ORIGINAL ARTICLE

Annals of Agricultural and Environmental Medicine 2013, Vol 20, No 2, 259–268
www.aaem.pl

Occupational exposure to airborne microorganisms, endotoxins and β-glucans in poultry houses at different stages of the production cycle

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Lawniczek-Walczyk A, Gorny RL, Golofit-Szymczak M, Niesler A, Wlazlo A. Occupational exposure to airborne microorganisms, endotoxins and β-glucans in poultry houses at different stages of the production cycle. Ann Agric Environ Med. 2013; 20(2): 259–268.

Abstract

The aim of the presented study was to assess the exposure of poultry workers to airborne microorganisms, endotoxins and β-glucans during different stages of the chicken production cycle in 3 commercially-operated poultry houses. Personal and stationary sampling was carried out to assess exposure to both viable and total microbial aerosols. The stationary measurements of PM_{10} were performed to establish the level of endotoxins and β-glucans. The concentrations of bacterial and fungal aerosols ranged from 2.5×10^2 CFU/m^3 – 2.9×10^6 CFU/m^3, and from 1.8×10^2 CFU/m^3 – 1.8×10^5 CFU/m^3, respectively. The number of culturable microorganisms was significantly lower than their total counts, constituting from 0.0004% – 6.4% of the total microbial flora. The level of PM_{10} in poultry facilities did not exceed 4.5 mg/m^3. After the flock entered the clean house, the level of endotoxins and β-glucans increased from below detection limit to 8,364 ng/m^3 and from 0.8 ng/m^3 to 6,886 ng/m^3, respectively. The presented study shows that professional activities in poultry farms are associated with constant exposure to bioaerosol, which may pose a health hazard to workers. It was found that workers' exposure to airborne microorganisms increased with consecutive stages of the chicken production cycle.

Key words

poultry farm, occupational exposure, bioaerosol, endotoxins, β-glucans; PM_{10}

INTRODUCTION

During the last decade, poultry farming has been found to be one of the most dynamically developing branches of modern agriculture. European poultry meat production reached nearly 12 billion tons in 2011 with a share of 12% of the global production volume. The growing demand for poultry causes an increase in the number of poultry farms in many countries [1]. The air in poultry houses is usually heavily contaminated by large quantities of dust particles of biological and non-biological origin, toxic gases (NH_3, CO_2, H_2S), and odors [2, 3, 4, 5, 6]. For poultry workers, the main health risk is most likely posed by biological aerosols. Bioaerosol in poultry houses contains particles released chiefly from settled dust, which originates from feed, manure, litter, feather fragments and animal skin, as well as microorganisms (bacteria, fungi, viruses), their bio-products and fragments [7, 8, 9, 10]. Numerous studies have been conducted to evaluate airborne microbial populations in poultry houses, hatcheries, and processing facilities. They have shown that poultry workers are usually exposed to high concentrations of airborne microorganisms that often exceed the level of 10^6 CFU/m^3 [6, 7, 11, 12, 13, 14, 15, 16, 17, 18, 19].

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Received: 20 October 2012; accepted: 27 February 2013

Human exposure to bioaerosols is associated with a wide range of adverse health effects [20, 21, 22, 23, 24]. The evidence from both epidemiological and experimental studies suggests that endotoxins, which are part of the outer membrane of Gram-negative bacteria, and (1"3)-β-D-glucans, a cell-wall component of molds, are important etiologic agents [11, 20, 25, 26, 27, 28].

Inhalation of endotoxins may result in respiratory tract inflammations and toxic pneumonitis due to a non-specific activation of alveolar macrophages which release inflammatory mediators. Endotoxins can also cause fever, shivering, cough, and influenza-like symptoms [29, 30]. Moreover, several occupational studies have linked exposure to (1"3)-β-D-glucans, present in organic dust, with both the development of diseases (atopy, allergy, asthma, airway inflammation, farmer's lung) and exacerbation of disorders (headache, dry cough, nasal and eye irritation) [22, 25, 26, 27, 30].

Exposure to airborne microorganisms in the occupational environment is usually evaluated by culture-based methods in which colony forming units (CFU) are counted on selective agar media. These traditional methods have several disadvantages. Biological particles can be present in the air as viable cells with an ability to produce colonies on proper medium, viable but non-culturable, non-viable or as microbial cell fragments. The structural components of microorganisms, such as endotoxins or b-glucans, can exert adverse health effects [30, 31]. Therefore, exposure

to bioaerosol particles is difficult to assess using a single sampling procedure or a method of analysis [32]. To date, many studies have focused on endotoxin and β-glucan concentration measurements in total dust. However, only a few studies in both occupational and non-occupational environments have shown that these immunologically reactive structures are usually carried on small dust particles [33, 34, 35, 36, 37]. Due to the fact that settling velocities of particles larger than 10 μm in diameter are relatively high and their subsequent half-lives are below 10 min., they are generally unable to penetrate into the human respiratory tract in large quantities [37]. Therefore, determination of endotoxin and β-glucan concentrations in PM_{10} is an important factor in the exposure assessment to biological aerosols.

OBJECTIVE

The aim of this study was to evaluate workers' exposure to bioaerosols in poultry houses at different stages of the production cycle. In particular, these investigations focused on determining the level of airborne microorganisms, endotoxins and (1→3)-β-D-glucans in PM_{10}. Personal (button aerosol sampler) and stationary (Andersen impactor) sampling were carried out to assess exposure to both viable and total microbial aerosols. The stationary measurements of PM_{10} (Harvard impactor) were performed to establish the level of endotoxins and (1→3)-β-D-glucans. Their usefulness as markers of microbiological contamination was also evaluated. To the best of our knowledge, this study is among the few investigations assessing exposure to endotoxins and (1→3)-β-D-glucans in PM_{10} in poultry houses.

