Air contaminants in animal production: the poultry case

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Abstract

A descriptive study was developed in order to assess air contamination caused by fungi and particles in seven poultry units. Twenty seven air samples of 25 litters were collected through impaction method. Air sampling and particle concentration measurement were performed in the pavilions' interior and also outside premises, since this was the place regarded as reference. Simultaneously, temperature and relative humidity were also registered.

Regarding fungal load in the air from the seven poultry farms, the highest value obtained was 24040 CFU/m^3 and the lowest was 320 CFU/m^3. Twenty eight species/genera of fungi were identified, being *Scopulariopsis brevicaulis* (39.0%) the most commonly isolated species and *Rhizopus* sp. (30.0%) the most commonly isolated genus. From the *Aspergillus* genus, *Aspergillus flavus* (74.5%) was the most frequently detected species. There was a significant correlation (r=0.487; p=0.014) between temperature and the level of fungal contamination (CFU/m^3).

Considering contamination caused by particles, in this study, particles with larger dimensions (PM5.0 and PM10) have higher concentrations.

There was also a significant correlation between relative humidity and concentration of smaller particles namely, PM0.5 (r=0.438; p=0.025) and PM1.0 (r=0.537; p=0.005).

Characterizing typical exposure levels to these contaminants in this specific occupational setting is required to allow a more detailed risk assessment analysis and to set exposure limits to protect workers' health.

Keywords: occupational health, poultry, air contamination, fungi, particles.

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1 Introduction

It is now appropriately recognized that exposures to biological agents in both the occupational and residential indoor environment are associated with a wide range of adverse health effects with major public health impact, including contagious infectious diseases, acute toxic effects, allergies and cancer. Therefore, the interest in bioaerosol exposure has increased over the last few decades. Several new industrial activities have emerged in recent years in which exposures to biological agents can be abundant [1]. However, dose–response relationships have often not been described and knowledge about threshold values is not available (with the exception of a few agents). This relative lack of knowledge is mainly due to the lack of valid quantitative exposure assessment methods [1].

In poultry houses, large-scale production has led to the increase of bird densities within buildings [2]. Such high densities of animals kept within confined spaces are a source of human health problems related to organic dust exposure [2, 3]. Respiratory impairment in poultry stockmen, which occurred as a result of such exposure, includes chronic bronchitis, hypersensitivity pneumonitis (allergic alveolitis), occupational asthma and toxin fever [4, 5].

Organic dust is composed by both of viable particulate matter (also called bioaerosols). Bioaerosols are comprised of airborne bacteria, fungi, viruses and their by-products, endotoxin and mycotoxin [2, 6] and also by non-viable particles, generated by such things as faeces, litter, feed, feather formation (which produces a high quantity of allergen dandruff).

Exposure to bioaerosols in broiler houses may vary depending on the stage of the birds' growth, since the biomass of feces and feather dandruff increase sharply during the fattening period. Moreover, during the collection of the fattened birds for transportation to the slaughterhouse the activity of the workers who catch birds and put them into boxes transiently generates a lot of supplementary bioaerosols. Furthermore, the forklift-truck operators, who load the boxes of chickens into the transportation, also may be exposed to those bioaerosols.

Evidence from both epidemiological and experimental studies supports the hypothesis that exposure to fungal spores is causally associated with the development of hypersensitivity pneumonitis, organic dust toxic syndrome, decline in lung function, severity of asthma, respiratory symptoms, and airway inflammation. Furthermore, a recent review document on fungal spores suggests an occupational exposure limit of 10^5 spores for diverse fungal species in non-sensitized populations[7]. In addition, (1/3)-β-D-glucan, glucose polymers and other constituents present in the mycelial and in the spores' walls of most fungi, have been shown to have a possible negative impact on health [1].

Dust concentrations in laying houses may vary according to many factors, which include external temperature, relative humidity, ventilation rate, presence or absence of air cleaning technologies, animal stocking density, type of bird, bird age, manure management methods, such as belt collection or scrapers, bird disturbance and lighting regimes, as well as the sampling methods used [6, 8, 9].

