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# Concentrations and Size Distributions of Airborne Particulate Matter and Bacteria in an Experimental Aviary Laying-Hen Housing System

## Abstract

High levels of airborne particulate matter (PM) and bacteria may exist in animal housing, which can be detrimental to the health of animals and workers. The sizes of these bioaerosols determine their aerial-transport behaviors and depositions in the respiratory tracts of animals and humans. However, little is known regarding the size distribution of airborne PM and bacteria in livestock houses, especially alternative animal housing systems that aim to enhance animal welfare, such as aviary hen-housing systems. The study reported here was therefore conducted to characterize the concentrations (both in count and in mass) and size distributions of airborne bacteria (in count) and PM (both in count and in mass) in a pilot-scale welfare-oriented aviary laying-hen setting. Thirty-four laying hens were kept in the environmentally-controlled aviary setting (L  $\times$  W  $\times$  H = 2.2  $\times$  2.3  $\times$  2.4 m) for 3 months. The hens were given a 16L:8D photoperiod (lights on at 6:00h and off at 22:00h); and access to the litter floor from 12:00h to 22:00h daily. Airborne bacteria and PM were simultaneously sampled for 15 min at 1.5 m height above the litter floor every fourth day at 5:45h, 9:45h, 13:45h, 17:45h, and 21:45h. Concentrations of airborne bacteria at six size ranges (0.65-1.1  $\mu$ m, 1.1-2.1  $\mu$ m, 2.1-3.3  $\mu$ m, 3.3-4.7  $\mu$ m, 4.7-7.1  $\mu$ m, and >7.1  $\mu$ m) and the PM concentrations (0.5-20  $\mu$ m) were determined. The daily mean ( $\bar{x}$  $\pm$ SD) concentrations of PM count, PM mass and total bacterial concentration were, respectively, 1.70 ( $\bar{x}$  $\pm$ 0.66)  $\times 10^7$  particles  $m^{-3}$ , 1.12 ( $\bar{x}$  $\pm$ 0.47)  $mg m^{-3}$  and 3.39 ( $\bar{x}$  $\pm$ 2.38)  $\times 10^5$  CFU  $m^{-3}$ . Concentrations of PM and total bacteria during the litter-access period (12:00h-22:00h) were significantly higher than those during the rest of the day when the hens were off the floor ( $P < 0.05$ ). Median diameters for the PM count and mass were, respectively, 2.11  $\mu$ m and 7.45  $\mu$ m. PM <10  $\mu$ m accounted for more than 95% of the total PM count, whereas PM >2.5  $\mu$ m accounted for more than 90% of the total PM mass. The majority (>95%) of the airborne bacteria were contained in particles >3.3  $\mu$ m. Airborne bacteria count was positively related to PM mass concentration ( $P < 0.05$ ) with a slope of 3.84( $\bar{x}$  $\pm$ 2.70)  $\times 10^5$  CFU  $mg^{-1}$  PM. Results of the study are useful for improving understanding of transport behaviors of aerosols in the aviary hen setting, assessing potential respiratory risks to humans and animals, and exploring mitigation techniques.

## Keywords

Airborne bacteria, Particulate matter, Cage-free, Size distribution

## Disciplines

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## Concentrations and Size Distributions of Airborne Particulate Matter and Bacteria in an Experimental Aviary Laying-Hen Housing System

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**Abstract.** High levels of airborne particulate matter (PM) and bacteria may exist in animal housing, which can be detrimental to the health of animals and workers. The sizes of these bioaerosols determine their aerial-transport behaviors and depositions in the respiratory tracts of animals and humans. However, little is known regarding the size distribution of airborne PM and bacteria in livestock houses, especially alternative animal housing systems that aim to enhance animal welfare, such as aviary hen-housing systems. The study reported here was therefore conducted to characterize the concentrations (both in count and in mass) and size distributions of airborne bacteria (in count) and PM (both in count and in mass) in a pilot-scale welfare-oriented aviary laying-hen setting. Thirty-four laying hens were kept in the environmentally-controlled aviary setting ( $L \times W \times H = 2.2 \times 2.3 \times 2.4$  m) for 3 months. The hens were given a 16L:8D photoperiod (lights on at 6:00h and off at 22:00h); and access to the litter floor from 12:00h to 22:00h daily. Airborne bacteria and PM were simultaneously sampled for 15 min at 1.5 m height above the litter floor every fourth day at 5:45h, 9:45h, 13:45h, 17:45h, and 21:45h. Concentrations of airborne bacteria at six size ranges (0.65-1.1  $\mu\text{m}$ , 1.1-2.1  $\mu\text{m}$ , 2.1-3.3  $\mu\text{m}$ , 3.3-4.7  $\mu\text{m}$ , 4.7-7.1  $\mu\text{m}$ , and  $>7.1$   $\mu\text{m}$ ) and the PM concentrations (0.5-20  $\mu\text{m}$ ) were determined. The daily mean ( $\pm$ SD) concentrations of PM count, PM mass and total bacterial concentration were, respectively,  $1.70 (\pm 0.66) \times 10^7$  particles  $\text{m}^{-3}$ ,  $1.12 (\pm 0.47)$  mg  $\text{m}^{-3}$  and  $3.39 (\pm 2.38) \times 10^5$  CFU  $\text{m}^{-3}$ . Concentrations of PM and total bacteria during the litter-access period (12:00h-22:00h) were significantly higher than those during the rest of the day when the hens were off the floor ( $P < 0.05$ ). Median diameters for the PM count and mass were, respectively, 2.11  $\mu\text{m}$  and 7.45  $\mu\text{m}$ . PM  $< 10$   $\mu\text{m}$  accounted for more than 95% of the total PM count, whereas PM  $> 2.5$   $\mu\text{m}$  accounted for more than 90% of the total PM mass. The majority ( $>95\%$ ) of the airborne bacteria were contained in particles  $> 3.3$   $\mu\text{m}$ . Airborne bacteria count was positively related to PM mass concentration ( $P < 0.05$ ) with a slope of  $3.84 (\pm 2.70) \times 10^5$  CFU  $\text{mg}^{-1}$  PM. Results of the study are useful for improving understanding of transport behaviors of aerosols in the aviary hen setting, assessing potential respiratory risks to humans and animals, and exploring mitigation techniques.

