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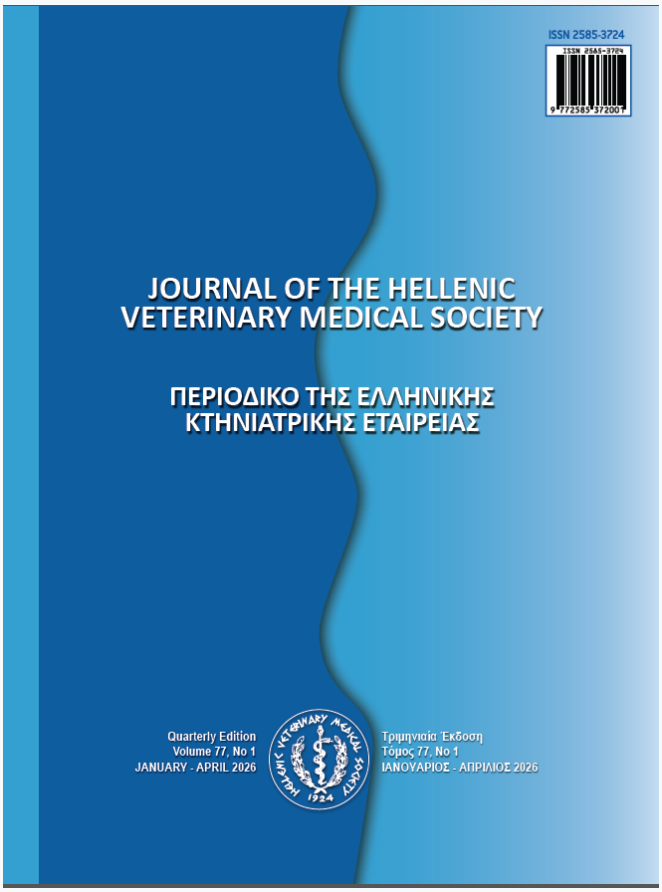


# The Impact of Seasonal Climate Conditions on Fungal Biodiversity in Barn Air

*G Giray, A Şimşek*

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Research article
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# The Impact of Seasonal Climate Conditions on Fungal Biodiversity in Barn Air

**G. Giray,[1] A. Şimşek[2]**

*[1,2]Kastamamu University, Department of Veterinary Medicine, Ihsangazi Technical Science Vocational School, Ihsangazi, Kastamonu-Türkiye*

**ABSTRACT:** This study examines the seasonal effects of climatic variables on fungal biodiversity in the indoor air of cattle barns in Turkiye. Over 12 months, air samples were collected twice a month from 10 cattle barns, resulting in isolating 72 fungal species from 18 genera by using air sampler for isolation and MALDI-TOF MS identification techniques. Dominant genera such as *Acremonium, Alternaria, Aspergillus, Cladosporium*, and *Penicillium* were detected throughout the year, underlining their continuous presence. Seasonal variability is evident, with *Cladosporium* and *Alternaria* in high amount in warmer months, while *Penicillium* is more prevalent in colder months. Environmental variables, including temperature and humidity, strongly correlated with fungal concentrations, and ideal ranges were identified for the major species. These findings highlight the health hazards of airborne fungal spores to cattle and barn workers and focus on the need for improved air quality management techniques in livestock facilities. The study constitutes a fundamental understanding of fungal distribution and environmental interactions and calls for further in-depth research to mitigate health hazards and improve barn air quality.

***Keyword:*** Airborne Fungi; Barn Indoor Air Quality; Seasonal Variation Microbial Bioaerosols; Veterinary Mycology

*Correspondence author:*
Şimşek Abdullah,
Kastamamu University, Department of Veterinary Medicine, Ihsangazi Technical Science Vocational School, Ihsangazi, Kastamonu-Türkiye
E-mail address: abdullahsimsek@kastamonu.edu.tr

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## INTRODUCTION

Microorganisms in the air, freely suspended or attached to particles, are called "bioaerosols." Their widespread presence in the air indicates that airborne microbial contamination is a potential hazard for barns and their workers (Stetzenbach et al., 2004). The diversity and concentration of these bioaerosols can lead to numerous infectious diseases in animals and humans. Air sampling for bioaerosols has been conducted for decades with classical monitoring which is based on collection through using forced air sampling devices and analysis by culture or microscopy in artificial growth environment (Stetzenbach et al., 2004). A significant proportion of airborne microbial pollutants include bacteria and fungi. Fungi are important regarding their allergenic, toxicogenic, and inflammatory features (Robbins et al., 2000; Özer & Kesenkas, 2015). Fungal spores can cause respiratory allergies, asthma, and aspergillosis (Pepeljnjak & Segvic, 2003). Genera such as *Cladosporium, Alternaria, Penicillium*, and *Aspergillus* are the main airborne microfungus in allergy reactions (Kalyoncu, 2010; Gelisken, 2008). Furthermore, toxic metabolites produced by molds have been found to have potential carcinogenic properties (Pekel, 2008).