Particles of all sizes may be deposited in the nose and pharyngeal region. However, only particles with an aerodynamic diameter of less than 15 μm can enter the tracheobronchial tree and only particles with an aerodynamic diameter of less than 7 μm can enter the alveoli. Respirable dust accounts for ~18% of total dust mass. The fraction of dust including particles less than 5 μm aerodynamic diameter is the respirable fraction [9]. Approximately 50% of those particles that enter into the respiratory system will reach the alveoli and furthermore, the particle size range with the largest percentage of deposition in the lungs is 1–2 μm in aerodynamic diameter. Particles smaller than 0.5 μm are respirable, but are exhaled and not deposited in the lungs. Therefore, interest lies in controlling respirable dust, 0.5–5 μm, and "modified" inhalable dust, >5 μm in mean aerodynamic diameter [10].

Gathering temporal information about the quantity and the composition of bioaerosols is necessary to better understand the relationship between factors that influence them and the adverse health symptoms of both workers and animals [1]. Therefore, the purpose of this research was to assess air contamination caused by fungi and particles in seven poultry units and also explore possible associations with independent variables.

2 Materials and methods

A descriptive study was developed in order to assess air contamination caused by fungi and particles in seven poultry units. This assessment was carried out in the winter, when ventilation rates were low, in order to measure the highest extent of exposure.

Twenty eight air samples of 25 litters were collected through impaction method. Air sampling and particles concentration measurement were performed in pavilions' interior and also outside premises, since this was the place regarded as reference.

Simultaneously, temperature and relative humidity were also monitored through the equipment Babouc, LSI Sistems and according to the International Standard ISO 7726 - 1998.

Air samples were collected at one meter tall with a flow rate of 140 L/minute, onto malt extract agar (MEA) supplemented with the antibiotic chloramphenicol (0.05%). After laboratory processing and incubation of the collected samples, quantitative (colony forming units - CFU/m^3) and qualitative results were obtained, with identification of the isolated fungal species. Whenever possible, filamentous fungi were identified to the species level, since adverse health effects vary according to fungal species within the same genera [11, 12]. Identification of filamentous fungi was carried out by macroscopic and microscopic observations, using lactophenol blue stain and achieved through comparison of morphological characteristics listed in illustrated literature [12].

Concerning particles, besides measurement of their concentration, differentiation between size fractions was also performed (PM0.5; PM1.0; PM2.5; PM5.0; PM10) due to the importance in health studies, aiming to

estimate dust penetration within the respiratory system and, consequently, their potential health effect.

Tables with frequency distribution of the isolated fungal species were made with the obtained data. Fungal concentration dependence of the two monitored environmental parameters – temperature and relative humidity – was analyzed. For each particle size, the dependence of particles' concentration and relative humidity was studied. Data analysis was performed with the statistical software SPSS 19.0 using the correlation analysis.

3 Results

Regarding fungal load in the air from the seven poultry farms, the highest value obtained was 24040 CFU/m^3 and the lowest was 320 CFU/m^3 (Table 1).

Table 1: Quantification of the fungal air load in the seven poultries studied.

Poultry farm	N*	Highest value CFU/m^3	Lowest value CFU/m^3	Mean value CFU/m^3
1	3	3680	880	1603,3
2	1	4040	4040	4040
3	3	2520	640	1586,6
4	3	1000	320	706,6
5	4	24040	1280	14350
6	3	3600	2000	2540
7	2	8120	2520	5320

* Number of samples/measurements.

Twenty eight species/genera of fungi were identified, being *Scopulariopsis brevicaulis* (39.0%) the most commonly isolated species and *Rhizopus* sp. (30.0%) the most commonly isolated genus (Table 2).

Table 2: Most frequent fungi identified in air.

Air	Frequency (%)
Scopulariopsis brevicaulis	40.5
Rhizopus sp.	30.0
Penicillium sp.	10.1
Aspergillus sp.	9.7
Others	9.7

From the *Aspergillus* genus, *Aspergillus flavus* (74.5%) was the most frequently isolated species, followed by *Aspergillus versicolor* (19.4%). Other *Aspergillus* species (6.1%) were also identified namely *A. fumigatus*, *A. niveus*, *A. glaucus* and *A. niger*.

