Medycyna Pracy 2012;63(1):1–10

http://medpr.imp.lodz.pl

ORIGINAL PAPER

Małgorzata Sowiak
Karolina Bródka
Anna Kozajda
Alina Buczyńska
Irena Szadkowska-Stańczyk

FUNGAL AEROSOL IN THE PROCESS OF POULTRY BREEDING – QUANTITATIVE AND QUALITATIVE ANALYSIS

AEROZOL GRZYBOWY W PROCESIE HODOWLI DROBIU – ANALIZA ILOŚCIOWA I JAKOŚCIOWA

Nofer Institute of Occupational Medicine, Łódź
Department of Environmental Health Hazards

Abstract

Background: The aim of this study was to assess fungal air contamination in the processes associated with poultry breeding depending on the season. The evaluation was based on the determined concentrations of fungi and qualitative identification of isolated microorganisms. **Materials and Methods:** The study covered 2 hatcheries and 3 hen buildings. The air was sampled in spring, summer and autumn directly onto a filter using air aspirator. For the quantitative analysis of fungi, the medium MEA with chloramphenicol and streptomycin was used. The qualitative identification of fungi was carried out based on macro- and microscopic analysis. **Results:** The concentrations of total airborne mesophilic fungi in breeding facilities ranged from 1.22×10^3 to 5.87×10^5 cfu/m^3 with the arithmetic mean value 1.60×10^5 cfu/m^3. In 45% of the taken samples, these levels exceeded the reference value recommended in Poland for occupational environment exposure. The fungi concentration in the air of poultry houses was significantly modified by season ($p = 0.04$). A higher concentration of fungi occurred in autumn ($p = 0.05$). The dominant fungal microflora in the air was composed of molds (88%), with the most prevalent genus *Acremonium*. Yeasts constituted another 10% of bioaerosol and were mainly represented by genus *Candida*. The fungal aerosol contained two species qualified to the 2 group of risk – *Aspergillus fumigatus* and *Candida tropicalis*. **Conclusions:** Facilities of poultry farms are contaminated with high concentrations of fungal aerosols, especially in a colder season, often exceeding the recommended limits. Among the fungi, there are also present pathogenic microorganisms that may pose a risk to farm workers' health. Med Pr 2012;63(1):1–10

Key words: fungal aerosol, poultry breeding, occupational exposure, season

Streszczenie

Wstęp: Celem badania była ocena zanieczyszczenia powietrza grzybami w procesach związanych z hodowlą drobiu w zależności od pory roku. Oceny tej dokonano na podstawie oznaczonych stężeń grzybów oraz identyfikacji jakościowej wyizolowanych mikroorganizmów. **Materiał i metody:** Badaniem objęto 2 wylęgarnie i 3 kurniki. Powietrze pobierano wiosną, latem i jesienią bezpośrednio na filtr za pomocą pompki. Do analizy ilościowej grzybów wykorzystano pożywkę MEA z chloramfenikolem i streptomycyną. Identyfikację jakościową grzybów prowadzono w oparciu o analizę makro- i mikroskopową. **Wyniki:** Ogólne stężenia grzybów mezofilnych w powietrzu pomieszczeń hodowlanych mieściły się w zakresie $1,22\times10^3$–$5,87\times10^5$ jtk/m^3 ze średnią wartością arytmetyczną $1,60\times10^5$ jtk/m^3. W 45% pobranych prób poziomy te przekraczały zalecaną w Polsce wartość referencyjną dla narażenia w środowisku pracy. Stężenie grzybów w pomieszczeniach hodowlanych dla drobiu było istotnie modyfikowane przez porę roku ($p = 0,04$). Wyższe stężenie grzybów występowało jesienią ($p = 0,05$). Dominującą mikroflorę grzybową w powietrzu stanowiły pleśnie (88%) z najczęściej występującym rodzajem *Acremonium*. Drożdżaki stanowiły kolejne 10% bioaerozolu i reprezentowane były głównie przez rodzaj *Candida*. Aerozol grzybowy zwierał dwa gatunki zakwalifikowane do drugiej grupy zagrożenia – *Aspergillus fumigatus* i *Candida tropicalis*. **Wnioski:** Pomieszczenia ferm drobiowych zanieczyszczone są wysokimi stężeniami aerozolu grzybowego, szczególnie w chłodniejszej porze roku, często przekraczającymi zalecane limity. Wśród grzybów obecne są również drobnoustroje patogeniczne, które mogą stanowić zagrożenie dla zdrowia pracowników ferm. Med. Pr. 2012;63(1):1–10

Słowa kluczowe: aerozol grzybowy, hodowla drobiu, narażenie zawodowe, pora roku

Authors' address: Department of Environmental Health Hazards, Nofer Institute of Occupational Medicine, ul. św. Teresy 8, 91-348 Łódź, e-mail: msowiak@imp.lodz.pl
Received: 13 December 2011
Accepted: 13 January 2012

The project was financed with a grant for statutory activity IMP 3.2/2009 titled "Assesment of the influence of biological agents levels and irritant gases levels on the activity of the respiratory system in workers employed in poultry breeding". Project leader: Dr. Alina Buczyńska.

