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Airborne fungi in a dairy barn with emphasis on microclimate and emissions

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ABSTRACT

The aim of the study was to determine fungi count in a dairy barn and its immediate environment and to help establish borderline values in line with EU recommendations on airborne emissions from animal housing. A Merck MAS-100 air sampler was employed with respective nutrient agar for the capture, incubation and counting of airborne fungi. Air temperature, relative humidity and air velocity were simultaneously determined by a Testo 400 device. Air sampling was done once a week in the morning (at 7:30), in the middle of the day (at 12:30) and in the evening (at 18:30), during two autumn months. Within the barn, measurements were performed in the animal housing area along the feedlot and outside the barn at a distance of 5 m, 25 m and 50 m, downwind and upwind from the barn. The mean values of total airborne fungi count in the barn air were 5.85×10^4 /m^3 in the morning, 5.52×10^4 CFU/m^3 at noon, and 6.01×10^4 CFU/m^3 in the evening. The fungi count showed a statistically significant decrease as close as 5 m to the barn ($P<0.05$). The microclimate parameters measured in the barn were within the standard values for dairy barn indoor atmospheres.

Key words: air temperature, relative humidity, air velocity, airborne fungi, dairy barn, environment

Introduction

Indoor livestock production leads to air contamination in the form of aerosols that may have potentially harmful effects on both human and animal health and animal productivity. Air emissions from barns may affect the hygiene quality of the immediate

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environment (WATHES, 1995; MATKOVIĆet al., 2006; MATKOVIĆ et al., 2007). HIRST, (1995) defines a bioaerosol as a cloud of biological particles, characterized by viability, infectivity, allergenicity, toxicity, and pharmacological properties. The air within and outside the barn represents a medium by which the bioaerosol particles migrate from one place to another (STETZENBACH, 1997). In animal housing, the sources of bioaerosols include feed, manure, litter and the animals themselves (JACOBSON et al., 2003; ANONYM., 2003). Bioaerosols contain viruses, bacteria, fungi, fungal spores, whole cells or cell parts, along with nonspecific, organic and inorganic dust (WATHES, 1995; LANGE et al., 1997). This obviously calls for in-depth quantitative and qualitative research, to indicate potential problems and offer possible solutions. The universal importance of the issue is reflected in the Integrated Pollution Prevention and Control Directive (ANONYM., 1996) issued by EU experts, requiring EU member countries to establish borderline levels of air pollution from livestock facilities. Fungi are just one, ubiquitous group of animal housing air pollutants, having the potential to induce allergologic and toxicologic effects on health. The rate of potential hazard depends on the fungi count, which in turn varies with microclimate parameters, animal housing construction, animal population density and keeping and feeding conditions (HILLMAN et al., 1992; LANGE et al., 1997; SEEDORF et al., 1998c; SEEDORF, 2004).

Therefore, the aim of the present study was to assess the effect of microclimate parameters on the fungi count in a dairy barn and their air emission into the environment. The results thus obtained would help in the interpretation of borderline fungi levels in the immediate environment of a dairy barn.

Materials and methods

The study was conducted in a dairy barn on a family-owned farm. The south and southwest boundaries of the farmstead border arable land, partially used as grazing land. The barn is built from materials usual for the area: the foundations and floor from concrete, the longitudinal walls from hollow brick, the front from boards, and the roof from rolling hardboard, with three air apertures of 0.6 m×0.4 m on the ridge of the roof. The ceiling of the barn is made of boards, the loft serving for hay and straw storage. The barn dimensions are 14 m×12.5 m×3 m. The central part of the barn is the feedlot corridor, with a concrete trough running alongside and connecting construction above the trough, with a watering facility and vacuum milking system. The lying area is a concrete surface, cleaned daily (at 07:00 and 18:30) and covered with fresh straw bedding. The barn is ventilated by natural airflow through interspaces between the front boards, four windows (120×120 cm) on the longitudinal walls, and air apertures on the ridge of the roof. During the study, there were 25 black-spotted lactating cows in the barn. The cows were fed their usual fodder, hay, haylage and concentrate at 06:30 and 18:00.

