ASSESSING ENVIRONMENTAL MITIGATION STRATEGIES IN CONTROLLED CAGE-FREE AVIARY HOUSING SYSTEMS: EFFECT ON AIR QUALITY AND BIOAEROSOLS

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HIGHLIGHTS

- Three mitigation strategies were tested in experimental chambers fitted as small-scale aviaries.
- Particle monitor and high-flow air samplers were used to sample airborne dust (PM) and bioaerosols.
- Oil emulsion sprinkling on the litter surface of aviaries was effective in reducing PM emissions in experimental chambers.

ABSTRACT. *Alternative housing systems for laying hens are gradually replacing battery cages. Though these systems provide more space per animal and enrichments, higher concentrations of ammonia and airborne dust have been reported in those environments. In this project, three environmental control strategies and a control condition were tested in twelve experimental chambers fitted as small-scale aviaries. The experiment was conducted during three periods spanning 18 weeks, during which PM and bioaerosols were sampled in each chamber in three separate sampling campaigns. Emissions of PM, culturable bacteria and molds, total bacteria, Penicillium, and Aspergillus molds and selected fecal indicators (Campylobacter coli, Campylobacter jejuni, Clostridium perfringens, Escherichia coli, Enterococcus spp., Salmonella spp.) were assessed in each experimental chamber. Strategies that included oil emulsion sprinkling on litter surface were effective at reducing PM emissions, while no significant effect ($p > 0.05$) was observed on ammonia emissions, and the effect on bioaerosol was variable under the evaluated conditions.*

Keywords. *Airborne dust, Aviaries, Bioaerosol, Laying hen alternative housing, Mitigation.*

Rising concerns for animal welfare have led to changes of practices in commercial laying egg production over the latest decades. A ban on conventional battery cages was implemented in the European Union by Directive 1999/74/EC for a gradual transition toward alternative housing systems and cage-free systems (CFS) (Shields et al., 2017). Similar changes were adopted by the Canadian egg industry in 2017, with a gradual retreat of battery cages awaited for 2036 with the new Code of Practice for the Care and Handling of Pullets and Laying Hens (National Farm Animal Care Council & Egg Farmers of Canada, 2017). Alternative housing systems (enriched cages or cage-free) allow more space for each animal and structures for the expression of natural behaviors such as wing stretching and perching (Hughes and Duncan, 1988). The presence of litter in some systems allows further display of natural behaviors such as dust bathing, foraging, and scratching (Gonzalez-Mora et al., 2021; Hartcher and Jones, 2017). Although alternative housing systems are considered better for animal welfare, higher concentrations of ammonia and airborne dust (particulate matter (PM)) have been previously reported in such environments, whose exposure can be hazardous to both animal and human respiratory health (Arteaga et al., 2015; Hayes et al., 2013; Le Bouquin et al., 2013; Nimmermark et al., Lund, Gustafsson, & Eduard, 2009).

Particulate matter (PM) is a mixture of aerosolized solid particles, organic matter, bioaerosols, and liquid droplets. The main sources of PM in laying hen facilities are the litter and dejections, feed, microorganisms, the skin, and the feather sheds (Ellen et al., 2000; Just et al., 2009; Kirychuk et al., 2006). In aviaries, the presence of litter surface and the higher mobility of the birds may contribute to higher concentrations of PM. Occupational exposure to PM in agricultural settings has been associated with the development of respiratory symptoms and illnesses in workers such as dyspnea, wheezing, cough, phlegm, asthma, and chronic bronchitis (Donham et al., 2000; Just et al., 2009; Kirychuk et al., 2003). PM can be categorized in different size fractions

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according to their aerodynamic diameter such as PM10 (particles with an aerodynamic diameter, lower than 10 µm) and PM2.5 (aerodynamic diameter lower than 2.5 µm). Particle sizes affect airborne behavior and deposition in the various regions of the human respiratory tract (Hinds, 1999).

The presence of manure and moisture within the litter in CFS provides suitable conditions for the production of ammonia (David et al., Mejdell, Michel, Lund, & Oppermann Moe, 2015; Pelletier and Godbout, 2016; Wood and Van Heyst, 2016). Exposure to concentration of ammonia equal to or above 25 ppm might lead to irritation of the mucous membranes and respiratory tract in both hens and humans (Nimmermark et al., 2009), while concentrations between 60 and 70 ppm seem to predispose to secondary infection, decreased feed intake, and higher mortality in birds. Donham et al. (2000) have established a threshold concentration for ammonia associated with a decrease in human pulmonary function to 12 ppm for 2 to 4 h work shifts in animal confinement buildings.

Laying hen facilities also contain bioaerosols, airborne components originating from living organisms that include microorganisms. Bioaerosols may play a role in the spread of infectious disease and surface contamination and may be a potential health hazard for both humans and fowl. Bacteria of the genera *Campylobacter, Clostridium, Enterococcus, Escherichia,* and *Salmonella* have been previously reported in the air of poultry operations (Just et al., 2012). Molds previously reported in such facilities include the genera *Cladosporium, Aspergillus, Penicillium, Alternaria, Fusarium,* and *Geotrichum* (Just et al., 2009).