Keeping the cattle for a long time in closed stalls leads to the accumulation of fungal spores and other microbial particles in the atmosphere. These particles can lead to respiratory diseases, allergic reactions, and feed spoilage in humid and poorly ventilated environments (Elfman et al., 2009; Wålinder et al., 2011). High concentrations of fungal spores can significantly affect animal health, leading to diseases such as bronchopneumonia, mastitis, and foulbrood (Tell, 2005). In addition, volatile organic chemicals emitted by these particles degrade indoor air quality and negatively affect health (Miller, 1992; Fischer, 2003). As a result, microbiological regulation of indoor air in animal shelters is significant for animal welfare and health protection.

Kastamonu province is one of the regions in Turkey where animal husbandry is intensively practiced, and family-type animal husbandry is a vital economic occupation. Therefore, regulating microbial populations in barn air is vital for protecting animal health and the health of people working in the barn. This study is a preliminary investigation designed to analyze the microfungal composition and concentrations in the indoor air of family cattle barns, considering seasonal variations. The study's main objective is to evaluate the impact of barn structural characteristics and ventilation systems on air quality and to provide recommendations to improve animal welfare.

## MATERIAL METHOD

Indoor air samples were taken from 10 barns in the Çay, Sipahiler, and Sevindik regions of the İhsangazi District of Kastamonu Province. The selected barns are family-type enterprises whose ventilation systems consist of only a few household windows. Sampling was carried out twice a month over 12 months (Figure 1).

Microfungus that presented in the indoor air of the barns were separated using a "Microbial Air Sampler." A Merck Millipore M Air T Microbiological Air Sampler was used to capture microfungal bioaerosols, and 100 L of air was aspirated for each sample. DRBC (Dichloran Rose Bengal Chloramphenicol Agar), CDA (Czapex Dox Agar), and MEA (Malt Extract Agar) were used for the isolation of microfungi. Identification of the microfungi included both macroscopic and microscopic studies.

Microfungi isolated from the indoor air of ten barns were cultured in the laboratory at 25 °C for seven days. Microfungal colonies grown in Petri plates were transferred to inclined cultures con-


**Figure 1.** The locations of Çay, Sipahiler, and Sevindik neighborhoods in the İhsangazi District of Kastamonu Province are shown with dots (adapted from Google Maps).

taining PDA (Potato Dextrose Agar) to create stock cultures stored at 4 °C. PDA, CDA, and MEA agar were used for identification. Microscopic analysis of the microfungus was performed using Lactophenol Cotton Blue solution, as Sime et al. (2002) recommended. The MALDI-TOF MS device available at Hatay Mustafa Kemal University Plant Health Clinic Application and Research Center was used for species that could not be identified microscopically.

Today, matrix-assisted laser desorption/ionization flight duration (MALDI-TOF) mass spectrometry (MS) has been recognized as a fast, easy-to-use, and successful analytical technique in microbiological studies, offering an accurate alternative to current identification techniques for the identification of plant-associated fungi, bacteria, and yeasts on a species basis. Microorganism analysis procedures by MALDI-TOF MS are technically simple and rapid. Furthermore, reproducible and commercial databases are available to identify a wide range of important microorganisms. MALDI-TOF MS analyzes specific peptides or proteins directly from intact bacteria, fungi, and yeast (Chalupová et al., 2014; Carolis et al., 2012).

In the microorganism identification device, the proteins obtained by the ethanol formic acid extraction method are identified by library screening using protein fingerprint matching to identify microorganisms (bacteria, fungi, yeast, mould). The spectra obtained with the flex control software program (Biotyper 3.0; Microflex LT; Bruker Daltonics GmbH, Bremen, Germany) were compared with the MALDI Biotyper Real-Time Classification (RTC) software (version 12). As a result of the analysis, the data determined as yellow/green colored between 1,700-3,000 is considered a reliable score value. (Uysal et al., 2018, Patel, 2019).