INTRODUCTION

The air inside buildings where intensive animal breeding takes place is usually contaminated with high concentrations of microorganisms (1–6). The highest levels of microorganisms among various sectors dealing with animal production were found in poultry breeding (4,5). According to the existing studies, bacteria are the dominant microorganisms in the poultry house bioaerosols, whose concentrations reach as much as 10^9 cfu/m^3 (4), but also fungi constitute a significant part of the airborne microflora in this sector. Their concentrations in stationary measurements usually range from 10^2 to 10^4 cfu/m^3 (2,5,7–10), whereas in personal measurements for poultry farm workers – they are contained within 10^4–10^8 cfu/m^3 (3,4). The fungal aerosol in breeding buildings often contains molds from the genera: *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Rhizopus*, *Scopulariopsis* and *Trichophyton* (2,8,11). Both viable forms of these fungi and their products (mycotoxins) or components ((1→3)-β-D-glucans), as well as fungal spores may cause a number of disorders in poultry breeding workers, concerning mainly the respiratory tract (mucous membrane irritation, invasive mycoses of lungs, allergic rhinitis, allergic pulmonary alveolitis, asthma) and the skin (dermatomycoses and onychomycosis) (12–15).

The presented article was aimed at evaluation of fungal aerosol in the processes related to poultry breeding taking into account the season. The evaluation was based on the determined concentrations of fungi and qualitative identification of isolated microorganisms.

MATERIALS AND METHODS

Facilities Characteristic

In the first part of the study, the owners of poultry farms from central Poland, associated in the regional organization of the poultry and pigs producers, were invited to participate in the study. Among the production facilities, whose owners have agreed to this participation, five buildings were included under the study. These buildings were indicated by a health and safety specialist representing the owners as the most typical ones for the industrial production of poultry and eggs in this region. Two of those buildings were used as hatcheries and the other three buildings – for hen fattening and for industrial production of eggs. The investigations were carried out from April to November 2009 in three measurement series. In April (spring), the average monthly temperature in the region under the study was 11°C and in sampling days 14.6°C, while in August (summer) and in November (autumn) these temperatures were respectively 18 and 23.4°C and 6.5 and 7.4°C. In the first series (spring), the measurements were carried out in all of the five buildings and at one point outside (background); in two consecutive series (summer, autumn) – only in three buildings and at one point outside. Hatchery buildings were characterized by: a small room (approximately 30 m^2) without a litter bed system, the average temperature of 23.2°C, and in a non-summer season additional central heating. In the hatchery, chickens stayed for 2 days, whereas in the other buildings – from 3 days to 64 weeks. The hen houses covered by the study were comparable in view of both construction and building materials. The rooms were equipped with an automatic drinking and feeding system. The litter bed system (straw) was used in the rooms. The floorage of each buildings amounted to approximately 1000 m^2 for 6500 hens. The buildings microclimate parameters (mainly temperature) were maintained by mechanical ventilation. The ventilators' efficiency in each of the buildings amounted to 4000 m^3/hour. In summer, mechanical ventilation was supported by natural ventilation (opened gates in the buildings). The hen buildings were not additionally heated. Each consecutive production cycle was preceded by removal of the bedding and cleaning and disinfection of the building.

Air Sampling and Measuring Microclimate Parameters

The sampling strategy was based on Polish Standards (16,17). In view of the expected high concentrations of fungal microflora in breeding facilities, a filtration method was used in this study. Indoor and outdoor air samples for determining concentrations of fungi were collected using the measuring sets consisting of a GilAir 5 pump (Sensidyne, Clearwater, Florida, USA) and the open-faced aerosol sampler (Two-Met, Zgierz, Poland), with a GF/A filter (Whatman International Ltd, Maidstone, Kent, UK) of a 37 mm diameter. The sets functioned at a flow rate of 2 l/min. The measuring sets were calibrated before each sampling procedure, using a Gillibrator 2 calibrator (Sensidyne, Clearwater, Florida, USA). The equipment was placed at the height of 1.5 meters above the floor. The sampling took 4–6 hours. Samples were collected in 2 repetitions.

After the sampling, the filters with collected biological material were put, using sterile tweezers, into tightly closed containers with Stuart-Ringertz Medium (Sigma-Aldrich Chemie GmbH, Munich, Germany) and transported to the laboratory. Then, the filters in the containers with the transport medium were covered with 10 ml of Phosphate Buffer Solution (BTL, Łódź, Poland) and by shaking on a platform shaker (shaking time: 50 minutes, shaking rate: 420 revolutions per minute) the biological material on the filters was eluted. A series of 10-fold dilutions was made from the obtained eluates. Plates with Malt Extract Agar supplemented with streptomycin and chloramphenicol (GRASO, Starogard Gdański, Poland) were inoculated with given volumes of eluates and their dilutions by a superficial method. In order to determine the total number of fungi, agar plates were incubated at 30°C for 5 days. The colonies which grew on the plates were calculated, and bearing in mind that the degree of the sample dilution and the volume of aspired air, the obtained fungi concentration was expressed as the number of colony forming units in 1 m^3 of the examined air (cfu/m^3). At least one colony of each visually apparently different type of colony from indoor air samples was selected for subculture and identification. The isolated fungi were identified to the genus and species level on the basis of colonial morphology on diagnostic media and on microscopic morphology by keys to identification (18–26) and also using biochemical tests API.