**Keywords.** Airborne bacteria, Particulate matter, Cage-free, Size distribution

## Introduction

Airborne bacteria are normally associated with particulate matter (PM) in livestock housing environments. Exposure to such airborne PM and bacteria can have negative impacts on the health of the animals and farmers (Whyte et al, 2002; Andersen et al., 2004; Mitchell et al., 2004). Aviary hen-housing system is an alternative egg production system that features certain enrichment elements, such as litter floor, perches and nest boxes. While hen's natural behaviors are accommodated, much higher dust and bacterial concentrations were found in aviary housing systems than in cage housing systems (Ellen et al., 2000; Protais et al., 2003; Hayes et al., 2012).

Airborne PM in livestock houses is a carrier of a large variety of microorganisms (Wang et al. 2000; Zhang, 2004; Zhang and Chen, 2006; Lee et al., 2006; Cambra-Lopez et al., 2010). Positive relationships between the airborne PM and bacteria have been previously reported (Bakutis et al., 2004; Lai et al., 2009; Verreault et al., 2010). Airborne PM with different aerodynamic sizes may harbor different colonies of bacteria. Airborne PM larger than 2  $\mu\text{m}$  in diameter was found to contain high amounts of bacteria in livestock houses (Lee et al., 2006). Little information is found on the relationship of airborne PM and bacterial concentrations in aviary laying-hen systems, and the association of bacteria with particle size distributions.

Knowledge of size distributions of airborne PM and bacteria in livestock housing is desirable for understanding the transport behaviors of bioaerosols and the health risk to animals and humans, and to improve the control techniques of the housing air environment. Several studies have investigated the size distributions of airborne PM or bacteria in broiler houses and pig houses (Heber et al., 1988; Roumeliotis and Heyst, 2007; Lai et al. 2012). However, with the continued trend toward alternative hen housing and increased use of aviary hen-housing systems, baseline information on size distributions of airborne PM and bacteria in such systems is desirable. Investigation of diurnal variations of the airborne PM and bacterial concentrations is also needed for improved environmental management in aviary laying-hen systems.

The objective of this research was to characterize the relationship of airborne PM and bacterial concentrations in six aerodynamic size ranges in an experimental aviary laying-hen setting. The size distributions of airborne PM and associated bacteria in the experimental setting and diurnal variations of airborne PM and bacterial concentration were also examined in this study.

## Materials and Methods

### Experimental aviary laying-hen setting

The 3-month experiment was conducted in a 2.2  $\times$  2.3  $\times$  2.4 m environmentally-controlled chamber at the Livestock Environment and Animal Physiology Laboratory of Iowa State University, IA, USA. Thirty-four 78 week-old (onset age) CV22 laying hens were kept in the environmental chamber that was equipped with an aviary housing system (fig. 1 and fig. 2). A two-tier aviary system (1.8  $\times$  1.0  $\times$  1.75 m) was placed in the chamber and the floor was covered with litter (sawdust + dry manure, 1.8  $\times$  1.8 m). The thickness of the litter (1-2 cm) in the aviary setting was based on that measured at the commercial farm where the hens were procured. Lighting was scheduled to be on at 6:00h and off at 22:00h (16L: 8D). Hens were given access to the litter from 12:00h to 22:00h (10 h) of each day. The feeders, drinkers, perches, and a nest box (0.6  $\times$  0.5  $\times$  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 ceiling air inlet. A manure collection tray was placed under the cage colony and the collected manure was scraped off and removed every 4 days.

