However, more studies regarding the duration of SAEW spray's airborne CB-suppressing effects and its influence on bird activity are desirable, including time-series measurements of airborne CB and more replicates.

CONCENTRATION OF AIRBORNE PM

Mean (±SD) airborne PM concentration ratios of before-spraying (14:45-15:00 h) to after-spraying (13:45-14:00 h) for the different size ranges and for the overall size range (0.65 to 20 μm) are given in table 4. According to the t-test, the mean PM mass concentration ratios over the entire size range (0.65 to 20 μm) for Trt, $Ctrl_{ns}$, and $Ctrl_w$ exceeded unity ($p < 0.05$), indicating temporal change in PM mass concentrations. The results thus indicate that spraying SAEW or tap water could not significantly reduce airborne PM compared to no spraying for the PM size range of 0.65 to 20 μm.

According to the one-way ANOVA, no difference was found among the mean PM concentration ratios of Trt, $Ctrl_w$, and $Ctrl_{ns}$ for the overall size range of 0.65 to 20 μm ($p = 0.38$). The same was true for the individual size ranges, except for the larger PM size range of 7.1 to 20 μm where Trt was shown to reduce PM concentration ($p <$

Table 4. PM concentration ratios between after-spraying and before-spraying in different size ranges.[a]

Spraying Regimen[b]	Size Range (μm)						
	0.65-1.1	1.1-2.1	2.1-3.3	3.3-4.7	4.7-7.1	7.1-20	0.65-20
No spraying ($Ctrl_{ns}$)	1.25 ±0.14	1.93 ±0.56	1.50 ±0.18	1.54 ±0.05	1.77 ±0.24	1.98 ±0.35 A	1.93 ±0.30
Spraying water ($Ctrl_w$)	1.57 ±0.67	1.34 ±0.41	1.42 ±0.32	1.61 ±0.36	1.65 ±0.54	1.23 ±0.61 AB	1.56 ±0.39
Spraying SAEW (Trt)	1.59 ±0.46	1.76 ±0.59	1.69 ±0.50	1.66 ±0.50	1.65 ±0.52	1.19 ±0.32 B	1.41 ±0.33

[a] PM mass concentration ratios were calculated using equation 4. Values are means ± standard deviations (n = 4). Values in the same column followed by different letters are significantly different ($p < 0.05$).

[b] Spraying was performed at 14:00-14:15 h.

0.05) and $Ctrl_w$ was shown to have the potential to do so ($p = 0.11$).

Some researchers have reported that spraying water could effectively reduce total airborne PM in livestock houses (Takai and Pedersen, 2000; Kim et al., 2006). Spraying electrolyzed water was also proven to reduce total dust in a laying-hen house (Zheng et al., 2012b). In this study, 0.65 to 20 μm PM was investigated instead of total PM due to the APS's measurement limits. Our results showed that airborne PM in the range of 0.65 to 20 μm was not remarkably reduced by spraying SAEW or tap water. An explanation for this outcome is that, compared to total (larger) dust, a limited amount of the smaller airborne PM in the 0.65 to 20 μm range could be settled out or suppressed by the sprayed SAEW or tap water aerosols. Moreover, sprayed SAEW or tap water aerosols could be enumerated by the APS, resulting in underestimation of the PM (0.65 to 20 μm) reduction. In other words, larger particles can be more effectively precipitated by sprayed SAEW or tap water aerosols than finer particles. Further studies may investigate airborne dust reduction by spraying SAEW and measuring dry PM mass for reduction in different size ranges.

Relationship of Airborne PM and CB Concentration Reductions

The PM mass-based airborne CB concentrations for the entire size range during the periods of 13:45-14:00 h and 14:45-15:00 h are given in table 5. According to the two-way ANOVA, the time intervals did not have an effect on the PM mass-based airborne CB concentration ($p = 0.74$), but the treatments and the interaction both significantly affected the response variable. The mean PM mass-based airborne CB concentration for Trt was lower than that for $Ctrl_{ns}$ or $Ctrl_w$ ($p < 0.05$).

As shown in table 5, the PM mass-based airborne CB concentrations in $Ctrl_w$ and $Ctrl_{ns}$ were not reduced from before-spraying to after-spraying ($p = 0.26$ and 0.10). However, the airborne CB concentration in Trt was effectively reduced during the same period. The results indicate that the airborne CB carried by airborne PM was reduced by spraying SAEW, which was not achieved by spraying tap water. Therefore, airborne CB reduction by spraying SAEW in the experimental chamber was predominantly caused by the bactericidal effect of SAEW, rather than the airborne PM reduction from spraying.

Table 5. PM mass-based airborne CB concentrations at 13:45-14:00 h and 14:45-15:00 h.[a]

Sampling Time	Airborne CB Concentration (10^5 CFU mg^{-1})		
	No Spraying ($Ctrl_{ns}$)	Spraying SAEW (Trt)	Spraying Water ($Ctrl_w$)
13:45-14:00 h (Before spraying)	1.44 ±0.12	1.37 ±0.14 A	1.75 ±0.17
14:45-15:00 h (After spraying)	1.60 ±0.16	0.89 ±0.13 B	2.20 ±0.27

[a] Spraying was performed at 14:00-14:15 h. Values are means ± standard deviations (n = 4). Values in the same column followed by different letters are significantly different ($p < 0.05$).

Conclusions

This study demonstrated that spraying SAEW reduced airborne CB for the aerodynamic size range of >0.65 μm in the aviary laying-hen housing chamber ($p < 0.05$), predominantly for the size range of >2.1 μm. Spraying SAEW and tap water potentially reduced airborne PM (0.65 to 20 μm) compared to no spraying, with an effective reduction in the size range of 7.1 to 20 μm. Airborne CB reduction by spraying SAEW in the aviary laying-hen chamber seems predominantly caused by the bactericidal effect of SAEW, instead of the airborne PM reduction from spraying. Spraying SAEW offers a potential means to improve indoor air quality in aviary laying-hen housing systems. Further studies are warranted to verify the study findings, especially under field conditions.

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