Detection and analysis of $PM_{2.5}$ microbial aerosol in Chicken Houses in Shandong Province, China

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To evaluate the environmental quality of different poultry houses, the concentrations and compositions of microbial aerosols and $PM_{2.5}$ were measured. Results showed that the concentrations of airborne bacteria, airborne fungi and airborne *Escherichia coli* in poultry houses were 0.167-4.484 × 10^4 CFU/m^3, 0.236-4.735 × 10^3 CFU/m^3, and 0-33.0 CFU/m^3, respectively. Distributions of bacteria and fungi at levels 5 and 6 of the Andersen sampler were 11.4%-34.3% and 16.8%-37.5%, respectively. Conditional pathogenic bacteria including *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Enterococcus* and *Aerococcus viridans*, were detected at the aforementioned level, in particle sizes similar to $PM_{2.5}$ and with $PM_{2.5}$ concentrations in poultry houses of 114-230 μg/m^3 . In $PM_{2.5}$, the chief bacteria genera were *Faecalibacterium*, *Bacteroides*, and *Escherichia*, whereas the dominant genus of fungus was *Aspergillus*. Importantly, the relative abundances of *Escherichia* and *Corynebacterium* in broiler houses were 3.1% and 1.94%, respectively, which were greater than those in layer houses. However, the percentages of *Aspergillus* and *Penicillium* were 13.5% and 0.56%, with a relatively high level in layer houses . Altogether, results revealed that the ambient air quality in poultry houses had a relatively high abundance of conditional pathogenic bacteria and concentration of $PM_{2.5}$, which could threaten the health of animals and workers.

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Abstract: To evaluate the environmental quality of different poultry houses, the concentrations and compositions of microbial aerosols and $PM_{2.5}$ were measured. Results showed that the concentrations of airborne bacteria, airborne fungi and airborne *Escherichia coli* in poultry houses were 0.167-4.484 × 10^4 CFU/m^3, 0.236-4.735 × 10^3 CFU/m^3, and 0-33.0 CFU/m^3, respectively. Distributions of bacteria and fungi at levels 5 and 6 of the Andersen sampler were 11.4%-34.3% and 16.8%-37.5%, respectively. Conditional pathogenic bacteria including *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Enterococcus* and *Aerococcus viridans*, were detected at the aforementioned level, in particle sizes similar to $PM_{2.5}$ and with $PM_{2.5}$ concentrations in poultry houses of 114-230 μg/m^3. In $PM_{2.5}$, the chief bacteria genera were *Faecalibacterium*, *Bacteroides*, and *Escherichia*, whereas the dominant genus of fungus was *Aspergillus*. Importantly, the relative abundances of *Escherichia* and *Corynebacterium* in broiler houses were 3.1% and 1.94%, respectively, which were greater than those in layer houses. However, the percentages of *Aspergillus* and *Penicillium* were 13.5% and 0.56%, with a relatively high level in layer houses. Altogether, results revealed that the ambient air quality in poultry houses had a relatively high abundance of conditional pathogenic bacteria and concentration of $PM_{2.5}$, which could threaten the health of animals and workers.

Keywords: Chicken house; $PM_{2.5}$; airborne bacteria; airborne fungus; conditional pathogenic bacteria; high-throughput sequencing

1. Introduction

Severe indoor environmental pollution arising from intensive poultry farming has caused serious harm to poultry and human health in those environments, primarily due to suspended particles in the air, including total suspended particulate and respirable particulate matter (PM_{10} and $PM_{2.5}$). Numerous studies have shown that long-term exposure to high concentrations of airborne particles increases both the morbidity and mortality of human diseases and reduces the life expectancy of humans (Hunt et al. 2003; Pope et al. 2009). In particular, $PM_{2.5}$ considerably threatens human health by entering the trachea and penetrating deep into the lungs, bronchi, and alveoli (Brook et al. 2004).

As a result of the normal activities of chickens and breeders, microorganisms and chemical particles in the environments of chicken farms can form aerosols, which can be divided into biological and non-biological components. Bioaerosol, primarily derived from animal dander and feces, as well as feed(Cambralópez et al. 2010; Radon et al. 2002a), account for more than 25% of aerosols (Jaenicke 2005). Among microbial components, fungal spores and bacteria with diameters of 1-30 μm and 0.25-8 μm, respectively, form part of respirable particulate matter(Comrie & Thomson 1906; Linskens & H. 1974).

Multiple studies have shown that concentrations of airborne microbes at poultry farms often exceed 10^6 CFU/m^3 (Bakutis et al. 2004; Radon et al. 2002b). For both poultry and humans, long-term exposure to high concentrations of microbial aerosols in particular can cause upper respiratory tract irritation, chronic bronchitis, and organic dust toxicity syndrome, among other respiratory symptoms (Donham et al. 2000). However, few studies have focused on $PM_{2.5}$ in poultry houses in China. Although the concentrations of $PM_{2.5}$ in the inlets and outlets of chicken houses in china were measured to be 360 ± 90 μg/m^3 and 540 ± 130 μg/m^3, respectively, other properties of the environmental quality remain unmeasured (Chen & He 2015). At the same time, at least one study has demonstrated that the impacts of particulate matter on humans and animals relate not only to the concentrations of particulate matter and microbes, but also the composition of micro-organisms (Aarnink et al. 2010).

In response to both strands of research, in this study concentrations and compositions of microbial aerosols and $PM_{2.5}$ at chicken farms in Shandong Province, China, using different feeding modes were monitored, the bacteria and fungi in the $PM_{2.5}$ were identified, and air quality was evaluated.

2. Materials and methods

2.1 Chicken farms

Six chicken farms (Table 1) were selected from the area surrounding Tai'an, Shandong,

China.

2.2 Content determination of airborne bacteria and *Escherichia coli* (*E. coli*)

Samples were collected on an Andersen-6 microbial sample collector (Andersen 1958) using 5% blood agar medium for airborne bacteria and eosin-methylene blue medium for airborne *E. coli* as the sampling media, and an airflow rate of 28.3 L/min. The sample collector was centered in each chicken house at a height of 30-40 cm above the ground (i.e., the height as the chicken's nasal cavity). Six replicate samples were collected from each chicken house. To accommodate different health conditions, the driving time was set at 1-5 min and 5-15 min for bacteria and *E. coli*, respectively, and the number of colonies per level was 30-300.