Measurements were performed once a week in the morning (at 8:00), in the middle of the day (12:30) and in the evening (18:30) over a two-month period (October - November). In the barn, air sampling was done in the area of animal accommodation along the feedlot, and outside the barn, at distances of 5 m, 25 m and 50 m, downwind and upwind from the barn. Cardinal points were chosen according to the predominant wind direction. Measurements were taken simultaneously. Air samples for fungi determination were collected by use of a Merck MAS-100 device (Merck KgaA, Darmstadt, Germany) on a commercially available Sabouraud maltose agar (Biolife, Milan, Italy) and incubated at 22 °C for 5 days in a thermostat. Air was sampled in volumes of 10 L, because preliminary studies showed this to be optimal for subsequent plate analysis and type of agar. Air temperature (t ºC), relative humidity (rh%) and airflow velocity (w m/s) were determined by use of a Testo 400 device (Testo Inc., Lenzkirch, Germany).

From 8 measurements at three sampling times, a total of 648 plates, 216 from the barn at three sampling times and 72 from each distance outside the barn at two cardinal points, were analysed. The quantitative fungi content in air samples was determined by calculating grown colonies (CFU/m^3) on a colony counter, the results being corrected by the respective mathematical procedure (ANONYM., 1998). Dominated fungi were identified by native preparation. The values of total fungi count and microclimate parameters thus obtained were analysed by use of Microsoft Excel and Statistica 6 software and Wilcoxon matched pair test at the level of statistical significance of $P<0.05$ (ANONYM., 1994).

Results

Mean values of total fungi count in the barn air are shown in Tables 1-3. The values ranged from 3.7×10^3 to 1.89×10^5 CFU/m^3 in the morning, from 1.02×10^4 to 1.93×10^5 CFU/m^3 in the middle of the day, and from 2.44×10^4 to 1.41×10^5 CFU/m^3 in the evening.

In the present study, the measured values of the analyzed microclimate parameters were within the recommended ranges (Tables 1-3).

The most dominated airborne fungi in the barn and in the nearby environment were genera *Penicillium*, *Aspergillus*, *Scopulariopsis*, *Rhisopus* and yeast.

Table 1. Descriptive statistics of fungi count and basic microclimate parameters within and outside the barn on morning air sampling

Morning			n	Arithmetic mean	Minimum	Maximum	Range	Variance	Standard deviation	Standard error
Fungi (CFU/m^3)	Indoor		8	$5.85^{a} \times 10^4$	3.17×10^3	1.89×10^5	1.86×10^5	5.54×10^9	7.44×10^4	2.63×10^4
	Upwind	5 m	8	$8.88^{b} \times 10^3$	5.00×10^2	3.02×10^4	2.97×10^4	1.07×10^8	1.04×10^4	3.66×10^3
		25 m	8	6.03×10^3	7.83×10^2	2.87×10^4	2.80×10^4	9.06×10^7	9.52×10^3	3.37×10^3
		50 m	8	4.85×10^3	3.66×10^2	2.50×10^4	2.47×10^4	6.90×10^7	8.31×10^3	2.94×10^3
	Downwind	5 m	8	$2.64^{b} \times 10^3$	6.66×10^2	9.20×10^3	8.53×10^3	7.56×10^6	2.75×10^3	9.72×10^2
		25 m	8	2.48×10^3	4.33×10^2	8.27×10^3	7.83×10^3	6.18×10^6	2.49×10^3	8.79×10^2
		50 m	8	2.25×10^3	1.33×10^2	7.73×10^3	7.60×10^3	6.26×10^6	2.50×10^3	8.84×10^2
Microclimate	Indoor	t °C	8	11.20	5.26	15.80	10.50	11.50	3.39	1.20
		rh%	8	78.60	74.40	82.30	7.90	6.06	2.46	0.87
		w m/s	8	0.11	0.07	0.15	0.08	0.00	0.02	0.01
	Outdoor	t °C	8	9.44	2.50	14.10	11.60	16.70	4.08	1.44
		rh%	8	77.10	73.30	80.80	7.46	10.10	3.19	1.13
		w m/s	8	0.22	0.06	0.59	0.54	0.03	0.17	0.06

n = number of measurements; CFU = colony forming unit; t = temperature; rh = relative humidity; w = airflow velocity; ^{a,b} = arithmetic means that do not share the same letter in superscript were statistically significantly different at $P<0.05$