During indoor bioaerosol sampling at the same point, the basic parameters of microclimate, such as: temperature, relative humidity, CO_2 concentration and airflow velocity were measured. The measurements were carried out using the microclimate multifunction meter Testo 435-2 (Testo AG, Lenzkirche, Germany) at the height of 1.5 m over the floor during 10 minutes. The values of individual parameters were read out every minute, then the result was averaged for a given measurement point.

Statistical Analysis

Concentrations of airborne fungi in breeding facilities depending on the type of building and season were characterized by using arithmetic mean (AM), standard deviation (SD) and the range of the observed values. The values of microclimate parameters were characterized by using arithmetic mean (AM). To determine the effects of seasonality on the level of fungal aerosol univariate analysis was used. Thus, the obtained dimensionless data was statistically analyzed using one-way ANOVA. Comparisons of the average concentrations of fungal microflora and the average values of microclimate parameters to the different seasons were made using the least significance difference test of Fischer. To analyze the seasonal differences data from the buildings from 3 to 5 was used. Statistically significant differences between the concentrations of fungi in hatcheries and hen buildings in spring season were also assessed. Significant differences between the groups were evaluated using post hoc analysis by means of the Tukey's test, at $p < 0.05$ selected for statistical significance (27). Statistical calculations were made with the package Statistica version 8.

RESULTS

In hatcheries, the average values of temperature, relative humidity, CO_2 concentration and airflow velocity were respectively 23.2°C, 57%, 1410 ppm and 0.03 m/s. The average temperature inside the hen buildings in the surveyed seasons reached 22.2°C, 23.7°C and 19.1°C respectively in spring, summer and autumn. In case of relative humidity and CO_2 concentration, the average values of those parameters were at the level of 47.0% and 1263 ppm in I measuring series (spring), 84.1% and 1128 ppm in II series (summer) and 67.5% and 1473 ppm in III series (autumn). The average airflow velocity in the hen buildings in spring amounted to 0.50 m/s, in summer – 0.87 m/s, and in autumn – 0.35 m/s. The statistical analysis indicated significant differences between the levels of relative humidity in hen buildings in the surveyed seasons ($p < 0.001$). A significantly higher level of relative humidity occurred in summer as compared to spring or autumn ($p < 0.01$) (tab. 1).

The concentrations of airborne culturable fungi determined in poultry breeding houses and outside of those facilities as related to the type of building and season are presented in Table 2.

The total concentration of airborne mesophilic fungi in poultry breeding houses was high – within 1.22×10^3–5.87×10^5 cfu/m^3 with the mean value 1.60×10^5 cfu/m^3, which was by one order of magnitude higher than the average concentration of these microorganisms outside the breeding facilities (2.67×10^4 cfu/m^3). The lowest average concentration of fungi were noted in the hatcheries (AM = $1.26\text{x}10^3$ cfu/m^3). The average levels of fungal concentrations in the hen buildings were from over 10-fold to 100-fold higher.

Table 1. Average values of microclimate parameters inside poultry breeding houses depending on the type of building and season
Tabela 1. Średnie wartości parametrów mikroklimatu wewnątrz pomieszczeń hodowlanych dla drobiu w zależności od typu budynku i pory roku

Season Pora roku	Type of building Typ budynku	N	Microclimate parameters Parametry mikroklimatu							
			T [°C]	*p*	H [%]	*p*	CO_2 [ppm]	*p*	AV [m/s]	*p*
			AM		AM		AM		AM	
Spring / Wiosna	hatchery / wylęgarnia	2	23.2	–	57.0	–	1410	–	0.03	–
	hen building / kurnik	3	22.2		47.0		1263		0.50	
Summer / Lato	hen building / kurnik	3	23.7		84.1		1128		0.87	
				> 0.05		< 0.01		> 0.05		> 0.05
Autumn / Jesień	hen building / kurnik	3	19.1		67.5		1473		0.35	

N – number of measurements / liczba pomiarów.
T – temperature / temperatura.
H – relative humidity / wilgotność względna.
CO_2 – concentration of CO_2 / stężenie CO_2.
AV – airflow velocity / prędkość przepływu powietrza.
AM – arithmetic mean / średnia arytmetyczna.
p – *p*-value for seasonal differences (without hatcheries) / wartość *p* dla różnic w sezonach (bez wylęgarni).