MATERIALS AND METHOD

Locations of poultry houses and sampling strategy. The measurements were carried out in 3 commercially-operated poultry houses located in southern Poland. The air samples were taken during 3 different stages of the chicken production cycle. The initial sampling session (S.1) was conducted before 1-day-old chicks entered the poultry houses. At this stage, the poultry houses were clean and disinfected. During the second sampling session (S.2), air samples were collected in the poultry houses when the chickens were 7-days-old. The third sampling session (S.3) was performed 1 day before the departure of the 49–56-day-old chickens to a slaughterhouse. All investigated buildings were equipped with automatic feeding, watering, heating, and mechanical ventilation systems. Population density of chickens in examined poultry houses was 16–17 birds/m^2 during each stages of chicken production cycle. Chickens were kept on deep litter. Measurements were carried out twice at each of the 2 sampling locations, i.e. inside and outside the examined poultry houses in each session. To determine the seasonal variation of bioaerosol concentration in poultry houses, each sampling session was repeated twice at all sampling locations during the winter and summer seasons.

For the presented study, aerosol particles were collected using stationary and personal samplers. For quantitative and qualitative analyses of microflora, the samples of airborne bacteria and fungi were taken using Andersen impactors (stationary sampling) and Button aerosol samplers (personal sampling). At the same time, the measurements of PM_{10} were performed using Harvard impactors (stationary sampling). All stationary measurements were carried out at a height of 1.5 m above ground level o simulate aspiration from the human breathing zone.

B**ioaerosol sampling and analysis.** Stationary sampling of viable microorganisms was carried out using a 6-stage Andersen impactor (model 10–710, Andersen Instruments, Atlanta, GA, USA) at a flow rate of 28.3 l/min for 0.5 to 2 min. Impactor samples were collected on 4 different nutrient media (BTL, Łódź, Poland): blood TSA (Trypticase Soy Agar with 5% sheep blood), SS Agar (Salmonella Shigella Agar), Endo Agar and MEA (Malt Extract Agar). Such combination of sampling media enables both enumeration and identification of the most common microorganisms in the groups of:

a) Gram-positive and Gram-negative mesophilic bacteria;
b) Gram-negative bacteria belonging to *Salmonella* and *Shigella* genera;
c) coliform and other enteric microorganisms;
d) fungi (including moulds and yeasts). The collected samples were incubated at the temperature of:
   bacteria – 1 day at 37°C, followed by 3 days at 22°C and 3 days at 4°C;
   fungi – 4 days at 30°C followed by 4 days at 22°C.

After incubation, the bioaerosol concentration was calculated as colony forming units per m^3 (CFU/m^3). The isolated bacterial colonies were identified to the genus and/or species level based on their morphology, microscopic structure and biochemical reactivity (using API tests; bioMérieux, Marcy-l'Etoile, France). The isolated fungal colonies were directly identified under stereo (SteREO Discovery V.12, Carl Zeiss, Göttingen, Germany) and light microscopes (Eclipse E200, Nikon, Tokyo, Japan) based on their macro- and micro-morphological characteristics. The analysis of yeasts was additionally supplemented by biochemical API tests (bioMérieux).

Simultaneously, with stationary measurements, the personal samples were taken using filter samplers (Button aerosol sampler, SKC Ltd., Eighty-Four, PA, USA) clipped onto a worker's collar. Bioaerosol samples were collected on gelatin filters (25 mm with a pore size of 3 μm; SKC Ltd.) at a flow rate of 4 l/min for 30 min. After sampling, each filter was removed from the sampler holder and dissolved in sterile water containing 0.01% Tween 80. Part of the suspension was plated on microbiological media (TSA, SS, Endo, MEA) and used for determination of culturable microorganisms (CFU/m^3). The rest of the suspension was used for examination of total microbial counts by a modification of the CAMNEA method [38]. The obtained samples were treated with formaldehyde (37%) (POCH S.A., Gliwice, Poland) and then stained with acridine orange (Sigma-Aldrich Chemie GmbH, Munich, Germany). After filtration of the resulted suspension through a black polycarbonate filter with a pore size of 0.8 μm (Whatman, Maidstone, UK), all microorganisms were counted using an epifluorescence microscope (Nikon) and their concentration expressed as the number of cells/m^3.

PM_{10} sampling and analysis. PM_{10} samples were obtained using Harvard impactors (Air Diagnostic and Engineering Inc., Naples, ME, USA) operated at a flow rate of 10 l/min for 4 h. Particles were collected on 37-mm Teflon filters with 1 μm pore size (SKC Ltd.). The mass of PM_{10} in all

samples were gravimetrically determined by weighting the filters before and after sampling, following in both cases by a 24 h equilibration period at constant air temperature and humidity. After gravimetric analysis, each filter was transferred into a 50-ml, pyrogen-free polypropylene tubes and stored in a dry state at -20°C until extracted for endotoxins and (1→3)-β-D-glucans.

Endotoxin analysis. All filters were analyzed first for endotoxin content. Dust collected on filters was extracted with 10 ml of sterile pyrogen-free water (PWF, LAL reagent water, Lonza, Basel, Switzerland) by shaking on a platform shaker (Promax 1020, Heidolph Instruments GmbH & Co., Schwabach, Germany) at room temperature for 1 h. The dust suspensions were centrifuged at 1,000×g for 10 min (5804 R, Eppendorf AG, Hamburg, Germany) and divided into 2 parts. The first part of the supernatant was analyzed in duplicate for endotoxins using Kinetic-QCL Limulus Amebocyte Lysate (LAL) assay (Lonza), following the manufacturer instructions. The assay had a potency of 12 EU/ng against *Escherichia coli* 055:B5 standard endotoxin. The concentration of airborne endotoxin was expressed in ng/m^3.

(1"3)-β-D-glucan analysis. The remaining part of the supernatant was vortexed (BioVortex V1 Plus, Biosan, Riga, Latvia) for 2 more minutes, followed by an additional 10 min agitation in an ultrasonic bath (Sonic 5, Polsonic, Warsaw, Poland). Directly afterwards, 0.6M NaOH was added and the suspension additionally shaken for 1 h at room temperature in order to unwind the triple-helix structure of the glucans and make them water soluble. The concentrations of (1→3)-β-D-glucans were assayed using the quantitative kinetic Glucatell assay (Associates of Cape Cod, East Falmouth, MA, USA) and expressed as ng/m^3.