Table 2. Descriptive statistics of fungi count and basic microclimate parameters within and outside the barn on noon air sampling

Noon			n	Arithmetic mean	Minimum	Maximum	Range	Variance	Standard deviation	Standard error
Fungi (CFU/m^3)	Indoor		8	$5.52^{a} \times 10^4$	1.02×10^4	1.93×10^5	1.83×10^5	3.45×10^9	5.87×10^4	2.08×10^4
	Upwind	5 m	8	$5.69^{b} \times 10^3$	1.53×10^3	1.53×10^4	1.37×10^4	2.54×10^7	$5.0\,4 \times 10^3$	1.78×10^3
		25 m	8	$2.71^{c} \times 10^3$	9.00×10^2	7.90×10^3	7.00×10^3	5.11×10^6	2.26×10^3	7.99×10^2
		50 m	8	2.00×10^3	4.33×10^2	4.53×10^3	4.10×10^3	1.69×10^6	1.30×10^3	4.59×10^2
	Downwind	5 m	8	$4.30^{b} \times 10^3$	8.66×10^2	1.14×10^4	1.06×10^4	1.54×10^7	3.92×10^3	1.39×10^3
		25 m	8	3.21×10^3	8.33×10^2	1.08×10^4	1.00×10^4	1.08×10^7	3.28×10^3	1.16×10^3
		50 m	8	2.95×10^3	4.66×10^2	1.44×10^4	1.39×10^4	2.17×10^7	4.66×10^3	1.65×10^3
Microclimate	Indoor	t °C	8	13.10	8.23	18.20	9.93	17.40	4.17	1.47
		rh%	8	74.70	64.00	84.30	20.30	69.30	8.32	2.94
		w m/s	8	0.10	0.04	0.17	0.13	0.00	0.04	0.01
	Outdoor	t °C	8	12.00	6.95	19.20	12.30	23.90	4.89	1.73
		rh%	8	73.70	57.00	85.20	28.20	124.00	11.10	3.94
		w m/s	8	0.48	0.11	1.49	1.38	0.19	0.44	0.15

n = number of measurements; CFU = colony forming unit; t = temperature; rh = relative humidity; w = airflow velocity; ^{a,b,c} = arithmetic means that do not share the same letter in superscript were statistically significantly different at $P<0.05$

Table 3 Descriptive statistics of fungi count and basic microclimate parameters within and outside the barn on evening air sampling

1			n	Arithmetic mean	Minimum	Maximum	Range	Variance	Standard deviation	Standard error
Fungi (CFU/m^3)	Indoor		8	$6.01^{a} \times 10^4$	2.44×10^4	1.41×10^5	1.16×10^5	1.90×10^9	4.36×10^4	1.54×10^4
	Upwind	5 m	8	$9.60^{b} \times 10^3$	5.00×10^2	3.67×10^4	3.62×10^4	1.39×10^8	1.18×10^4	4.17×10^3
		25 m	8	5.64×10^3	8.33×10^2	9.77×10^3	8.93×10^3	1.52×10^7	3.90×10^3	1.38×10^3
		50 m	8	6.23×10^3	2.66×10^2	2.23×10^4	2.20×10^4	5.46×10^7	7.39×10^3	2.61×10^3
	Downwind	5 m	8	$4.58^{b} \times 10^3$	3.33×10^2	1.02×10^4	9.83×10^3	1.43×10^7	3.78×10^3	1.34×10^3
		25 m	8	$2.71^{c} \times 10^3$	2.00×10^2	6.07×10^3	5.87×10^3	4.54×10^6	2.13×10^3	7.54×10^2
		50 m	8	2.76×10^3	4.66×10^2	6.83×10^3	6.37×10^3	4.87×10^6	2.21×10^3	7.80×10^2
Microclimate	Indoor	t °C	8	12.90	5.50	22.50	17.00	33.40	5.78	2.04
		rh%	8	71.30	54.50	88.00	33.50	140.00	11.80	4.18
		w m/s	8	0.12	0.03	0.25	0.22	0.01	0.08	0.03
	Outdoor	t °C	8	11.40	3.29	22.20	18.90	36.90	6.08	2.15
		rh%	8	69.80	54.00	85.20	31.20	103.00	10.10	3.59
		w m/s	8	0.36	0.12	0.70	0.58	0.05	0.22	0.08

