atmosphere

MDPI

Article

Effects of Season and House Microclimate on Fungal Flora in Air and Broiler Trachea

Danijela Horvatek Tomić [1,*], Ivica Ravić [2], Anamaria Ekert Kabalin [3], Matija Kovačić [4], Željko Gottstein [1] and Mario Ostović [5,*]

1 Department of Poultry Diseases with Clinic, Faculty of Veterinary Medicine, University of Zagreb, 10000 Zagreb, Croatia; gottstei@vef.hr
2 Veterinary Department, 88000 Mostar, Bosnia and Herzegovina; iravic@hotmail.com
3 Department of Animal Breeding and Livestock Production, Faculty of Veterinary Medicine, University of Zagreb, 10000 Zagreb, Croatia; akabalin@vef.hr
4 Kovačić Family Farm, Šopron, 48267 Orehovec, Croatia; matija.kovacic.kole@gmail.com
5 Department of Animal Hygiene, Behaviour and Welfare, Faculty of Veterinary Medicine, University of Zagreb, 10000 Zagreb, Croatia
* Correspondence: horvatek@vef.hr (D.H.T.); mostovic@vef.hr (M.O.); Tel.: +385-1-2390-281 (D.H.T.); +385-1-2390-294 (M.O.)

Abstract: Fungi are present in abundance in poultry housing. The aim of the study was to assess the effect of season and microclimate parameters in poultry housing on fungal flora in the air and broiler trachea in commercial fattening conditions. The study was conducted in summer and winter. Study results indicated seasonal impact and association between fungal flora composition in housing air and broiler trachea. However, the total fungal count in housing air was significantly higher in summer and in broiler trachea in winter, both significantly correlated with indoor relative humidity and ammonia concentration. There was no significant correlation between outdoor and indoor air temperature, relative humidity and airflow rate, respectively. Study results suggested that environmental determination of fungi should be accompanied by their determination in broilers. In addition, seasonal impact on fungal contamination should be associated with microclimate conditions in the poultry house rather than the season itself. The fungi detected and the results obtained have implications not only for broiler health but also for the health of humans working in such environments.

Keywords: poultry; mycoflora; season; microclimate; health

Citation: Horvatek Tomić, D.; Ravić, I.; Kabalin, A.E.; Kovačić, M.; Gottstein, Ž.; Ostović, M. Effects of Season and House Microclimate on Fungal Flora in Air and Broiler Trachea. *Atmosphere* **2021**, *12*, 459. https://doi.org/10.3390/atmos12040459

Academic Editor: Ferdinando Salata

Received: 22 February 2021
Accepted: 2 April 2021
Published: 6 April 2021

1. Introduction

Diseases occurring in intensive livestock production that are directly associated with the environment of animal farming are influenced by a number of factors including air quality. Fungal spores are a constituent of bioaerosol. Comparative studies of air quality in housing intended for various farm animals have shown that poultry housing air contains the highest rate of fungi [1,2], broiler houses in particular [3,4]. Like bacteria, fungi may originate from the soil, dust, feed and litter, and to a lesser extent from the poultry [5], although some species such as *Aspergillus* sp. may utilise and degrade keratin from feathers [6]. Although fungal diseases are less common in poultry as compared with bacterial and viral diseases, they should not be neglected because when occurring, they can cause considerable economic losses either by direct infestation or via mycotoxin production [7].

Moulds of the genus *Aspergillus* are ubiquitous saprophytic microorganisms that induce clinically manifest infections in poultry under specific conditions, primarily involving respiratory system [8]. Aspergillosis is a severe disease in poultry farming all over the world [9], associated with high morbidity and mortality, along with poor feed conversion and weight gain in recovering birds, and carcass condemnation [7,10]. Young birds are more susceptible to acute aspergillosis, whereas chronic form of the disease is sporadic and

more common in older poultry. Although the chronic form is by far less frequent, it causes enormous losses by affecting older and thus economically higher-price poultry [10,11]. The disease is also important from the occupational and public health aspects [12–14].

Aspergillosis is mostly caused by *A. fumigatus*, the most pathogenic fungus affecting poultry [7,15], however, the role of other *Aspergillus* species in the disease should not be ignored [9]. Aspergillosis is not a contagious disease but develops upon inspiring a large number of spores, or in cases of reduced resistance in poultry. The factors favouring disease development include long-term exposure and heavily contaminated environment, stress, immunosuppression, poor ventilation and sanitation, wet litter, malnutrition, and prolonged feed storage [8,16]. Season and microclimate parameters are known to be important factors that influence the occurrence of fungi and fungal diseases in poultry production. However, there is no strict consensus on their effects. Sajid et al. [17] report on a higher incidence of aspergillosis in poultry during warm and humid season as compared with cold season, which is consistent with the results reported by Sultana et al. [18]. Viegas et al. [19] found that fungal air contamination in poultry houses increased with higher indoor air temperature, whereas the study conducted by Popescu et al. [20] showed negative correlation of fungal count in the house air with air temperature but positive correlation with relative humidity inside houses. Lawniczek-Walczyk et al. [21] recorded no significant correlation of indoor air temperature or relative humidity with fungal count in the house air, and no seasonal differences in fungal count. Debey et al. [22] reported a higher yeast count in poultry house air in winter as compared with summer, whereas the total mould count showed no significant seasonal difference. However, seasonal differences were recorded in particular mould counts, with higher *Aspergillus* sp. concentration recorded in winter as compared with summer. The increased rate of *Aspergillus* sp. isolation correlated negatively with relative humidity. Wójcik et al. [23] found a higher fungal count in poultry house air in winter as compared with summer.

