

Assessing the Abundance of Airborne Fungi in a Dairy Cattle Barn

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Received: 3 July 2014

Accepted: 3 August 2014

Abstract

The presented research aimed at determining the species composition and concentration of colony forming units (CFU) in two types of cattle barns for dairy cows and in a calf barn. The study was carried out in winter and summer 2012. The measurements took place in a free stall and tie stall barn and, additionally, in a calf barn. The mycological analysis of the air was conducted using the impact method, with an Air Ideal 3P apparatus. Spores of 45 species of fungi were found in cattle barn air, among them 36 species were collected in winter and 30 in summer. The fungal population was characterized by considerable variation across the seasons of the year and between barn types. The species most abundant irrespective of the season was *Cladosporium herbarum*. Winter conditions favored *Penicillium nigricans*, whereas summer was conducive to the development of *Rhodotorula glutinis*. The concentrations of fungal spores determined in the study were not a direct hazard to barn workers or animals.

Keywords: airborne fungi, mycobiota, bioaerosol, mycotoxins, cattle barns, animal hygiene

Introduction

Workers employed in animal farms are exposed to a number of factors negatively affecting their health [1-3]. One of the most serious problems in this context is the high frequency of disorders of the upper respiratory routes, mainly allergies and asthma [4, 5]. These disorders are most probably related to exposure to airborne fungal spores as well as to those present in substrate, including barn bedding. It has been estimated that approximately 80 fungal taxa can be made responsible for allergic conditions in humans [6]. Many species of fungi-producing airborne spores can also produce detrimental mycotoxins, i.e. aflatoxins, ochratoxins, and trichotecenes [3, 7-9]. The high concentration of these airborne spores is usually associated

with particular working environments and tasks, like uploading of grain or hay [10-13]. While the agricultural produce is being stored, the so-called “storage fungi” may develop and it happens most often at those instances when the stored material is not adequately dried prior to storing, or the storage conditions (e.g. air humidity) are irrelevant. The taxa that are most importantly included in the storage pathogen category are those belonging to the genera of *Aspergillus* and *Penicillium* [14]. They occur commonly in animal husbandry rooms, where they are specifically found in animal feed and in barn bedding [2, 3, 15, 16], but they are also present in miscellaneous kinds of human living space [17] as well as in public-use environments such as lecturing halls [18], banks, pharmacy shops [19], and even in health care institutions [20].

Species of *Aspergillus* and *Penicillium* genera are capable of producing mycotoxins, products of the secondary

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metabolism of the mycelial fungi. These are compounds of low molecular mass characterized by low polarity and high thermostability, which makes them resist decomposition in the pasteurization process or even in higher temperatures. Interestingly, they become degraded at basic environments and once they are exposed to UV radiation. In structural terms they are classified as aromatic or – less frequently – as aliphatic hydrocarbons [21]. While entering the human body through the alimentary tract, respiratory route, or through dermal contact, mycotoxins exert their detrimental effect on the health of exposed humans [22]. *Aspergillus* spp. produce the following mycotoxins: aflatoxin B1, G1, and M1, ochratoxin A, and sterigmatocystine. The taxa of *Penicillium* spp. produce ochratoxine A, cytrynine, pauline, penitrem A. Moreover, *Aspergillus* spp. and *Penicillium* spp. are able to produce large numbers of conidia, which can also negatively affect the health status of humans and animals because these spores may themselves contain mycotoxins and as such are the most allergenic fungal organs [23]. The fungal genera in question can cause severe immunological disorders as well as infections of internal and external organs. *Aspergillus* spp. may induce invasive aspergilloses, otomycoses, onychomycoses, and allergic inflammatory conditions of the pulmonary alveoli, as well as other allergic reactions. They can be a factor of lung infections in patients showing immune deficiencies. These fungi are known to be the cause of sinusitis, dermatitis, and nail and cornea infections. They may as well induce deep infections of internal organs such as kidneys, endocardium, or the auditory canal. Exposure to species of *Aspergillus* genus may also result in hepatitis or arthritis. On the other hand, the taxa counted into *Penicillium* genus are mostly responsible for infections of skin, bone marrow, and intestines, but they are also known to infect kidneys and cornea, and cause fungal pneumonia, endocarditis peritonitis, and urinary tract infections and allergies [24].