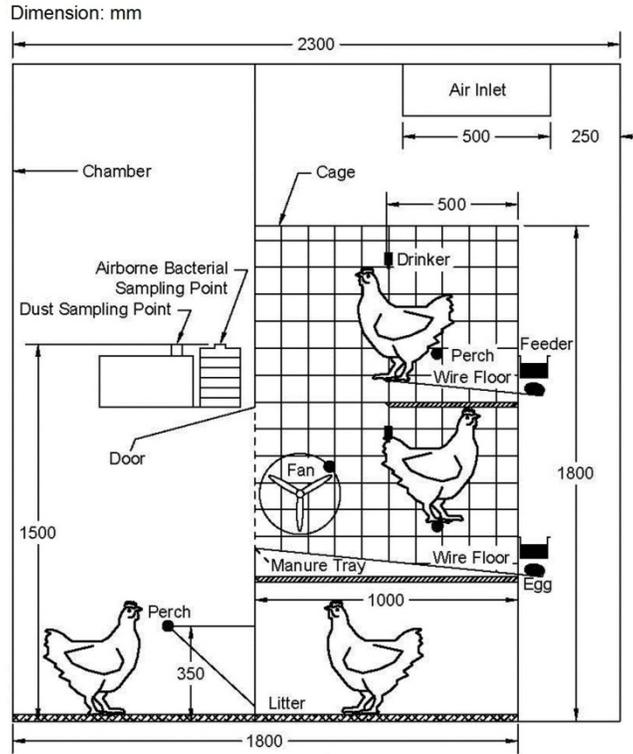
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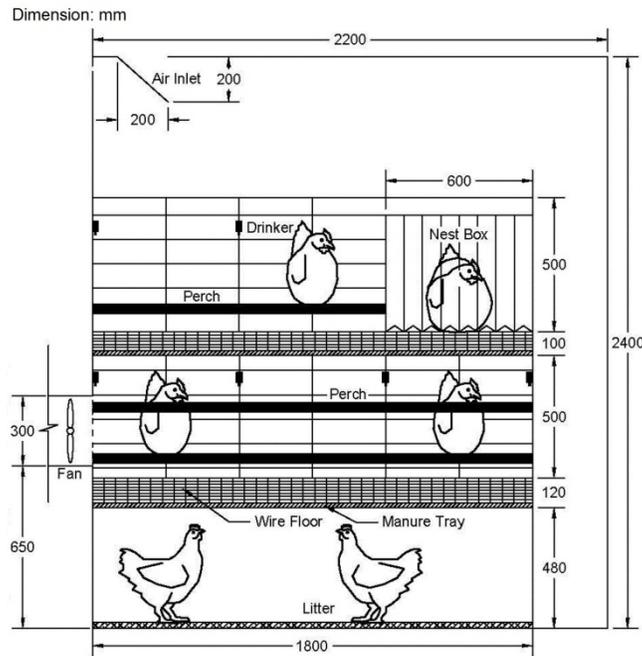
**Table 1. Resource allowance in the aviary laying-hen setting.**

Wire floor (cm <sup>2</sup> bird <sup>-1</sup> )	Litter floor (cm <sup>2</sup> bird <sup>-1</sup> )	Nest space (cm <sup>2</sup> bird <sup>-1</sup> )	Perch (cm bird <sup>-1</sup> )	Drinker (birds drinker <sup>-1</sup> )	Feed through (cm bird <sup>-1</sup> )
794	953	88	14 <sup>[a]</sup>	5.7	10

<sup>[a]</sup> Only the perches in the cage colony were included.



**Figure 1. A cross-sectional view of the aviary laying-hen setting.**



**Figure 2. A longitudinal view of the aviary laying-hen setting.**

## Experimental design

Concentrations and size distributions of airborne bacteria and PM in the aviary laying-hen setting were measured at 1.5 m height above the litter floor (fig. 1). The airborne PM and total bacteria were simultaneously sampled at 5:45-6:00h, 9:45-10:00h, 13:45-14:00h, 17:45-18:00h, and 21:45-22:00h every fourth day (6 repetitions in total).

### Airborne bacteria sampling and analysis

A bioaerosol impactor (Six-Stage Viable Andersen Cascade impactor; Thermo Fisher Scientific Inc., Franklin, MA, USA) was used for sampling airborne bacteria in this experiment. The impactor collects airborne microorganisms using an agar Petri dish in each of its six stages, which differentiate the collected microorganisms according to their size. From the first to sixth stages of the impactor, airborne microorganisms in the sizes of  $>7.1 \mu\text{m}$ ,  $4.7\text{-}7.1 \mu\text{m}$ ,  $3.3\text{-}4.7 \mu\text{m}$ ,  $2.1\text{-}3.3 \mu\text{m}$ ,  $1.1\text{-}2.1 \mu\text{m}$  and  $0.65\text{-}1.1 \mu\text{m}$  were collected. The impactor was operated at an air flow rate of  $28.3 \text{ L min}^{-1}$  calibrated using a rotameter (Dwyer RMC-123-SSV Rate-Master Flow Meter; Michigan City, IN) before the experiment. Each Petri dish was filled with 27 mL of sterilized nutrient agar (Trypticase Soy-Yeast Extract Agar, Fisher Scientific, Pittsburgh, PA, USA).

After sampling, each Petri dish with airborne bacteria collected on the medium was immediately rinsed with 2 mL sterilized 0.9% physiological saline using a sterilized spreader for three times in a biosafety cabinet following the same method described by Zhao et al. (2011b). The rinsing-off liquid received 20  $\mu\text{L}$  of Tween 85 (Fisher Scientific, Pittsburgh, PA, USA) to disrupt any cell-particle aggregates (Krometis et al., 2009) followed by 30 s of vortex mixing at a speed of 3000 rpm. The volume of the rinsing-off liquid sample was recorded. The liquid sample was then serially diluted (1:10) in physiological saline and 0.5 mL of the original and the diluted samples were plated in duplicate on TSA agar. Then the Petri dishes and the glass Petri dish used in the impactor were incubated at  $37^\circ\text{C}$  for 24 h. After incubation, colonies in the Petri dishes with 30-300 colonies are enumerated. The airborne bacteria concentration in each range was calculated using equation 1. The airborne bacteria concentrations calculated based on the duplicate counting were averaged.

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

Where

C = airborne bacteria concentration at one of the six size range (colony-forming unit,  $\text{CFU m}^{-3}$ )

$N_1$  = number of colonies in a Petri dish with 30-300 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 plated on TSA agar (0.5 mL)

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

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

t = sampling duration (15 min).