After aerobically culturing the culture medium 37 °C for 24-48 h, colonies were counted, and the bacteria and *E. coli* content (CFU/m^3) was calculated according to Andersen's (1958) correction table.

2.3 Content determination of airborne fungi

Samples were collected on an Andersen-6 microbial sample collector (Andersen 1958) using Sabouraud agar as the sampling medium, and an airflow rate of 28.3 L/min. The sample collector was centered in in each chicken house at a height of 30-40 cm above the ground. Six replicate samples were collected from each chicken house. To accommodate different health conditions, the driving time was set at 1-5 min. After aerobically culturing the culture medium at 25 °C for 5-7 d, colonies were counted, and fungus content (CFU/m^3) was calculated according to Andersen's (1958) correction table.

2.4 Identification of airborne bacteria at the levels 5 and 6 of the Andersen-6 sampler

Single colonies at levels 5 and 6 of the Andersen-6 sampler were isolated and cultured in 5% blood agar. The bacteria were identified by the 16S rRNA sequence analysis. The primers were 27F-AGAGTTTGATCCTGGCTCAG and 1492R-ACGGCTACCTTGTTACGACTT. The 16S rRNA sequences were blasted in NCBI(Clarridge 2004; Kolbert & Persing 1999; Relman 1999).

2.5 Sample Collection and analysis of $PM_{2.5}$

Samples of $PM_{2.5}$ were collected with waterproof air sampling filters (HaoChenTianCheng Ltd., Beijing, China) using a ZR-3920 ambient air particulate matter (total suspended particulate/PM_{10}/$PM_{2.5}$) sampler (Zhongrui Ltd., Qingdao, China). Airflow rate was set at 100 L/h, the sampling height was 1 m, and the acquisition time was 48 h.

Total genomic DNA was extracted directly from $PM_{2.5}$ samples using FastDNA® spin kit (MP biomedicals, Santa Ana, CA, USA) following the manufacturer's protocol. The V3-V4 region of bacterial 16S rRNA and the Internal Transcribed Spacer (Lawniczekwalczyk et al.) regions of fungi were sequenced using the upgraded HiSeq sequencing platform, and sequences were analyzed with the Quantitative Insights Into Microbial Ecology software package (http://qiime.org/index.html) and UPARSE pipeline (http://drive5.com/uparse/). The reads were

first filtered with Quantitative Insights Into Microbial Ecology quality filters using default settings for Illumina processing. The UCLUST method was used to cluster sequences into operational taxo-nomic units at an identity threshold of 97%, while the RDP classifier was used to assign each operational taxonomic unit to a taxonomic level. Additional analyses for rarefaction curves and Shannon index were performed with Quantitative Insights Into Microbial Ecology.

2.6 Data analyses

The median of concentrations was used to represent the aerosol concentration, whereas the maximum and minimum values were used for the range of aerosol concentrations. A Student's *t*-test was performed to examine significant differences among treatments using Statistical Package for the Social Sciences 19.0 software (IBM, Chicago, USA).

3. Results

3.1 Airborne bacteria and fungi

The concentration of the airborne aerobic bacteria were 0.385-4.484 × 10^4 CFU/m^3 in the broiler houses and 0.167-0.742 × 10^4 CFU/m^3 in the layer houses (Fig.1A), whereas the concentrations of airborne *E. coli* were 0-33.0 CFU/m^3 in the broiler houses and 0-14.1CFU/m^3 in the layer houses (Fig. 1B). Concentrations of airborne fungi were 0.236-4.735 × 10^3 CFU/m^3 in the broiler houses and 1.319-2.326 × 10^3 CFU/m^3 in layer houses (Fig. 1C).

3.2 Distribution of airborne bacteria and fungi on the Andersen sampler

Results of measuring the distribution of airborne bacteria and fungi on the Andersen sampler in the six chicken farms revealed 17.6%-49.7% distribution of aerobes at level 1 (> 7 μm) and level 2 (4.7-7 μm), 29.8%-51.2% at level 3 (3.3-4.7 μm) and level 4 (2.1-3.3 μm), and 11.4% -34.3% at level 5 (1.1-2.1 μm) and level 6 (0.6-1.1μm), as Fig. 2A shows. Distributions of fungi were 15.6%-32.0% at levels 1 and 2, 39.6%-54% at levels 3 and 4, and 16.8% -37.5% at levels 5 and 6 (Fig. 2B).

3.3 Bacteria composition at levels 5 and 6 of the Andersen sampler

Bacteria collected at levels 5 and 6 of the Andersen sampler included 25 strains from House A, 27 strains from House B, 25 from House C, 18 from House D, 25 from House E, and 16 from House F, all composed of 10 Gram-negative and 103 Gram-positive bacteria. The predominant genera of bacteria were *Staphylococcus*, *Corynebacterium*, and *Macrococcus*, which accounted for 50.0%-81.5% of identified strains (Table 2).

3.4 Identification of bacteria with 16S rRNA high-throughput sequencing

As shown in Fig. 3A, 21 species of known bacteria, five species of unknown bacteria, and a species of Archaea were identified at the phylum level in $PM_{2.5}$ samples. By quantity, the top 10 species were of *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Cyanobacteria*,

