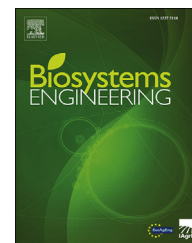




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Research Paper

Mitigating airborne bacteria generations from cage-free layer litter by spraying acidic electrolysed water



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Cage-free (CF) hen housing has an inherent air quality challenge of high airborne bacteria (AB) concentrations arising from hens' activities (e.g., scratching, dustbathing, and social interactions) on the litter floor. Acidic electrolysed water (AEW) is an effective disinfectant that has been widely used in the food industry. Information on AEW application to mitigate AB and litter bacteria (LB) in CF housing is lacking. This lab-scale study evaluated reduction of AB and LB by spraying AEW at three dosages (25, 50, and 75 mL [kg dry litter]⁻¹ d⁻¹, or designated as D25, D50, and D75, respectively), three pH values (3, 5, and 7) and two free-chlorine (FC) concentrations (100 and 200 mg L⁻¹, or FC100 and FC200). Treatment combinations were compared with control (no spray). Three replicates were conducted for testing the effect of spray dosage and pH and four replicates for testing the effect of FC on AB and LB reduction. The results showed that a lower pH AEW yielded a greater AB and LB reduction efficiency. Spraying a lower-dosage AEW (e.g., D25) at pH of 3, 5, or 7 significantly reduced airborne total bacteria ($P < 0.001$) as compared to control; however, D75 resulted in higher airborne total bacteria due to litter moisture accumulation that promoted bacteria growth ($P < 0.001$). Under the D25-pH3 regimen, FC200 resulted in 45.2% lower AB emissions from the hen litter as compared to FC100. This study provides a foundation for conducting the subsequent field test to verify the efficacy of this promising mitigation technique (AEW spray) to improve the indoor air quality and air emissions of CF hen houses.

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1. Introduction

Public concerns or perception over animal welfare led to the banning of conventional cage egg production in the European

Union (EU) as of 2012 and a shift to enriched colony or cage-free (CF) systems (Alberdi, Arriaga, Calvet, Estellés, & Merino, 2016; Appleby, 2003). In the United States, a number of restaurant chains, retailers, and grocers have pledged to source CF eggs only in the foreseeable future (e.g., by 2025 or

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Nomenclature

AB	Airborne bacteria, CFU m ⁻³ or log CFU m ⁻³
AEW	Acidic electrolysed water
ATB	Airborne total bacteria, CFU m ⁻³ or log CFU m ⁻³
CF	Cage-free
CFU	Colony-forming unit
D25	AEW spray dosage of 25 mL (kg dry litter) ⁻¹ d ⁻¹
D50	AEW spray dosage of 50 mL (kg dry litter) ⁻¹ d ⁻¹
D75	AEW spray dosage of 75 mL (kg dry litter) ⁻¹ d ⁻¹
DEC	Dynamic emission chamber
EW	electrolysed water
FC	free chlorine, mg L ⁻¹
FC100	free chlorine concentration of 100 mg L ⁻¹
FC200	free chlorine concentration of 200 mg L ⁻¹
LB	Litter bacteria, CFU g ⁻¹ or log CFU g ⁻¹
LMC	Litter moisture content, %
LTB	Litter total bacteria, CFU g ⁻¹ or log CFU g ⁻¹
MP	measurement period
NH ₃	Ammonia, ppm;
PM	particulate matter, mg m ⁻³
PM2.5	PM that have a diameter less than 2.5 μm
PM10	PM with a diameter less than 10 μm
TC	Treatment combination
TSP	Total suspended particulate, mg m ⁻³
VR	Ventilation rate, L min ⁻¹ or m ³ h ⁻¹
VDC	DC voltage, V.

2030) (Xin, 2016). According to the current number of pledges, it would take more than 70% of the current US layer inventory to meet the pledged CF egg demand by 2025 (Xin, 2016; UEP, 2016). While CF housing allows hens to better perform their natural behaviours (foraging, dustbathing, wing-flapping, etc.) which are limited in conventional housing systems, an inherent challenge with CF housing is the poor indoor air quality, i.e., high concentrations of ammonia, particulate matter (PM) and airborne bacteria (AB), especially during cold weather (when ventilation rate is low) and higher emissions of these aerial pollutants from litter to air (Shepherd et al., 2015; Xin et al., 2011; Hayes et al., 2013; Zhao, Xin, Shepherd, Hayes, & Stinn, 2013; Zhao et al., 2016, 2015). The high PM and AB concentrations in CF hen houses primarily stem from the hens' activities on the litter floor (Zhao et al., 2015, 2016). Litter/manure is the primary source of bacteria in hen houses. Disinfecting the litter could potentially reduce the contamination of litter bacteria (LB) such as Gram negative bacteria to floor eggs (Quarles, Gentry, & Bressler, 1970; Hannah et al., 2011). In addition, controlling litter Gram negative bacteria may reduce the transportation of faecal bacteria such as *E. coli* (*Escherichia coli*) to crop or pasture land (Soupir, Mostaghimi, Yagow, Hagedorn & Vaughan, 2006).

