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Scientific Paper

AIRBORNE FUNGI IN DWELLINGS FOR DAIRY COWS AND LAYING HENS*

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The air of animal dwellings can contain great amounts of bioaerosol composed of dust, bacteria, fungi, and endotoxins. The composition may depend on animal species, building construction, animal accommodation, and microclimate parameters, to name just a few factors. Pathogens contained may be a serious threat to animal and human health.
The aim of our study was to analyse the fungi aerosol content in a stable housing dairy cows and in a coop for laying hens over the three autumn months of 2007. The air was sampled on Petri dishes with Sabouraud glucose agar. After laboratory treatment, we identified the most common fungi. Their count in the stable ranged from 3.98×10^{3} CFU m^{-3} to 5.11×10^{4} CFU m^{-3} and in the coop from 6.89×10^{4} CFU m^{-3} to 1.13×10^{5} CFU m^{-3}. The difference between the two animal dwellings was statistically different at the level of $p<0.05$. In both dwellings, the most common were the fungi *Aspergillus* sp., *Penicillium* sp., and yeasts, followed by *Cladosporium* sp., *Fusarium* sp., *Mucor* sp., *Scopulariopsis* sp., *Alternaria* sp., and *Rhizopus* sp.
Our results are entirely in line with values reported in literature and are at the lower end of the range. They call for further investigation that would eventually lead to setting air quality standards for animal dwellings and to developing reliable monitoring systems in order to ensure safe food and safe environment.

KEY WORDS: *air hygiene, airborne microorganisms, animal housing, environment*

The air of animal dwellings can have considerable amounts of bioaerosols containing dust, bacteria, fungi, and endotoxins. Their composition depends on the type of construction, animal population, temperature, moisture, and activities performed indoors such as feeding, milking, and collecting eggs.

Intensive animal production is a significant source of air contaminants that may greatly influence animal health and production. In addition, it may also pose occupational and environmental health risk (1-3). The air in any housing type is contaminated with various microorganisms and gases. In addition to the mechanical effects, these pollutants may cause infection, affect the immune system or cause allergies in animals and humans. These effects may be additionally aggravated by poor microclimate, the temperature-humidity complex in particular.

Saprophytes are the most common in these settings, but they may also contain pathogenic bacteria, fungi, and viruses. Eighty percent of the fungi found in animal dwellings air belong to the *Aspergillus* sp. and *Penicillium* sp., followed by *Fusarium* sp., *Cladosporium* sp., *Mucor* sp., and *Alternaria* sp. (4).

The aim of our study was to analyse the content and the composition of fungi in indoor air of a stable

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housing dairy cows and of a laying hen coop, and to establish indoor temperature and humidity as the basic microclimate indicators. This study continues our investigation of airborne pollutants whose aim is to establish acceptable levels and composition of airborne fungi in animal dwellings.

MATERIAL AND METHODS

The stable was 6 m x 8 m x 3 m, classically built, and accommodating 12 cows. The cows stayed in the stable all day long, received the usual fodder (hay, haylage, and concentrate), and were milked twice a day. Milk was instantly taken to the collection point, because this husbandry did not have a cooling device. Fungi were measured at three spots in the feeding corridor.

The coop consisted of conventional cages housing about 17,000 Shaver hybrid laying hens. Feeding, watering, ventilation, lighting, and manure removal were regulated automatically. Fungi measurements started at week 25 of production. Air was sampled at five spots at the second floor level along the central corridor.

Measurements in both dwellings were done 12 times, once a week, between 8:00 h and 12:00 h in the morning, for three months. Ten-litre air samples for determining the fungi content were collected using the air sampler MAS-100 (Merck KgaA, Darmstadt, Germany). Airborne particles were collected on Petri dishes (9 cm in diameter) with a commercially available Sabouraud glucose agar (Biolife, Milan, Italy) and incubated at 22 °C for 5 days in a incubator. Grown colonies (CFU m^{-3}) were counted using a digital colony counter (Selecta, Spain).