Fusobacteria, *Tenericutes*, *Acidobacteria*, and *Chloroflexi*. Among them, *Firmicutes* accounted for the highest proportion (48.41%-78.90%), followed by *Bacteroidetes* (3.05%-21.15%), *Proteobacteria* (5.38%-15.28%) and *Actinobacteria* (6.27%-13.24%). At the family level, 146 species were known bacteria and 16 species were unknown bacteria. The top 10 species were of *Ruminococcaceae*, *Lachnospiraceae*, *Bacteroidaceae*, *Enterobacteriaceae*, *Lactobacillaceae*, *Micrococcaceae*, *Fusobacteriaceae*, *Coriobacteriaceae*, *Veillonellaceae*, and *Erysipelotrichaceae*. *Ruminococcaceae*, *Lachnospiraceae*, and *Lactobacillaceae* accounted for 15.14%-44.36%, 10.15%-16.88% and 1.09%-10.94% of the species, respectively (Fig. 3B). At the genus level, 224 were of known strains and nine were of unknown strains. By quantity, the top 10 species were of *Faecalibacterium*, *Bacteroides*, *Escherichia*, *Lactobacillus*, *Micrococcus*, *Oscillospira*, *Kocuria*, *Ruminococcus*, *Corynebacterium*, and *Megamonas*. The dominant species were *Faecalibacterium* (5.77%-31.63%), *Bacteroides* (1.08%-10.83%), *Lactobacillus* (4.03%-6.49%) and *Escherichia* (0.80%-6.92%), as shown in Fig. 3C. The relative abundance of *Escherichia* in the broiler houses was 5.17%, which was greater than its 0.99% in layer houses ($p < 0.05$), as Fig. 3D shows. Similarly, the relative abundance of *Corynebacterium* in broiler houses was 1.94%, which was higher than that in layer houses ($p > 0.05$; Fig. 3E).

3.5 Identification of fungi with ITS1 high-throughput sequencing

At the phylum level, 5 species were known, whereas five were unknown. By quantity, the top 10 species were of *Ascomycota*, *Basidiomycot*a, *un--s-Fungi sp*, *un--s-fungal sp K6*, *un--s-fungal sp 38 CC 06_28*, *Chytridiomycota*, *Glomeromycota*, *Zygomycota*, *un--s-fungal sp APA_2013*, *un--s-Cystobasidium*, and *Pallidum*. The dominant fungi were of Ascomycota (39.49%-68.22%) and *Basidiomycota* (3.54%-37.49%), as shown in Fig. 4A. At the family level, 83 species were known and 110 species were unknown. By quantity, the top 10 species were of *Trichocomaceae*, *un-s-Agaricomycetes sp*, *un-s-Agaricales sp*, *Davidiellaceae*, *Pleosporaceae*, *un-s-Polyporalessp*, *un-s-Ascomycota sp*, *un-s-Hypocrealessp*, *un-s-Hymenochaetales sp* and *Hypocreaceae* (Fig. 4B). Lastly, at the genus level, 75 species were known and 175 species were unknown. Also by quantity, the top 10 main species were of *Aspergillus*, *un-s-Agaricomycetes sp*, *un-s-Agaricalessp*, *un-S-Pleosporaceae sp RS_5*, *un-s -Polyporalessp*, *Davidiella*, *un-s-Ascomycota sp*, *un-s-Trichocomaceae sp, Cladosporium*, and *un-s-Hypocreales sp*. (Fig. 4C). The relative abundance of *Aspergillus* was 19.35% in layer houses, compared to 4.74% in broiler houses (Fig. 4D), whereas the relative abundance of *Penicillium* was 0.88% in layer houses, which was higher than that in the broiler houses (Fig. 4E).

3.6 $PM_{2.5}$ concentration and microbial diversity analysis

Concentrations of $PM_{2.5}$ in chicken houses ranged from 114 to 230 $\mu g/m^3$ (Table 3), and there was no significant difference between layer and broiler houses ($p > 0.05$). Per Shannon diversity

analysis, bacterial diversity was greater in broiler houses, for a Shannon value of 7.83, compared to 6.92 in layer houses ($p < 0.05$). By contrast, the diversity of the fungal species in layer houses was greater than in broiler houses (Table 4).

4. Discussion

The concentrations of airborne bacteria and fungi in the sample of chicken houses in Shandong Province, China, were 0.167-4.484 × 10^4 CFU/m^3 and 0.236-4.735 × 10^3 CFU/m^3, respectively. In previous studies, concentrations of airborne bacteria and fungi in poultry houses in the southern Netherlands were greater, ranging from 2.5×10^2 CFU/m^3 to 2.9 × 10^6 CFU/m^3 and from 1.8 × 10^2 CFU/m^3 to 1.8 × 10^5 CFU/m^3, respectively (Lawniczekwalczyk et al. 2013). The concentration of aerosols obtained in all houses except House A was slightly lower than the limit recorded (2.5 × 10^4 CFU/m^3) in the Farmland Environmental Quality Evaluation Standards for Livestock and Poultry Production (N/YT 388-1999) by the Ministry of Environmental Protection of the People's Republic of China. Of all chicken houses, the concentrations of airborne bacteria, fungi, and *E. coli* were greatest in House A, perhaps because sampling in House A occurred in early spring, when the ambient temperature was low and the ventilation poor.

The concentration of bacteria in the air was generally greater than that of fungi, which aligns with the findings of previous study (Lawniczekwalczyk et al. 2013). The concentration of bacteria in broiler houses was greater than that in layer houses, related to not only the sampling conditions (e.g., warmer temperature, better ventilation), but also the cleaning frequency. Feces is the primary source of aerosols in livestock and poultry houses and thus an important factor of the concentration of bacteria in air inside the houses. The breeding mode of poultry is another important factor for the formation of microbial aerosols; whereas laying hens are cage-rearing, broilers are floor-rearing. Broilers have a broader range of activity, which facilitates microbial aerosol formation. However, the concentration of fungi in layer houses was greater than in broiler houses, which requires further examination.