New colonies obtained from microfungus samples were sent to Hatay Mustafa Kemal University Plant Health Clinic Application and Research Center for identification by MALDI-TOF. The microorganism identification approach adhered to the methodology outlined by Uysal et al. (2018). Protein extractions were performed from pure microfungus cultures, and genus and species identification was obtained using spectral analysis generated by the instrument.

The MALDI-ToF MS plate is placed in the instrument chamber. Each point to be analyzed is hit with a laser, resulting in the desorption and ionization of bacteria or fungi and matrix molecules from the target plate. The cloud of ionized molecules is accelerated towards the time-of-flight mass analyzer, a detector. Lighter molecules move faster, followed by progressively heavier ones. A mass spectrum is produced; this indicates the number of ions that hit the detector over time. The separation is based on the mass-to-charge ratio because, for this application, the charge is usually sole, and the separation is based on molecular weight (Patel, 2015).

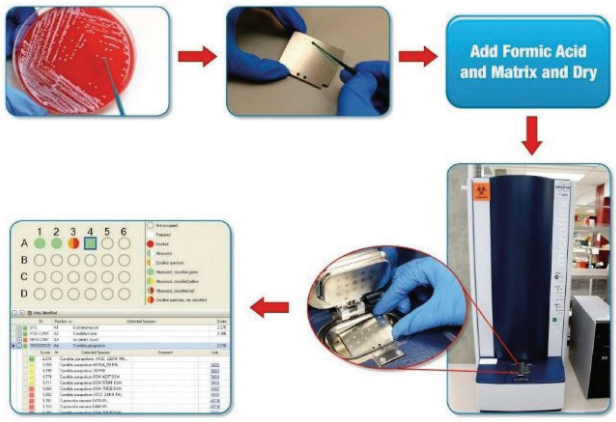


**Figure 2.** Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-ToF MS) process for microfungi identification (Patel, 2015). A colony is picked from a culture plate to a spot on a MALDI-ToF MS target plate (a disposable or reusable plate with multiple spots, each of which can be used to test different colonies). For yeast applications, cells are usually treated with formic acid on the target plate and then dried. The spot is coated with 1-2 µL of matrix and dried. The plate is placed in the ionization chamber of the mass spectrometer. A mass spectrum is generated and compared by the software to a library of mass spectra, resulting in the identification of the yeast (in the example *Candida parapsilosis* at position A4). Used with permission from Mayo Medical Education and Research Foundation. All rights reserved.

### Culture Medium and Incubation

Microfungi isolated from barn indoor air were cultured in the laboratory at 25 °C for seven days. After incubation, fungal colonies grown on Petri plates were transferred to various culture media, including PDA, CDA, CYA, 25% Glycerol Nitrate Agar (G25N), and MEA for storage as stock cultures.

### Microscopic Identification and Statistical Analysis of Microfungi

Correlation and multiple regression analyses were performed to assess the relationship between collected microfungi samples and environmental factors. The effect of environmental variables, including temperature and humidity, on microfungus species and density was analyzed using IBM SPSS Statistics (Version 22) and Graphpad (Version 10) software.

## CONCLUSION

This study analyzed air samples from 10 stables in the İhsangazi District of Kastamonu Province and identified 72 different microfungi species of 18 genera. The most frequently observed genera were *Acremonium, Alternaria, Cladosporium, Aspergillus*, and *Penicillium*. A comprehensive list of the identified species is presented below (Table 1).

**Figure 3.** Pre-purification image of some microfungus in the barn interior environment selected from the İhsangazi District of Kastamonu Province (Photographed by Giray and Şimşek).

**Figure 4.** Kastamonu Province, Ihsangazi District, the image of the sampled family-type barn (Photographed by Giray and Şimşek).

**Figure 5.** Styrofoam ceiling and ventilation of the sampled family barn in the İhsangazi District of Kastamonu Province (Photographed by Giray and Şimşek).

### Seasonal Distribution

Significant differences were observed in the seasonal distribution of microfungi. The density of *Alternaria* and *Cladosporium* species increased in summer and fall. Acremonium and Penicillium species were observed to be more abundant in winter (Table 2).