The presented research was aimed at determining the species composition and the CFU concentration in two types of cattle barns for dairy cows and in a calf barn, in two seasons of the year.

Experimental Procedures

The study was carried out in two distinct terms: every week in January and in July of 2012. The airborne fungal spores were sampled in two types of barns: a free stall barn (203 animals) and a tie stall one (126 animals), and additionally in a calf barn (107 calves), all the property of the Cattle Breeding Center in Kamieniec Ząbkowicki (Lower Silesia). Sampling always took place between 9 and 10 a.m. Analysis employed the impact method using an Air Ideal 3P apparatus and PDA (potato dextrose agar) culturing medium (Biocorp). The volumes of the samples were 50 L, 100 L, and 150 L. In any one of the buildings the measurements were taken in 3 replicates, at 3 different spots of the barn: at the entrance, in the interior, and near the exit. The sampling device was positioned 1.5 m above the floor level of the barn. The sampled fungi were cultured for 7 days at 22°C.

After incubation the appearing colonies were counted and fungi were identified taxonomically based on their morphology, using available monographic keys [25, 26]. Then the number of CFU per 1000 L (1 m³) of the air was calculated based on the number of the colonies grown on a Petri dish, according to the formula:

$$X = (a \times 1000) / V$$

...where: a stands for the total number of fungal colonies that had grown on a Petri dish exposed to a sample of the air and V is the volume of the air taken by the sampler (L).

Out of all the sampled material, 16 species that occurred most frequently were selected for statistical analysis: *Alternaria alternata*, *A. brasiliensis*, *Aspergillus flavus*, *Botrytis cinerea*, *Cladosporium cladosporioides*, *C. herbarum*, *Epicoccum nigrum*, *Fusarium oxysporum*, *Giberella avenacea*, *Penicillium notatum*, *P. lanosocoeruleum*, *P. nigricans*, *P. purpurogenum*, *P. velutinum*, *Rhodotorula rubra*, and *R. glutinis*. Relations between the number of fungal species, the season, and barn type were assessed using log-linear analysis. Significant deviations from the expected values of the incidence of particular species calculated according to formula (1), point at an interaction between the variables. After the logarithmic transformation of the expected values the model assumes linear form, which may be described in the following formula:

$$\text{Ln}(E_{ij}) = M + \lambda_i^X + \lambda_j^Y + \lambda_{ij}^{XY} \quad (1)$$

...where E_{ij} – expected values, M – general mean, λ_i^X – the effect of i th value of variable X , λ_j^Y – the effect of j th value of the variable Y , λ_{ij}^{XY} – the effect of interactions of i th value of the variable X , and j th value of the variable Y .

The log-linear model allows for verification of hypothesis assuming no interactions between two or more experimental factors. It also enables assessment of effects of particular factors on the variability of the investigated population of fungi. In the next part of the study the structure and variability of the fungal population, as depending on the season of the year and barn type, was analyzed using correspondence analysis.

Results

The microclimatic conditions during the air sampling process were similar in the free stall and tie stall barn (Table 1).

In total, spores of 43 species of fungi were found in the air, 36 of them being sampled in winter and 30 in summer (Table 2). In the free stall barn the winter sampling resulted in collection of spores representing 25 species, whereas from the summer sampling spores belonging to 19 species were recorded. In the tie stall barn these numbers were 23 species in winter and 22 in summer. In the calf barn these numbers amounted to 26 and 23, respectively.

The log-linear analysis has demonstrated that in high temperatures and in the high relative humidity prevailing in summer the number of the investigated taxa was higher

Table 1. Microclimate conditions in the dairy houses.

Location of measurement	Winter		Summer	
	Temperature [°C]	Relative air humidity [%]	Temperature [°C]	Relative air humidity [%]
Free stall	18.0	78.2	21.0	67.2
Tie stall barn	17.0	71.9	22.0	65.0
Calf barn	19.5	88.9	22.0	72.0

compared to the winter results (Table 3). Moreover, in the free stall barn lower spore concentration was found compared to the tie stall type or to calf barn, and that was true irrespective of the season. Significant interaction between barn type and season points to the variable density of the investigated fungal population depending on the prevailing atmospheric conditions. In winter, considerably higher spore density was found in the tie stall barn compared to the other objects (Tables 4, 5). Conversely, in summer higher CFU density was observed in the calf barn.