Measurement of microclimate parameters. During every sampling session, the influence of microclimate conditions on bioaerosol levels in the poultry houses was checked. The air temperature and relative humidity were recorded with hytherograph (Omniport 20, E+E Elektronik GmbH, Engerwitzdorf, Austria).

Statistical analysis. All statistical analyses were performed using Statistica (data analysis software system), version 7.1 (StatSoft, Inc., Tulsa, OK, USA). The geometric mean (GM) and geometric standard deviation (GSD) were used to characterize the obtained data. After their log-normal transformation, the subsequent statistical analyses were carried out based on *t*-test and Pearson correlation.

RESULTS

Concentration of viable airborne microorganisms. The concentrations of bacterial and fungal aerosols obtained by stationary sampling are shown in Table 1. Taking into account all sampling sessions, the range of culturable bacteria concentrations was 7.1×10^2–1.3×10^6 CFU/m^3 in winter and 2.5×10^2–2.9×10^6 CFU/m^3 in summer. It was found that bacterial aerosol concentrations in examined poultry houses varied greatly at different stages of production cycle. The highest concentration was found in S.3 (with GM of 1.2×10^6 CFU/m^3 in winter and 2.6×10^6 CFU/m^3 in summer) and was approximately 7 to 9 times higher than in S.2 ($p<0.00001$ and $p<0.000001$, in winter and summer, respectively) and 488 to 6418 times higher than in S.1 ($p<0.000001$ and $p<0.0000001$, in winter and summer, respectively). The lowest bacterial concentration was observed in S.1 in which GM did not exceed 2.5×10^3 CFU/m^3.

Table 1. Bacterial and fungal concentrations (CFU/m^3) in outdoor air and poultry houses determined with a six-stage Andersen impactor (stationary sampling).

Sampling session		Bacteria		Fungi	
		GM	GSD	GM	GSD
Winter	S.1	2,542	2.5	773	2.4
	S.2	1,36,839	1.5	1,346	3.0
	S.3	12,41,223	1.1	23,494	5.0
	Outdoor	130	8.0	64	2.7
Summer	S.1	399	1.5	374	1.7
	S.2	363,052	1.9	2,801	1.9
	S.3	2,564,082	1.1	15,817	1.7
	Outdoor	271	3.1	318	1.9

The range of culturable fungi concentrations observed during all sampling sessions was 2.1×10^2–1.8×10^5 CFU/m^3 in winter and 1.8×10^2–3.0×10^4 CFU/m^3 in summer. The contamination of airborne fungi reached the highest level in S.3 (with GM of 2.3×10^4 CFU/m^3 in winter and 1.6×10^4 CFU/m^3 in summer) and was about 6 to 17 times higher than in S.2 ($p<0.05$ and $p<0.01$, in winter and summer, respectively) and 30 to 42 times higher than in S.1 ($p<0.01$ and $p<0.0001$, in winter and summer, respectively). The concentration of fungal aerosol in S.2 was also significantly higher than in S.1 in summer sampling period ($p<0.00001$).

The comparison of indoor and outdoor bioaerosol concentrations showed that indoor bacterial and fungal levels were higher than outdoor ones ($p<0.0001$ and $p<0.001$, for bacteria and fungi, respectively). Regarding the samples measured using Andersen impactor, a significantly higher exposure to bacterial aerosol was observed in S.2 and S.3 in summer than in winter ($p<0.05$ and $p<0.001$, respectively). In case of fungal aerosol, no significant seasonal variation was found.

The concentrations of culturable bacterial and fungal aerosols in personal samples are shown in Table 2. The highest concentrations of airborne bacteria were measured in S.3, in both winter and summer (GM: 8.4×10^5 CFU/m^3 and 6.8×10^5 CFU/m^3, respectively). These concentrations

Table 2. Bacterial and fungal concentrations (CFU/m^3) and total counts of airborne microorganisms (×10^5 cells/m^3) in poultry houses, determined with Button aerosol sampler (personal sampling).

Sampling session		Bacteria		Fungi		Total counts	
		GM	GSD	GM	GSD	GM	GSD
Winter	S.1	4,508	1.4	1,934	1.1	5	1.8
	S.2	124,145	7.2	19,104	3.1	1,286	1.6
	S.3	842,402	2.1	30,444	2.1	70,649	48.1
Summer	S.1	179	1.6	60	1.0	0.1	1.1
	S.2	49,437	2.7	7,688	3.5	17,803	13.5
	S.3	678,184	1.5	13,242	2.9	1,893,691	10.1

were 7 to 14 times higher than in S.2 ($p<0.0001$ and $p<0.01$, in winter and summer, respectively) and 187 to 3796 times higher than in S.1 ($p<0.000001$ and $p<0.0000001$, in winter and summer, respectively). During this study, the peak fungal concentration was observed in S.3 with winter GM of 3.0×10^4 CFU/m^3 and summer GM of 1.3×10^4 CFU/m^3. However, no significant difference was noted between fungal aerosol concentrations in S.3 and S.2. The concentrations of fungal aerosol in S.1 were considerably lower than in S.2 ($p<0.05$ and $p<0.001$) and S.3 ($p<0.01$ and $p<0.0000001$, in both cases: in winter and summer, respectively). No seasonal variation was observed for bacterial and fungal aerosols in all studied sessions.

In studied poultry houses, the bioaerosol concentrations measured using personal and stationary samplers were different from each other. In summer sessions S.1, S.2 and S.3, bacterial concentrations obtained using Andersen impactor were significantly higher than those measured using Button aerosol sampler ($p<0.05$, $p<0.01$, and $p<0.001$, respectively). Moreover, higher fungal aerosol concentrations were observed in summer S.1 when measured using stationary sampler than using personal aspirator ($p<0.01$). In winter, no significant difference between bioaerosol concentrations obtained by personal and stationary sampling was found.