To improve air quality by reducing PM and ammonia in livestock operations, environmental control strategies (ECS) have been previously studied, such as the sprinkling of electrolyzed water (Lilong Chai et al., 2018; Ellen et al., 2000) or oil (Takai and Pedersen, 1999), the addition of litter amendment (Janczak et al., 2017), and the control of litter moisture. In collaboration with the Research and Development Institute for the Agri environment (Quebec, Canada), the effect of three strategies on PM, ammonia, and bioaerosol emissions was studied in independently-ventilated hermetic experimental chambers. ECSs were expected to improve air quality and reduce the emissions of bioaerosols as well. This study complements the results presented by Gonzalez-Mora et al. (2020), who investigated the effects of the ECS on the spatial occupancy and natural behaviors of hens. This work presents the effect of the evaluated strategies on dust, bioaerosols concentration, and emissions.

Materials and Methods

Experimental Chambers and Ambient Conditions Monitoring

The study was conducted in the BABE facility ("*Laboratoire sur le bilan agroenvironnemental des bâtiments d'élevage":* laboratory on the agri-environmental balance assessment of livestock buildings*)* at the Research and Development Institute for the Agri-environment (IRDA) in Deschambault (Quebec, Canada) (Létourneau et al., 2020). The BABE facility includes twelve identical hermetic, independently ventilated experimental chambers (1.2 m width, 2.44 m depth, 2.44 m height). Fresh air from outside is warmed or cooled before entering the chambers to reach 22±1°C (Gonzalez-Mora et al., 2021). Airflow was set to 2 m^3*min^{-1} and assessed with an air pressure monitor combined with a variable aperture diaphragm (IRIS damper, 204 mm diameter, model 200, accuracy ± 5%, Continental Fan Manufacturing Inc., NY, USA). The temperature and relative humidity of each chamber were monitored using probes (Model CS500, Campbell Scientific Canada, AB, Canada) located at the air outlets of each chamber.

Chambers, experimental aviaries, were fitted out with a mesh floor, a litter box, longitudinal feeders, nipple drinkers, a perch, and a nesting place, as described in Gonzalez-Mora et al. (2021). Aviary disposition and dimensions followed the guidelines stated in the new *Code of Practice for the Care and Handling of Pullets and Laying Hens* (National Farm Animal Care Council & Egg Farmers of Canada, 2017). The litter area represented 17% or 33% of the total usable space, excluding the nesting place, each filled with 900 g and 1800 g of sawdust, respectively. Litterbox content was renewed at the end of each measurement period.

The Lohmann White hens were 19 weeks old upon arrival. Fourteen hens were randomly assigned to a chamber. An adaptation period of 2 weeks was allowed for the acclimatization of the animals before any mitigation strategies were applied. Experimental protocols took place with twelve hens in each chamber.

Experimental Setup and Timeline of the Study

Chambers, experimental aviaries, were fitted out with a mesh floor, a litter box, longitudinal feeders, nipple drinkers, a perch, and a nesting place (fig. 1). The three environmental control strategies were randomly assigned to experimental chambers and evaluated in triplicate as described in Gonzalez-Mora et al. (2021) (table 1). Three chambers were used as controls, and no strategy was applied to them during the experimentation. Oil emulsion consisted of a mix of canola oil and an organic acid solution (Viscosity (cp): 31; density: 0.962 g*mL^{-1}) and was sprayed on the litter box's surface on a bi-weekly basis (1.17 L*m^{-3}*$week^{-1}$). Litter box's floor of HFOS (Heated floor and oil sprinkling) chambers were maintained at 27°C. Lastly, activated biochar (AirEX Energy, BiocharFX, Laval, QC, Canada) was used as an

Figure 1. Interior of an experimental chamber with a litterbox (dust bath), which occupies 17% or 33% of available surface excluding nesting place (17% on fig. 1), a mesh floor, a perch, a longitudinal feeder, nipple drinkers, and a nesting place.

Table 1. Description of the studied environmental control strategies.

Mitigation Strategies	Abbreviation	Description
None (Control)	Ctrl	33% of usable space occupied by litter (Chambers 4, 8, and 12)
Reduction of litter space	T17	17% of usable space occupied by litter (Chambers 1, 5, and 11)
Heating floor under litter and spraying of oil emulsion on litter	HFOS	33% of usable space occupied by litter Spraying 1.17 L/m³/week of oil emulsion on litter. Floor under the litter maintained at 27°C (Chambers 2, 6, 10)
Adding of absorbent in litter and spraying of oil emulsion on litter	AOS	33% of usable space occupied by litter Spraying 1.17 L/m³/week of oil emulsion on litter. 10% (m/m) of absorbent (biochar) added to the litter(Chambers 3, 7, 9)

absorbent and mixed with the litter box's contents, representing 10% of the litter's initial weight.