A positive correlation was observed (r=0.487; *p=0,014*) between temperature and the level of fungal contamination (CFU/m^3), therefore our study showed that in the settings with higher temperatures the fungal load was higher. Temperature

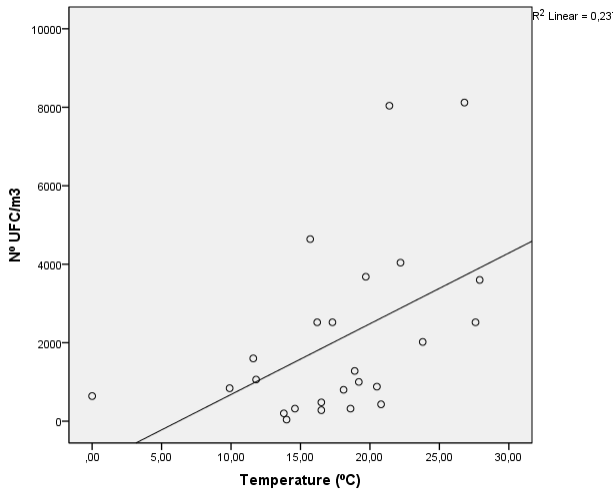

Figure 1: Correlation between temperature and fungal load.

variation contributes 23.7% to the explanation of the number of CFU/m^3 variation.

Considering particles' contamination, the ones with larger size were detected in higher concentrations, particularly PM5.0 (particles of dimension 5.0 μm or less) and PM10 (particles of dimension 10 μm or less). Median results are shown for the 7 poultry farms (Table 3).

Table 3: Median values for particles concentration in each size.

Poultry Unit	PM0.5 (mg/m^3)	PM1.0 (mg/m^3)	PM2.5 (mg/m^3)	PM5.0 (mg/m^3)	PM10 (mg/m^3)
A	$3.4x10^{-4}$	$6.7x10^{-4}$	$59.3x10^{-4}$	$1.0\ x10^{4}$	$6.0\ x10^{4}$
B	$9.4x10^{-4}$	$18.6\ x10^{-4}$	$82.8\ x10^{-4}$	$8.4\ x10^{2}$	$3.2\ x10^{5}$
C	$6.8\ x10^{-4}$	$1.2\ x10^{-3}$	$7.4\ x10^{-3}$	$1.1\ x10^{5}$	$8.0\ x10^{5}$
D	$2.7x10^{-4}$	$4.6\ x10^{-4}$	$1.9\ x10^{-3}$	$3.4\ x10^{-2}$	$2.1\ x10^{5}$
E	$1.4\ x10^{-3}$	$3.1\ x10^{-3}$	$21.6\ x10^{-3}$	$2.1\ x10^{5}$	$5.8\ x10^{5}$
F	$2.3\ x10^{-3}$	$2.8\ x10^{-3}$	$7.9\ x10^{-3}$	$6.5\ x10^{-2}$	$2.6\ x10^{5}$
G	$5.0\ x10^{-4}$	$7.5\ x10^{-4}$	$2.3\ x10^{-3}$	$2.2\ x10^{-2}$	$1.4\ x10^{5}$

Particularly, in units A, B, C and E sizes PM5.0 and PM10 were mainly responsible for contamination.

Higher levels of relative humidity lead to lower numbers of particles. Nevertheless, a positive correlation was found between relative humidity and concentration of some particles sizes, namely PM0.5 (r=0.438; p=0.025) and PM1.0 (r=0.537; p=0.005) meaning that relative humidity variation contributes 19.2% to explain PM0.5 variation and 28.8% for PM1.0 variations.

4 Discussion

Fungi pose potential health risks because of the production of allergens, a wide range of mycotoxins, and inflammatory substances such as beta-D-glucan. In fact, several studies have demonstrated the relationship between increased spore counts and fungal antigen levels with the presence of allergic [13]. Not surprisingly, respiratory symptoms are present among the fungi-exposed workers, such as the poultry workers. Early reports from poultry rearing or slaughter houses described cases of hypersensitivity pneumonitis and later epidemiological studies revealed the presence of extensive chest symptoms and changes in the respiratory function [3]. Considering the fungal load detected, a study performed in two poultry farms in Zagreb [14] presented much higher counts than the ones found in the seven poultry farms analyzed in our study (31200 CFU/m^3 – 4900 CFU/m^3 and 68400 CFU/m^3 – 7600 CFU/m^3 *versus* 240 CFU/m^3– 24040 CFU/m^3). These quantitative differences may be due to many factors such as environmental variables, ventilation rate, presence or absence of air cleaning technologies, animal stocking density, type of bird, bird age, manure management methods and others [2]. In accordance with our results, also in Rimac *et al*. [14] species belonging to the genera *Scopulariopsis*, *Rhizopus*, *Aspergillus* and *Penicillium* were the most prevalent. Moreover, some of the most prevailing fungal species identified in our study (*Penicillium* and *Aspergillus* spp.) have been described to cause hypersensitivity reactions in humans, with clinical manifestations such as allergic rhinitis, asthma and extrinsic alveolitis.