### Airborne PM measurement

The count concentration of airborne PM was determined at 5-min intervals using an Aerodynamic Particle Sizer (APS) spectrometer (Model 3321, TSI Inc., Shoreview, MN, USA) which measured the particles count concentration in 51 channels (or consecutive size range) over the size range from  $0.523$  to  $20.535 \mu\text{m}$  with lower limits of  $0.523$ ,  $0.562$ ,  $0.604$ ,  $0.649$ ,  $0.698$ ,  $0.750$ ,  $0.806$ ,  $0.866$ ,  $0.931$ ,  $1.000$ ,  $1.075$ ,  $1.155$ ,  $1.241$ ,  $1.334$ ,  $1.433$ ,  $1.540$ ,  $1.655$ ,  $1.778$ ,  $1.911$ ,  $2.054$ ,  $2.207$ ,  $2.371$ ,  $2.548$ ,  $2.738$ ,  $2.943$ ,  $3.162$ ,  $3.398$ ,  $3.652$ ,  $3.924$ ,  $4.217$ ,  $4.532$ ,  $4.870$ ,  $5.233$ ,  $5.623$ ,  $6.043$ ,  $6.494$ ,  $6.978$ ,  $7.499$ ,  $8.058$ ,  $8.660$ ,  $9.306$ ,  $10.00$ ,  $10.746$ ,  $11.548$ ,  $12.409$ ,  $13.335$ ,  $14.330$ ,  $15.339$ ,  $16.548$ ,  $17.783$ , and  $19.110 \mu\text{m}$ . The PM mass concentration in different size range was also given by APS.

## Data analysis

The daily average PM count concentration, PM mass concentration and bacterial concentration were calculated based on the 30 measurements for 5 different sampling periods. For each of the 5 sampling period, airborne bacterial concentrations (in the entire size range, >0.65 µm for bacteria and 0.65-20µm for PM) on the 6 sampling days were averaged. Statistical analysis was performed using the Statistical Analysis System (SAS 9.2, SAS Institute Inc., Cary, NC, USA). Tukey's test was used to determine the significant differences among the means of airborne bacterial concentrations for the 5 sampling periods at the 5% significance level. The same methodology was used to investigate the diurnal airborne PM mass concentration variations.

The count and mass PM concentrations measured by APS in all ranges were averaged and plotted, as is, to show the concentration distributions of PM. Since the 51 different size ranges in which airborne PM was measured by APS are not of the same width, it is necessary to present standardized fractions in line graphs. Therefore, a spectrum of standardized PM fraction in these ranges, i.e., fraction distribution, was derived and plotted as well.

The midpoint diameter was given in each size range by the APS, which is used in the distributions of standardized PM count and mass fraction. The standardized PM count and mass fraction of a size range was calculated using equations 2 and 3, respectively:

$$f_{i,count} = \frac{n_i / \Delta d_i}{N} \quad (2)$$

$$f_{i,mass} = \frac{m_i / \Delta d_i}{M} \quad (3)$$

Where

$f_{i,count}$  = PM count fraction of the  $i^{th}$  size range ( $\mu\text{m}^{-1}$ )

$n_i$  = particle population of the  $i^{th}$  size range (particles  $\text{m}^{-3}$ )

$\Delta d_i$  = the  $i^{th}$  size range ( $\mu\text{m}$ )

$N$  = total particle population of all size ranges (particles  $\text{m}^{-3}$ )

$f_{i,mass}$  = PM mass fraction of the  $i^{th}$  size range ( $\mu\text{m}^{-1}$ )

$m_i$  = particle weight of the  $i^{th}$  size range ( $\text{mg m}^{-3}$ )

$M$  = total particle weight of all size ranges ( $\text{mg m}^{-3}$ ).

The count median diameter (CMD) and mass median diameter (MMD) of PM over the size range of 0.523-20.535 µm were calculated by equations 4 and 5, respectively:

$$CMD = \exp\left(\frac{\sum N_i \ln MD_i}{N}\right) \quad (4)$$

$$MMD = \exp\left(\frac{\sum M_i \ln MD_i}{M}\right) \quad (5)$$

Where

CMD = PM count median diameter ( $\mu\text{m}$ )

$N_i$  = PM count number in the  $i^{th}$  size range (particles  $\text{m}^{-3}$ )

$MD_i$  = midpoint diameter of PM in the  $i^{th}$  size range ( $\mu\text{m}$ )

$N$  = total particle population of all size ranges (particles  $\text{m}^{-3}$ )

MMD = PM mass median diameter ( $\mu\text{m}$ )

$M_i$  = PM mass concentration in the  $i^{th}$  size range ( $\text{mg m}^{-3}$ )

$M$  = total PM mass of all size ranges ( $\text{mg m}^{-3}$ ).