The experimentation was conducted over 18 weeks and included the adaptation period (from 12 February 2019 to 25 February 2019) and two measurement periods: from 16 February 2019 to 23 April 2019 and from 23 April 2019 to 18 June 2019. The hens were taken out of the rooms for a moment to allow disinfection and dust cleaning of the chambers between the two measurement periods. All the parameters were measured before implementing the environmental control strategies in both trials as a reference.

Measurement of Ammonia Concentrations in Experimental Chambers and Calculations of Ammonia Emissions

Ammonia concentrations were assessed in each chamber using an infrared spectrometer (FTIR, model DX4040, Gasmet, Helsinki, Finland). Air samples were taken in alternance of chambers every 15 minutes and sent through Teflon tubes to a mobile laboratory housing the spectrometer. Ammonia concentrations were then converted into emission values (g * year^{-1} * hen^{-1}) as described in Gonzalez-Mora et al. (2022).

PM Monitoring and Air Sampling in Experimental Chambers

Air sampling was conducted on 25 February (Adaptation Period), before any application of mitigation strategies, and on 23 April (Campaign A) and 18 June (Campaign B), both following an 8-week application of the ECS. During each sampling campaign, monitoring and air sampling were performed in each experimental chamber for 10 minutes. Particulate matter concentrations (PM10, PM4, PM2.5, PM1; mg*m^{-3}) were measured in real-time using a DustTrak DRX Aerosol Monitor (model 8534, 3 L*min^{-1}, TSI Incorporated, Shoreview, MN, USA). Data were extracted using the TrakPro Data Analysis software (Version 4.6.1.0, TSI), and results were transformed in mg*day^{-1}*hen^{-1}. Bioaerosols were collected in 15 mL of sterile phosphate-buffered saline solution using the wet cyclone Coriolis Micro (200 L*min^{-1}, Bertin Corp., Rockville, MD, USA) and on an electret filter with the SASS 3100 Dry Air Sampler (300 L*min^{-1}, Research International, Monroe, WA, USA). Particles were extracted from the filters using the SASS 3010 Particle Extractor (Research International) and 5 mL of extraction buffer (138 mM NaCl, 2.7 mM KCl, 0.05% Triton X-100, <0.1% NaN_3, 10 mM sodium phosphate buffer).

Quantification of Culturable Bacteria and Fungi

The volume of samples collected with the Coriolis Micro was completed to 15 mL with a solution of phosphate-buffered saline 1 X and 0.01% Tween 20. One hundred microliters of 1:10 serially diluted samples were then plated in triplicate on Tryptic Soy Agar (TSA, BD Difco) supplemented with 5 µg/ml amphotericin (Sigma-Aldrich) and Rose Bengal Agar (RBA, BD Difco) with 50 µg/mL chloramphenicol (Sigma-Aldrich) for quantification of culturable mesophilic bacteria and fungi respectively. TSA plates were incubated at 25°C for two days and RBA at 25°C for 7 days. Mean colony forming unit (CFU) counts from triplicates were used to calculate mean CFU emissions (CFU* day^{-1} * hen^{-1}) from each chamber. Mold colonies were identified by the genus by microscopic observations using the lactophenol cotton blue dye (20 mL demineralized water, 20 ml lactic acid, 20 g phenol crystal, 0.05 g aniline blue, 40 mL glycerol) (Dufresne and St-Germain, 2018).

DNA Extraction From Air Samples

One ml of each SASS 3100 air sample was filtered on a hydrophilic 0.2 µm polycarbonate membrane (Merck Millipore Ltd., Tullagreen, Carrigtwohill, Co. Cork, Ireland) using a vacuum filtration system as described by Mbareche et al. (2019). Each filter was then inserted in a sterile 1.7 mL polypropylene tube filled with 750 µL of Bead Solution from the kit DNeasy Powerlyser Powersoil (QIAGEN, Hilden, North Rhine-Westphalia, Germany) and a sterile 0.3 mm tungsten bead. Tubes were kept frozen at -20°C until DNA extraction.

For DNA extraction, frozen filters were pulverized in a bead beater homogenizer (Mixer Mill MM301; Retsch, Düsseldorf, Germany) set at 30 revolutions per second for 20 minutes. The liquid content of the tubes and filter fragments was used for DNA extraction using QIAGEN DNeasy Powerlyser Powersoil, following instructions provided by the manufacturer. Extracted samples were then stored at -20°C until use.

Detection and Quantification of Total Bacteria, *Penicillium* sp., *Aspergillus* sp., and *Paecilomyces variotii*, and Bacterial Human Pathogens and Fecal Indicators by Quantitative PCR

PCR protocols shown in table 2 were used for the quantification of total bacteria, *Penicillium* sp., *Aspergillus* sp., and *Paecilomyces variotii* (PenAsp) as well as various bacterial human pathogens and fecal indicators.