Table 2. Concentrations of airborne fungi inside and outside of the poultry breeding houses depending on the type of building and season
Tabela 2. Stężenia grzybów w powietrzu wewnątrz i na zewnątrz pomieszczeń hodowlanych dla drobiu w zależności od typu budynku i pory roku

Season Pora roku	Type of building Typ budynku	Indoor concentration of total fungi ×10² [cfu/m³] Stężenie grzybów ogółem wewnątrz budynków ×10² [jtk/m³]						Outdoor concentration of total fungi ×10² [cfu/m³] Stężenie grzybów ogółem na zewnątrz budynków ×10² [jtk/m³]	
		N	AM (SD)	MIN	MAX	P^1	P^2	N	AM
Spring / Wiosna	hatchery / wylęgarnia	2	12.56 (0.44)	12.24	12.87		–	1	0.35
	hen building / kurnik	3	920.99 (1278.74)	68.35	2 391.30	0.41			
Summer / Lato	hen building / kurnik	3	810.16 (784.74)	263.93	1 709.40	–	0.04	1	150.94
Autumn / Jesień	hen building / kurnik	3	4 121.85 (1 849.70)	2 182.08	5 865.92	–		1	650.68
Total / Ogółem	hatcheries and hen buildings / wylęgarnie i kurniki	11	1 598.56 (1 967.70)	12.24	5 865.92	–	–	3	267.33

N – number of measurements (each in 2 repetitions) / liczba pomiarów (każdy w 2 powtórzeniach).
AM – arithmetic mean / średnia arytmetyczna.
SD – standard deviation / odchylenie standardowe.
MIN – minimal value of the range / minimalna wartość zakresu.
MAX – maximal value of the range / maksymalna wartość zakresu.
p – level of statistical significance: p^1 – *p*-value for hatcheries versus hen buildings in spring season, p^2 – *p*-value for seasonal differences (without hatcheries) / poziom istotności statystycznej: p^1 – wartość *p* dla wylęgarni w porównaniu do kurników w okresie wiosennym, p^2 – wartość *p* dla różnic pomiędzy porami roku (bez wylęgarni).

The analysis of the levels of fungal microorganisms depending on the season indicated that the average airborne fungi concentration in the breeding facilities (without hatcheries) was at a similar level in spring and summer (respectively 9.21×10^4 cfu/m^3 and 8.10×10^4 cfu/m^3) with a slight increase of that value during spring. The average concentration of fungal aerosol in autumn was by one order of magnitude higher than in spring and summer and amounted to 4.12×10^5 cfu/m^3. In comparison with the concentrations of fungi obtained in the outdoor air, the level of these microorganisms in hatcheries in spring was 100-fold, and in hen houses – 1000-fold higher than in the bioaerosol of the background (3.55×10^1 cfu/m^3), and in autumn – 10-fold higher (the outdoor fungal concentration: 6.50×10^4 cfu/m^3). In summer, the concentration of airborne fungi inside was only 5-fold higher than outside the facilities.

In the spring season, no statistically significant differences were observed between the concentrations of fungi in hatcheries and hen buildings ($p = 0.41$). The variance analysis indicated a significant impact of season on the concentrations of total fungi ($p = 0.04$) in the environment of the poultry breeding facilities. A comparison of fungal microflora concentrations for different seasons revealed that the fungal aerosol was characterized by higher concentration in autumn, as compared to spring or summer (on the border of statistical significance $p = 0.05$). The p-value for significance differences in the concentrations of fungi depending on the type of building and season are shown in Table 2.

The results of qualitative identification of fungal aerosol in poultry breeding facilities are presented in Table 3.

Table 3. The qualitative composition and percentage structure of fungal aerosol in poultry breeding facilities
Tabela 3. Skład jakościowy i procentowa struktura aerozolu grzybowego w pomieszczeniach hodowlanych dla drobiu

Genus/species Rodzaj/gatunek	Average concentration of genus/ /species in fungal aerosol $\times10^2$ [cfu/m^3] Średnie stężenie rodzaju/gatunku w aerozolu grzybowym $\times10^2$ [jtk/m^3] (N = 11)	Percentage of genus/species in fungal aerosol Procent rodzaju/gatunku w aerozolu grzybowym [%] (N = 11)	Risk group* Grupa ryzyka
Yeasts / Drożdże	–	9.69	–
Candida famata	12.00	0.75	–
Candida pelliculosa	53.86	3.37	–
Candida tropicalis	3.95	0.25	2
Candida zeylanoides	75.97	4.75	–
Cryptococcus humicola	2.67	0.17	–
Cryptococcus laurentii	1.31	0.08	–
Rhodotorula mucilaginosa	3.90	0.24	–
Sporobolomyces salmonicolor	1.21	0.08	–
Mold fungi / Grzyby pleśniowe	–	87.92	–
Acremonium strictum	755.28	47.25	–
Acremonium spp.	57.24	3.58	–
Alternaria sp.	0.98	0.06	–
Arthrinium spp.	1.64	0.10	–
Aspergillus candidus	0.09	0.01	–
Aspergillus fumigatus	6.50	0.41	2 A
Aspergillus niger	0.48	0.03	–

Table 3. The qualitative composition and percentage structure of fungal aerosol in poultry breeding facilities – cont.
Tabela 3. Skład jakościowy i procentowa struktura aerozolu grzybowego w pomieszczeniach hodowlanych dla drobiu – cd.