Regarding the *Aspergillus* genus, *A. flavus* (74.5%) was the most frequent species isolated and it is a well-known producer of potent mycotoxins (aflatoxins) [15]. Also noteworthy in these settings, is the detection of *Aspergillus fumigatus*, one of the saprophytic fungi most widespread in air but capable of causing severe or sometimes fatal aspergillosis [16]. Furthermore, according to American Industrial Hygiene Association (AIHA), in 1996, for determination of biological contamination in environmental samples, the confirmed presence of the species *Aspergillus flavus, Aspergillus versicolor* and *Aspergillus fumigatus* requires implementation of corrective measures [17].

There was a significant correlation (r=0.487; p=0.014) between temperature and the level of fungal contamination (CFU/m^3), similar to other studies [18, 19], and corroborating the temperature influence in the fungal growth.

Particles measurement was performed in our study allowing the achievement of information about particles size distribution in these settings. This characterization is a key factor in dust production in poultry facilities since rate of aerosolization, setting velocity and resuspension rate of airborne particles differ depending on particle size [20].

Ellen and colleagues [8] have verified that dust concentrations in these settings can range from 0.02 to 81.33 mg/$m^3$for inhalable dust and 0.01 to 6.5 mg/m^3 for respirable dust. Our results showed the same tendency: higher density in size PM5.0 and, mainly in size PM10. The presence of particles belonging to the respirable range (<5-7 μm) means that the found poultry dust

particles can penetrate into the region of the lung where gas exchange occurs. Larger particles (PM10) can also cause disease by impacting in the upper and larger airways below the vocal cords. Donham and colleagues reported evidence of a dose-related decline in lung function in poultry workers. Dose-response trends were found for cross-shift declines in FEV_1 with total (PM10) and respirable dust (PM5.0 and lower sizes) [21].

A study developed in nine different settings involving the textile industry (cotton spinning, wool combing and weaving), agricultural activities (mushroom cultivation and picking, saw mills, grain handling and animal feed manufacture) and animal handling (swine confinement and poultry catching and shackling) obtained the highest median exposures to particles in the animal handling industries (poultry: 11.5 mg/m^3, swine: 6.7 mg/m^3) [22]. Our results regarding particles count were lower than those, probably due to the fact that the tasks mentioned in this study were not possible to define in our study.

The significant correlation found between the small dimension particles (PM0.5 and PM1.0) and the relative humidity may be explained by the fact that relative humidity affects the inhalable dust (increase in relative humidity levels decreases dust contamination), but not the respirable dust (the fraction below 5 μm) [8]. Considering that low ventilated buildings (normally in winter season) have higher relative humidity levels and lower dust aerosolization than highly ventilated buildings [6, 20] we demonstrate that this is not observed for smaller particles in our results.

A study developed in Texas [23] reported concentrations for PM10 ranging from 0.1 to 0.3 mg/m^3. Our results showed higher concentrations in particles of these sizes probably due to the lower ventilation rates detected in our poultry facilities. These lower ventilation rates in the winter season are created aiming to protect the birds' health. In Texas, higher ventilation rates are typically used, as well as evaporative cooling systems. Higher ventilation rates may dilute the PM10, and the evaporative coolers may suppress PM10 emissions by maintaining a higher relative humidity in the buildings. Consequently, seasonal effects on concentration and, ventilation rate should be more closely evaluated by collecting additional data during summer season.

Some previous studies [24, 25] have mentioned that there is a less ground disturbance in the facilities where birds are not housed on the floor and their movement is restricted. Our study only relates to poultry units where the movement was not restricted and this fact probably also contributes to the obtained results regarding particles of higher sizes (PM5.0 and PM10).

Additionally, it should be noticed that environmental monitoring for this study was limited to a single day measurement in each poultry unit, and variations in the levels of the measured pollutants (fungi and particles) should be expected due to variations in animal and working activities.

5 Conclusions

Characterizing typical exposure levels to bioaerosols and non-viable particles in this occupational setting are required to establish exposure limits and to find

means of reduction to their exposure. Moreover, for health risk assessment studies, we need to consider that simultaneous exposure to more than one contaminant, such as exposure to fungi and particles, is common in this setting.

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