Reaction mixtures contained 10 µL 2X iQ SuperMix (Bio-Rad Laboratories, Hercules, USA) or 2x iQ SYBR Green Supermix (Bio-Rad) for probeless assays, 0.1 µL of reverse and forward primer mix (250 nM of each primer), 0.1 µL of probe (50 nM), 2 µL genomic DNA, and nuclease-free water for a total volume of 20 µL. Assays were run on a CFX384 Touch Real-Time PCR Detection System

Table 2. PCR protocols used for the detection and quantification of total bacteria, PenAsp, and bacterial human pathogens and fecal indicators.

Targeted Microorganisms	Targeted Gene	Primers and Probes (5’-3’)	References
Total bacteria	rRNA 16S	F-5’-GGTAGTCYAYGCMSTAAACG- 3’ R-5’-GACARCCATGCASCACCTG-3’ P-5’-/6-FAM/TKCGCGTTG/ZEN/CDTCGAATTAAWCCAC/1ABkFQ/-3’	(Bach et al., 2002)
Penicillium sp., *Aspergillus* sp. and *Paecilomyces variotii*	ITS1- 5.8S	F-5’-CGGAAGGATCATTACTGAGTG-3’ R-5’-GCCCGCCGAAGCAAC-3’ P-5’-FAM-CCA ACC TCC CAC CCG TG-TAMRA-3’	(Haugland et al., 2004)
Campylobacter coli	*GlyA*	F-5’-CATATTGTAAAACCAAAGCTTATCGG-3’ R-5’-AGTCCAGCAATGTGTGCAATG-3’ P- 5’-/6FAM/TAAGCTCCA/ZEN/ACTTCATCCGCAATCTCTCTAAATTT/1ABkFQ/ -3’	(LaGier, Joseph, Passaretti, Musser, & Cirino, 2004)
Campylobacter jejuni	*HipO*	F-5’-TGCTAGTGAGGTTGCAAAAGAATT-3’ R-5’-TCATTTCGCAAAAAAATCCAAA-3’ P-5’-/6-FAM/– ACGATGATT/ZEN/AAATTCACAATTTTTTTCGCCAAA/1ABkFQ/-3’	
Clostridium perfringens	rRNA 16s	F- 5’-CGCATAACGTTGAAAGATGG-3’ R-5’-CCTTGGTAGGCCGTTACCC-3’	(Wise and Siragusa, 2005)
Escherichia coli	rRNA 16s	F-5’-GTTAATACCTTTGCTCATTGA-3’ R- 5’-ACCAGGGTATCTAATCCTGTT-3’	(Malorny et al., 2004)
Enterococcus sp.	rRNA 16S	F-5’-CCCTTATTGTTAGTTGCCATCATT-3’ R- 5’-ACTCGTTGTACTTCCCATTGT-3’	(Rinttilä, Kassinen, Malinen, Krogius, & Palva, 2004)
Salmonella sp.	*invA*	F- 5’-CGTTTCCTGCGGTACTGTTAATT-3’ R- 5’-AGACGGCTGGTACTGATCGATAA-3’ P-5’-6-FAM-CCACGCTCT/ZEN/TTCGTCT/1ABkFQ/-3’	(Shannon, Lee, Trevors, & Beaudette, 2007)
	ttrRSBCA	F: 5’-CTCACCAGGAGATTACAACATGG-3’ R: 5’-AGCTCAGACCAAAAGTGACCATC-3’ P: 5’-Fam-CACCGACGGCGAGACCGACTTT-tamra-3’	(Malorny et al., 2004)

(Bio-Rad) using following the thermoprotocols of 95°C for 3 minutes followed by 40 cycles of 95°C for 15 seconds, and 60°C for 1 minute; or 95°C for 3 minutes followed by 40 cycles of 95°C for 15 seconds, 55°C for 30 seconds, and 75°C for 30 seconds for probeless assays. The reaction mixture for PenAsp contained 7.5 µL iQ SuperMix (Bio-Rad), 0.3 µL of reverse and forward primer mix (500 nM of each primer), 0.1 µL of probe (100 nM), 2 µL genomic DNA, and nuclease-free water for a total volume of 15 µL. Data were expressed as number of copies per day per hen.

Quantification of Total Endotoxins Using Kinetic LAL Assay

Samples retrieved from the SASS 3100 Dry Air Sampler were used for quantification of total airborne endotoxins with the Kinetic-QCL Limulus Amebocyte Lysate (LAL) Assay (Lonza, Basel, Switzerland). Serial dilutions of 055: B5 endotoxin of *Escherichia coli* were used as standards. The assay had a sensitivity range of 0.005 EU*ml^{-1} to 5 EU*ml^{-1}. A representative group of samples was tested for inhibition/stimulation of the LAL reaction through a spike recovery assay. Data were converted into EU*m^{-3} of air, then into EU emissions per day per hen.