Genus/species Rodzaj/gatunek	Average concentration of genus/ /species in fungal aerosol $\times 10^2$ [cfu/m^3] Średnie stężenie rodzaju/gatunku w aerozolu grzybowym $\times 10^2$ [jtk/m^3] (N = 11)	Percentage of genus/species in fungal aerosol Procent rodzaju/gatunku w aerozolu grzybowym [%] (N = 11)	Risk group* Grupa ryzyka
Aspergillus paradoxus	63.54	3.98	–
Aspergillus versicolor	45.04	2.82	–
Aspergillus spp.	6.97	0.44	–
Cladosporium sphaerospermum	0.10	0.01	–
Eurotium amstelodami	0.05	0.003	–
Fusarium solani	1.30	0.08	–
Geotrichum spp.	204.48	12.79	–
Chaetomium spp.	0.63	0.04	–
Mucor racemosus	28.50	1.78	–
Nigrospora sp.	0.33	0.02	–
Oidiodendron sp.	1.52	0.09	–
Penicillium aurantiogriseum	117.01	7.32	–
Penicillium brevicompactum	0.05	0.003	–
Penicillium chrysogenum	0.24	0.01	–
Penicillium citrinum	0.14	0.01	–
Penicillium glabrum	1.31	0.08	–
Penicillium spp.	13.06	0.82	–
Scopulariopsis brevicaulis	93.71	5.86	–
Scopulariopsis candida	5.29	0.33	–
Other / Pozostałe	–	2.39	–
Total / Ogółem	–	100.00	–

N – number of measurements / liczba pomiarów.
* Classification according to the ordinance issued by the Minister of Health on 22 April 2005 („–" – not classified as 2–4 risk group; „2" – 2 risk group – agent which can cause human disease and might be a hazard to workers; it is unlikely to spread to the community; there is usually effective prophylaxis or treatment available; A – possible allergic effects) (28) / klasyfikacja według Rozporządzenia Ministra Zdrowia z dnia 22 kwietnia 2005 r. („–" – niesklasyfikowany jako 2.–4. grupa zagrożenia; „2" – 2. grupa zagrożenia – czynnik, który może wywoływać choroby wśród ludzi i może być niebezpieczny dla pracowników; jego rozprzestrzenianie się w społeczeństwie jest mało prawdopodobne; zazwyczaj istnieją skuteczne metody profilaktyki lub leczenia; A – możliwe efekty alergiczne) (28).

The analysis of fungal aerosol in breeding facilities indicated the presence of 34 species of fungi belonging to 18 genera. 4 genera of yeasts were identified: *Candida*, *Cryptococcus*, *Rhodotorula* and *Sporobolomyces,* as well as 14 genera of molds: *Acremonium*, *Alternaria*, *Arthrinium*, *Aspergillus*, *Cladosporium*, *Eurotium*, *Fusarium*, *Geotrichum*, *Chaetomium, Mucor*, *Nigrospora*, *Oidiodendron*, *Penicillium* and *Scopulariopsis*. While the share of yeasts in the entire pool of the determined fungi reached only 9.7%, molds constituted 87.9% in that pool. Furthermore, some environmental species were found, which could not be explicitly identified.

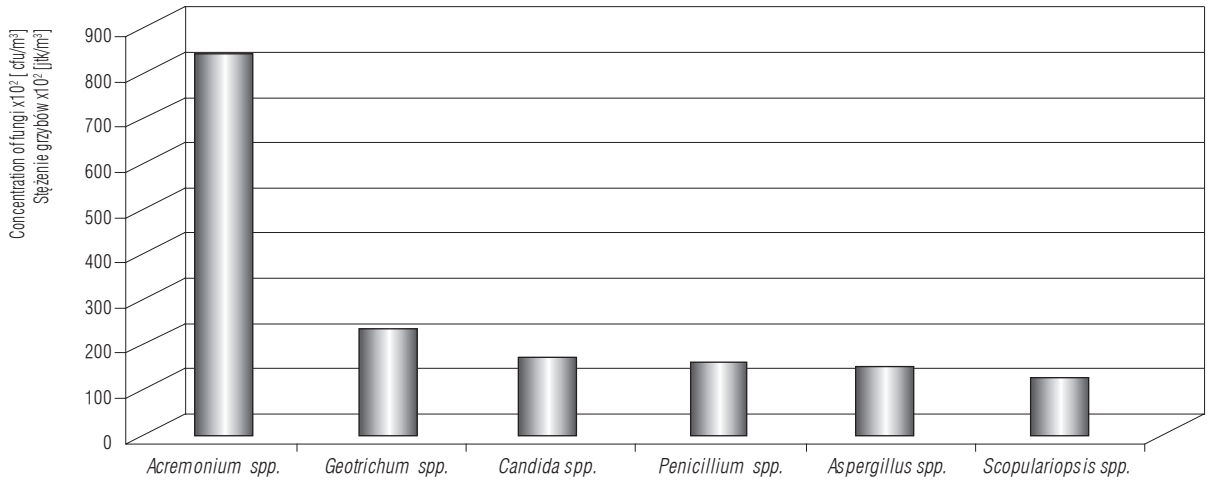

Fig. 1. The average concentration of the dominant fungi genera in the bioaerosol of poultry breeding houses
Ryc. 1. Średnie stężenie dominujących rodzajów grzybów w bioaerozolu pomieszczeń hodowlanych dla drobiu

In the fungal aerosol of poultry breeding houses, the molds of *Acremonium* genus prevailed, comprising over 50% of all the determined species. In addition to this, a significant part constituted the fungi of genera: *Geotrichum* (12.8%), *Candida* (9.1%), *Penicillium* (8.3%), *Aspergillus* (7.7%) and *Scopulariopsis* (6.2%). The share of the other isolated genera did not exceed 2%. Figure 1 presents the average concentrations of the dominant fungi genera in the bioaerosol of breeding facilities. The concentration of *Acremonium* species reached 8.13×10^4 cfu/m^3, whereas the concentrations of *Geotrichum*, *Candida*, *Penicillium*, *Aspergillus* and *Scopulariopsis* genera were several times lower (9.90×10^3–2.04×10^4 cfu/m^3). The average concentration of the other identified genera was within 4.64––2.85×10^3 cfu/m^3.