To investigate the relationship between PM and airborne bacteria, concentrations of PM in the similar size range as the bacteria (i.e. 0.65-1.1  $\mu\text{m}$ , 1.1-2.1  $\mu\text{m}$ , 2.1-3.3  $\mu\text{m}$ , 3.3-4.7  $\mu\text{m}$ , 4.7-7.1  $\mu\text{m}$ , and 7.1-20  $\mu\text{m}$ ) were calculated. PM count and mass concentrations in the range of 0.65-1.1  $\mu\text{m}$  were calculated using equation 6 and equation 7, respectively. PM count and mass concentrations in ranges of 1.1-2.1  $\mu\text{m}$ , 2.1-3.3  $\mu\text{m}$ , 3.3-4.7  $\mu\text{m}$ , 4.7-7.1  $\mu\text{m}$ , and 7.1-20  $\mu\text{m}$  were calculated similarly. These values were averaged based on the 30 measurements.

$$N_{0.65-1.1} = N_{0.649-1.075} - \frac{DL_1 - 0.649}{MD_{0.649-0.698} - MD_{0.604-0.649}} (N_{0.649-0.698} - N_{0.604-0.649}) + \frac{DL_2 - 1.075}{MD_{1.075-1.555} - MD_{1.000-1.075}} (N_{1.075-1.555} - N_{1.000-1.075}) \quad (6)$$

$$M_{0.65-1.1} = M_{0.649-1.075} - \frac{DL_1 - 0.649}{MD_{0.649-0.698} - MD_{0.604-0.649}} (M_{0.649-0.698} - M_{0.604-0.649}) + \frac{DL_2 - 1.075}{MD_{1.075-1.555} - MD_{1.000-1.075}} (M_{1.075-1.555} - M_{1.000-1.075}) \quad (7)$$

Where

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

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

$DL_1$  and  $DL_2$  = lower and upper diameter boundaries of the size range for bacteria, i.e. 0.65  $\mu\text{m}$  and 1.1  $\mu\text{m}$

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

The percent of PM count and mass in each size range to those in the entire size range (0.65-20  $\mu\text{m}$ ) was calculated using equation 8 and equation 9:

$$P_{i,\text{count}} = \frac{N_i}{N} \quad (8)$$

$$P_{i,\text{mass}} = \frac{M_i}{M} \quad (9)$$

Where

$P_{i,\text{count}}$  = percent of  $i^{\text{th}}$  size range in the entire size range in count and in mass (%)

$N_i$  = number of PM count in the  $i^{\text{th}}$  size range (particles  $\text{m}^{-3}$ )

$N$  = sum of PM count in the entire size range (particles  $\text{m}^{-3}$ )

$P_{i,\text{mass}}$  = percent of  $i^{\text{th}}$  size range in the entire size range in count and in mass (%)

$M_i$  = PM mass in the  $i^{\text{th}}$  size range ( $\text{mg m}^{-3}$ )

$M$  = sum of PM mass in the entire size range ( $\text{mg m}^{-3}$ ).

For each size range of 0.65-1.1  $\mu\text{m}$ , 1.1-2.1  $\mu\text{m}$ , 2.1-3.3  $\mu\text{m}$ , 3.3-4.7  $\mu\text{m}$ , 4.7-7.1  $\mu\text{m}$ , and >7.1  $\mu\text{m}$  (7.1-20  $\mu\text{m}$  for airborne PM), airborne PM and bacterial concentrations based on the 30 measurements (5 times for each day, 6 days total) were recorded, respectively. To investigate the relationship between airborne PM and bacterial concentration, bivariate correlation analysis was performed using SAS software (SAS 9.2, SAS Institute Inc., Cary, NC, USA) at the 5% significance level. Linear regression equations were produced with Microsoft Excel software (Microsoft Corporation, Redmond, WA, USA).

The bacterial concentrations related to airborne PM mass in each size range were calculated using equation 10. For each size range, the bacterial concentrations related to airborne PM mass based on 30 measurements

were averaged. Statistical analysis was performed using the SAS (SAS 9.2, SAS Institute Inc., Cary, NC, USA). Tukey's test was used to determine the significant differences among the means of airborne bacterial concentrations related to airborne PM weight in the 6 size ranges at 5% significance level.

$$C_i = \frac{C_b}{C_p} \quad (10)$$

Where

$C_i$  = bacterial concentration related to airborne PM mass in the  $i^{\text{th}}$  size range (CFU  $\text{mg}^{-1}$ )

$C_b$  = airborne bacterial concentration in the  $i^{\text{th}}$  size range (CFU  $\text{m}^{-3}$ )

$C_p$  = airborne PM concentration in the  $i^{\text{th}}$  size range ( $\text{mg m}^{-3}$ ).

## Results and Discussion

### Thermal Environment

During the experiment, ventilation rate was maintained at about  $3.0 \text{ m}^3 \text{ h}^{-1}$  per bird. Air temperature was from  $19.0^\circ\text{C}$  to  $26.3^\circ\text{C}$  (averaging  $21.6^\circ\text{C}$ ), and relative humidity was from 22% to 68% (averaging 37%) in the environmental chamber.

### Concentrations of Airborne PM and Bacteria

#### Total Concentration

The daily average PM count concentration, PM mass concentration and bacterial concentration were  $1.70 (\pm 0.66) \times 10^7$  particles  $\text{m}^{-3}$ ,  $1.12 (\pm 0.47) \text{ mg m}^{-3}$  and  $3.39 (\pm 2.38) \times 10^5$  CFU  $\text{m}^{-3}$ , respectively.