Total counts of airborne microorganisms. The total concentrations (viable and non-viable together) of airborne microorganisms in poultry houses are shown in Table 2. The range of total count was 2.1×10^5–2.1×10^{11} cells/m^3 in winter and 1.1×10^4–3.6×10^{12} cells/m^3 in summer. Significantly higher level of total microbial aerosols was observed in S.3 and was approximately 54–106 times higher than in S.2 ($p<0.05$ for both winter and summer seasons) and 1,460-14,442,757 times higher than in S.1 ($p<0.001$ and $p<0.00001$, in winter and summer, respectively). When sampling seasons were compared, higher total concentrations of airborne microorganisms were noted in S.1 in winter than in summer ($p<0.0001$). In the case of S.2 and S.3, no significant seasonal variation was found within these sessions.

A comparison of total counts with concentrations of culturable airborne microorganisms collected by stationary sampling indicated that the number of culturable microorganisms were significantly lower than total counts constituting in winter, from 0.02% – 0.7%, and in summer from 0.001% – 6.4% of the total (p values ranged from 0.0000001 – 0.01). The level of culturable microorganisms determined by personal sampling was also considerably lower than total counts, constituting in winter from 0.01% – 1.2%, and in summer from 0.0004% – 1.9% of the total (p values ranged from 0.000001 – 0.01).

Qualitative analysis of bacterial and fungal aerosols. The percentage distributions of microorganisms identified in the air of examined poultry houses showed differences between 3 sampling sessions (Fig. 1). Prior to the entry of 1-day old chickens, fungal microorganisms were the most prevalent in the air of studied facilities, making up to 34.8% of all identified isolates. The second most numerous group was Gram-positive cocci constituting 21.7% of the total microflora, followed by Gram-positive bacilli, non-sporing Gram-positive rods, and mesophilic actinomycetes (14.7%, 13.7%, and 13.7%, respectively). Airborne concentrations of Gram-negative rods were very low (1.4%). After the entry of the flocks of

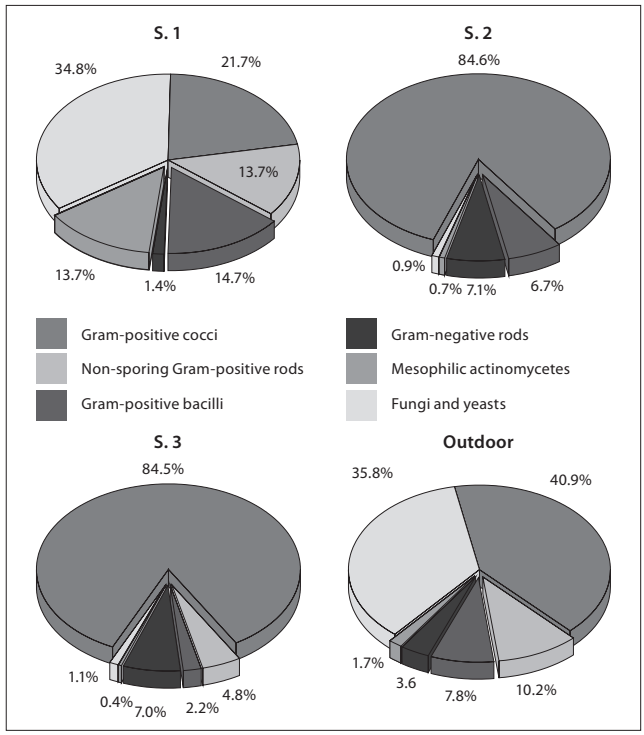

Figure 1. Percentage distributions of microbial groups identified in poultry houses during 3 subsequent sampling sessions.

chickens into the poultry houses, the composition of the air microflora changed significantly. The domination of Gram-positive cocci that accounted for about 85% in S.2 and S.3 was observed. The results also indicated a significant increase in concentrations of Gram-negative rods (constituting about 7% in S.2 and S.3) with simultaneous decrease in the percentage of other microbial groups contributing to the total microflora. Qualitative composition in each of these indoor environments differed substantially from the outdoor air where the most numerous were Gram-positive cocci (40.9%) and fungi (35.8%) (Fig. 1).

Sixty-two bacterial species from 30 genera and 75 fungal species from 20 genera were isolated from the air of the poultry houses (Tab. 3). Gram-positive cocci from *Staphylococcus* (13 species) and *Enterococcus* (4 species) genera predominated among the identified bacteria. Among Gram-negative rods prevailed species from *Acinetobacter*, *Enterobacter*, *Escherichia*, *Pantoea*, and *Klebsiella* genera. The most frequently isolated fungi belonged to *Penicillium* (17 species), *Aspergillus* (16 species) and *Scopulariopsis* (5 species) genera.

Qualitative analysis demonstrated that bacterial and fungal strains obtained by stationary sampling (impaction on agar) differed from those determined by personal sampling (filtration). In all sampling sessions, a significantly higher number of species was identified by impaction than by filtration (62 bacterial and 75 fungal vs. 20 bacterial and 27 fungal species, respectively). In personal samples, very few Gram-negative rods and yeasts were detected.

Exposure to PM_{10}, endotoxins and (1→3)-β-D-glucans. The concentrations of PM_{10}, endotoxins and (1→3)-β-D-glucans in the examined poultry houses are shown in Table 4. The PM_{10} concentrations ranged between 73 μg/m^3 and 4095 μg/m^3 in winter and between 28 μg/m^3 and 4511 μg/m^3 in summer.

Table 3. Microorganisms isolated from air of poultry houses using Andersen impactor and Button aerosol sampler.