The aim of the study was to compare mycoflora in the house air and broiler trachea between summer and winter, with special reference to *Aspergillus* sp. The following hypotheses were tested: (i) fungal count and composition in poultry house air will differ between summer and winter, (ii) fungal flora in poultry house air will be correlated with fungal flora in broiler trachea, (iii) indoor microclimate conditions will influence fungal flora, and (iv) outdoor climate conditions will influence indoor microclimate.

2. Materials and Methods

2.1. Location and Climate

The study was carried out in Koprivnica-Križevci County, Republic of Croatia, characterized by temperate continental climate with precipitation spread throughout the year, 850–900 mm on average. There are two precipitation peaks, in July and November, whereas February has the lowest precipitation level. The highest summer temperatures are accompanied by the highest precipitation levels. There are no extremely dry periods. Winds blow throughout the year, thus the area is characterised by gentle winds. Maximal air humidity is recorded in November and December, and minimal humidity in April and May, with 82% mean annual relative humidity [24].

2.2. Experimental Design

The study included broiler fattening cycles in summer (July–August 2016) and winter (December–January 2016/2017). In each season, fattening cycle lasted for five weeks, with 18,000 Ross hybrid broilers kept under commercial housing conditions in a closed house with controlled microclimate, at up to 33 kg/m^2 stocking density, and a mixture of chopped straw and sawdust (10 cm depth) used as litter. Heating was provided by oil heater and ventilation by a negative pressure system. The lighting programme was set in accordance with the Ross broiler manufacturer's recommendations [25]. Broilers were fed complete feed mixture from round pan feeders and watered from nipple drinkers with cups, with

ad libitum feeding and watering. The broiler house is cleaned and disinfected after each production cycle, with two-week house rest between the cycles.

Microclimate conditions, air temperature, relative humidity and airflow rate inside the house, ammonia concentration in house air, and fungal concentration in house air and in broiler trachea were determined once a week during fattening period in both seasons. Air temperature, relative humidity and airflow rate were also measured at 5 m outdoor weekly. Air temperature (°C), relative humidity (%), airflow rate (m/s) and ammonia concentration (ppm) were measured by portable digital instruments (Testo SE & Co. KGaA, Lenzkirch, Germany, and Dräger Safety AG & Co. KGaA, Lübeck, Germany). For determination of fungal count, the air was sampled onto Petri dishes with Sabouraud dextrose agar (Biolife, Milan, Italy) using a SAS 100^{TM} device (PBI International, Milan, Italy). Plates were incubated at 25 °C for five to seven days and grown colonies were expressed as colony-forming units per m^3 air (CFU/m^3), with result correction according to the table and formula supplied with the device. Both indoor and outdoor measurements were performed from 9.00 to 12.00 a.m. at nine sites. The presence of fungi in broilers was determined in tracheal swabs obtained weekly from 30 randomly chosen, apparently healthy birds. Tracheal swabs were taken with a sterile stick soaked with sterile saline (1 mL), then 100 µL was smeared on agar. Agar and incubation conditions were the same as for airborne fungi. Results were expressed as CFU/swab. Fungal identification was carried out by macroscopic observation of grown colonies and microscopic examination of spores using lactophenol blue stain [26].

2.3. Statistical Analysis

Data analysis was performed by Statistica v. 13.5 reference software (TIBCO Software Inc., Palo Alto, CA, USA, 2018). Data were processed by standard descriptive statistics methods and expressed as mean and standard deviation or median and minimum-maximum values, depending on the normality of distribution estimated by the Kolmogorov–Smirnov test. Student's t-test was used to test the significance of differences in the mean values of air temperature, relative humidity and airflow rate inside and outside the house, ammonia concentration in house air, and total fungal count in house air between two seasons. The significance of differences in the median count of total fungi in broiler trachea and particular fungal counts in house air and broiler trachea between the seasons were tested by Mann–Whitney U-test, and so were between-season differences in the values of all parameters observed according to fattening weeks. Differences among fattening weeks within a season were tested by Friedman ANOVA and Wilcoxon matched pairs test for post hoc analysis. Correlations among study parameters were assessed by Spearman rank order correlations. The level of statistical significance was set at $p < 0.05$, although differences at the levels of $p < 0.01$ and $p < 0.001$ are also reported in tables.

3. Results and Discussion

Study results on the average values of air temperature, relative humidity and airflow rate inside and outside the house, ammonia concentration in house air, and fungal count in house air and broiler trachea (total and particular fungal count) in summer and winter, total fungal count in house air and broiler trachea according to fattening weeks in each season, and correlations among study parameters are shown in Tables 1–6 and Figures 1 and 2. The figures showing indoor and outdoor air temperature, relative humidity and airflow rate, and ammonia concentration in house air according to fattening weeks in each season are presented in Supplementary Materials. During the study period, no cases of fungal diseases were recorded, while the rate of broiler deaths was within the technologically predicted rate in both seasons.

Table 1. Values of house microclimate parameters during five-week broiler fattening in summer and winter.