Significant interaction between the species to fungi and the seasons of the year testifies to the differential effect of the fluctuating atmospheric conditions on the incidence of particular taxa. Out of the whole investigated population, *Cladosporium herbarum* shows higher CFU density irrespective of the seasons. On the other hand, winter conditions apparently favoured *Penicillium nigricans*, whereas the highest air concentration of *Rhodotorula glutinis* CFU was observed in the summer season.

Using correspondence analysis (CA), our study further determined the structure of relations between spore density of particular fungi and factors such as season of the year and barn type. Eigenvalues shown in the graph axes reflect the variance of the distribution of datapoints (i.e. of fungi species) for the first and second dimensions. The term inertia, as used within the CA, is a measure of datapoints distribution – variance of the original, 6-dimensional space. Based on Fig. 1, one may distinguish four groups of fungi according to their response to the barn type and the season of the year. The first group would include *Aspergillus flavus*, *P. nigricans*, and *R. rubra*, seen in the first part of the graph (Fig. 1). The second group is made of *R. glutinis*, *P. velutinum*, *B. cinerea*, and *A. alternata*. The third association is apparently distant from the previous two, and it contains only two species: *P. purpurogenium* and *P. lanosocoeruleum*. The fourth group, except for *F. oxysporum*, is located near the beginning of the coordinate system of the graph.

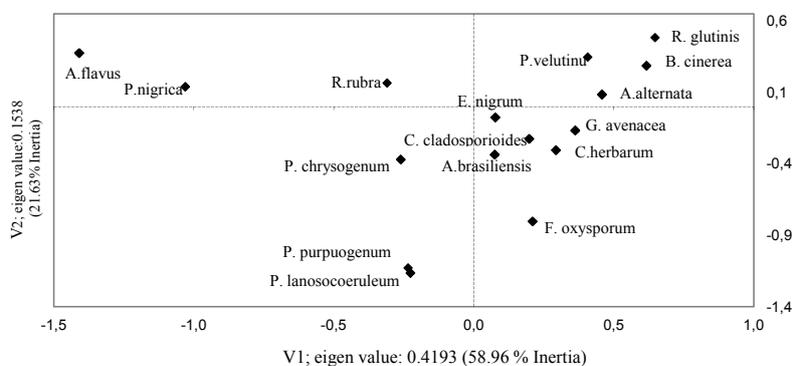


Fig. 1. Correspondence analysis of variability of individual species of fungi, depending on the type of barn and season.

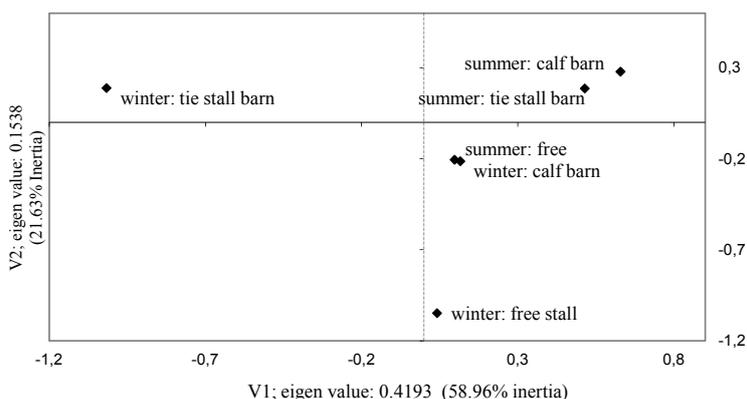


Fig. 2. Correspondence analysis of variability of the test population of fungi in individual barns, depending on the season.

Table 2. The average value of fungal CFU/m³ in the dairy houses.