As regards the species structure of the fungal aerosol in the poultry breeding houses, the molds of *Acremonium strictum* species prevailed (47.3%). The average concentration of these fungi was high, at the level of 7.55×10^4 cfu/m^3. The *Geotrichum* species came second (12.8%) with a 4-fold lower concentration in bioaerosol (2.04×10^4 cfu/m^3), to be followed (7.3%) by molds *Penicillium aurantiogriseum*. The concentration of this species amounted to 1.17×10^4 cfu/m^3. These were followed by molds *Scopulariopsis brevicaulis* (5.9%) and yeasts *Candida zeylanoides* (4.8%) exhibiting only slightly lower average concentrations, respectively: 9.37×10^3 cfu/m^3 and 7.60×10^3 cfu/m^3. The concentration of the other identified species was within 4.64–6.35×10^3 cfu/m^3. Within this group, two species of fungi were isolated which were qualified to the 2 group of hazardous biological agents which can pose risk to the health in occupational environment (according to the ordinance issued by the Polish Ministry of Health on 22 April 2005 (28) and harmonized to the EU Directive 2000/54/EC (29)). These were *Aspergillus fumigatus* and *Candida tropicalis*. Their average concentrations were respectively at the level of 6.50×10^2 cfu/m^3 and 3.95×10^2 cfu/m^3, which constituted 0.4% and 0.3% of the total airborne fungal microflora in the breeding facilities.

DISCUSSION

The performed study indicates that the air inside the breeding facilities connected with industrial breeding of poultry contained high concentrations of mesophilic fungi of the order of 10^3–10^5 cfu/m^3. In 45% of the taken samples, these levels considerably exceeded the reference value recommended in Poland for occupational environment exposure (5.0×10^4 cfu/m^3), as proposed by Dutkiewicz and Mołocznik (30). These concentrations were also higher than the levels found with various measuring methods in poultry houses by other researchers and which ranged from 5.00×10^2 to 8.50×10^4 cfu/m^3 (2,5,7–10,31).

Considering the type of a breeding building, the lowest concentrations of total fungi were observed, similarly to the findings of other authors (31), in hatcheries (buildings with the 1- and 2–day old chickens).

In the facilities with older flocks (hen buildings), the level of microorganisms was increased by one or two orders of magnitude. In those facilities, the reference value of fungi concentrations was exceeded even by 10-fold. A probable explanation for this may be the fact that in the hen houses (in contrast to the hatcheries) the litter bed system was used. According to the results obtained in poultry breeding houses by Witkowska et al. (32), the bedding material can be an excellent medium for the development of fungi and constitute their source.

The performed variance analysis demonstrated a significant impact of a season on fungi concentrations in poultry breeding houses. In autumn, a significantly higher concentration of fungal aerosol was found, as compared to spring or summer. In spring and summer, the average level of fungal microflora only slightly exceeded the above-mentioned reference value recommended in Poland for occupational environment exposure, but in autumn it was by as much as one order of magnitude higher than that value. A reason for this could be higher humidity (above 67%) and moderate temperature (19°C) prevalent in poultry breeding houses in autumn, considering that these conditions are particularly good for the development of fungi. Although in summer in similar temperatures the relative humidity value in breeding facilities was even higher (above 84%), in that period the mechanical ventilation of the facilities was additionally supported by the air exchange through opened doors of poultry houses, which increased the airflow rate. That procedure could contribute to a more intensive removal of any fungal contaminants from the poultry houses air and consequently to a decrease in their level in the breeding facilities bioaerosol.

The fungal microflora isolated in poultry breeding facilities was largely diversified. A similar diversity was observed by other researchers who surveyed poultry houses (4,8,10). Furthermore, consistently with the data presented in their studies, the filamentous fungi prevailed in the breeding facilities bioaerosol. The dominant fungal microflora in the air inside the investigated facilities were mainly molds of *Acremonium* and *Geotrichum* genera. These fungi are associated with humid environment or soil and comprise mainly saprophytic species. Only in special cases, in subjects with particularly deficient immunity, some species may induce an invasive infection in the pathologically changed epidermis, as well as infections of oral cavity, respiratory tract or lungs (18,21,23,25,26). These results differ from the results presented in other studies where these genera were not identified at all (8), or they were not included in the dominant fungal microflora in the air of the breeding facilities (2,7,9,10).