Parameter	Summer	Winter
	Mean ± SD (Min–Max)	
Air temperature (°C)	28.16 ± 2.59 (22.60–32.10)	27.12 ± 2.93 (22.20–32.00)
Relative humidity (%)	61.87 ± 4.78 (54.00–70.50)	68.63 ** ± 9.21 (52.00–80.70)
Airflow rate (m/s)	0.18 ± 0.09 (0.03–0.32)	0.15 ± 0.07 (0.05–0.29)
Ammonia (ppm)	6.52 ± 2.63 (1.00–11.00)	10.96 *** ± 5.62 (3.00–21.00)

Values in the same row differ significantly at ** $p < 0.01$ or *** $p < 0.001$.

Table 2. Values of air temperature, relative humidity and airflow rate outside house during five-week broiler fattening in summer and winter.

Parameter	Summer	Winter
	Mean ± SD (Min–Max)	
Air temperature (°C)	24.90 ± 5.40 (14.00–29.00)	−0.02 * ± 3.71 (−6.70–4.60)
Relative humidity (%)	55.99 ± 14.74 (37.90–84.10)	66.80 * ± 15.96 (40.20–87.20)
Airflow rate (m/s)	0.59 ± 0.42 (0.18–1.50)	0.98 * ± 0.72 (0.43–3.36)

Values in the same row differ significantly at * $p < 0.05$.

Table 3. Total fungal count in house air and broiler trachea during five-week fattening in summer and winter.

Parameter	Summer	Winter
Airborne fungi (CFU/m^3) Mean ± SD (Min–Max)	$2.82 \times 10^4 \pm 2.81 \times 10^2$ (4.9×10^3–8.1×10^4)	1.92 ** $\times 10^4 \pm 1.27 \times 10^4$ (2×10^2–3.7×10^4)
Tracheal fungi (CFU/swab) Median (Min–Max)	2.70×10^2 (50–4.01×10^3)	1.91 ** $\times 10^3$ (0–1.71×10^4)

Values in the same row differ significantly at ** $p < 0.01$.

Table 4. Correlation between house microclimate parameters, and airborne and tracheal fungi.

Parameter	Air Temperature (°C)	Relative Humidity (%)	Airflow Rate (m/s)	Ammonia (ppm)	Airborne Fungi (CFU/m^3)
Airborne Fungi (CFU/m^3)	−0.164	0.437 *	0.195	0.335 *	-
Tracheal Fungi (CFU/swab)	−0.437 *	0.691 *	0.197 *	0.491 *	0.208 *

* $p < 0.05$.

Table 5. Composition of airborne fungal flora in broiler house during five-week fattening in summer and winter.

Fungi	Summer	Winter
	CFU/m^3 Median (Min–Max)	
Aspergillus sp.	1.20×10^3 (0–4.04×10^4)	1.00 *** $\times 10^2$ (0–9.00×10^2)
A. flavus	1.00×10^3 (0–3.99×10^4)	1.00 *** $\times 10^2$ (0–6.00×10^2)
A. fumigatus	Not detected	0 (0–3.00×10^2)
A. niger	2.00×10^2 (0–2.00×10^3)	0 *** (0–3.00×10^2)
A. terreus	Not detected	0 (0–3.00×10^2)
Cladosporium sp.	0 (0–1.00×10^3)	0 (0–8.00×10^2)
Fusarium sp.	0 (0–2.00×10^2)	0 (0–1.00×10^2)
Mucor sp.	0 (0–3.00×10^2)	1.00 *** $\times 10^2$ (0–2.60×10^4)
Penicillium sp.	0 (0–8.00×10^2)	2.50 *** $\times 10^3$ (0–1.00×10^4)
Rhizopus sp.	0 (0–1.00×10^2)	0 (0–1.00×10^2)
Yeasts	2.14×10^4 (4.00×10^3–3.99×10^4)	5.00 *** $\times 10^3$ (0–3.40×10^4)
Unidentified	1.00×10^2 (0–6.00×10^2)	0 *** (0–2.00×10^2)

Values in the same row differ significantly at *** $p < 0.001$.

Table 6. Composition of tracheal fungal flora during five-week broiler fattening in summer and winter.

Fungi	Summer	Winter
	CFU/m^3 Median (Min–Max)	
Aspergillus sp.	0 (0–1.00 × 10^2)	0 *** (0–40)
A. flavus	0 (0–1.00 × 10^2)	0 *** (0–40)
A. fumigatus	Not detected	0 (0–30)
A. niger	0 (0–30)	0 (0–20)
A. terreus	Not detected	0 (0–10)
Cladosporium sp.	0 (0–1.80 × 10^2)	0 *** (0–20)
Fusarium sp.	0 (0–20)	0 (0–10)
Mucor sp.	0 (0–30)	0 *** (0–3.10 × 10^2)
Penicillium sp.	10 (0–1.70 × 10^2)	10 * (0–6.60 × 10^2)
Rhizopus sp.	0 (0–20)	0 (0–20)
Trichophyton sp.	Not detected	0 (0–10)
Yeasts	2.20 × 10^2 (0–3.99 × 10^3)	1.65 ** × 10^3 (0–1.71 × 10^4)
Unidentified	0 (0–1.00 × 10^2)	0 *** (0–10)

Values in the same row differ significantly at * $p < 0.05$, ** $p < 0.01$ or *** $p < 0.001$.