Andersen impactor	Button aerosol sampler
Gram-positive cocci	
Aerococcus viridians, *Enterococcus* (*E. avium*, *E. durans*, *E. faecium*, *E. faecalis*), *Globicatella sanguinis*, *Kocuria varians*, *Micrococcus* spp., *Rothia mucilaginosa*, *Staphylococcus* (*S. auricularis*, *S. aureus**, *S. capitis*, *S. chromogenes*, *S. cohnii*, *S. epidermidis* , *S. gallinarum*, *S. haemolyticus*, *S. intermedius*, *S. lentus*, *S. saprophyticus*, *S. sciuri*, *S. simulans*, *S. xylosus*)	*Kocuria varians*, *Micrococcus* spp., *Staphylococcus* (*S. capitis*, *S. chromogenes*, *S. cohnii*, *S. epidermidis*, *S. gallinarum*, *S. lentus*, *S. sciuri*, *S. simulans*, *S. xylosus*)
Non-sporing Gram-positive rods	
Arthrobacter spp., *Brevibacterium* spp., *Corynebacterium* (*C. jeikeium*, *C. pseudodiphteriticum*, *C.* spp.*), *Cellulomonas cellulans*, *Microbacterium* spp.	*Brevibacterium* spp., *Cellulomonas cellulans*, *Microbacterium* spp.
Gram-positive bacilli	
Bacillus (*B. brevis*, *B. cereus*, *B. circulans*, *B. coagulans*, *B. firmus*, *B. licheniformis*, *B. megaterium*, *B. mycoides*, *B. subtilis*, *B.* spp.)	*Bacillus* (*B. cereus*, *B. firmus*, *B. mycoides*)
Gram-negative rods	
Acinetobacter baumannii, *Aeromonas caviae*, *Burkholderia cepacia*, *Cedecea daviseae*, *Enterobacter* (*E. asburiae*, *E. cloacae**, *E. sakazaki*), *Escherichia coli*, *Hafnia alvei*, *Klebsiella* (*K. pneumoniae ssp. pneumonia**, *K. pneumonia**, *K. oxytoca**, *K.* spp.*), *Pantoea* spp., *Proteus mirabilis**, *Pseudomonas oryzihabitans*, *Serratia plymuthica*, *Shigella* spp., *Salmonella* spp.*	*Escherichia coli*, *Pseudomonas oryzihabitans*
Mesophilic actinomycetes	
Nocardia spp., *Rhodococcus* spp., *Streptomyces* spp.	*Streptomyces* spp.
Filamentous fungi	
Absidia spp., *Acremonium* (*A. charticola*, *A. strictum*, *A.* spp.), *Alternaria* (*A. alternata*, *A. tenuissima*, *A.* spp.), *Aspergillus* (*A. candidus*, *A. clavatus*, *A. flavus*, *A. fumigatus**, *A. glaucus*, *A. niger*, *A. melleus*, *A. ochraceus*, *A. oryzae*, *A. ostianus*, *A. parasiticus*, *A. penicillioides*, *A. sydowii*, *A. ustus*, *A. versicolor*, *A.* spp.,) *Aureobasidium* (*A. pullulans*, *A.* spp.), *Byssochlamas fulva*, *Chaetomium* (*C. elatum*, *C.* spp.), *Cladosporium* (*C. cladosporioides*, *C. herbarum*, *C. macrocarpum*, *C.* spp.), *Chrysosporium* spp., *Fusarium* (*F. sambucinum*, *F. verticillioides*, *F.* spp.), *Mucor* (*M. hiemalis*, *M. plumbeus*, *M.* spp.), *Penicillium* (*P. aurantiogriseum*, *P. chrysogenum*, *P. citrinum*, *P. commune*, *P. coprobium*, *P. crustosum*, *P. cyclopium*, *P. digitatum*, *P. expansum*, *P. griseofulvum*, *P. oxysporum*, *P. polonicum*, *P. rugulosum*, *P. variable*, *P. verrucosum*, *P. viridicatum*, *P.* spp.) *Phialophora* (*P. fastigiata*, *P.* spp.), *Rhizopus* (*R. oryzae*, *R. stolnifer*, *R.* spp.), *Sporotrichum* spp., *Scopulariopsis* (*S. brevicaulis*, *S. brumptii*, *S. candida*, *S. fusca*, *S.* spp.), *Trichophyton* spp.*	*Acremonium strictum*, *Alternaria* (*A. alternata*, *A.* spp.), *Aspergillus* (*A. clavatus*, *A. flavus*, *A. melleus*, *A. penicillioides*, *A. ustus*) *Aureobasidium* (*A. pullulans*, *A.* spp.), *Byssochlamas fulva*, *Cladosporium macrocarpum*, *Chrysosporium* spp., *Fusarium* (*F. sambucinum*, *F.* spp.), *Mucor* (*M. hiemalis*, *M.* spp.), *Penicillium* (*P. chrysogenum*, *P. crustosum*, *P. expansum*, *P. rugulosum*, *P. viridicatum*, *P.* spp.) *Phialophora fastigiata*, *Rhizopus* spp., *Scopulariopsis brevicaulis*
Yeasts	
Candida (*C. dubliniensis*, *C. guilliermondii*, *C. laurentii*, *C.* spp.), *Cryptococcus* (*C. albidus*, *C.* spp.), *Rhodotorula minuta*	*Candida laurentii*

* – microorganisms classified by Directive 2000/54/EC into group 2 according to level of risk of infection

Table 4. Concentration of endotoxins (ng/m³), (1→3)-β-D-glucans (ng/m³) and PM_{10} (μg/m³) in air of poultry houses and outdoor air in winter and summer sampling seasons.

Sampling session		PM_{10}		Endotoxins		(1→3)-β-D-glucans	
		GM	GM	GM	GSD	GM	GSD
Winter	S.1	253	2.2	BD	BD	1	1.2
	S.2	1,342	1.7	756	2.5	646	2.5
	S.3	1,883	2.1	1,897	2.7	582	1.8
	Outdoor	144	2.2	BD	BD	3	3.4
Summer	S.1	203	0.7	0.1	1.4	74	1.1
	S.2	833	1.8	281	3.5	291	1.6
	S.3	1,168	12.1	3,406	1.3	1,164	3.7
	Outdoor	77	21.0	0.1	17.2	8	1.1

Notes: BD – below detection limit

The lowest indoor values (GM of 253 μg/m³ in winter and 203 μg/m³ in summer) were noted in the first sampling session, whereas the highest were measured in S.3 (with GM of 1,883 μg/m³ in winter and 1,168 μg/m³ in summer). There were no statistically significant differences between PM_{10} concentrations in S.2 and S.3.