Calculations of PM and Bioaerosol Emissions

Emissions of PM and bioaerosols were estimated using the concentrations of those respective compounds and air flow rate in each chamber, as seen in Bottcher et al. (2004) and similar to the simplified calculation of gas emissions in livestock operations (Hassouna and Eglin, 2015), as in:

$$Emission\left(x*day^{-1}*hen^{-1}\right)= \frac{(unit)}{m^3}*ventilation\ rate\left(\frac{m^3}{min}\right)*\frac{1440\ min}{day}*\frac{1}{\#hens} \quad (1)$$

Statistical Analysis

PM and bioaerosol emission data were analyzed using the Mixed Procedure in SAS (ver. 9.4 SAS Institute, Inc., Cary, N.C.). Data were transformed in log10 prior to analysis.

Results and Discussion

Temperature and Ambient Relative Humidity

The ambient temperature within the experimental aviaries (chambers) was set at 22±1°C throughout the 8-week experiment. As presented in Gonzalez-Mora et al. (2022), measured ambient temperatures were on average between 21.1°C and 22.5°C in all chamber types for all three sampling campaigns (Adaptation period, Campaigns A and B). However, ambient relative humidity (RH) statistically differed ($p < 0.05$, One-Way ANOVA, Tukey Method) between each sampling campaign, with the lowest RH measured during the Adaptation period (8.96%-10.7%) in February and the highest during Campaign B (37.6%-41.0%) in June. Relative humidity was lower than targeted values for commercial systems (50%-70%) (National Farm Animal Care Council & Egg Farmers of Canada, 2017) due to air conditioning to reach the setpoint temperature and also because the airflow rate was higher than required due to the BABE-laboratory characteristics. In the BABE facility, the fresh incoming air is drawn from outside the facility, and it is the same for all the rooms. The incoming air is preconditioned in a duct, which has an air conditioning unit and a heating device. The incoming air is cooled or heated before entering the rooms to help keep the same setpoint temperature inside the rooms. The air conditioning allows the adjustment of the desired temperature in the rooms, but it can decrease at the same time as the relative humidity. The BABE facility has been adapted to integrate a humidification system to adjust the desired relative humidity values in the rooms for future experiences.

Ammonia Emissions

Ammonia (NH3) emissions from both trials were estimated and presented in Gonzalez-Mora et al. (2022). No significant differences in NH3 emissions were observed between treatments T17 ($p = 0.46$; $p = 0.1391$), HFOS ($p = 1.00$; $p = 0.3615$), and AOS ($p = 0.46$; $p = 0.3282$) and the control under the evaluated conditions (period 1; period 2). Average values of NH3 emissions ranged from 7.99 ± 0.95 to 10.46 ± 1.66 g yr^{-1} hen^{-1} and from 21.03 ± 2.89 to 34.56 ± 5.91 g yr^{-1} hen^{-1} (±, 95% C.I.) for batches 1 and 2, respectively. The differences in NH3 emissions were attributed to the higher relative humidity measured during batch 2 compared to batch 1, as higher relative humidity may contribute to higher litter moisture, which provides conditions for more ammonia emissions. Relative humidity was not controlled (adjusted), and it was dependent on external relative humidity and temperature; the setpoint temperature and the airflow rate were defined by the ventilation systems after air conditioning. Thus, assessed ammonia emissions might be underestimated due to the air conditioning system reducing the air humidity.

PM Emissions and Reduction

Figure 2 shows particulate matter emissions for each treatment for all sampling campaigns. During the Adaptation period, no oil sprinkling, adsorbent, or heat was applied to the litter in the experimental aviaries. For all size fractions, PM emissions from the 17% chambers were similar to those from the control chambers. For example, total dust emissions were 62.850 ± 16.164 mg*day^{-1}*hen^{-1} in 17% chambers and 69.352 ± 5.307 mg*day^{-1}*hen^{-1} in control chambers. However, PM emissions of all size fractions during the Adaptation period were slightly higher ($p < 0.05$) in chambers with HFOS and in those with AOS (for total dust: 86.013 ± 4.111 mg*day^{-1}*hen^{-1} and 118.244 ± 10.022 mg*day^{-1}*hen^{-1}, respectively).