The seasonal distribution of microfungus species in the indoor air of animal shelters was analyzed. *Alternaria, Aspergillus, Cladosporium, Mucor,* and *Penicillium* species were common in all seasons. In contrast, *Acremonium*, *Botrytis*, and *Beauveria* were recorded only during certain seasons. Also, certain species were more common in the winter season. The study's findings include microfungus species that were consistently present throughout the year and showed seasonal fluctuations in their distribution (Table 2).

### Impact of Weather Conditions

The influence of meteorological variables on the distribution of microfungus was also studied. The density of *Aspergillus* and *Penicillium* species increased under high temperatures and low humidity, while *Cladosporium* species showed a significant increase under high humidity conditions. Figure 3 shows the monthly average temperature and relative humidity trends documented over the trial period.

**Table 1.** Table of microfungus species and genera detected in barns

| Genus | Species |
|---|---|
| *Acremonium* | *Acremonium charticola, A. strictum, A. zonatum* |
| *Alternaria* | *Alternaria alternata, A. alternarina, A. brassiciola, A. citri, A. tenuissima* |
| *Aspergillus* | *Aspergillus awamori, A. candidus, A.carbonarius, A. flavus, A. fumigatus, A. iizukae, A. montevidensis, A. niger, A. ochraceus, A. parasiticus, A. tamarii, A. terreus, A. tubigensis A. tubingensis, A. versicolor, A. wentii* |
| *Botrytis* | *Botrytis cinerea* |
| *Beauveria* | *Beauveria bassiana* |
| *Cladosporium* | *Cladosporium butyri, C. cladosporioides, C. cucumerinum, C. herbarum, C. macrocarpum, C. ramotenellum, C. teniussinum* |
| *Drechslera* | *Drechslera australiensis* |
| *Fusarium* | *Fusarium oxalicum, F. oxysporum* |
| *Geosmithia* | *Geosmithia pallida* |
| *Mucor* | *Mucor circinelloides, M. hiemalis, M. racemosus, M. pulumbeus* |
| *Penicillium* | *Penicillium aurantiogriseum, P. brevicompactum, P. camemberti, P.chrysogenum, P. commune, P. crustosum, P. decumbens, P. digitatum, P. expansum, P. fellutanum, P. glabrum, P. griseofulvum, P. italicum, P. mangini, P. nalgiovense, P. oxalicum, P. pasqualense, P. polanicum, P. roqueforti, P. verrucosum, P. viridicatum* |
| *Rhizopus* | *Rhizopus stalonifer* |
| *Scopulariopsis* | *Scopulariopsis brevicaulis* |
| *Sporothrix* | *Sporothrix schenckii* |
| *Trichoderma* | *Trichoderma harzianum, T. viride* |
| *Trichothecium* | *Trichothecium roseum* |
| *Trichophyton* | *Trichophyton sp.* |
| *Ulocladium* | *Ulocladium atrum, U. chartarum* |

**Table 2.** Distribution of common microfungus species according to seasons.

| Types | Spring | Summer | Autumn | Winter |
|---|---|---|---|---|
| *Acremonium* | | | October, November | January, February |
| *Alternaria* | All months | All months | All months | All months |
| *Aspergillus* | All months | All months | All months | All months |
| *Botrytis* | | | | December, January |
| *Beauveria* | | | September, November | |
| *Cladosporium* | All months | All months | All months | All months |
| *Drechslera* | May | | | |
| *Fusarium* | | July, August | | |
| *Geosmithia* | April | | | December |
| *Mucor* | All months | All months | All months | All months |
| *Penicillium* | All months | All months | All months | All months |
| *Rhizopus* | All months | | | |
| *Scopulariopsis* | March, May | | | |
| *Sporothrix* | | | September | |
| *Trichoderma* | | | October | December, January |
| *Trichothecium* | March | June | September, October | December |
| *Trichophyton* | | | | December |
| *Ulocladium* | March, April | | | December |

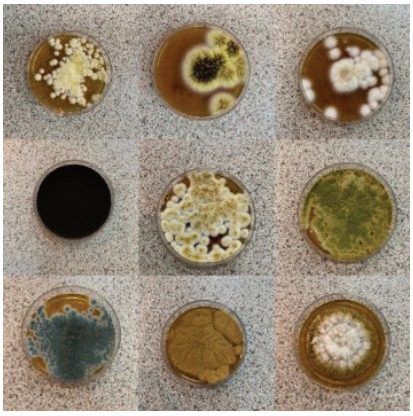

**Figure 6.** Pictures of some of the species isolated and identified from the barn interior (Photographed by Giray and Şimşek).