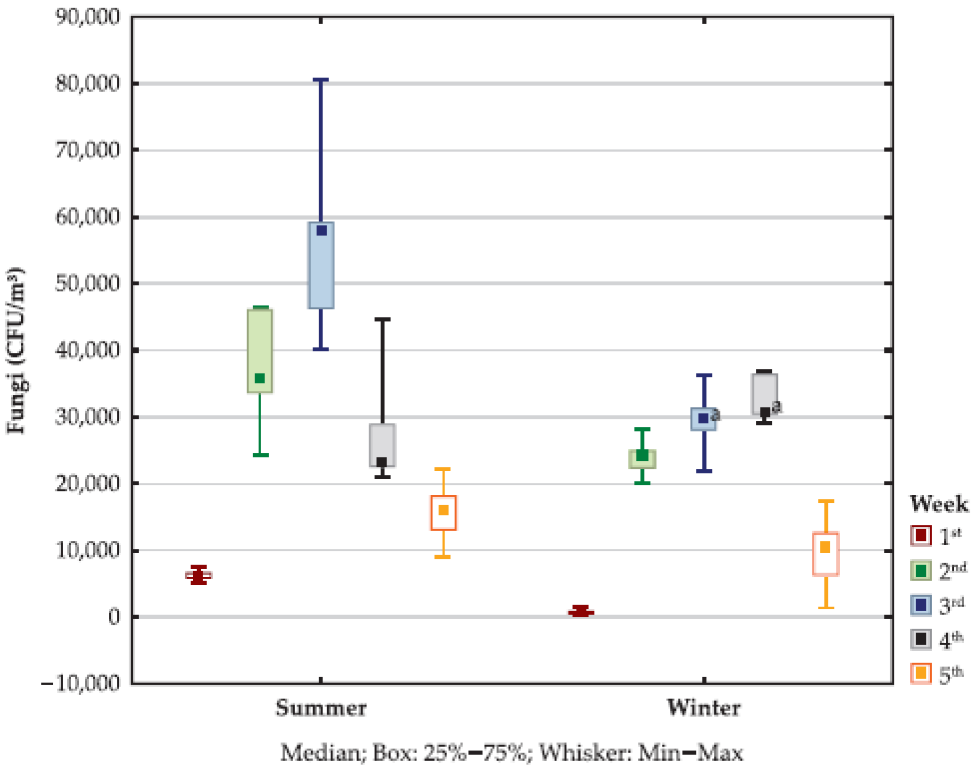

Figure 1. Airborne fungi in broiler house according to fattening weeks in summer and winter. [a] All values within the same season differ significantly ($p < 0.05$), except for the marked ones. All values within the same weeks differ significantly between the seasons ($p < 0.05$).

The measured values of house microclimate parameters were comparable with the results reported elsewhere [21,27–29]. Air temperature inside the house was highest in the first fattening week, then generally decreased significantly ($p < 0.05$) during the fattening period in both summer and winter. In comparison to winter, higher indoor air temperature was recorded in all fattening weeks in summer, yielding significant differences ($p < 0.05$) in the second, fourth and fifth weeks; however, there was no significant between-season difference in the mean indoor air temperature during fattening period (Table 1). In summer, relative humidity inside the house increased until the mid-fattening period, followed by a decrease (the lowest value being recorded in the last, fifth fattening week), unlike winter when air humidity increased until the end of the fattening period (the highest value

being recorded in the last, fifth fattening week). In comparison to summer, significantly higher values of indoor relative humidity ($p < 0.05$) were recorded in the last two fattening weeks in winter, with a significantly higher mean value ($p < 0.01$) during the fattening period in winter (Table 1). In both summer and winter, airflow rate in the broiler house generally increased with fattening weeks. However, unlike winter, in summer there was no significant difference at the end as compared with the beginning of fattening. There was no significant between-season difference in indoor airflow rate either according to fattening weeks or in the mean values (Table 1).