The proportions of bacteria and fungi at levels 5 and 6 of Andersen-6 sampler were 11.4%-34.3%, and 16.8%-37.5%, respectively. The particle size of specimens at levels 5 and 6 was 0.6-2.1μm, which was similar to that of $PM_{2.5}$ (0-2.5μm). Fine particles ($PM_{2.5}$) are more likely to penetrate and deposit deeper into the tracheobronchial and alveolar regions of humans and animals. According to the analysis of bacteria at levels 5 and 6 on the Andersen-6 sampler, the dominant bacteria were of *Staphylococcus*, *Corynebacterium*, and *Macrococcus*. The pathogenic bacteria were *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, species of *Enterococcus*, and *Aerococcus viridans*. For one, *Klebsiella pneumoniae* can cause high mortality from systemic infection, pneumonia, meningitis, liver abscess, endophthalmitis, urinary system inflammation, wound infection, and systemic sepsis(Zhang et al. 2008). By contrast, *Enterococcus* widely exists

209 in the intestinal tract in humans and animals, where it causes urinary tract infection, endocarditis,
210 and wound and abdominal infection (H et al. 2004). By still greater contrast, *Aerococcus viridans*
211 is a model species of *Aerococcus* (Zaria 1993), that can cause endocarditis, urinary tract
212 infection, bacteremia, sepsis, meningitis, septic arthritis, and other diseases, particularly when
213 immune function is low or dysfunctional(Jiang et al. 2013; Untereker & Hanna 1976). Lastly,
214 *Staphylococcus epidermidis* often exists on the surface of human or animal skin and mucous
215 membrane, where is causes dermatitis in humans and animals(El-Asrar et al. 2000). On the
216 whole, such conditional pathogens pose a serious risk to the health of animals and workers in and
217 around chicken houses.

218 The concentration of $PM_{2.5}$ in the chicken houses ranged from 114 μg/m^3 to 230 μg/m^3.
219 Studies have shown that the concentration of $PM_{2.5}$ in the inlets and outlets of chicken houses can
220 be 360 ± 90 μg/m^3 to 540 ± 130 μg/m^3, respectively (Chen & He 2015). The data obtained in the
221 present study were slightly lower than those values, which indicates that the air pollution had
222 reached moderate and severe levels, according to Ambient Air Quality Standards (2012), and thus
223 poses a potential threat to human and animal health.

224 The most prominent bacteria in $PM_{2.5}$ were *Firmicutes*, *Ruminococcaceae* and
225 *Faecalibacterium*. Analyses of the bacterial diversity of pig houses revealed that the dominant
226 species were *Firmicutes*, *Bacteroidetes* and *Actinobacteria*at the phylum level (Kristiansen et al.
227 2011), which were also detected as predominant species in poultry houses in the present study. At
228 the genus level, the relative abundance of *Escherichia* was 5.17% in broiler houses and thus
229 greater than that in layer houses ($p > 0.05$), which resembled the results of concentration of
230 *Escherichia* from the Andersen sample collector. The high content of *Escherichia* poses a
231 potential threat to the health of breeders and chickens, and might have factored into the high rate
232 of *E. coli* infection in broiler houses. At the same time, the relative abundance of
233 *Corynebacterium*, as a conditional pathogenic bacterium, was greater in broiler houses than in
234 layer houses. The predominant fungi of $PM_{2.5}$ in the chicken houses were *Ascomycota*,
235 *Trichocomaceae*, and *Aspergillus*. Notably, the content of *Aspergillus* was 13.5% at the genus
236 level, which was significantly greater in layer houses than in broiler houses. Other studies have
237 reported that, among a dozen poultry fungal diseases, the most dangerous pathogen is
238 *Aspergillus(Pinello et al. 1977; Sauter et al. 1981; SO et al. 1978)*, which can also infect humans.
239 Similarly, the relative abundance of *Penicillium* was greater in layer houses than in broiler
240 houses, which aligns with the antibacterial ability of *Penicillium* as the source of penicillin and a
241 killer of bacteria.

242 Although the aerodynamic diameters of bacteria according to the $PM_{2.5}$ sampler were similar
243 to those collected at levels 5 and 6 of the Andersen sampler, nonculturable bacteria and anaerobic

bacteria were also detected in the $PM_{2.5}$ samples via high-throughput sequencing. Bacteria can be systematically analyzed at the phylum, class, order, family, genus, and species levels and quantitatively characterized using a method involving the Andersen sampler, yet qualitatively assessed by high-throughput sequencing only. Therefore, the Andersen sampler is necessary for the detection of airborne microbes.

Altogether, in the $PM_{2.5}$ samples, bacteria were more diverse in broiler houses, whereas fungi were more diverse in layer houses. The composition of microbial communities also differed between layer and broiler houses. As mentioned, the chief source of micro-organisms in the air was feces, and the composition of microbial communities in the intestinal tracts of animals related closely to the species, given different nutritional requirements, feed composition, and feed modes for different species. Therefore, the composition of the microbial community differs in layer and broiler houses, and the communities have certain specie-related characteristics.

5. Conclusions

The air environment of chicken house is relatively closed. And the concentrations and compositions of airborne microorganisms were multifarious in poultry house. The concentrations of airborne bacteria were 0.167-4.484 × 10^4 CFU/m^3, with the concentrations of airborne fungi of 0.236-4.735 × 10^3 CFU/m^3, the concentrations of airborne Escherichia coli of 0-33.0 CFU/m^3. We identified some conditional pathogenic bacteria including *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Enterococcus* and *Aerococcus viridans* at respirable part of Andersen-6 sampler. In addition, the *Faecalibacterium*, *Bacteroides*, and *Escherichia* are the chief bacteria genera, with Aspergillus of the dominant genus of fungus in $PM_{2.5}$. What's more, pathogenic microorganisms including *Escherichia*, *Corynebacterium* and *Aspergillus* were detected in the air of the tested poultry houses. In conclusion, the result is that the multifarious bacteria and fungi of respirable part were identified in poultry house. Some of them are pathogenic, with threatening the health of animals and human beings.

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Figure 1

Box plot of the concentration of airborne bacteria and fungi in poultry houses.

(A) The concentration of airborne aerobic bacteria, (B) airborne *E. coli*, (C) airborne fungi. Boxes correspond to the interquartile range between the 25th and 75th percentiles, and central lines represent the 50th percentile. Whiskers correspond to the maximum and minimum values.