n = number of measurements; CFU = colony forming unit; t = temperature; rh = relative humidity; w = airflow velocity; ^{a,b,c} = arithmetic means that do not share the same letter in superscript were statistically significantly different at $P<0.05$

Discussion

Only satisfactory microclimate conditions can ensure good animal health and productivity. Any distortion in the microclimate, keeping, feeding and other requirements acts as a stressor. More intensive animal utilisation provokes stronger stress effect that induce diseases development, mostly of multicausal etiology (MÜLLER and WEISER, 1987b; WATHES, 1994; WATHES et al., 1998). Therefore, the pathogenesis of these diseases includes both external and internal factors, that do not cause disease by themselves.

The thermoneutral zone of ambient temperature for dairy cows is between 5 °C and 20 °C, in combination with relative humidity of less than 70% and a desirable airflow velocity from 0.50 m/s to 1 m/s. Similar experiences in relation to these requirements in dairy barns have been reported by MARTHI et al. (1990), KADZERE et al. (2002), VUČEMILO et al. (2003) and others. The arithmetic means of the measured values obtained suggested indoor parameters to be influenced by outdoor conditions. Differentiation in these values pointed to the relatively good studied barn construction properties.

The total fungi count in barn air depends on the animal species, housing conditions, and procedures of animal feeding and grooming. The animals, their feed, bedding and faeces are the sources of microorganisms. The exact number of microorganisms, including fungi, in barn air is difficult to determine because aerial microorganisms are liable to sedimentation, aggregation, ventilation, dehydration, radiation and other stressors influencing their viability (COX, 1989; WILSON et al., 2002). It may also vary according to the sampling technique employed. Airborne fungi are disseminated from the barn to its immediate environment. The distance the fungi will travel and the rate of their count reduction depend on their original count within the barn, airflow, position of air outlets, barn lot ground configuration, local climate conditions, and fungi biological life (MÜLLER, 1987; MÜLLER and WEISER, 1987a; CHARLES, 1994; SEEDORF et al., 1998a; SEEDORF et al., 1998b).

In the present study, the fungi count was determined at three sites at distances of 5 m, 25 m and 50 m from the barn, at two opposite cardinal points. The greatest distance of 50 m was chosen because small family-run farms are the predominant type of farming facility in Croatia, the barns generally being constructed on house lots. At greater distances, the mixing and cumulation of microorganisms from neighbouring farms can be presumed.

The values obtained are consistent with those described by EDUARD (1997), stating the total count of these microorganisms in cattle barns to be up to 10^5 CFU/m^3. Furthermore, the results are also consistent with literature data, where the total fungi count in animal housing is in a range of 10^3-10^9 CFU/m^3 (WATHES, 1994; HARTUNG, 1994 and 1998; SEEDORF et al., 1998a; DUCHAINE et al., 1999; SEEDORF, 2004). Comparable results have also been reported by VINKOVIĆ et al. (2003), VINKOVIĆ et al. (2004), GUTZMIRTL et al. (2004), MATKOVIĆ et al. (2006).

The study results showed the mean fungi count to decrease 6 times at 5 m upwind and 22 times at the same distance downwind from the barn in the morning. A Wilcoxon matched-pair test yielded a statistically significant ($P<0.05$) difference in the fungi count measured inside the barn and at 5 m downwind and upwind (Table 1). The other two outdoor sampling sites (at 25 m and 50 m) showed a slight fungi count decrease.