Fungal species	Winter			Summer		
	Free stall	Tie stall barn	Calf barn	Free stall	Tie stall barn	Calf barn
<i>Absidia glauca</i> Hagem	20				60	
<i>Alternaria alternata</i> (Fr.) Keissl.	400	420	660	420	480	2260
<i>Apiospora montagnei</i> Sacc.		20				
<i>Aspergillus brasiliensis</i> Varga, Frisvad & Samson	20	40	220		40	20
<i>Aspergillus candidus</i> Link				40	40	
<i>Aspergillus flavus</i> Link	2960	40	190	20	20	20
<i>Aspergillus fumigatus</i> Fresen.	100		20			
<i>Aspergillus foetidus</i> Thom & Raper						
<i>Aspergillus versicolor</i> (Vuill) Tirab.			10		160	160
<i>Botrytis cinerea</i> Pers.		40	200	200	300	620
<i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries	420	400	260	400	520	620
<i>Cladosporium herbarum</i> (Pers.) Link	1280	740	1280	1140	1380	1900
<i>Cylindrocarpon radicola</i> Wollenw.						20
<i>Epicoccum nigrum</i> Link	100	340	260	460	320	300
<i>Exerophilum</i> sp.			10			60
<i>Fusarium oxysporum</i> Schlecht.	40		130	60		20
<i>Gibberella avenacea</i> R.J. Cook	20	20	220		20	120
<i>Gibberella intricans</i> Wollenw.		20	20	20	20	
<i>Mucor hiemalis</i> Wehmer	20				200	
<i>Penicillium vulpinum</i> (Cooke & Massee) Seifert & Samson	20					
<i>Penicillium frequentans</i> Westling			60			20
<i>Penicillium implicatum</i> Biourge		20	20			20
<i>Penicillium janthinellum</i> Biourge	20		20			
<i>Penicillium lanoso-coeruleum</i> Thom	80	40	40	40		
<i>Penicillium nigricans</i> K.M. Zalesky	160	3240	440	580	120	40
<i>Penicillium notatum</i> Westling	560	820	220	80	280	380
<i>Penicillium purpurogenum</i> Flerov	800	440	40	160	100	140
<i>Penicillium urticae</i> Bainier	60	20		100		
<i>Penicillium velutinum</i> J.F.H. Beyma		140	200	60	60	560
<i>Penicillium waksmanii</i> K.M. Zalesky		120	40		100	20
<i>Penicillium vermiculatum</i> P.A. Dang.					20	20
<i>Penicillium aurantiogriseum</i> Dierckx	20					
<i>Penicillium</i> sp.				80	60	20
<i>Phoma leveillei</i> Boerema & G.J. Bollen						
<i>Rhodotorula rubra</i> (Schimon) F.C. Harrison	20	620	200	280	260	200
<i>Rhodotorula glutinis</i> (Fresen.) F.C. Harrison	40	480	220	380	2080	3320
<i>Sarocladium strictum</i> (W. Gams) Summerb.	40	140	80			
<i>Talaromyces funiculosus</i> (Thom) Samson, Yilmaz, Frisvad & Seifert			20			
<i>Talaromyces rugulosus</i> (Thom) Samson, Yilmaz, Frisvad & Seifert	40	20				
<i>Talaromyces variabilis</i> (Sopp) Samson, Yilmaz, Frisvad & Seifert	20					
<i>Trichoderma harzianum</i> Rifai	20					
<i>Truncatella hartigii</i> (Tubeuft) Steyaert	20					
<i>Verticillium lecanii</i> (Zimm.) Viégas				60		
Total	7300	8180	5080	4580	6640	10860

Table 3. Tests of main effects, marginal and partial associations, and interactions between experimental factors.

Effect	Degrees of freedom	Chi ² partial association	Significant level (p)	Chi ² marginal association	Significant level (p)
Fungal species (1)	15	9,839.47	<0.001	9839.47	<0.001
Period of year (2)	1	15.13	<0.001	15.13	<0.001
Type of barn (3)	2	1,077.58	<0.001	1077.58	<0.001
1 × 2	15	3,500.73	<0.001	4420.84	<0.001
1 × 3	30	33,355.49	<0.001	4275.60	<0.001
2 × 3	2	238.88	<0.001	1158.99	<0.001

Fig. 2 reflects variation in fungal spore density between the different barn types, in winter or summer. Comparing species distribution as seen in Fig. 1 to the distribution, in Fig. 2, of the points that represent barn types, one may observe some connections between them. In the free stall barn conditions in winter season the highest density of the spores of *P. purpurogenum* and *P. lanosocoeruleum* has been demonstrated. On the contrary, conditions prevailing in winter inside the tie stall barn were most conducive to the development of *A. flavus*, *P. nigricans*, and *R. rubra*. Furthermore, *R. glutinis*, *P. velutinum*, *B. cinerrea*, and

A. alternata had been showing higher spore density inside the tie stall and calf barns in summer. Microclimatic conditions of the winter in the calf barn and of the summer in free stall barn favored development of the other sampled species of fungi.