Airborne bacteria may be present either as individual bacterial particles or attachments to PM (Cambra-López, Aarnink, Zhao, Calvet, & Torres, 2010; Cambra-López, Hermosilla, Lai, Aarnink, & Ogink, 2011; Zhao, Aarnink, De Jong, & Groot Koerkamp, 2014a, Zhao et al., 2016; Smets, Moretti, Denys, & Lebeer, 2016). Reducing AB levels in

animal houses is conducive to achieving healthy working conditions and animal growing environment (Davies & Breslin, 2003; Seedorf et al., 1998; Wales, Breslin, & Davies, 2006; Zucker, Trojan, & Müller, 2000). The generation and migration of AB could contaminate eggs in laying hen houses and lead to food safety problems (Ahmed, Schulz, & Hartung, 2013; Hannah et al., 2011). De Reu et al. (2005) reported that egg shell contamination is positively correlated to AB concentration in laying hen houses. The recently completed Coalition for Sustainable Egg Supply (CSES) study in the United States revealed that AB concentrations in CF hen house were significantly higher than in conventional cage or enriched colony systems (Zhao et al., 2016). Emissions of AB and other pollutants such as PM and NH₃ from animal feeding operations could affect ambient air quality and pose a health risk to the neighbouring residents (Huijskens, Smit, Rossen, Heederik & Koopmans 2016). Radon et al. (2007) reported that animal feeding operations may contribute to the burden of respiratory diseases (e.g., asthma symptoms, nasal allergies) among their neighbours. Schinasi et al. (2011) reported that air pollutants such as PM, odour, and endotoxin released from swine operations could be related to acute physical symptoms in a longitudinal study. Smit et al. (2017) found that the risk of community-acquired pneumonia significantly increased when living near poultry farms within 1.15 km, possibly resulting from changes in oropharyngeal microbiota composition in susceptible individuals; exposure to air pollutants such as PM and endotoxin may contribute to dysbiosis of upper respiratory tract microbiota in susceptible individuals.

A variety of liquid agents, i.e., tap water, oil, formalin dilution, and electrolysed water (EW), have been tested to reduce PM and AB generation in poultry houses (Adell et al., 2015; Carrique-Mas, Marin, Breslin, McLaren, & Davies, 2009; Ogink, van Harn, van Emous, & Ellen, 2012; Ru, Zhao, Hadlcon, Zhu, & Ramdon, 2017; Winkel, Cambra-López, Koerkamp, Ogink, & Aarnink, 2014; Winkel, Mosquera, Aarnink, Koerkamp, & Ogink, 2016a; Winkel et al., 2016b). EW is produced by electrolysing dilute NaCl or KCl-MgCl₂ solution in an electrolysis cell (Hricova, Stephan, & Zweifel, 2008), and the primary germicidal component in EW is the free chlorine (FC) formed by electrolysis. EW with different FC concentrations can be produced by either adjusting the concentration of NaCl or KCl-MgCl₂ or controlling the time of electrolysis process (Hricova et al., 2008; Zhao et al., 2014b). Acidic EW (AEW) has been shown to be effective in inactivating bacteria on shell egg and disinfecting food products (Casteel, Schmidt, & Sobsey, 2008; Izumi, 1999; Park, Hung, & Chung, 2004; Park, Hung, Lin, & Brackett, 2005).

Therefore, identifying an optimal range of pH, spray dosage, and FC concentration of AEW as an effective spray agent would be conducive to disinfecting CF hen house and reducing the discharge of AB and LB to the environment and ecosystems. Zheng et al. (2014) investigated spray of tap water or slightly AEW (SAEW) on chicken litter at the same spray dosage of 80 mL m⁻² to disinfect laying hen house. The results showed spraying SAEW reduced 50% more AB than using tap water. Liquid agents with lower pH value or higher FC concentration tend to have higher disinfection efficiency. However, spraying agent with high acidity (e.g., pH < 3) or high FC concentration (e.g., >500 mg L⁻¹) may cause corrosion to the

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