In the middle of the day, the mean fungi count also showed a statistically significant decrease at 5 m from the barn at two opposite cardinal points. A Wilcoxon matched-pair test yielded a statistically significant ($P<0.05$) difference between the fungi count measured inside the barn and at 5 m upwind, the total fungi count at 5 m upwind and 25 m upwind, and the fungi count inside the barn and at 5 m downwind (Table 2).

The mean total fungi count measured in evening samples exceeded that recorded at the other two sampling times (Tables 1-3). Likewise, the morning sample total fungi count decreased several times at 5 m from the barn at both cardinal points, being greater downwind (13 times), as confirmed by a statistically significant Wilcoxon matched-pair test result ($P<0.05$). The same level of statistical significance was recorded at both 5 m and 25 m downwind (Table 3).

The fungi count increased in the evening, which could be related to diurnal animal activity and barn manipulation. The outdoor fungi count decreased several fold at 5 m from the barn, as demonstrated by a statistically significant Wilcoxon matched-pair test result ($P<0.05$). This could be due to the absence of physical barriers to natural airflow in the immediate barn environment, allowing for rapid and thorough barn air dilution. At distances of 25 m and 50 m from the barn, the mean fungi count showed a further slight decrease, however, their air concentration may also depend on the distance from neighbouring farm barns.

Dominated airborne fungi were the genera *Penicillium*, *Aspergillus*, *Scopulariopsis*, *Rhizopus* and yeasts, both in the barn and nearby environment. These results are similar to the findings of HARTUNG, (1992 and 1994), SEEDORF et al., (1998), and MATKOVIĆ et al. (2007).

Conclusion

The mean values of total fungi count in the barn air was 5.85×10^4 CFU/m^3 in the morning, 5.52×10^4 CFU/m^3 in the middle of the day, and 6.01×10^4 CFU/m^3 in the evening. The fungi count showed a statistically significant decrease as close as 5 m to the barn ($P<0.05$). The total fungi count was observed to increase in the evening, which could be attributed to daily animal and human activities in the barn.

In establishing the borderline value in line with EU recommendations on airborne emissions from animal housing, further research into dairy barns with different housing types and numbers of cows number is necessary.

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SAŽETAK

Svrha ovoga istraživanja bila je odrediti brojnost gljivica u zraku staje za muzne krave i njezinom neposrednom okolišu da bi se mogle postaviti granične vrijednosti sukladno s preporukom stručnjaka EU o zračnim onečišćenjima iz životinjskih nastambi. Uzorci zraka u kojem je određivan broj gljivica bili su uzimani uređajem MERCK MAS-100 (MERCK KgaA, Darmstadt) na gotove podloge hranjivoga agara. U isto vrijeme mjerena je temperatura, relativna vlaga i brzina strujanja zraka pomoću uređaja TESTO 400. Mjerenja su obavljana u jutro (7:30), u podne (12:30) i na večer (18:30) jedanput tjedno tijekom dva jesenska mjeseca. U

staji su mjerenja obavljana u zoni boravka životinja duž hranidbenoga hodnika, a izvan staje na udaljenostima od 5 m, 25 m i 50 m istočno i zapadno od staje. Izmjerene srednje vrijednosti ukupnog broja gljivica u staji iznosile su $5,85\times10^4$ CFU/m^3 u jutarnjem terminu, $5.52\times S10^4$ CFU/m^3 u podnevnom mjerenju te 6.01×10^4 CFU/m^3 u večernjem terminu mjerenja. Izvan staje brojnost gljivica značajno se smanjivala već na 5 metara udaljenosti od staje, na obje strane svijeta, što je dokazano Wilcoxonovim testom ekvivalentnih parova na razini statističke značajnosti $P<0,05$. Izmjereni mikroklimatski pokazatelji u staji bili su unutar standardnih vrijednosti.

Ključne riječi: zrak, temperatura, relativna vlaga, brzina strujanja, gljivice, staja, okoliš