A considerable percentage share in the fungal aerosol of poultry breeding houses constituted also *Aspergillus* and *Penicillium* genera, which in most studies are mentioned as the dominant microflora in the hen house air (2,4,7–10). These genera comprise many saprophytic species, as well as pathogens. *Penicillium* genus seldom constitutes an etiological factor of human organism infections, however their conidia may exhibit allergic effects. Similarly, some filamentous fungi of *Aspergillus* genus may induce aspergilloses and allergic symptoms (18,21,23,26,33).

Other fungi genera, distinguished by the levels of concentrations in bioaerosol of the investigated breeding facilities, i.e. *Candida* and *Scopulariopsis* genera, according to literature data were also isolated from the air inside hen houses, but they not always made the dominant fungal microflora (4,9). Yeasts of genus *Candida* are ranked among opportunistic pathogens which only in specific conditions (e.g. deficient immunity system) may induce various types of infections and diseases (23,25). On the other hand, the *Scopulariopsis* genus comprises a dozen or so species occurring mainly in soil and on decaying plant remains, as well as on the surface of stored cereal grains. In people, the molds of this genus may induce various skin diseases (dermatomycoses) and nail infections (18,23,25,26).

The fungal aerosol in the investigated breeding facilities was found to contain two species belonging to the 2 group of biological agents that can pose risks in the working environment according to the ordinance issued by the Minister of Health on 22 April 2005 on occupational biological agents and health protection of people occupationally exposed to such agents (28). These were the species of *Aspergillus fumigatus* and *Candida tropicalis*. These fungi may induce diseases (mycoses) in humans but the possibilities of them spreading in human population are limited. Besides, there usually are some relevant effective methods to prevent or treat them (28). These species constitute a real risk to people with deficient immunity (e.g. those with AIDS or following a recent chemotherapy) or people particularly susceptible to mycotic infections. *Aspergillus fumigatus* is a thermophilic fungus which in natural environment occurs in soil and on decaying plants. Therefore, it is often isolated from compost and humid hay. In human environment, it was also found on stored humid grain, on damp buildings and finishing materials, on the sur-

face of ventilators and in settled dust. This species is a well-known human and animal pathogen. In people, it is a cause of systemic mycoses resulting from infections of lungs or other parts of respiratory tract induced by spores of this fungus. *A. fumigatus* also induces allergic effects. In animals, it is often isolated from wounds of birds and mammals, whereas in poultry respiratory organs it causes tuberculosis-like diseases. Moreover, this species produces various mycotoxins, including a very toxic gliotoxin, which may induce mycotoxicosis (hemoragia) in cattle (18,23–26,33). *Candida tropicalis* is a common human pathogen which causes many candidiases and infections, especially in people with a deficient immunity system, following long treatment with antibiotics, diabetic patients and people intravenously taking drugs (25).

The other species of fungi identified in the air of the breeding facilities were characterized by low hazardous effects or no such effects at all, so they cannot constitute any significant risks for the health of people with correct immunity levels.

The number of samples taken in our study was limited. Therefore it is not possible to generalize the conclusions about the differences in bioaerosols concentrations in the poultry houses. However, after careful analysis we drew the below-presented, though limited, conclusions.

CONCLUSIONS

- The air inside the facilities where industrial poultry breeding was conducted contained high concentrations of fungi, often exceeding the reference limit value for working facilities.
- The level of fungal aerosol in the poultry breeding houses was significantly modified by a particular season. The highest concentration of fungi was found in autumn.
- The dominant fungal microflora in the air of poultry breeding facilities were molds (88%), with the most abundant *Acremonium* genus. Yeasts constituted another 10% of fungal aerosol and were mainly represented by *Candida.*
- The fungal aerosol in the breeding facilities contained two species qualified to the 2 group of risk – *Aspergillus fumigatus* and *Candida tropicalis*.
- The work connected with intensive production of poultry, poses a risk for workers and requires the use of personal protection measures, especially for the respiratory tract and skin.

ACKNOWLEDGMENTS

We want to give special thanks to our colleague Marcin Cyprowski, PhD, who worked in the Nofer Institute of Occupational Medicine, for participation and assistance in carrying out the measurements in the poultry houses as well as to the owners of poultry farms and a health and safety specialist representing them for enabling the measurements to be conducted and creating a favorable atmosphere during their implementation.

REFERENCES

1. Dutkiewicz J, Pomorski ZJH, Sitkowska J, Krysińska-Traczyk E, Skórska C, Prażmo Z et al. Airborne microorganisms and endotoxin in animal houses. Grana 1994;33(2):85–90. DOI: 10.1080/00173139409427837
2. Jo WK, Kang JH: Exposure levels of airborne bacteria and fungi in Korean swine and poultry sheds. Arch Environ Health 2005;60(3):140–6
3. Lee S-A, Adhikari A, Grinshpun SA, McKay R, Shukla R, Reponen T: Personal Exposure to Airborne Dust and Microorganisms in Agricultural Environments. J Occup Environ Hyg 2006;3:118–130. DOI: 10.1080/15459620500524607
4. Radon K, Danuser B, Iversen M, Monso E, Weber C, Hartung J et al.: Air contaminants in different European farming environments. Ann Agric Environ Med 2002;9:41–8
5. Seedorf J, Hartung J, Schröder M, Linkert KH, Phillips VR, Holden MR et al: Concentrations and emissions of airborne endotoxins and microorganisms in livestock buildings in Northern Europe. J Agric Eng Res 1998;70:97–109
6. Sowiak M, Bródka K, Buczyńska A, Cyprowski M, Kozajda A, Sobala W et al: An assessment of potential exposure to bioaerosols among swine farm workers with particular reference to airborne microorganisms in the respirable fraction under various breeding conditions. Aerobiologia. DOI: 10.1007/s10453-011-9216-0
7. Vučemilo M, Matković K, Vinković B, Jakšić S, Granić K, Mas N: The effect of animal age on air pollutant concentration in a broiler house. Czech J Anim Sci 2007;52(6):170–74
8. Lugauskas A, Krikstaponis A, Sveistyte L: Airborne fungi in industrial environments – potential agents of respiratory diseases. Ann Agric Environ Med 2004;11(1):19–25
9. Rimac D, Macan J, Varnai VM, Vučemilo M, Matković K, Prester L et al: Exposure to poultry dust and health effects in poultry workers: impact of mould and mite allergens. Int Arch Occup Environ Health 2010;83(1):9–19. DOI: 10.1007/s00420-009-0487-5