#### Diurnal Variations of Airborne PM and Bacteria Concentrations

As shown in figure 4, the airborne PM and total bacterial concentrations showed similar diurnal patterns during the sampling periods. The highest average airborne PM and total bacterial concentrations were found at 13:45-14:00h, followed by 17:45-18:00h, 21:45-22:00h, 9:45-10:00h, and 5:45-6:00h. The airborne PM and total bacterial concentrations during the litter-access period (12:00h-22:00h) were significantly higher than those during the off-litter period ( $P < 0.05$ ). Bird activity is a major cause of airborne PM concentration changes in poultry houses (Heber et al., 2006; Mitchell et al., 2004; Zheng et al., 2012). Litter is a major source of airborne PM and bacteria (Vucemilo et al., 2007, Zhao et al. 2013). Barker et al. (2010) reported aerobic bacterial concentration in the poultry litter was more than  $7.0 \log_{10}$  CFU  $\text{g}^{-1}$ . These high concentrations of airborne bacteria and PM during the litter-access period are attributable to the high bird activities on litter.

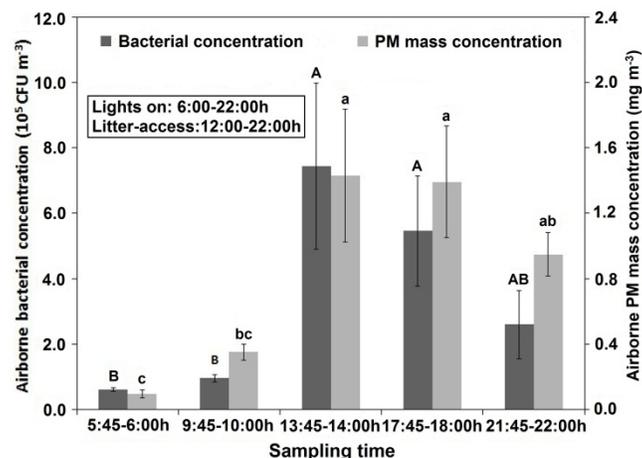


Figure 4. Diurnal variations of airborne PM and bacterial concentrations of the experimental aviary hen housing. Vertical bars represent SE. Vertical bars labeled with different letters in the same series indicate significant difference ( $P < 0.05$ ).

## Size Distributions of Airborne PM and Bacteria

### Particulate Matter (PM)

Size distributions of airborne PM in count and in mass (0.523-20.535  $\mu\text{m}$ ) are shown in figure 5 and figure 6, respectively. It is apparent that the two distributions differ considerably. The standardized count fraction was high in the size range of 0.523-1.0  $\mu\text{m}$ , and then reduced with increasing diameter. Specifically,  $\text{PM}_{1-10}$ ,  $\text{PM}_{1-2.5}$ ,  $\text{PM}_{2.5-10}$ , and  $\text{PM}_{10-20}$  accounted for 26.6%, 28.5%, 42.4%, and 2.5% over the range of 0.523-20.535  $\mu\text{m}$ , respectively, with  $\text{PM}_{2.5}$  dominating the distribution (55.1%). Lai et al. (2012) reported particle counts in animal houses accounted for 87% in the size range of  $< 1.0 \mu\text{m}$ . The high  $\text{PM}_{1-10}$  proportion in aviary hen housing results from the presence and use of litter by the hens for scratching and dust-bathing. Over the entire size range of 0.523-20.535  $\mu\text{m}$ , the PM count had a median diameter of 2.11  $\mu\text{m}$ , the PM mass had a median diameter of 7.45  $\mu\text{m}$ .

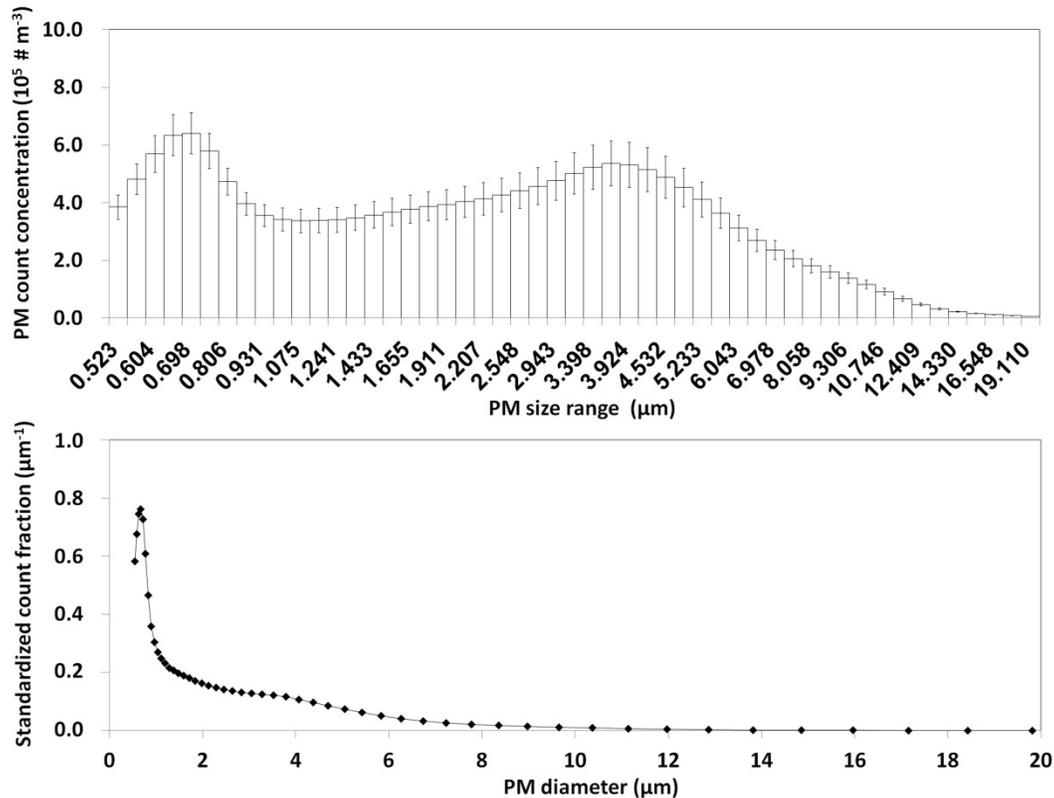


Figure 5. Airborne PM count concentrations in the 51 size ranges and standardized count fraction in the range of 0-20  $\mu\text{m}$ . Vertical bars represent standard errors.