Discussion of the Results

Fungi enter the animal husbandry space as airborne particles travelling through ventilation systems, but they are also transferred by humans. Conditions prevalent indoor,

Table 4. Abundance of species of fungi in the winter, depending on the type of barn.

Species	Type of barn			Total
	Free stall	Tie stall barn	Calf barn	
<i>A. alternata</i>	133	140	220	493
<i>A. brasiliensis</i>	6	14	73	93
<i>A. flavus</i>	14	987	64	1065
<i>B. cinerea</i>	0	14	67	81
<i>C. cladosporioides</i>	140	134	87	361
<i>C. herbarum</i>	427	247	427	1101
<i>E. nigrum</i>	35	113	87	235
<i>F. oxysporum</i>	14	0	44	58
<i>G. avenacea</i>	6	6	74	86
<i>P. notatum</i>	187	273	73	533
<i>P. lanosocoeruleum</i>	27	14	14	55
<i>P. nigricans</i>	54	1046	146	1246
<i>P. purpurogenum</i>	267	146	14	427
<i>P. velutinum</i>	0	47	67	114
<i>R. rubra</i>	6	206	67	279
<i>R. glutinis</i>	14	160	73	247
Total	1330	3547	1597	6474

Table 5. Abundance of species of fungi in the summer, depending on the type of barn.

Species	Type of barn			Total
	Free stall	Tie stall barn	Calf barn	
<i>A. alternata</i>	140	160	753	1053
<i>A. brasiliensis</i>	0	14	6	20
<i>A. flavus</i>	6	6	6	18
<i>B. cinerrea</i>	67	100	207	374
<i>C. cladosporioides</i>	134	173	207	514
<i>C. herbarum</i>	380	460	634	1474
<i>E. nigrum</i>	154	107	100	361
<i>F. oxysporum</i>	20	0	6	26
<i>G. avenacea</i>	0	6	40	46
<i>P. chrysogenum</i>	27	93	126	246
<i>P. lanosocoeruleum</i>	14	0	0	14
<i>P. nigricans</i>	193	40	14	247
<i>P. purpurogenum</i>	54	32	46	132
<i>P. velutinum</i>	20	20	187	227
<i>R. rubra</i>	93	87	67	247
<i>R. glutinis</i>	127	693	1106	1926
Total	1429	1991	3505	6925

mostly high relative air humidity, may affect their development there. Nevertheless, some authors have found no variation in CFU value between the summer and winter seasons [23]. Our results do not conform to that notion. Conversely, they apparently indicate that, in the winter, higher CFU concentrations occur in tie stall barns, whereas in free stall and in calf barns these values are lower in winter compared to summer. Calf barn results from Denmark confirm such a conclusion [28]. In turn, during a study conducted in cattle barns in India [29], maximum concentrations of fungal spores were recorded during periods of humid weather, i.e. during the monsoon season. There, the taxa of the genus *Aspergillus* predominated, such as *Penicillium*, making up >50% of all the collected spores, as well as those of *Cladosporium*, with their mean density fluctuating around 900 CFU. The CFU density oscillated around this 900 mean value between 350 and even as much as 2200 CFU. Inside the barns in India the CFU density was higher in winter season, when the prevailing outside temperatures were relatively low. The factors that most importantly promote the increase in CFU concentration are temperature and air relative humidity prevailing indoors [29]. In our study, the microclimatic conditions were similar at the time of air sampling from the free stall and tie stall barns.

Many authors report on CFU density in cattle barns being orders of magnitude higher than that found in our study. Blom et al. [28] have determined CFU concentrations in calf barns as 10^3 - 10^5 CFU/m³. Authors from Finland show even higher concentrations, amounting to 10^5 - 10^7 CFU/m³ [15]. The spore densities found in our study were as high as 1,693 CFU/m³ in winter and 3,614 CFU/m³ during summer. Such a discrepancy may likely reflect some versatility in cattle barn design, including the design, working conditions, and the resulting efficacy of the ventilation systems used [29]. Barns in which the present study were carried out were renovated in the course of recent years, which have made their ventilation systems work efficiently.