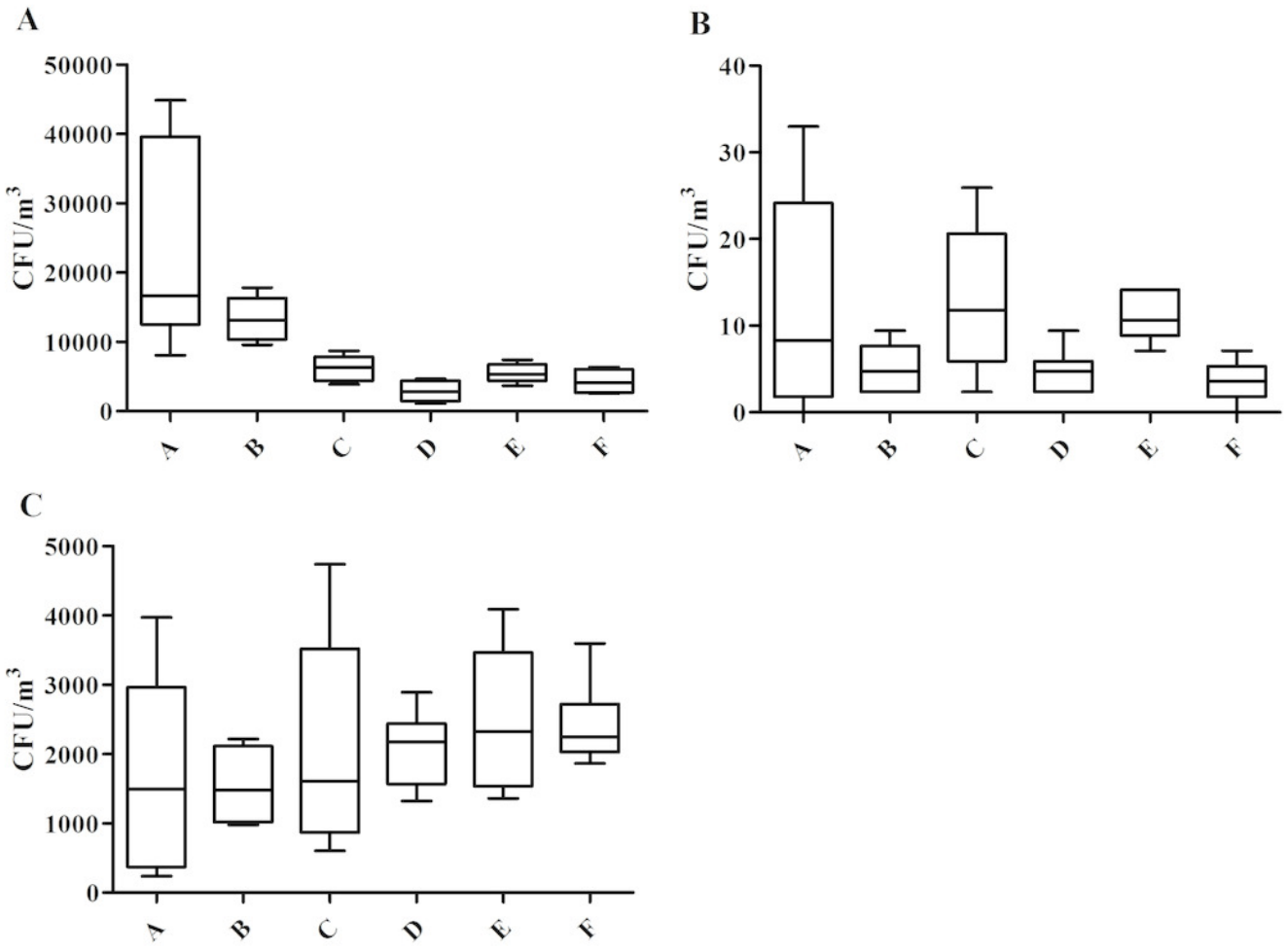

Figure 2

Size distributions of airborne bacteria and fungi at sampling locations in the poultry house.

(A) Size distributions of airborne bacteria and (B) airborne fungi; aerodynamic diameter ranges for the viable particle sizing sampler were >7.0 μm (first stage), 4.7-7.0 μm (second stage), 3.3-4.7 μm (fourth stage), 2.1-3.3 μm (fourth stage), 1.1-2.1 μm (fifth stage), and 0.6-1.1 μm (sixth stage).

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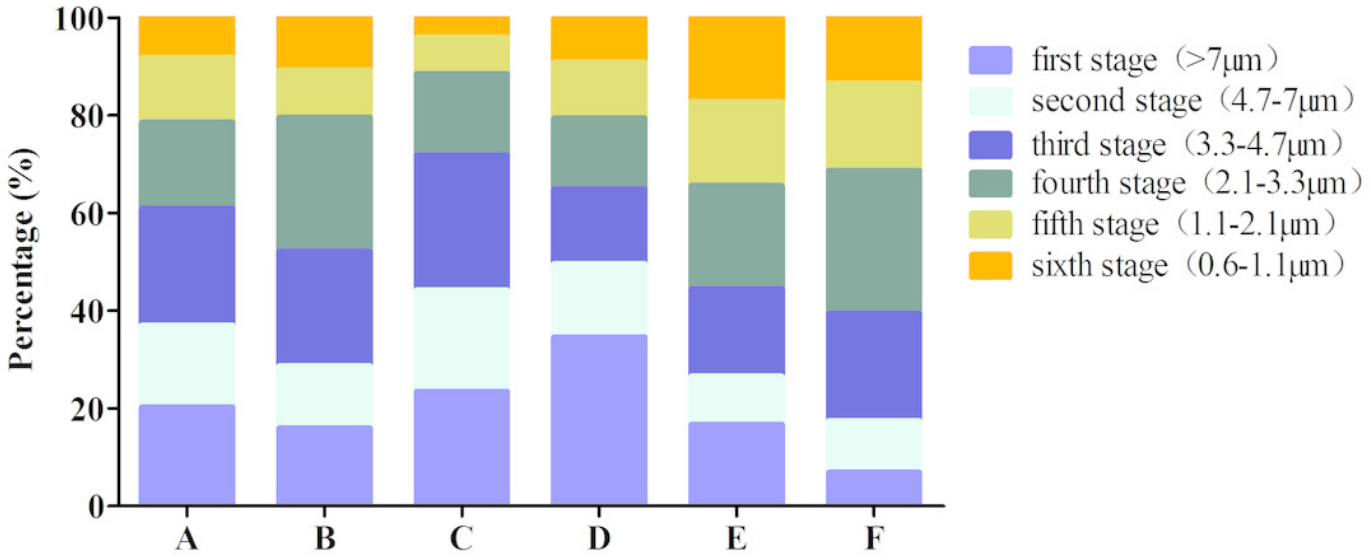
A