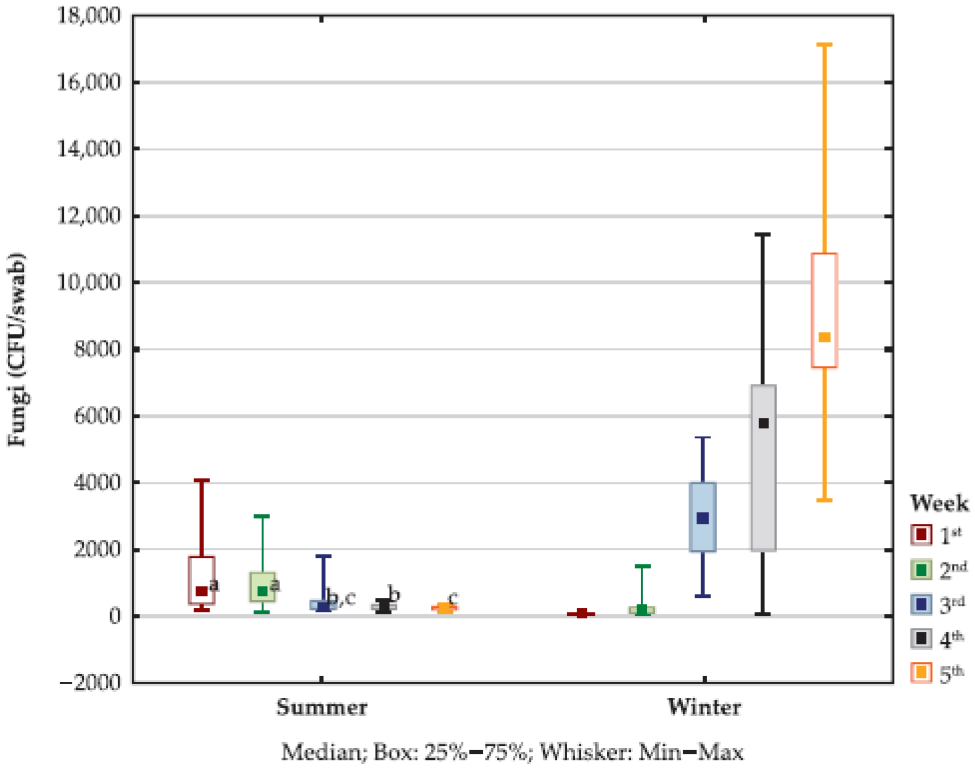

Figure 2. Tracheal fungi according to broiler fattening weeks in summer and winter. [a,b,c] All values within the same season differ significantly ($p < 0.05$), except for those marked with the same letter. All values within the same weeks differ significantly between the seasons ($p < 0.05$).

Mean air temperature outside the house was significantly higher ($p < 0.05$) during fattening period in summer, whereas the mean relative humidity and airflow rate were significantly higher ($p < 0.05$) in winter (Table 2). There was no significant correlation between either outdoor or indoor values of air temperature ($r = 0.233$; $p > 0.05$), relative humidity ($r = -0.095$; $p > 0.05$) and airflow rate ($r = 0.216$; $p > 0.05$), indicating controlled production conditions. Both indoor and outdoor mean relative humidity was higher in winter (Tables 1 and 2, respectively); however, unlike outdoor relative humidity, indoor relative humidity in winter increased with fattening weeks. This could be due to reduced ventilation in winter to prevent heat losses and avoid additional heating costs. That is why the concentrations of gaseous air pollutants in poultry housing usually are higher in winter months as well [30,31], as also confirmed by the results of this study. Ammonia concentration in house air generally increased significantly ($p < 0.05$) during fattening period in both seasons, except for a decrease in the last fattening week in winter. Comparison of the two seasons showed higher ammonia concentrations, with significantly higher values ($p < 0.05$) in the third and fourth fattening weeks and a significantly higher ($p < 0.001$) mean ammonia concentration during fattening in winter (Table 1).

The concentrations of airborne fungi in broiler houses commonly range from 10^3 to 10^5 CFU/m^3, with *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Fusarium* and *Scopulariopsis* as predominant fungal genera. The following genera also are quite commonly found:

Rhizophus, *Mucor* and *Geotrichum* [21,23,27,32]. In our study, total fungal count in broiler house air was consistent with previous research, having increased significantly ($p < 0.05$) by the mid-fattening period in both seasons (Figure 1), which could be explained by higher broiler activity in the initial fattening period, whereafter their activity decreased due to greater body mass and reduced mobility, thus decreasing the level of air pollution [27]. With the exception of the fourth week, total fungal count in the house air was significantly higher ($p < 0.05$) throughout the fattening period in summer as compared with winter (Figure 1), and so was the mean total fungal count ($p < 0.01$) in the house air during summer (Table 3). Although a significant positive correlation was found between fungal count in the air and relative humidity inside house (r = 0.437; $p < 0.05$) (Table 4), the results obtained could be explained by the lower mean indoor humidity in summer (Table 1). It is known that dust particles serve as microorganism carriers, and dust concentrations in poultry housing are higher at lower humidity [19,33]. Lawniczek-Walczyk et al. [21] did not find significant correlation between air humidity and fungal count in broiler housing or seasonal differences in fungal count, whereas Wójcik et al. [23] report on a higher airborne fungal count in winter as compared with summer. However, in their study, relative humidity was lower in winter, suggesting that fungal contamination should not be associated exclusively with season but rather with the real house microclimate. Contrary to the findings of Viegas et al. [19] and Popescu et al. [20], there was no significant correlation of fungal count in house air with indoor air temperature and airflow rate (Table 4); yet, in line with our report, the latter authors [20] found significant positive correlation between relative humidity and fungal count in poultry house air.

Investigating the effect of environmental conditions on mycoflora in poultry housing, Debey et al. [22] found the air to contain higher *Aspergillus* sp. concentration in winter as compared with summer, which was associated with a higher dust level and lower relative humidity, indicating that the spores of these fungi were more frequently found in dry than in humid air. This was supported by the results of our study. Although *A. fumigatus* and *A. terreus* were not isolated in the house air or broiler trachea in summer, *Aspergillus* sp. including *A. flavus* and *A. niger* was significantly more frequently detected ($p < 0.001$) both in the house air and broiler trachea in summer (Tables 5 and 6). However, in summer, the mean indoor relative humidity was lower as compared with winter. On the other hand, *Mucor* sp. and *Penicillium* sp. were significantly more commonly detected (at least at $p < 0.05$) in house air and broiler trachea in winter. In addition, *Cladosporium* sp. was more frequently isolated in the air and broiler trachea in summer, although its count in broiler trachea showed significant seasonal difference ($p < 0.001$). Although identified in house air and broiler trachea in both seasons, only the concentrations of *Fusarium* sp. and *Rhizopus* sp. did not yield seasonal differences. Thus, six mould genera including four *Aspergillus* species and *Trichophyton* sp. isolated in broiler trachea in winter were isolated in house air and broiler trachea in both seasons (Tables 5 and 6).