Fungal species were identified by their culture properties and micromorphology. Air temperature (°C), relative humidity (%) and airflow rate (m s^{-1}) were determined using a TESTO device (Testo Inc., Lenzkirch, Germany).

For statistical analysis we used Microsoft Excel and Statistica 7 software, and it included descriptive statistics and Wilcoxon's test. The level of significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Reports of air concentrations of microorganisms in poultry housing greatly vary, which may to some extent be related to different sampling methods used in different studies. The concentration of airborne microorganisms in layer housing reported by Hartung (5) ranged from 360 to 3,781 colony forming units per litre (CFU L^{-1}) of air, and by Müller (6) between 17 CFU L^{-1} and 5,860 CFU L^{-1} air. Seedorf et al. (7) reported that the total airborne microorganism concentration in animal housing ranged from 8.3 log CFU h^{-1} per 500 kg body weight (b.w.) in layer houses to 6.5 log CFU h^{-1} per 500 kg b.w. in yearling cattle and milk cow housings. The emission of fungi ranged from 7.7 log CFU h^{-1} per 500 kg b.w. in broiler housing through 5.8 log CFU h^{-1} per 500 kg b.w. in weaned piglet housing. Wathes (8) says that usually there are more than 10^9 CFU m^{-3} of fungi in the air of animal dwellings. Eduard (9) reports that the total count of fungi in a cattle barn can reach 10^5 CFU m^{-3}.

Cows are not as susceptible to environmental influences as other farm animals. Their optimal ambient temperature is between 5 °C and 20 °C at relative humidity between 60 % and 80 % (10, 11) and air flow preferably exceeding 0.30 m s^{-1}. In contrast, the optimum air temperature for laying hens is between 15 °C and 22 °C (12). Temperature outside these limits significantly decreases or can entirely stop egg production. Optimal relative humidity for hens is around 65 % (13).

Fungi counts depend on the sampling method and refer to live fungi. Fungi viability in turn depends on the microclimate, especially on relative humidity. At 55 % to 75 %, most of the fungi can survive for a short time (14).

Ventilation system is the most responsible for air quality in animal dwellings. Objects with artificial microclimate such as layer housings are expected to have a higher number of airborne microorganisms. Microclimate in our dwellings were in the recommended range for these animals (15). Moreover, concentrations of ammonia and carbon dioxide were below reports in literature (16, 17). This suggests good building construction and ventilation system.

Our comparison has shown a statistically significant difference in air quality between the stable and the coop with the conventional cage system (Table 1), particularly in respect to the fungi content, air flow, and ammonia and carbon dioxide concentrations ($p<0.05$). These results are consistent with international data, where fungi counts in animal dwellings range between 10^3 CFU m^{-3} and 10^9 CFU m^{-3} (5, 7-9, 18-

Table 1 *Mean counts of airborne fungi and microclimate parameters in animal dwellings*

Parameters	n	Dairy stable			Layer coop		
		September	October	November	September	October	November
Fungi / CFU m^{-3}	12	$3.98x10^{3(a)}$	$4.12x10^{4(a)}$	$5.11x10^{4}$	$6.89x10^{4(b)}$	$7.13x10^{4(b)}$	$1.13x10^{5(b,c)}$
Air temperature / °C	12	18.4	17.4	15.3	18.9	17.9	17.1
Relative humidity / %	12	74.5	62.7	62.5	65.9	62.8	68.8
Air flow / m s^{-1}	12	0.20^{a}	0.21^{a}	0.16^{a}	0.08^{b}	0.07^{b}	0.08^{b}
Ammonia / %	12	0^{a}	0^{a}	0^{a}	0.0011^{b}	0.0010^{b}	0.0011^{b}
Carbon dioxide / %	12	0.06^{a}	0.09^{a}	0.07^{a}	0.12^{b}	0.17^{b}	0.18^{b}
Lighting / lx	12	20	22	23	17	21	19

n=number of measurements; CFU=colony forming unit; ^{a,b,c}=arithmetic means that do not share the same letter in superscript were statistically significantly different at $p<0.05$

20). Comparable results have also been reported by Croatian authors (11, 21-23). The dominant species in our study, that is, *Aspergillus*, *Penicillium*, and yeasts (Table 2) confirm other reports (4, 5, 7, 19, 24). Both the species and the levels of exposure to them raise a certain concern for the health of animals and humans who in these dwellings (25, 26). This will be the subject of further investigation.