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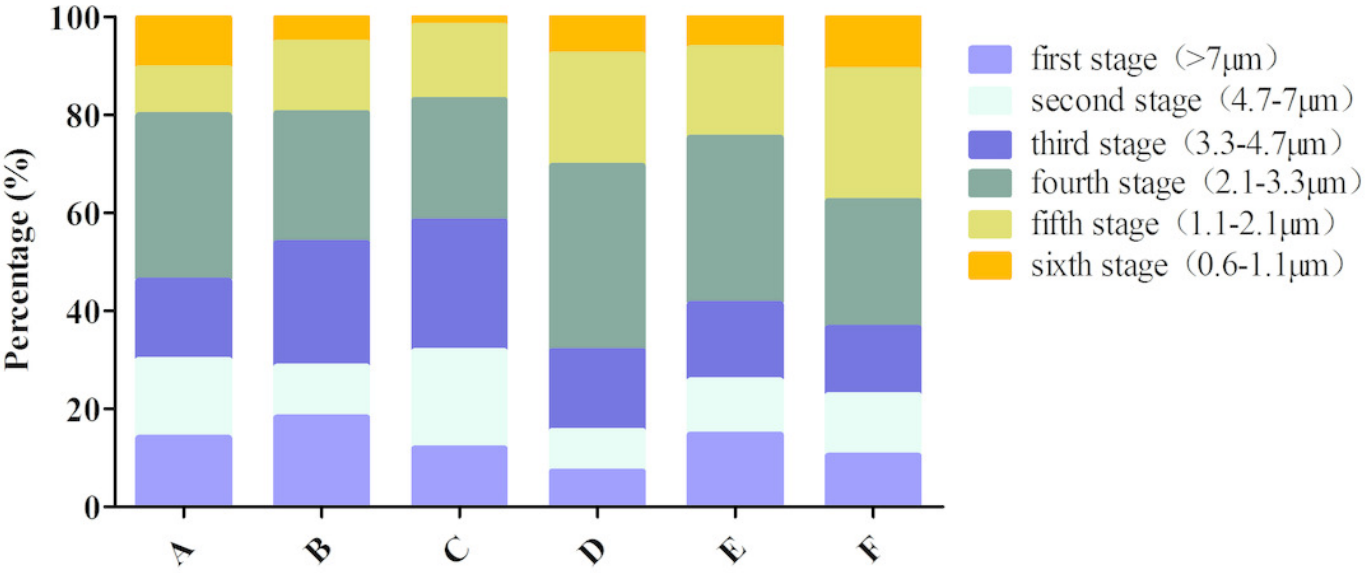
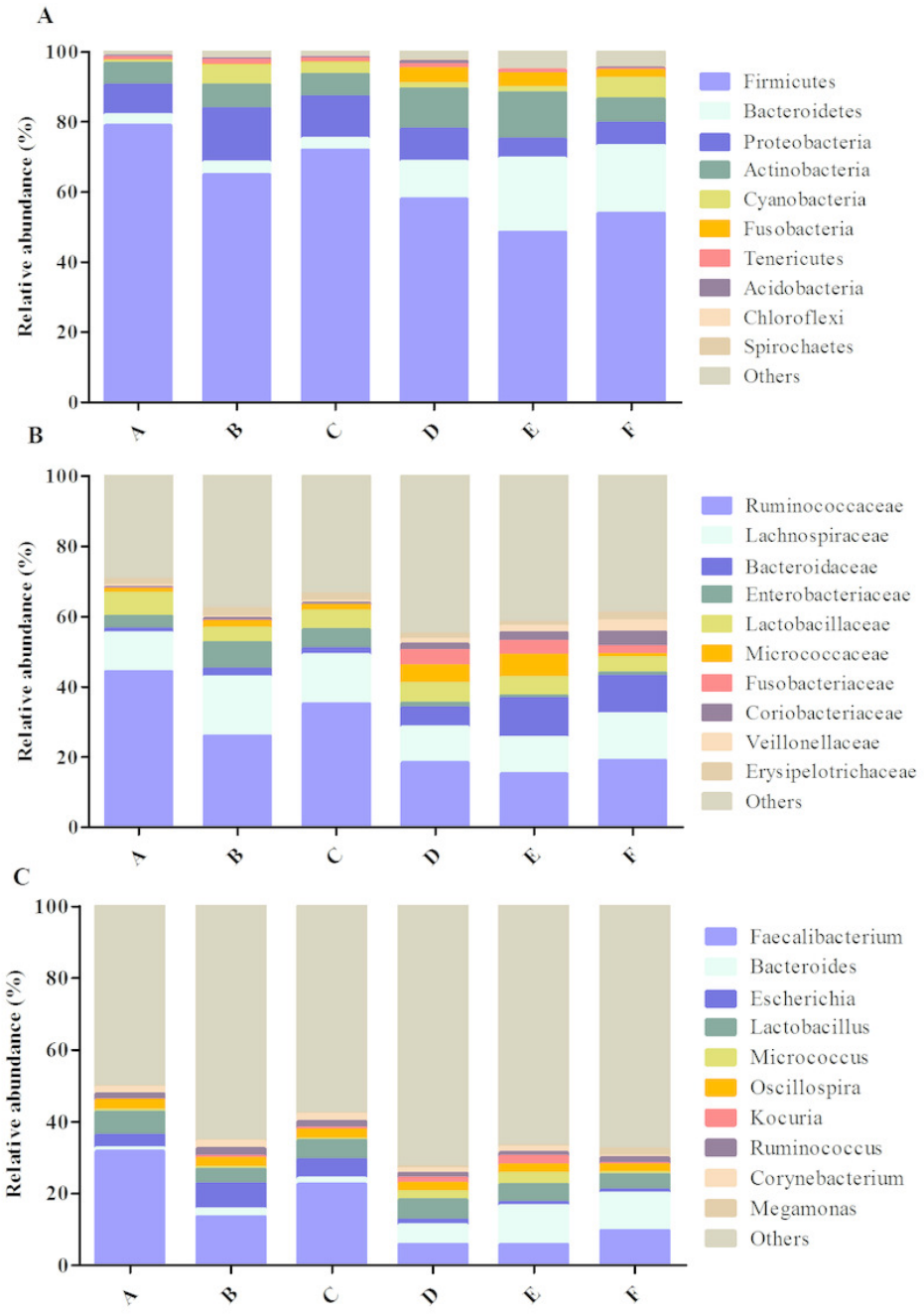


Figure 3

Relative abundance of bacteria in poultry houses.