The studied environmental characteristics did not significantly vary over the months (P=0.10). The ideal range of high and low humidity levels and high and low temperatures for each fungal species studied were defined in detail in Table 3.

In order to determine the minimum and maximum temperature values of the microfungus detected in the indoor air of the barn depending on the indoor air temperature, TFA Heat, and Humidity Meter Device was measured in the middle parts of the barn and in the same place each time.

The 18 species of microfungi analyzed in this study show differences influenced by environmental conditions. *Alternaria, Aspergillus, Mucor, Penicillium,* and *Ulocladium* can grow over a broad temperature spectrum (5.5°C to 34.7°C) and at high humidity levels (83%). In contrast, *Acremonium, Botrytis, Scopulariopsis,* and *Trichophyton* prefer cooler environments with moderate humidity levels (70-75%). *Beauveria, Cladosporium, Fusarium*, and *Trichoderma* thrive at high temperatures (around 30°C) and high humidity levels (80%). *Drechslera, Geosmithia, Rhizopus, Sporothrix*, and *Trichothecium* live in low temperatures and humidity levels. Microfungal species respond to a wide range of environmental conditions, showing various responses to fluctuations in temperature and humidity.

Correlation study showed a positive relationship between microfungus density and temperature and a negative relationship with humidity. Multiple regression analysis showed that temperature and humidity significantly affected microfungi density ($p < 0.05$).

## DISCUSSION

This study constitutes comprehensive research in Turkiye that examines the seasonal distribution of airborne microfungal flora in cow stables and the environmental factors affecting this flora. Our findings make important contributions to the literature by affecting animal welfare and barn workers' health and occupational safety.

Throughout the study, species such as *Mucor, Alternaria, Aspergillus, Trichothecium, Cladospori-*

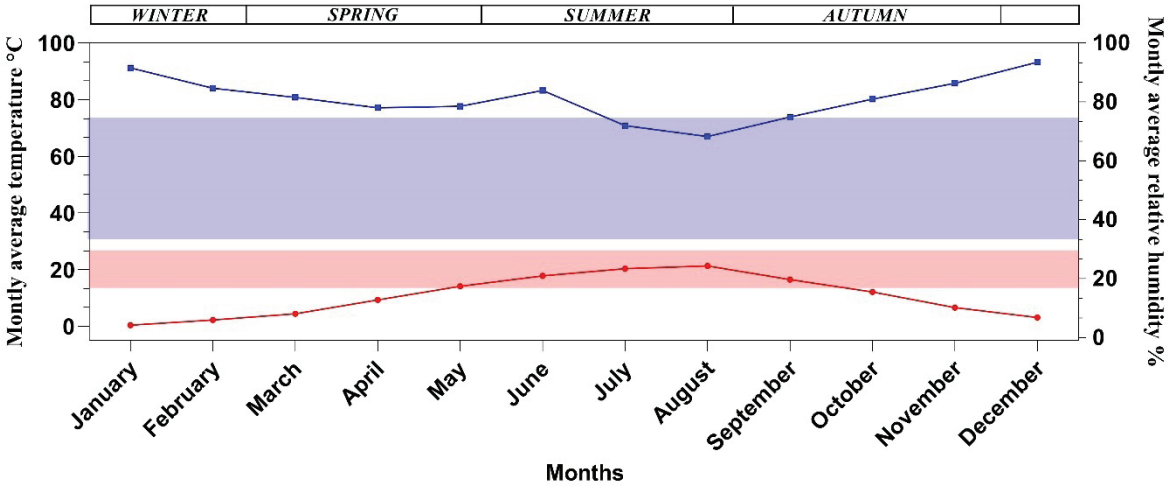


**Figure 7.** The monthly average temperature (Red line) and humidity (Blue line) trend, expressed in divided standard units for each year's season. The red band represents the optimal range of average high and low temperatures for the studied microfungus species, while the blue band represents the optimal range of average high and low humidity for the studied microfungus species. (URL-1 2023).