During both Campaign A and Campaign B, oil-based mitigation strategies contributed to the reduction from 90% to 97% of PM1, PM2.5, PM4, PM10, and total dust emissions. Total dust emissions in control chambers were on average 296.955 ± 134.784 mg*day^{-1}*hen^{-1} during Campaign A and 172.840 ± 71.536 mg*day^{-1}*hen^{-1} during Campaign B, while total dust emissions in HFOS chambers were 6.147 ± 1.389 mg*day^{-1}*hen^{-1} during Campaign A and 8.267 ± 5.887 mg*day^{-1}*hen^{-1} for Campaign B. Total dust emissions in AOS chambers were 23.004 ± 16.477 mg*day^{-1}*hen^{-1} during Campaign A. Compared to control experimental aviaries, the reduction of litter space in 17% chambers also contributed to reducing emissions of all size PM size-fractions (from 55% to 77%), with total dust emissions of 68.436 ± 43.473 mg*day^{-1}*hen^{-1} for Campaign A and 65.502 ± 53.753 mg*day^{-1}*hen^{-1} for Campaign B. Overall, the combination of oil sprinkling and heated floor allowed for the highest reduction of PM emissions. This was attributed to the use of a heated floor at a low temperature (27°C) combined with the oil emulsion sprinkling. The heated floor at this temperature produced a better adhesion of the emulsion layer on the litter. Oil emulsion sprinkling contributed to a slight agglomeration of the litter particles. The litter moisture content was 17.60% in the control treatment and 16.27% in the heated floor plus oil emulsion treatment (data not shown). Simultaneous use of oil sprinkling onto the litter with a heated floor at a low temperature combined the benefits to decrease the aerosolization of PM. The analyses of litter particle

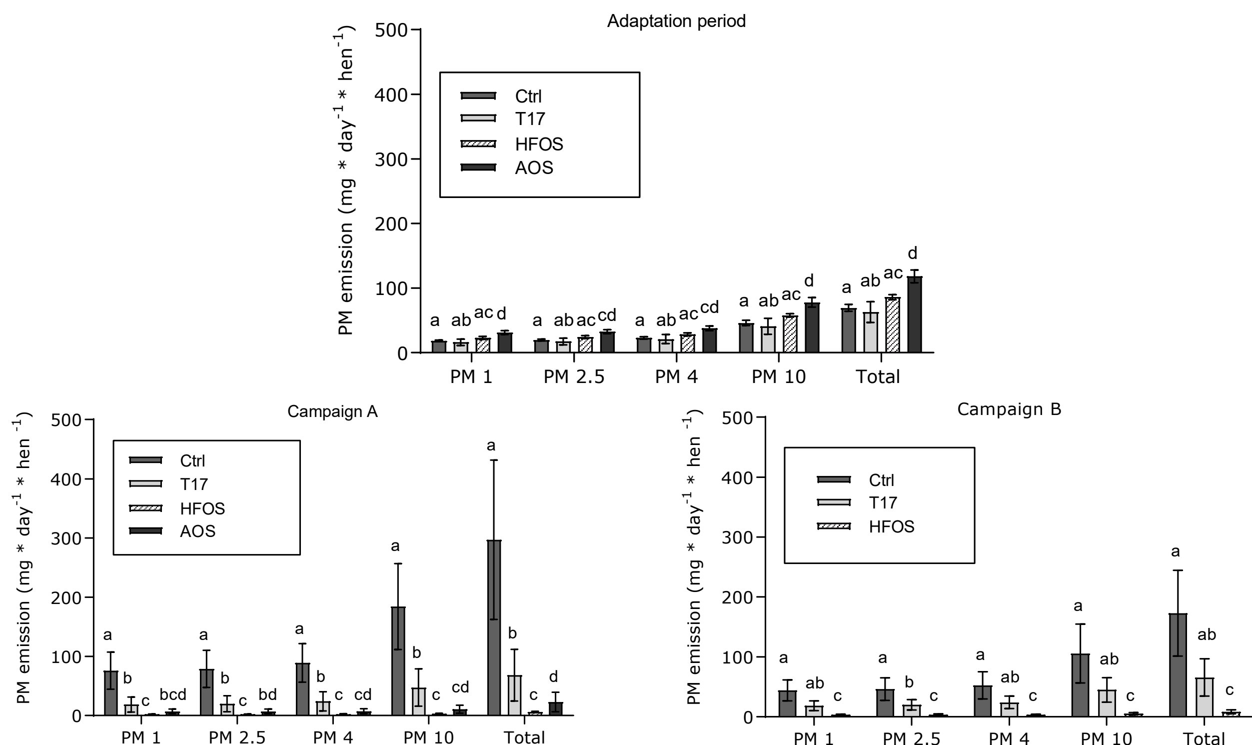

Figure 2. Emissions of PM fractions for each chamber type during the three sampling campaigns (n = 3, mean ±SD): Bars sharing letters are not significantly different from each other ($p > 0.05$). CTRL = control conditions; T17 = reduction of litter space; HFOS = Heated floor under the litter floor and oil sprinkling on the litter floor; and AOS = Adding of absorbent in litter and spraying of oil emulsion on litter.

characteristics would allow for further assessment to better describe the effect of the treatments on the sources of PM and bioaerosol and their effect on litter quality.