(A) Relative abundance of sequences belonging to the top 10 bacterial at the phylum level. (B) Relative abundance at the family level. (C) Relative abundance at the genus level. (D) Relative abundance of genera *Escherichia* and (E) *Corynebacterium.* Bars were expressed as means ± SD (n=3). Student's *t* test was conducted to examine differences. *, $p < 0.05$.



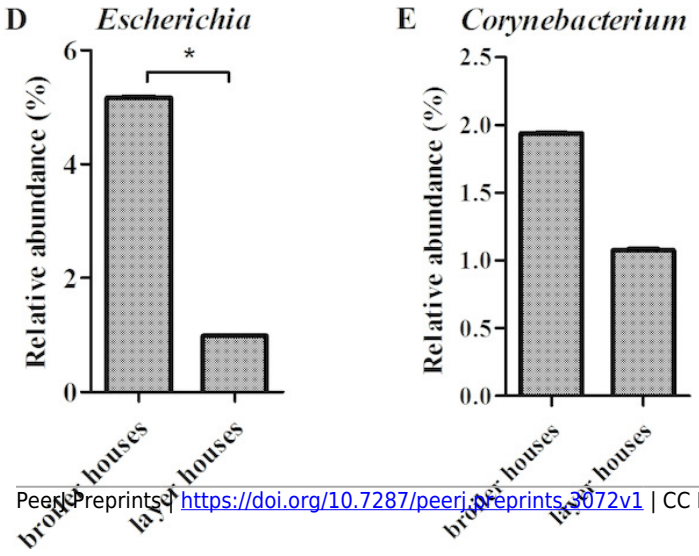
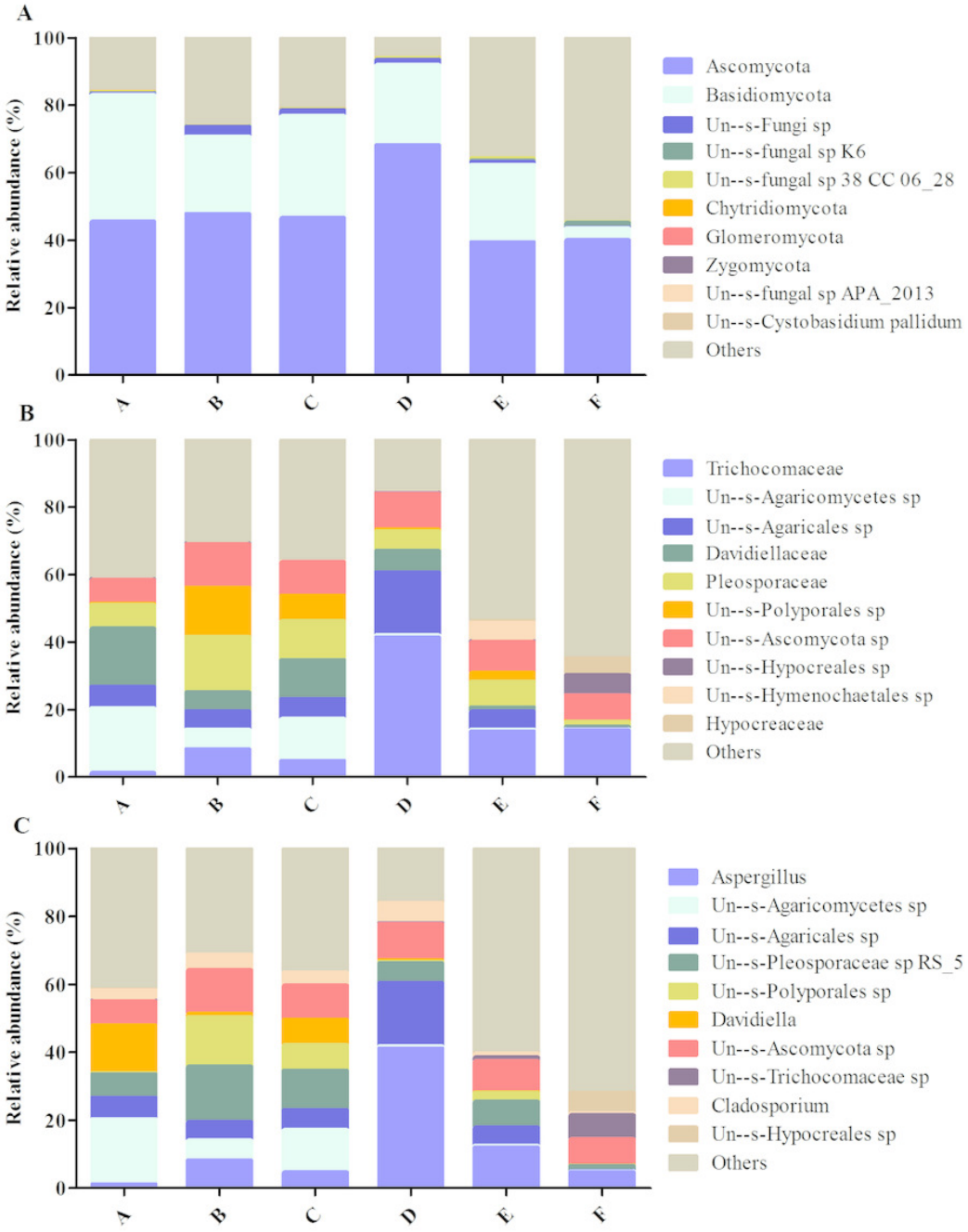


Figure 4

Relative abundance of fungi in the poultry houses.