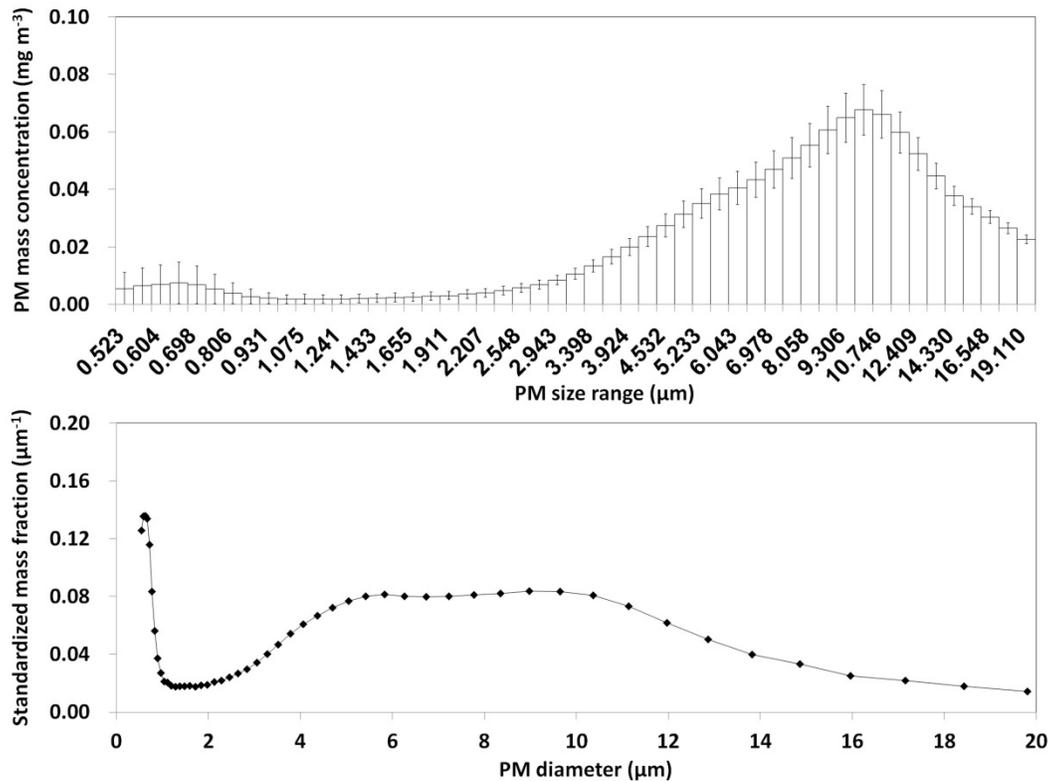


Figure 6. Airborne PM mass concentrations in the 51 size ranges and standardized count fraction in the range of 0-20  $\mu\text{m}$ . Vertical bars represent standard errors.

#### Airborne bacteria

Concentrations of PM count, PM mass, and airborne bacteria in each sub-size range and the corresponding percentage of each sub-range relative to the entire size range are listed in Table 2. The distributions of PM count were by and large uniform, with the exceptions of the lower (0.65-1.1  $\mu\text{m}$ ) and upper (7.1-20  $\mu\text{m}$ ) sub-ranges accounting for a larger and smaller proportion (24.7%, 8.0%), respectively. In contrast, the distributions of PM mass depended on the particle size, with the smaller diameter range having the least share (0.1%) and the largest diameter range having the most share (61.5%). As shown in Table 2, size distribution of airborne bacteria essentially mirrored PM mass distribution. This result was consistent with the study done for pig housing by Zhao et al. (2011a) who found that airborne bacteria were predominantly associated with particles >3.3  $\mu\text{m}$ . It is speculated that airborne PM (carriers of airborne bacteria) with larger aerodynamic sizes and higher mass contain more bacteria.

Table 2. Size distributions of airborne bacterial concentration, airborne PM mass, and PM count concentration in the six ranges.

Size range ( $\mu\text{m}$ )	Bacterial concentration ( $10^4$ CFU $\text{m}^{-3}$ )		PM mass concentration ( $10^{-2}$ mg $\text{m}^{-3}$ )		PM count concentration ( $10^6$ particles $\text{m}^{-3}$ )	
	Mean $\pm$ SE	Percent	Mean $\pm$ SE	Percent	Mean $\pm$ SE	Percent
0.65-1.1	0.018 $\pm$ 0.004	0.1 %	0.092 $\pm$ 0.019	0.1 %	3.16 $\pm$ 0.71	24.7 %
1.1-2.1	0.176 $\pm$ 0.034	0.5 %	0.463 $\pm$ 0.068	0.5%	2.12 $\pm$ 0.31	16.6 %
2.1-3.3	0.70 $\pm$ 0.15	2.0 %	1.89 $\pm$ 0.31	2.0 %	1.81 $\pm$ 0.29	14.2 %
3.3-4.7	4.14 $\pm$ 1.31	11.9 %	7.72 $\pm$ 1.91	8.3 %	2.33 $\pm$ 0.57	18.2 %
4.7-7.1	8.36 $\pm$ 2.52	24.1 %	25.70 $\pm$ 6.61	27.6 %	2.36 $\pm$ 0.61	18.4 %
>7.1 <sup>[a]</sup>	21.31 $\pm$ 3.87	61.4 %	57.18 $\pm$ 9.82	61.5 %	1.02 $\pm$ 0.19	8.0 %

<sup>[a]</sup> The size range of airborne PM is 7.1-20  $\mu\text{m}$ .