**Table 3.** Density of Microfungus Species Depending on Weather Conditions

| Genus | High Temperature | Low Temperature | High Humidity (%) | Low Humidity (%) |
|---|---|---|---|---|
| *Acremonium* | 25,6 | 5,5 | 75 | 37 |
| *Alternarıa* | 34,7 | 5,5 | 83 | 27 |
| *Aspergillus* | 34,7 | 5,5 | 83 | 27 |
| *Botrytis* | 24,5 | 5,5 | 75 | 59 |
| *Beauverıa* | 30 | 18,2 | 80 | 34 |
| *Cladosporıum* | 34,7 | 5,5 | 83 | 27 |
| *Drechslera* | 27,2 | 20 | 76 | 36 |
| *Fusarıum* | 34,2 | 22,9 | 61 | 27 |
| *Geosmıthıa* | 26,3 | 8,1 | 83 | 36 |
| *Mucor* | 34,7 | 5,5 | 83 | 27 |
| *Penicillium* | 34,7 | 5,5 | 83 | 27 |
| *Rhızopus* | 34,7 | 5,5 | 83 | 27 |
| *Scopularıopsis* | 27,2 | 15,3 | 76 | 36 |
| *Sporothrix* | 30 | 20 | 52 | 34 |
| *Trıchoderma* | 26,2 | 12,9 | 74 | 43 |
| *Trıchothecıum* | 34,7 | 12,9 | 80 | 34 |
| *Trıchophyton* | 19,5 | 12,2 | 74 | 44 |
| *Ulocladıum* | 26,5 | 5,5 | 83 | 36 |

*um,* and *Penicillium* were consistently detected and formed permanent elements of the barn air throughout all seasons. High levels of these species pose significant risk factors for respiratory allergies, asthma, and health complications associated with mycotoxins (Adhikari, 2000; Elfman et al., 2009). *Aspergillus flavus*, known for its capacity to produce the carcinogen aflatoxin, has been detected throughout the year, raising significant public health concerns (Pekel, 2008). This underlines that barn air poses a danger not only for animals but also for barn workers and people in the environment.

The fact that species such as *Fusarium, Rhizopus, Scopulariopsis, Sporothrix, Drechslera, Beauveria, Botrytis*, and *Acremonium* are only observed during specific periods indicates that these microfungi are more sensitive to environmental fluctuations and exhibit a transient occurrence depending on seasonal changes. The higher occurrence of these species, especially in summer, has been associated with plant materials, indicating that agricultural environments can serve as fungal reservoirs (Ghasian et al., 2011; Adhikari et al., 2004). This study confirmed the significant effect of temperature and relative humidity on the production and spread of microfungal spores in indoor environments. Increased temperature and humidity significantly increased the cultivable concentrations of fungal spores, leading to a significant increase in bioaerosol load, especially during the rainy season (Herrero & Zaldivar, 1997; Larsen & Frisvad, 1994). This discovery highlights that microbial load in barn air affects both animal health and the quality of products such as milk and feed. Seasonal findings show that *Cladosporium* and *Alternaria alternata* were the most common species detected in the air during summer, probably originating from plant components and contributing to significant spore dispersal. In contrast, *Aspergillus* and *Penicillium* species were primarily detected during winter. The findings suggest species diversity across several seasons is significantly linked to barn structural features and environmental conditions (Adhikari et al., 2004; Ghasian et al., 2011).

## CONCLUSION

This study has provided a detailed map of the microfungal flora of 10 barns in Ihsangazi District, Kastamonu Province. It has highlighted the importance of indoor ventilation management in cattle barns. Consistent assessment of barn air quality, improvement of ventilation systems, and implementation of preventive strategies against microfungal

proliferation are essential for the welfare of animals and the health of workers. The high levels of *Aspergillus, Penicillium,* and *Cladosporium* detected in this study are alarming for animal health and may lead to contamination of milk and dairy products (Ranjan & Sinha, 1991; Cabral et al., 2012).

In conclusion, this study provides a solid basis for improving indoor air quality in animal barns and reducing microfungal-related health problems. Our findings provide an important framework for strategic decision-making to ensure the sustainability and safety of the cattle sector at both local and global levels. However, comprehensive and in-depth research is required to gain a deeper understanding of the effects of microfungal flora on health.

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**Data statement**

Data are available on request or on a public repository.

**Credit authorship contribution statement**

Abdullah ŞİMŞEK: Writing – Original draft, Conceptualization, Formal analysis, Investigation, Gülay GİRAY: Methodology, Investigation, Project Administration, Validation.

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