(A) Relative abundance of sequences belonging to top 10 fungi at the phylum level. (B) Relative abundance at the family level. (C) Relative abundance at the genus level. (D) Relative abundance of genera *Aspergillus* and (E) *Penicillium.* Bars were expressed as means ± SD (n=3). Student's *t* test was conducted to examine differences. *, $p < 0.05$.



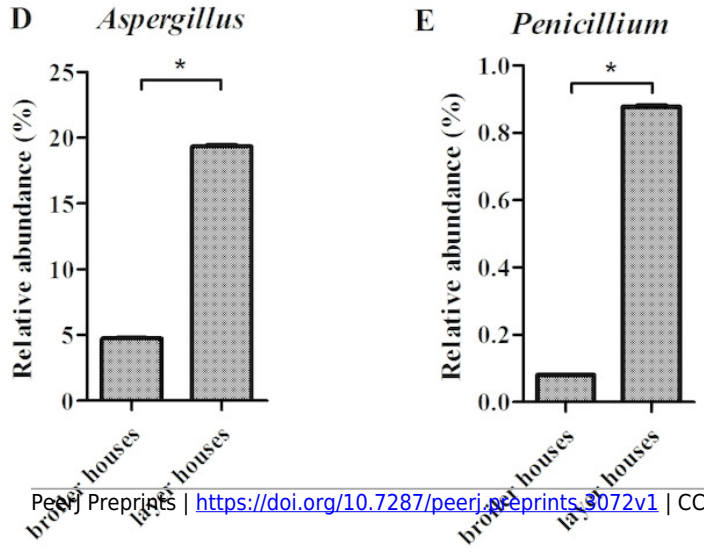


Table 1(on next page)

Description of poultry houses

a: Heated by coal stove; b:Wetted by water curtain.

1

Poultry house	Time（year month）	Animals	Temperature	Relative humidity（%）	Ventilation	Clean up feces
A[a]	15.04	broiler	26 °C	80	bad	4-5 d/time
B	15.06	broiler	25 °C	70	good	3-4 d/time
C	15.07	broiler	35 °C	78	good	2-3 d/time
D[b]	15.07	layer	25 °C	85	good	1 d/time
E	15.08	layer	32 °C	76	good	1 d/time
F	15.10	layer	21 °C	40	good	1 d/time

2

Table 2 (on next page)

Bacteria of the levels 5 and 6 of the Andersen sampler

1

A		B		C		D		E		F	
Bacterial family	number	Bacterial family	number	Bacterial family	number	Bacterial family	number	Bacterial family	number	Bacterial family	number
Staphylococcus epidermidis	2	Corynebacterium jeikeium	1	macrococcus caseolyticus	11	Staphylococcus saprophyticus	2	Brevibacterium Otitidis	1	Corynebacterium xerosis canis	2
Staphylococcus	4	Brevibacterium	1	Enterococcus faecalis	1	Enterococcus faecalis	1	Corynebacterium	2	Rothia nasimurium	1
Klebsiella pneumoniae	1	Corynebacterium	9	Staphylococcus kloosii	1	Bacillus simplex	1	Staphylococcus	1	Staphylococcus sciuri	1
Enterococcus faecalis	1	Corynebacterium amycolatum	2	Rhodococcus ruber	1	Micrococcus luteus	2	Micrococcus luteus	1	Exiguobacterium acetylicum	1
Corynebacterium	6	Staphylococcus saprophyticus	1	Staphylococcus hominis	2	Staphylococcus equorum	2	Bacillus subtilis	1	Micrococcus luteus	1
Brachybacterium	1	Aerococcus viridans	1	Staphylococcus epidermidis	2	macrococcus caseolyticus	3	Brachybacterium	2	Brachybacterium nesterenkoii	1
Bacillus licheniformis	1	Bacillus	1	Exiguobacterium acetylic	1	Corynebacterium	2	Staphylococcus saprophyticus	3	Microbacterium	1

				um							
Exiguobacterium acetylicum	1	Staphylococcus epidermidis	3	Streptomyces zaomyceticus	1	Exiguobacterium acetylicum	1	Staphylococcus gallinarum	1	Corynebacterium jeikeium	1
Staphylococcus caprae	2	Staphylococcus chromogenes	2	Psychrobacter cibarius	1	Staphylococcus sciuri	2	macrococcus caseolyticus	4	Staphylococcus saprophyticus	1
Corynebacterium xerosis	2	Staphylococcus	1	Acinetobacter radioresistens	1	Aerococcus viridans	1	Micrococcus	2	Bacillus licheniformis	1
macrococcus caseolyticus	3	Corynebacterium xerosis	1	Acinetobacter calcoaceticus	1	Klebsiella pneumoniae	1	Staphylococcus caprae	1	Brevibacterium luteolum	1
Brevibacterium	1	Staphylococcus hominis	2	Streptomyces netropsis	1			Corynebacterium jeikeium	3	Staphylococcus chromogenes	2
		Enterococcus cecorum	1	Acinetobacter radioresistens	1			Staphylococcus lentus	1	Corynebacterium xerosis	1
		paracoccus solventivorans	1					Staphylococcus epidermidis	1	Streptomyces griseoaurantiacus	1
								Pseudomonas stutzeri	1		

Table 3(on next page)

Concentration of $PM_{2.5}$ in poultry house

1

Poultry house	A	B	C	D	E	F
Concentration (μg/m^3)	159	163	170	114	150	230

2

Table 4(on next page)

Shannon and Simpson indexes of bacteria and fungi in broiler and layer houses

Data are presented as means ± SD (n=3).

[a, b] Values with different superscripts in the same column differ significantly ($P < 0.05$). Student's *t* test was conducted to examine differences.

1

	bacteria		fungus	
	Shannon	Simpson	Shannon	Simpson
Broiler house	7.83±0.30[a]	0.99	4.15±0.57	0.86±0.04
Layer house	6.917±0.57[b]	0.9465±0.04	4.23±0.51	0.90±0.01

2

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