On average, yeasts predominated in house air and broiler trachea in both seasons, being significantly higher in house air in summer ($p < 0.001$), and in broiler trachea in winter ($p < 0.01$) (Tables 5 and 6), confirming association between the fungal flora composition in house air and broiler trachea, as well as the seasonal effect of fungal composition. In addition, these findings suggest that yeasts contribute significantly to total fungal count. Thus, the average total fungal count was higher in house air in summer and in broiler trachea in winter, although a significant positive correlation was found between fungal count in house air and in broiler trachea (r = 0.208; $p < 0.05$) (Table 4). Debey et al. [22] found yeast count to be higher in poultry house air in winter, which could be due to lower relative humidity, as previously discussed. Accordingly, higher airborne yeast concentration in our study in summer could be explained by lower mean relative humidity inside the house found in this season.

In summer, fungal count in broiler trachea decreased significantly ($p < 0.05$) after the second fattening week and maintained the level achieved until the end of the fattening period, whereas in winter it increased significantly ($p < 0.05$) (Figure 2). Accordingly, in

spite of the lower fungal count in house air in winter, fungal count in broiler trachea was higher as compared with summer. These results could be explained by the significant positive correlation (r = 0.691; $p < 0.05$) between relative humidity inside house and fungal count in broiler trachea (Table 4), i.e., by the higher mean indoor relative humidity in winter, whereby air humidity favoured deposition of fungi and tracheal infestation. Thus, more fungal genera were detected in broiler trachea in winter than in summer (Table 6). Besides this, ammonia concentration in house air was higher in winter than in summer. Ammonia has been demonstrated to be one of the main precursors of secondary particles [30,34]. Kumari et al. [35] found a significant positive correlation of air ammonia and secondary particles with fungal abundance. In our study, a significant positive correlation was also recorded between ammonia concentration and fungal count both in house air (r = 0.335; $p < 0.05$) and broiler trachea (r = 0.491; $p < 0.05$) (Table 4). In addition, tracheal fungal count was significantly correlated with air temperature (r = −0.437; $p < 0.05$) and airflow rate in the broiler house (r = 0.197; $p < 0.05$) (Table 4).

Potentially harmful fungi were identified in both seasons. *Aspergillus flavus* and *A. niger* are known to produce mycotoxins and can have a role in the outbreak of aspergillosis, *A. flavus* in particular [16,36]. Although *Aspergillus* sp., predominantly *A. flavus*, prevailed both in house air and in broiler trachea in summer, *A. fumigatus* and *A. terreus* were found in house air and in broiler trachea in winter. Also, *Trichophyton* sp. was isolated from broiler trachea in winter. According to the Commission Directive (EU) 2019/1833 [37], *Aspergillus* sp. and *Trichophyton* sp. are classified into group 2 biological agents considering the risk of infection in occupational environment. *Penicillium* sp., also known as a mycotoxin producer [36], was also more frequently identified in broiler trachea, as well as in house air in winter. *Fusarium* sp. is another mycotoxin producing fungus [36], but there were no seasonal differences in its concentrations either in house air or in broiler trachea. In addition, *Fusarium* sp. has been reported among the emerging causes of opportunistic mycoses in humans and animals [38,39].

4. Conclusions

The results of our study revealed a seasonal impact on fungal count and composition in poultry house air and in broiler trachea, demonstrating the association of fungal flora in house air and in broiler trachea. Indoor relative humidity and ammonia concentration were found to influence total fungal count in both poultry house air and broiler trachea; yet, in order to obtain exact results on seasonal effects, fungal determination should be performed both in the birds and in their environment. Nonsignificant correlation between outdoor and indoor air temperature, relative humidity and airflow rate indicated that seasonal differences in fungal contamination should not be related to season alone but rather to the housing microclimate conditions. The fungi detected in the study pose a health risk for both the poultry and the humans working in poultry environment.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/atmos12040459/s1, Figure S1: Air temperature inside broiler house according to fattening weeks in summer and winter, Figure S2: Relative humidity inside broiler house according to fattening weeks in summer and winter, Figure S3: Airflow rate inside broiler house according to fattening weeks in summer and winter, Figure S4: Air ammonia concentration in broiler house according to fattening weeks in summer and winter, Figure S5: Air temperature outside broiler house according to fattening weeks in summer and winter, Figure S6: Relative humidity outside broiler house according to fattening weeks in summer and winter, Figure S7: Airflow rate outside broiler house according to fattening weeks in summer and winter.

Author Contributions: Conceptualization, D.H.T., M.O. and I.R.; methodology, D.H.T., M.O. and I.R.; validation, D.H.T., M.O. and I.R.; formal analysis, A.E.K.; investigation, D.H.T., M.O., I.R. and M.K.; resources, D.H.T., M.O., I.R. and M.K.; data curation, A.E.K.; writing—original draft preparation, D.H.T., M.O. and I.R.; writing—review and editing, D.H.T., M.O., I.R., A.E.K., M.K. and Ž.G.; visualization, I.R., A.E.K. and Ž.G.; supervision, D.H.T. and M.O.; project administration, D.H.T.

and M.O.; funding acquisition, I.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was approved by the Ethics Committee of the Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia (class: 640-01/16-17/43; record no.: 251-61-01/139-16-2; April 21, 2016).