## Relationship between Airborne PM and Bacteria

The airborne bacteria concentration (Colony Forming Units or CFU m<sup>-3</sup>) and PM mass concentration (mg m<sup>-3</sup>) followed linear relationships ( $P < 0.05$ ) for all the ranges. No significant differences in such relationships were detected among the sub-ranges; hence the data were pooled to plot the relationship for the entire range (fig. 7). Airborne PM is considered as the carrier of airborne bacteria, and airborne PM in livestock buildings contains a large variety of bacteria (Zhang and Chen, 2006; Lee et al., 2006). PM >20  $\mu\text{m}$  was not detected in this experiment due to the instrument limit, realizing that PM >20  $\mu\text{m}$  can be hardly suspended in the air. It has been demonstrated that the overall PM spectrum is contained within the range of 0.015-20  $\mu\text{m}$  (Gloster et al., 2007). PM of 7.1-20  $\mu\text{m}$  was taken as PM >7.1  $\mu\text{m}$  when assessing the relationship between airborne PM and bacteria concentrations in this study. As shown in Table 3, specific bacterial concentration related to airborne PM mass was 3.84 ( $\pm 2.70$ )  $\times 10^5$  CFU mg<sup>-1</sup>. It shows that equal mass of airborne PM (0.65-20  $\mu\text{m}$ ) would carry similar amount of airborne bacteria.

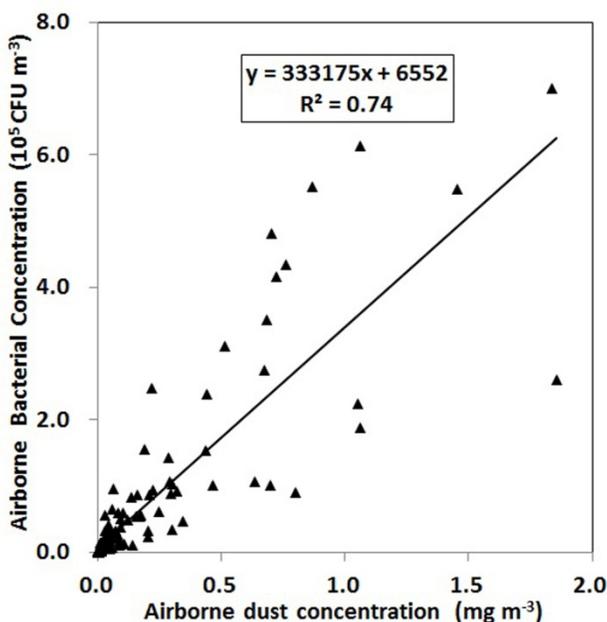


Figure 7. Relationship between airborne bacterial concentration and airborne PM concentration in the range of 0.65-20 $\mu\text{m}$ .

Table 3. Airborne bacterial concentrations related to airborne PM mass in the 6 size ranges

Size ranges ( $\mu\text{m}$ )	0.65-1.1	1.1-2.1	2.1-3.3	3.3-4.7	4.7-7.1	>7.1	>0.65
Bacterial concentration ( $10^5$ CFU mg <sup>-1</sup> )	3.19	3.50	3.27	4.54	4.00	4.45	3.84
	$\pm 2.99$	$\pm 2.07$	$\pm 2.34$	$\pm 2.98$	$\pm 3.22$	$\pm 2.39$	$\pm 2.70$

Values reported as means  $\pm$  standard deviation (n=30, n=180 for >0.65  $\mu\text{m}$ ).

No significant differences detected among the sizes ( $P > 0.05$ ).

## Conclusions

This study demonstrates that airborne bacteria concentration and airborne PM mass concentration follow linear positive relationships in the aerodynamic size range of 0.65-20  $\mu\text{m}$  in the aviary laying-hen house setting ( $P < 0.05$ ). Specific bacteria concentration relative to airborne PM mass was found to be 3.84 ( $\pm 2.70$ )  $\times 10^5$  CFU mg<sup>-1</sup>, being independent of the size ranges under consideration (0.65-20  $\mu\text{m}$ ).

PM and total bacterial concentrations during the litter-access period (12:00h-22:00h) were significantly higher than those during the off-litter period ( $P < 0.05$ ).

Airborne PM of 0.65-1.1  $\mu\text{m}$  and 7.1-20  $\mu\text{m}$  accounted for highest and lower percentage (24.7% vs. 8.0%) in the total particle count, respectively. The size distribution of airborne bacteria closely follows the PM mass distribution. Airborne PM mass of 3.3-20  $\mu\text{m}$  in size and airborne bacteria of >3.3  $\mu\text{m}$  in size were the respective majority.

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