During the winter season sampling the species of *Aspergillus* and *Penicillium* genera showed the highest percentage proportion in the sampled fungal material, whereas in the summer season the relative proportion altered. The species of *Cladosporium* genus and *Alternaria alternata* were isolated the most in summer sampling. These results conform to the findings of other authors [2, 3, 15, 16]. Fungi of *Cladosporium* sp. and the species of *A. alternata* are ubiquitous by the end of plant growth season within the canopies of cereals and rape, causing black rot in both the crops and disseminating their spores. This may likely contribute to their abundant incidence in the air of cattle barns. Ghasian and Maghsood [3] reported the presence of 17 species of these fungi in cattle barns. In our study 45 species of fungi were found in total. On the other hand, Adhikari et al. [2] found only 10 fungi species in dairy cattle barns.

The majority of the fungi found in the air in Finnish barns belong to the genera of *Aspergillus* and *Penicillium*,

although *Cladosporium* sp. had been sampled as well. Taxa of *Cladosporium* sp. had been also isolated from the straw used as barn bedding. This may explain considerably higher concentrations of *Cladosporium herbarum* recorded in the calf barn, where the bedding is relatively often replaced with new material [15]. A similar situation is observed with genus *Fusarium*; concentrations of the spores of *Fusarium* sp. in calf barn are higher compared to free stall or tie stall barn air. The straw used as barn bedding should always be dry and show no symptoms of infestation by fungi [29].

The most numerous fraction of the fungi associations recorded from the air of either conventional or industrial barns in Iran was made up of *Aspergillus* sp. (>35%) and *Penicillium* sp. (23%). *Fusarium*, *Cladosporium*, and *Alternaria* had been found there as well. Of all the species of *Aspergillus*, *A. flavus* was the most abundant taxon, sampled from conventional barns, where straw was used as bedding. In the present study this same fungus was mostly determined in the air samples coming from the tie stall barn and calf barn, where large volumes of straw were used on the barn floor. This species creates a direct risk of pathological conditions of hands and mucous membranes in animal husbandry workers [16].

Interestingly, our results do not conform fully to those obtained by Ghasian and Maghsood [3]. These authors have shown that in industrial barns the CFU concentration is twice as high compared to conventional ones, both in winter and summer. Our results demonstrate the opposite: in the tie stall barn, representing conditions close to conventional barn design, we have recorded CFU concentrations nearly two-fold higher than in the free stall barns, which more closely resemble industrial husbandry.

The presence in the atmosphere of a barn of the spores of *Aspergillus* and *Penicillium* sp. may represent a direct health threat for animal husbandry workers. For the sheer fact that they are capable of producing mycotoxins [30] or able to cause mycoses and other respiratory system diseases [31], these fungi are considered one of the important components of indoor bioaerosol. Nevertheless, in order to confirm such a causative relationship in any individual case it is necessary to conduct a series of molecular tests, as not all of the isolates derived from air sampling show the ability to synthesize mycotoxins [32, 33].

Last but not least, the spores of species belonging to the genus *Rhodotorula* had been found in the atmosphere of the investigated dairy and calf barns. The highest number of colonies of these taxa had been cultured from the air samples taken in the calf barn in summer. This finding remains in agreement with results of some other authors [34, 35]. The fungi were isolated from air samples in such barns in which large amounts of hay had been both stored and used as floor bedding. There, the taxa of *Rhodotorula* spp. made up to 30% of all total number of collected fungal spores [34]. Fungi of *Rhodotorula* spp. directly compromise the health status of cattle of different age groups, as they cause mycoses of ear and other organs in animals [35].

Conclusions

1. The investigated atmospheric fungal association was characterized by considerable variability depending on the season of the year and barn type.
2. Within the association, the species of *Cladosporium herbarum* showed higher air concentration of its CFU, irrespective of the season.
3. Winter conditions favored development of *Penicillium nigricans*, whereas summer was conducive to *Rhodotorula glutinis*.
4. During winter the variability in CFU density of particular species of fungi was rather high. On the other hand, the spore counts of individual species in samples taken in summer differed less from one another.
5. In winter the variation in abundance of individual species is highly dependent on barn type. On the contrary, in the summer the associations sampled from different types of barns differed less in their species composition.
6. We consider it necessary that in order to prevent potential threats to life and health status of both husbandry workers and animals, regular monitoring of the aeromycological composition of bioaerosol in large dairy barns should be carried out.

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