10. Karwowska E: Microbiological air contamination in farming environment. Pol J Environ Stud 2005;14(4):445–49
11. Gemeinhardt H, Wallenstein G: Occurrence of molds in airborne dusts in poultry farms. Zentralbl Mikrobiol 1985;140:375–79
12. Śpiewak R: Zoophilic and geophilic fungi as a cause of skin disease in farmers. Ann Agric Environ Med 1998;5:97–102
13. Lacey J, Dutkiewicz J: Bioaerosols and occupational lung disease. J Aerosol Sci 1994;25(8):1371–404. DOI: 10.1016/0021-8502(94)90215-1
14. Dutkiewicz J, Śpiewak R, Jabłoński L: Classification of occupational biohazards and exposed worker groups [in Polish]. Lublin: Instytut Medycyny Wsi; 1999
15. Schenker MB, Christiani D, Cormier Y, Dimich-Ward H, Doekes G, Dosman J et al: Respiratory health hazards in agriculture. Am J Respir Crit Care Med Suppl 1998;158(5):1–76
16. Polish Standard PN-EN 13098:2007. Workplace atmosphere – Guidelines for measurement of airborne microorganisms and endotoxin. Warszawa: Polish Standard (Polski Komitet Normalizacyjny); 2007
17. Polish Standard PN-EN 14042:2004. Workplace atmospheres – Guide for the application and use of procedures for the assessment of exposure o chemical and biological agents. Warszawa: Polish Standard (Polski Komitet Normalizacyjny); 2004
18. Fassatiová O: The microscopic fungi in technical microbiology [in Polish]. Warszawa: Wydawnictwo Naukowo-Techniczne; 1983
19. Watanabe T: Pictorial Atlas of soil and seed fungi: morphologies of cultured fungi and key to species., Boca Raton, FL (USA): Lewis Publishers; 1994
20. Fisher F, Cook NB: Fundamentals of Diagnostic Mycology. Philadelphia, PA (USA): WB Saunders; 1998
21. Piontek M: Moulds [in Polish]. Wydawnictwo Politechniki Zielonogórskiej, Zielona Góra 1999
22. Pitt JI: A laboratory guide common *Penicillium* species. 3rd edition. North Ryde (Australia): Food Science Australia; 2000
23. Flannigan B, Samson RA, Miller JD [eds.]: Microorganisms in home and indoor work environments. Boca Raton, FL (USA): CRC Press LLC; 2001
24. Klich MA: Identification of common *Aspergillus* species. Centraalbureau voor Schimmelcultures, Utrecht (The Netherlands) 2002
25. Larone DH: Medically important fungi. A guide to identification. 4th edition. Washington D.C.: ASM Press; 2002
26. Samson RA, Hoekstra ES, Frisvad JC: Introduction to food- and airborne fungi. 7th edition. Utrecht (The Netherlands): Centraalbureau voor Schimmelcultures; 2004
27. Scheffe H: Analysis of Variance. New York: John Wiley & Sons Inc.; 1959
28. Ordinance issued by the Minister of Health on 22 April 2005 on occupational biological hazards and health protection of people occupationally exposed to such hazards [in Polish]. Journal of Laws of 2005, no. 81, item 716 with subsequent amendments (DzU z 2005 r. nr 81, poz. 716)
29. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). OJEC 2000 L 262/21
30. Górny RL: Biohazards: standards, guidelines, and proposals for threshold limit values [in Polish]. Podst Met Oceny Srod Pr 2004;3(41):17–39
31. Sharma D, Dutta BK, Singh AB: Exposure to indoor fungi in different working environments: A comparative study. Aerobiologia 2010;26(4):327–37. DOI: 10.1007/s10453-010-9168-9
32. Witkowska D, Chorąży Ł, Mituniewicz T, Makowski W: Microbiological contamination of litter and air during rearing of broiler chickens [in Polish]. Water Environ Rural Areas 2010;10(2):201–10
33. Libudzisz Z, Kowal K, Żakowska Z [sci. eds.]: Technical Microbiology. Vol. 1. Wydawnictwo Naukowe PWN, Warszawa 2007