Comparison between indoor and outdoor particulate aerosol concentrations showed that indoor PM_{10} levels in S.2 and S.3 were higher than in outdoor air ($p<0.001$). In session S.1, no significant difference was observed. Moreover, PM_{10} concentrations in winter were higher than those in summer; however, these differences were not statistically significant.

The results of the presented study also reveal that the endotoxin content in PM_{10} increased simultaneously with subsequent stages of the production cycle. The concentrations of endotoxins ranged from below detection limit to 8,364 ng/m³ in winter, and from 0.04 ng/m³ to 4372 ng/m³ in summer. Their concentrations reached the highest levels in S.3 (with GM of 1,897 ng/m³ in winter and 3,406 ng/m³ in summer), and were significantly higher than in S.2 ($p<0.05$ and $p<0.01$) and S.1 ($p<0.001$ and $p<0.00001$, in both cases for winter and summer, respectively). The comparison between indoor and outdoor endotoxin concentrations showed that their indoor air levels in S.2 and S.3 were higher than outdoor levels (in both cases $p<0.001$). Regarding S.1, no significant differences were observed. The comparison between different sampling seasons demonstrated that endotoxin concentrations in S.1 were much higher in summer than in winter ($p<0.01$). In S.2 and S.3, no significant seasonal variations were found.

The concentrations of (1→3)-β-D-glucans in PM_{10} ranged from 0.8 ng/m³ – 6886 ng/m³. The lowest levels of (1→3)-β-D-glucans were observed in S.1 (GM did not exceed 74 ng/m³). After the entry of the flocks into the poultry houses, the concentrations of (1→3)-β-D-glucans increased considerably ($p<0.05$–0.001) and ranged between 195 ng/m³ and 1371 ng/m³ in S.2 and between 297 ng/m³ and 6886 ng/m³ in S.3. No significant differences were noted between concentrations of (1→3)-β-D-glucans in S.2 and S.3. The comparison of indoor

and outdoor concentrations showed that (1→3)-β-D-glucan levels in indoor air, in all sampling sessions, were higher than in outdoor air (p<0.001), with the exception of winter S.1. Significantly higher levels of (1→3)-β-D-glucans in S.1 were recorded in summer than in winter (p<0.001); however, no significant seasonal variations of β-glucan concentrations were observed for S.2 and S.3, nor for outdoor measurements.

Microclimate Parameters. Air temperature and relative humidity recorded inside the poultry houses during different stages of production cycle are presented in Table 5. The lowest air temperature (between 14–22°C) and humidity (between 38–53%) were noted during S.1. After the chickens had entered the poultry houses (S.2), the air temperature and humidity was raised to 29–33°C and 51–67%, respectively. During S.3, the air temperature decreased to 21–26%, whereas relative humidity rose again to 66–78%.

Table 5. Results of air temperature and relative humidity measurements inside and outside poultry houses in winter and summer sampling seasons.

Sampling session		Temperature (°C)		Relative humidity (%)	
		GM	GSD	GM	GSD
Winter	S.1	14	1.6	45	1.1
	S.2	30	1.1	61	1.1
	S.3	21	1.1	68	1.1
	Outdoor	5	1.4	66	1.2
Summer	S.1	15	1.1	66	1.0
	S.2	29	1.1	62	1.1
	S.3	25	1.1	69	1.1
	Outdoor	23	1.5	45	1.5

Relationships between bioaerosol constituents and environmental parameters. The correlation between bioaerosol constituents and environmental parameters is analyzed in Table 6. The concentrations of endotoxins showed a strong positive correlation with PM_{10} levels in both winter and summer sampling seasons (r=0.88 at p<0.01 and r=0.86 at p<0.001, respectively), as well as with Gram-negative rods (r=0.53 at p<0.05 and r=0.96 at p<0.0001, respectively). The concentrations of (1→3)-β-D-glucans revealed the same dependency with PM_{10} (r=0.78 at p<0.001 and r=0.68 at p<0.05, respectively). However, the occurrence of fungi in the air correlated positively with concentrations of (1→3)-β-D-glucans during summer sampling season only (r=0.86 at p<0.001). It was also found that PM_{10} concentrations were significantly associated with the levels of Gram-negative rods, total bacteria (in both cases: r=0.64 at p<0.01 and r=0.73 at p<0.01 in winter and summer, respectively), and fungi (however, a significant correlation with fungi was noted for summer measurements only: r=0.75 at p<0.01). In winter, a significant correlation was recorded between relative humidity and total bacteria (r=0.71 at p<0.01), Gram-negative rods (r=0.69 at p<0.01) and β-glucan concentration values (r=0.67 at p<0.05). During the summer sampling session, relative humidity values correlated significantly with the concentration of (1→3)-β-D-glucans only (r=0.72 at p<0.05).

DISCUSSION

To the best of our knowledge, this study is the first to evaluate endotoxin and (1→3)-β-D -glucan concentrations in PM_{10} at different stages of the poultry production cycle. It shows that the poultry workers are simultaneously exposed to high concentrations of airborne microorganisms as well as endotoxins and (1→3)-β-D-glucans.