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article and the Supplementary Materials; further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Radon, K.; Danuser, B.; Iversen, M.; Monso, E.; Weber, C.; Hartung, J.; Donham, K.J.; Palmgren, U.; Nowak, D. Air contaminants in different European farming environments. *Ann. Agric. Environ. Med.* **2002**, *9*, 41–48. [PubMed]
2. Matković, K.; Vučemilo, M.; Vinković, B. Airborne fungi in dwellings for dairy cows and laying hens. *Arh. Hig. Rada Toksikol.* **2009**, *60*, 395–399. [CrossRef] [PubMed]
3. Seedorf, J.; Hartung, J.; Schröder, M.; Linkert, K.H.; Phillips, V.R.; Holden, M.R.; Sneath, R.W.; Short, J.L.; White, R.P.; Pedersen, S.; et al. Concentrations and emissions of airborne endotoxins and microorganisms in livestock buildings in northern Europe. *J. Agric. Eng. Res.* **1998**, *70*, 97–109. [CrossRef]
4. Jo, W.-K.; Kang, J.-H. Exposure levels of airborne bacteria and fungi in Korean swine and poultry sheds. *Arch. Environ. Occup. Health* **2005**, *60*, 140–146. [CrossRef]
5. Lonc, E.; Plewa, K. Microbiological air contamination in poultry houses. *Pol. J. Environ. Stud.* **2010**, *19*, 15–19.
6. Kim, J.-D. Keratinolytic activity of five *Aspergillus* species isolated from poultry farming soil in Korea. *Mycobiology* **2003**, *31*, 157–161. [CrossRef]
7. Dhama, K.; Chakraborty, S.; Verma, A.K.; Tiwari, R.; Barathidasan, R.; Kumar, A.; Singh, S.D. Fungal/mycotic diseases of poultry—diagnosis, treatment and control: A review. *Pak. J. Biol. Sci.* **2013**, *16*, 1626–1640. [CrossRef]
8. Kapetanov, M.C.; Potkonjak, D.V.; Milanov, D.S.; Stojanov, I.M.; Živkov Baloš, M.M.; Prunić, B.Z. Investigation of dissemination of aspergillosis in poultry and possible control measures. *Zb. Matice Srp. Prir. Nauk.* **2011**, *120*, 269–278. [CrossRef]
9. Girma, G.; Abebaw, M.; Zemene, M.; Mamuye, Y.; Getaneh, G. A review on aspergillosis in poultry. *J. Vet. Sci. Technol.* **2016**, *7*, 382. [CrossRef]
10. Arné, P.; Thierry, S.; Wang, D.; Deville, M.; Loc'h, G.L.; Desoutter, A.; Féménia, F.; Nieguitsila, A.; Huang, W.; Chermette, R.; et al. *Aspergillus fumigatus* in poultry. *Int. J. Microbiol.* **2011**, *2011*, 746356. [CrossRef]
11. Dykstra, M.J.; Charlton, B.R.; Chin, R.P.; Barnes, H.J. Fungal infections. In *Diseases of Poultry*, 13th ed.; Swayne, D.E., Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L., Nair, V.L., Eds.; Wiley-Blackwell: Ames, IA, USA, 2013; pp. 1077–1096.
12. Pattron, D.D. *Aspergillus*, health implication & recommendations for public health food safety. *Internet J. Food Saf.* **2006**, *8*, 19–23.
13. Cafarchia, C.; Camarda, A.; Iatta, R.; Danesi, P.; Favuzzi, V.; Paola, G.D.; Pugliese, N.; Caroli, A.; Montagna, M.T.; Otranto, D. Environmental contamination by *Aspergillus* spp. in laying hen farms and associated health risks for farm workers. *J. Med. Microbiol.* **2014**, *63*, 464–470. [CrossRef]
14. Sabino, R.; Veríssimo, C.; Viegas, C.; Viegas, S.; Brandão, J.; Alves-Correia, M.; Borrego, L.-M.; Clemons, K.V.; Stevens, D.A.; Richardson, M. The role of occupational *Aspergillus* exposure in the development of diseases. *Med. Mycol.* **2019**, *57* (Suppl. 2), S196–S205. [CrossRef]
15. Cheng, Z.; Li, M.; Wang, Y.; Chai, T.; Cai, Y.; Li, N. Pathogenicity and immune responses of *Aspergillus fumigatus* infection in chickens. *Front. Vet. Sci.* **2020**, *7*, 143. [CrossRef]
16. Beernaert, L.A.; Pasmans, F.; van Waeyenberghe, L.; Haesebrouck, F.; Martel, A. *Aspergillus* infections in birds: A review. *Avian Pathol.* **2010**, *39*, 325–331. [CrossRef]
17. Sajid, M.A.; Khan, I.A.; Rauf, U. *Aspergillus fumigatus* in commercial poultry flocks, a serious threat to poultry industry in Pakistan. *J. Anim. Plant Sci.* **2006**, *16*, 79–81.
18. Sultana, S.; Rashid, S.M.H.; Islam, M.N.; Ali, M.H.; Islam, M.M.; Azam, M.G. Pathological investigation of avian aspergillosis in commercial broiler chicken at Chittagong district. *Int. J. Innov. Appl. Stud.* **2015**, *10*, 366–376.
19. Viegas, C.; Viegas, S.; Monteiro, A.; Carolino, E.; Sabino, R.; Verissimo, C. Air contaminants in animal production: The poultry case. In *Air Pollution XX.*; WIT Transactions on Ecology and the Environment; Longhurst, J.W.S., Brebbia, C.A., Eds.; WIT Press: Ashurst, Southampton, UK, 2012; Volume 157, pp. 315–323. [CrossRef]
20. Popescu, S.; Borda, C.; Diugan, E. Microbiological air contamination in different types of housing systems for laying hens. *ProEnvironment* **2013**, *6*, 549–555.