The reduction efficiency of PM of oil-emulsion combined with either a heated floor under the litter (27°C) or biochar added to the litter was similar to that reported by Chai et al. (2017). Chai et al. sprayed dosages of 75 ml of Acidic Electrolyzed Water $*kg^{-1}$ of dry $litter*day^{-1}$ (89%±1% of reduction for all PM size fractions 30 minutes after spraying, 83%±1% for all PM size fractions 24 h after spraying). Lower PM reduction efficiency resulted from applying 25 ml of neutral electrolyzed water $*kg^{-1}$ of dry $litter*day^{-1}$ (70% PM reduction after the first spraying) and from adding sulfate (93.2% sodium hydrogen sulfate and 6.5% sodium sulfate) (Chai et al., 2018) or 600 ml of water $*m^{-2}$ to the litter (64% reduction efficiency for PM10 and PM2.5) (Nico et al., 2012). However, the authors also mention that the sole use of water contributed to increased ammonia emissions, which may have an effect on air quality (Nico et al., 2012).

Bioaerosol Emissions

Airborne emissions of endotoxins, culturable bacteria and mold, total bacteria (culturable and non-culturable bacteria), *Penicillium-Aspergillus*, and fecal indicator bacteria (*C. perfringens*, *Enterococcus*) from experimental aviaries are shown in table 3. During Campaign A and Campaign B, endotoxin emissions found in HFOS chambers were approximately 10^1 to 10^2 lower than those in control aviaries. The difference, however, was not statistically significant ($p = 0.42$ for the fixed effects of the mitigation strategies). A 10^1 reduction of culturable molds were also observed in HFOS chambers ($p < 0.05$). However, the impact of these strategies on culturable bacteria emissions couldn't be accurately assessed since the number of colonies recovered from air samples was below 30 $CFU*plate^{-1}$, which is below the limit of quantification of the analytical method (Breed and Dotterrer, 1916; Tomasiewicz et al., 1980). Counts below 30 $CFU*plate^{-1}$ may underestimate the real concentration of bacteria and result in higher data variability. For the present study, the lower limits for accurate quantification of culturable bacteria emission were 4.60 x 10^5, 5.40 x 10^5, and 2.70 x 10^5 $CFU*day^{-1}*hen^{-1}$ during the adaptation period, Campaign A and Campaign B, respectively. Low relative humidity during campaigns A and B and high ventilation rates (10%-37% RH, 2 m^3*min^{-1}) within the chambers may have contributed to low initial concentrations of airborne bacteria and molds since high ventilation rates contribute to the dilution of airborne contaminants (Takai and Pedersen, 2000; Takai et al., 1998) and low ambient relative humidity reduces the cultivability and survival of microorganisms (Delort and Amato, 2017).

Observed molds on cultured air samples for all treatments were predominantly *Penicillium* sp., *Aspergillus* sp., *Cladosporium* sp., and yeasts. Which are found in various environments and are decomposers of plant matter (Domsch et al., 1993).

Total bacteria emissions from chambers were between 10^8 and 10^9 *E. coli* $equivalent*day^{-1}*hen^{-1}$ during all sampling campaigns, while *Clostridium perfringens* and *Enterococcus* sp. emissions were at 10^4 and 10^6 $genes*day^{-1}*hen^{-1}$, respectively. A ten-fold reduction of total bacteria was only observed during campaign B for both 17% chambers and HFOS chambers ($p < 0.05$). Similar reduction of *C. perfringens* was observed in HFOS chambers during Campaign B ($p < 0.05$), while *Enterococcus* sp. emissions remained similar regardless of the applied mitigation strategy.

Frequencies of detection of the molds *Penicillium* sp., *Aspergillus* sp., and *Paecilomyces variotii,* and of *E.coli* for each sampling campaigns are shown in table 4.

Thus, results showed that the combination of oil-emulsion sprinkling on litter surfaces has contributed to the reduction of airborne dust emissions in experimental aviaries. However, no notable reduction of culturable bacteria, endotoxin ($p = 0.42$), or *Enterococcus* sp. ($p = 0.58$) emissions

Table 3. Emissions of endotoxins, culturable bacteria and molds, total bacteria, and fecal indicator bacteria for each chamber types during the three sampling campaigns (mean ±SD, n = 3, except indicated otherwise).[a]