Many environmental investigations have shown that bacterial and fungal aerosol concentrations in poultry houses are higher than in others animal houses [6, 7, 11, 13, 15, 17, 18, 19], with which the results of the presented study are in good agreement. After the flock entered the clean poultry house, the concentrations of culturable bacteria increased

Table 6. Correlation between concentrations of aerosol components and values of microclimate parameters in poultry houses during winter and summer sampling sessions

		Endotoxins	β-glucans	PM_{10}	Total bacteria	Gram (–) rods	Fungi	Temperature	Relative humidity
Winter	Endotoxins	1							
	β-glucans	-	1						
	PM_{10}	***r*=0.88****	***r*=0.78*****	1					
	Total bacteria	-	-	***r*=0.64****	1				
	Gram (–) rods	***r*=0.53***	-	***r*=0.64****	***r*=0.99******	1			
	Fungi	-	*r*=0.39	*r*=0.51	-	-	1		
	Temperature	*r*=0.15	*r*=0.55	*r*=0.32	*r*=–0.01	*r*=–0.02	*r*=–0.04	1	
	Relative humidity	*r*=0.32	***r*=0.67***	*r*=0.19	***r*=0.71****	***r*=0.69****	*r*=0.31	-	1
Summer	Endotoxins	1							
	β-glucans	-	1						
	PM_{10}	***r*=0.86*****	***r*=0.68***	1					
	Total bacteria	-	-	***r*=0.73****	1				
	Gram (–) rods	***r*=0.96******	-	***r*=0.73****	***r*=0.99*******	1			
	Fungi	-	***r*=0.86*****	***r*=0.75****	-	-	1		
	Temperature	*r*=0.11	*r*=–0.03	*r*=0.53	*r*=0.13	*r*=0.13	*r*=0.19	1	
	Relative humidity	*r*=0.45	***r*=0.72***	*r*=–0.57	*r*=0.48	*r*=0.49	*r*=0.41	-	1

Significant relationships (* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001, ***** p<0.00001) expressed as Pearson correlation coefficients (*r*) in bold

up to the level of 2.6×10^6 CFU/m^3, which exceeded by about 1.2–25 times the Polish proposals for threshold limit value (TLV), which is 1.0×10^5 CFU/m^3 [32, 39]. However, the concentrations of Gram-negative rods and fungi were still below the Polish TLV proposals (i.e. below 2.0×10^4 CFU/m^3 and 5.0×10^4 CFU/m^3, respectively).

The prevalence of Gram-positive cocci among the airborne microflora of poultry facilities is closely related to their reservoirs, i.e. skin, feathers and faeces, as well as the respiratory tract of birds [40]. In the presented study, only a few (9 bacterial and 1 fungal species) among all detected microorganisms were classified into risk group 2 according to Directive 2000/54/EC, and based on what might be recognized as hazards to workers [41]. On the other hand, non-pathogenic and/or saprophytic species (e.g. some Gram-positive cocci) present in the air may become an opportunistic pathogen under certain conditions (for example, when they are in extremely high concentrations). It is well known that peptidoglycan, the major component of the Gram-positive bacterial cell wall, could have a negative effect on human and animal health, and may cause infection, endotoxaemia, and other systemic inflammations with organ failure [42].

During all sampling sessions, the concentrations of PM_{10} in poultry facilities did not exceed 4.5 mg/m^3. To date, only a few studies have been conducted to evaluate PM_{10} concentrations in poultry houses, and the values obtained in the presented study are similar to those reported by other authors [43, 44, 45]. Particulate matter is widely recognized as a serious indoor contaminant. In poultry houses, the particulate matter comes mainly from skin, feathers, faeces, uric acid crystals, feed, and litter [2, 46, 19]. It was observed that occupational exposure to PM was associated with the presence of chronic cough, chronic bronchitis and chest tightness [2, 47]. High level of PM inside livestock buildings has a negative influence not only on human, but also on animal health. Poor air quality can result in respiratory diseases and mortality among animals, thereby decreasing livestock production [2, 48].

The results of the presented study show that the air in the poultry houses was heavily polluted with bacterial endotoxins. Their concentrations ranged from 281 ng/m^3 – 3406 ng/m^3 and were on the same level as those reported by Seedorf *et al.* [18] in inhalable dust fractions. They were also similar to the concentrations noted in total dust in livestock farms by Bakutis *et al.* [7], Rylander and Carvalheiro [22], Pomorska *et al.* [49], and Rimac *et al.* [17]. The endotoxin concentrations measured in PM_{10} in the presented study, however, were higher than those reported by Kirychuk *et al.* [34] for the particulate matter fraction <9.8 μm. These differences may be attributed to the number of chickens in a flock, type of ventilation system and type of litter. The endotoxin concentrations in the examined poultry facilities exceeded 31–378 times the TLV of 9 ng/m^3 (90 EU/m^3) suggested by the Dutch Expert Committee for Occupational Standards [50], and were 3–34 times higher than the safety level of 0.1 μg/m^3 proposed by Clark [51] and Rylander [27], and 1.4–17 times greater than the Polish TLV proposal of 200 ng/m^3 (2,000 EU/m^3) [39]. In almost all sampling sessions, the concentrations of endotoxins exceeded 200 ng/m^3, i.e. the level suggested by Rylander [52] as a limit value for toxic pneumonitis. Based on the above data, it can be stated that occupational activities in the environment heavily contaminated by endotoxins (as in the examined poultry farms) pose a significant threat to exposed individuals.

In the air of occupied chicken houses, the high concentrations of (1→3)-β-D-glucans (ranging from 291 ng/m^3 – 1164 ng/m^3) were also detected. There are only a few studies concerning an exposure to (1→3)-β-D-glucans in poultry houses. The results of the presented study are in a good agreement with those reported by Rylander *et al.* [22] and Sander *et al.* [53]. However, these authors focused on β-glucan levels in total dust only. Unlike endotoxins, there are neither exposure standards nor TLV proposals for (1→3)-β-D-glucans. Nevertheless, Rylander *et al.* [54] demonstrated that exposure to low levels of (1→3)-β-D-glucans (0.1–5.2 ng/m^3) may increase the frequency of various non-specific adverse health outcomes, such as headache, dry cough, throat irritation, nasal irritation, and fatigue.