21. Lawniczek-Walczyk, A.; Górny, R.L.; Golofit-Szymczak, M.; Niesler, A.; Wlazlo, A. Occupational exposure to airborne microorganisms, endotoxins and β-glucans in poultry houses at different stages of the production cycle. *Ann. Agric. Environ. Med.* **2013**, *20*, 259–268.
22. Debey, M.C.; Trampel, D.W.; Richard, J.L.; Bundy, D.S.; Hoffman, L.J.; Meyer, V.M.; Cox, D.F. Effect of environmental variables in turkey confinement houses on airborne *Aspergillus* and mycoflora composition. *Poult. Sci.* **1995**, *74*, 463–471. [CrossRef]
23. Wójcik, A.; Chorąży, Ł.; Mituniewicz, T.; Witkowska, D.; Iwańczuk-Czernik, K.; Sowińska, J. Microbial air contamination in poultry houses in the summer and winter. *Pol. J. Environ. Stud.* **2010**, *19*, 1045–1050.
24. County Development Strategy of Koprivnica-Križevci County 2014–2020. (in Croatian). Available online: https://www.pora.com.hr/images/doc/2017/ZRS_KKZ-14-20.pdf (accessed on 12 March 2021).
25. Aviagen. Ross Broiler Management Handbook 2018. Available online: http://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-BroilerHandbook2018-EN.pdf (accessed on 16 November 2020).
26. Brown, A.E. *Benson's Microbiological Applications: Laboratory Manual in General Microbiology*, 9th ed.; McGraw-Hill: New York, NY, USA, 2005; pp. 57–64.
27. 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*, 170–174. [CrossRef]
28. Vučemilo, M.; Matković, K.; Vinković, B.; Macan, J.; Varnai, V.M.; Prester, L.; Granić, K.; Orct, T. Effect of microclimate on the airborne dust and endotoxin concentration in a broiler house. *Czech J. Anim. Sci.* **2008**, *53*, 83–89. [CrossRef]
29. Matković, K.; Marušić, D.; Ostović, M.; Pavičić, Ž.; Matković, S.; Ekert Kabalin, A.; Lucić, H. Effect of litter type and perches on footpad dermatitis and hock burn in broilers housed at different stocking densities. *S. Afr. J. Anim. Sci.* **2019**, *49*, 546–554. [CrossRef]
30. Brouček, J.; Čermak, B. Emission of harmful gases from poultry farms and possibilities of their reduction. *Ekol. Bratisl.* **2015**, *34*, 89–100. [CrossRef]
31. David, B.; Mejdell, C.; Michel, V.; Lund, V.; Moe, R.O. Air quality in alternative housing systems may have an impact on laying hen welfare. Part II—Ammonia. *Animals* **2015**, *5*, 886–896. [CrossRef]
32. Lonc, E.; Plewa, K. Comparison of indoor and outdoor bioaerosols in poultry farming. In *Advanced Topics in Environmental Health and Air Pollution Case Studies*; Moldoveanu, A., Ed.; IntechOpen: Rijeka, Croatia, 2011; Available online: https://www.intechopen.com/books/advanced-topics-in-environmental-health-and-air-pollution-case-studies/comparison-of-indoor-and-outdoor-bioaerosols-in-poultry-farming (accessed on 18 December 2020). [CrossRef]
33. Matković, K.; Vučemilo, M.; Vinković, B. Dust and endotoxin in laying hen dwellings. *Turk. J. Vet. Anim. Sci.* **2012**, *36*, 189–195. [CrossRef]
34. Erisman, J.W.; Schaap, M. The need for ammonia abatement with respect to secondary PM reductions in Europe. *Environ. Pollut.* **2004**, *129*, 159–163. [CrossRef]
35. Kumari, P.; Woo, C.; Yamamoto, N.; Choi, H.-L. Variations in abundance, diversity and community composition of airborne fungi in swine houses across seasons. *Sci. Rep.* **2016**, *6*, 37929. [CrossRef]
36. Filazi, A.; Yurdakok-Dikmen, B.; Kuzukiran, O.; Sireli, U.T. Mycotoxins in poultry. In *Poultry Science*; Manafi, M., Ed.; IntechOpen: Rijeka, Croatia, 2017; Available online: https://www.intechopen.com/books/poultry-science/mycotoxins-in-poultry (accessed on 17 December 2020). [CrossRef]
37. Publications Office of the European Union. Commission Directive (EU) 2019/1833 of 24 October 2019 amending Annexes I, III, V and VI to Directive 2000/54/EC of the European Parliament and of the Council as regards purely technical adjustments. *O. J.* **2019**, *L 279*, 54–79.
38. Van Diepeningen, A.D.; Brankovics, B.; Iltes, J.; van der Lee, T.A.J.; Waalwijk, C. Diagnosis of *Fusarium* infections: Approaches to identification by the clinical mycology laboratory. *Curr. Fungal Infect. Rep.* **2015**, *9*, 135–143. [CrossRef] [PubMed]
39. Batista, B.G.; de Chaves, M.A.; Reginatto, P.; Saraiva, O.J.; Fuentefria, A.M. Human fusariosis: An emerging infection that is difficult to treat. *Rev. Soc. Bras. Med. Trop.* **2020**, *53*, e20200013. [CrossRef] [PubMed]