Table 2 *Most common airborne fungi*

Fungi	Dairy stable %	Layer coop %
Alternaria sp.	0.50	0.50
Aspergillus sp.	31.00	30.00
Cladosporium sp.	2.60	5.00
Fusarium sp.	6.30	8.30
Yeasts	23.00	22.00
Mucor sp.	2.30	1.30
Penicillium sp.	25.00	25.00
Rhizopus sp.	8.00	7.83
Scopulariopsis sp.	1.30	0.07

CONCLUSION

The levels and the spectrum of airborne fungi in the investigated dwellings were at the lower end of ranges reported in literature. Our findings call for further investigation that would eventually lead to setting air quality standards for animal dwellings and to developing reliable monitoring systems in order to ensure safe food and safe environment.

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Sažetak

GLJIVICE KAO SASTAVNI DIO BIOAEROSOLA U NASTAMBAMA ZA MUZNE KRAVE I NESILICE KONZUMNIH JAJA

U zraku nastambi za držanje životinja stvaraju se znatne količine bioaerosola. Njega čine prašina, bakterije, gljivice, endotoksini i plinovi. Brojnost im ovisi o građevinsko-tehničkim značajkama nastambi, naseljenosti životinjama, načinu držanja, temperaturno-vlažnim odnosima u staji i aktivnostima oko hranjenja, mužnje, skupljanja jaja i drugih poslova. Ove čestice, ako su patogene, mogu biti ozbiljna prijetnja za zdravlje ljudi.
Mjerenja su obavljana u staji muznih krava te u objektu za nesilice, 2007. godine, tijekom tri jesenja mjeseca.
Zrak je uzorkovan na Petrijeve zdjelice sa Sabouraudovim glukoznim agarom, uređajem MAS 100. Nakon obrade u laboratoriju, prema osnovnim i mikromorfološkim osobinama poraslih kolonija identificirani su najčešće zastupljeni rodovi gljivica.
Srednja vrijednost broja gljivica u zraku staje za muzne krave kretala se od $3,98 \times 10^{3}$ CFU m^{-3} do $5,11 \times 10^{4}$ CFU m^{-3}. Broj gljivica u zraku objekta za nesilice kretao se od $6,89 \times 10^{4}$ CFU m^{-3} do $1,13 \times 10^{5}$ CFU m^{-3}. Ove vrijednosti statistički su se značajno razlikovale na razini $p<0,05$.
U obje pretraživane nastambe najčešće su bili zastupljeni rodovi *Aspergillus*, *Penicillium* i kvasnice. U manjem postotku utvrđene su gljivice iz rodova *Cladosporium* sp., *Fusarium* sp., *Mucor* sp., *Scopulariopsis* sp., *Alternaria* sp. i *Rhizopus* sp.
Rezultati ovih istraživanja o kvantitativnom i kvalitativnom sastavu gljivica u zraku pretraženih nastambi potpuno su u skladu s vrijednostima zabilježenim u literaturi te se nalaze na donjim granicama opisanih raspona. Utvrđeni broj i rodovi gljivica ukazuju na nužnost daljnjih istraživanja te potrebu postavljanja standardnih vrijednosti glede kvalitete zraka u nastambama za životinje, kao i razvoj vjerodostojnog sustava praćenja navedenih čimbenika, s ciljem stvaranja sigurne hrane i sigurnog okoliša.

KLJUČNE RIJEČI: *higijena zraka, mikroorganizmi iz zraka, okoliš, smještaj životinja*

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