	Adaptation Period				Campaign A				Campaign B		
	Ctrl	T17	HFOS	AOS	Ctrl	T17	HFOS	AOS	Ctrl	T17	HFOS
Endotoxin (10^4) [b]	0.149 ±0.055	0.170 ±0.137	0.396 ±0.145	5.86 ±9.24 (n = 2)	0.783 ±0.893 (n = 2)	3.47 ±3.83 (n = 2)	0.0866 ±0.0150	0.436 ±0.240	1.46 ±0.65	2.31 ±3.31	0.0369 ±0.0134 (n = 2)
Culturable Bacteria (10^5) [c]	0.451 ±0.171 a	0.142 ±0.0274 b	1.75 ±1.38 c	4.17 ±3.12cd	8.49 ±8.09	2.34 ±3.56	0.252 ±0.225	0.641 ±0.731	0.570 ±0.446	5.26 ±7.77	13.6 ±19.6
Culturable mold (10^4) [c]	0.525 ±0.284 a	0.609 ±0.512 abcd	0.289 ±0.120 c	0.717 ±0.765 d	13.2 ±7.70 a	11.1 ±14.8 abcd	1.92 ±2.31 c	19.0 ±29.3 d	12.0 ±10.4 a	33.2 ±28.5 abc	1.533 ±0.95 c
Total Bacteria (10^9) [d]	2.44 ±2.51	1.14 ±0.60	3.42 ±2.64	2.72 ±1.62	9.84 ±10.7	4.40 ±5.25	3.27 ±1.92	3.35 ±3.85	9.99 ±1.81 a	0.136 ±0.043 b	0.314 ±0.259 c
C. perfringens (10^4) [d]	9.92 ±3.08 a	4.00 ±2.08 b	6.10 ±8.56 c	14.50 ±4.30 (n = 2) ad	61.6 ±96.7 a	45.7 ±57.3 (n = 2) b	3.38 ±0.17 c	14.8 ±3.60 (n = 2) ad	33.3 ±20.1 a	7.84 ±2.63 b	3.11 ±2.19 (n = 2) c
Enterococcus (10^6) [d]	2.97 ±0.57	1.95 ±1.46	2.08 ±1.64	4.01 ±2.64	2.51 ±2.87	3.32 ±2.52 (n = 2)	1.03 ±0.56	2.82 ±2.78	2.30 ±1.10	3.81 ±4.73	2.63 ±3.10

[a] CTRL = control conditions; T17 = reduction of litter space; HFOS = Heated floor under the litter floor and oil sprinkling on the litter floor; and AOS = Adding of absorbent in litter and spraying of oil emulsion on litter. Different letters show significant difference between samples ($p < 0.05$).

[b] $EU*day^{-1}*hen^{-1}$, [c] $CFU*day^{-1}*hen^{-1}$, and [d] $gene*day^{-1}*hen^{-1}$ are emissions units.

Table 4. Frequency of detection of *Penicillium* sp. *Aspergillus* sp. and *E.coli*.[a]

	Adaptation Period				Campaign A				Campaign B		
	Ctrl	T17	HFOS	AOS	Ctrl	T17	HFOS	AOS	Ctrl	T17	HFOS
Pen.Asp	0/3	0/3	0/3	0/3	1/3	1/3	1/3	1/3	1/3	0/3	0/3
E. coli	3/3	1/3	2/3	2/3	1/3	1/3	0/3	1/3	1/3	0/3	0/3

[a] CTRL = control conditions; T17 = reduction of litter space; HFOS = Heated floor under the litter floor and oil sprinkling on the litter floor; and AOS = Adding of absorbent in litter and spraying of oil emulsion on litter.

has been observed, and results are variable regarding culturable molds and total bacteria. Other studies have shown the effect of other spraying solutions, such as slightly or acidic electrolyzed water and neutral electrolyzed water, on reducing culturable airborne bacteria in experimental hen facilities (Chai et al., 2017; Zheng et al., 2013; Zheng et al., 2014). However, those studies mainly look at culturable airborne bacteria concentrations in the short term after the application of the treatment, which may not be an indication of the persistence of the effect over time. It will be necessary to evaluate this effect of oil emulsion in future works, taking into account the characteristic of environmental conditions in animal production systems (Zheng et al., 2013).

CONCLUSIONS

Three dust and ammonia mitigation strategies were studied in twelve experimental aviaries. The combination of oil-emulsion sprinkling and heated floors under the litter contributed to the greatest reductions of PM. The drying effect of the heated floor under the litter did not have significant effects on PM concentrations when used in combination with oil emulsion sprinkling onto the litter. The combination of oil-emulsion sprinkling and the addition of activated biocharcoal in the litter floor contributed to PM emission reductions of a similar magnitude, thus making them promising environmental control strategies to improve air quality in cage-free housing systems for laying hens. However, the impact of the studied strategies on bioaerosols was variable, and their analysis was limited by the environmental parameters of the experimental setting. It is suggested that future works include the evaluation of a combination of oil emulsion, sprinkling, heating floor, and litter amendments in a commercial aviary as mitigation strategies for ammonia, airborne PM, and bioaerosols to elucidate the effect of the strategies to improve air quality under commercial scale conditions. Higher gas, dust, and bacteria concentrations typically found in commercial aviaries may allow us to better evaluate and confirm mitigation strategies' effects on airborne contaminants.

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INSTITUTIONAL REVIEW BOARD STATEMENT

The handling and care of the hens for the present study were approved by the Comité de protection des animaux (Animal Welfare Committee) of the Centre de recherche en sciences animales de Deschambault (CRSAD, Deschambault Animal Sciences Research Center, Quebec, Canada) (authorization number 19AVCPA01).

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NOMENCLATURE

CFS = cage-free systems
CFU = colony forming units
ECS = environmental control strategies
EU = endotoxin units
PM = Particulate matter
NH3 = ammonia