In the presented study it was observed that the concentrations of bacterial and fungal aerosols, as well as endotoxins, increased significantly with the consecutive stages of poultry production cycle. This finding may be explained by the phase of bird growth and specific conditions of the fattening cycle. Numerous studies have shown that the concentration of airborne particles increases with the age of chickens [8, 11, 16, 19, 49, 55, 56]; however, in case of both β-glucan and PM_{10} concentrations measured in the presented study, this association was not significant. Furthermore, other factors, including type of ventilation, bedding, feeding, as well as temperature, air humidity and lighting programme may also significantly influence the particle concentrations. The type of litter and its moisture content are also very important. Generally, by the end of the production cycle, the litter is usually dry and heavily polluted by microorganisms and faeces which, when airborne, may become a major source of different biological indoor contaminants [2, 49, 55, 56].

A significant correlation between particulate matter and bioaerosol constituents was also widely observed in the presented study, which revealed that high concentrations of PM_{10} were associated with high levels of total bacteria, Gram-negative rods, fungi, endotoxins, and (1→3)-β-D-glucans. Similar observations were reported for endotoxins and dust by Bakutis *et al.* [7] and Rosas *et al.* [57], for (1→3)-β-D-glucans and dust by Madsen *et al.* [36] and Stuurman *et al.* [58]. The significant positive correlations between endotoxin and Gram-negative rod concentrations, as well as between β-glucan and fungal aerosol concentrations, were also observed in the presented study. These relationships are in a good agreement with the data collected in different stock-rising as well as non-agricultural working environments by Bakutis *et al.* [7], Madsen *et al.* [36], Mandryk *et al.* [59] and Lee *et al.* [60].

This indicates that both (1→3)-β-D-glucans and endotoxins may be used as markers of exposure for fungal and (Gram-negative) bacterial contaminants in those types of occupational environments. As both of the above-mentioned microbial structural elements persist in the environment much longer than viable cells, and are able to maintain biological activity even after a death of parental cells [30], they may also serve as indicators of long-term exposure.

Seasonal variation analysis of bioaerosols in poultry houses revealed significantly higher concentrations of airborne bacteria in summer sampling sessions than in winter ones; however, this relationship was not observed for other measured bioaerosol components. In poultry houses,

microclimate parameters have to be adapted to a specific stage of the bird growth cycle. Recommended temperature and relative humidity for chickens should be between 30–33°C and 40–70% within the first 1–2 weeks, and 18–20°C and 65–70% within the next 5–8 weeks [61, 62]. During all sampling sessions, the environmental conditions in poultry houses were close to the optimal values, with one exception, i.e. in summer S.3, both indoor temperature and humidity exceeded the recommended values. It is well known that microclimate parameters are important factors affecting the level and composition of microbiological contaminants of both the litter and the air. As shown in the presented study, a significant correlation was found between the values of relative humidity and concentrations of total bacteria, Gram-negative rods, and (1→3)-β-D-glucans. Similar results were reported by Vučemilo *et al.* [19, 56] and Banhazi *et al.* [63]. Regarding the air temperature, no association was observed between this factor and bacterial as well as fungal concentrations.

As the bacterial and fungal community in the air of poultry houses can be qualitatively very diverse, a choice of proper bioaerosol sampling method for exposure assessment is of high great importance. In the presented study, the measurements of microbial aerosols were carried out with 2 different sampling techniques, i.e. impaction – using a 6-stage Andersen sampler, and filtration – using a Button aerosol sampler. As shown, the concentrations of viable bacteria and fungi obtained using both samplers did not significantly differ from one another. This finding is similar to the results reported by Rautiala *et al.* [64] and Ławniczek *et al.* [65]. In the presented study, however, the filter sampler gave incomplete information regarding qualitative composition of airborne microflora. A significantly higher number of bacterial and fungal species was isolated from the impactor plates than from filters. Furthermore, there were very few Gram-negative rods and yeasts detected in the filter samples. The lower number of microbial taxa identified from filter sampler was probably caused by biological stress during both the sampling process (desiccation) and analytical elaboration of the samples (filter dissolution) [65, 66, 67, 68].

Despite these disadvantages, the filtration method provides accurate data regarding for the total (viable and non-viable together) microbial counts. Based on that, it is clear that only a combination of culture-based and direct microscopy methods allows a precise determination of the 'real' microbial exposure. Moreover, it was demonstrated that the number of culturable microorganisms was significantly lower than their total counts, constituting from 0.0004% – 6.4% of the total microbial flora. These results, being in a good agreement with other studies [6, 64, 65, 67, 68, 69], indicate that only simultaneously performed stationary (qualitative) and personal (quantitative) sampling allows the exact definition of an exposure to microbial agents in the poultry farm environment.

CONCLUSIONS

The presented study shows that professional activities carried out in poultry farms are associated with constant exposure to large quantities of airborne microorganisms and their bio-products. As shown, the concentrations of endotoxins and (1→3)-β-D-glucans in PM_{10}, as well as Gram-negative rods and fungi, increased significantly in consecutive stages of the poultry production cycle. Such working conditions may pose a serious risk of respiratory diseases and/or the appearance of non-specific symptoms in the exposed individuals. Hence, a proper strategy of airborne contaminant reduction inside the poultry houses needs to be applied to reduce both animal and human exposure.

Significant positive correlations between endotoxin and Gram-negative rod concentrations, as well as between (1→3)-β-D-glucan and fungal aerosol concentrations documented in the presented study, confirm that endotoxins and (1→3)-β-D-glucans may be used as markers of microbiological contamination in this type of occupational environment. Moreover, the measurements of endotoxins and (1→3)-β-D-glucans in PM_{10} could assist in more precisely assessing the exposure to microbial hazards.

Due to extremely high levels of biologically-active particles (such as endotoxins and (1→3)-β-D-glucans), both proper prevention and control measures (including personal and stationary bioaerosol sampling) need to be applied to efficiently protect workers' health. Nevertheless, further investigations are required to establish widely-accepted guidelines for hygienic evaluation in this type of farming environment.

Acknowledgements

This study was funded by CIOP-PIB research project no. I-34 "Exposure assessment to immunologically reactive polymeric microbial compounds in poultry farms and in office buildings". The authors thank G. Ściagała, G. Sąkol, B. Łudzeń-Izbińska and A. Harkawy for their valuable assistance in this research.

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