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Identification of Microbial and Gaseous Contaminants in Poultry Farms and Developing Methods for Contamination Prevention at the Source

Dorota Witkowska and Janina Sowińska

Abstract

Microbial concentrations in poultry houses increase over time and contribute to the sick building syndrome. Very high and often logarithmic growth rates are reported for aerobic mesophilic bacteria, which account for the majority of known pathogenic bacteria. Bioaerosols suspended in air also contain mold spores and mold fragments, mostly fungi of various genera, including pathogenic fungi that produce mycotoxins. Microbiological mineralization of organic compounds, processes that involve litter and fecal microbes, produces toxic gases, including ammonia, carbon dioxide (CO_2), as well as volatile toxic and aroma compounds. The above threats have led to the initiation of various measures to limit pollution at the source, including legal regulations and methods aiming to neutralize the adverse effects of pollution (dietary, production, and hygiene standards). Hygienic methods are recommended as alternative methods of reducing contamination in poultry houses. Essential oil mist, organic and organic-mineral biofilters, litter additives, such as aluminosilicates (bentonite, vermiculite, halloysite), microbiological and disinfecting preparations, herbal extracts, and calcium compounds may improve hygiene standards in poultry farms.

Keywords: bacteria, fungi, gases, poultry farm, reduction methods

1. Introduction

The specific microclimate of farm buildings promotes the accumulation of bioaerosols containing harmful biological substances. Microbial concentrations in poultry houses increase over time and contribute to the sick building syndrome. Very high and often logarithmic

growth rates are reported for aerobic mesophilic bacteria, which account for the majority of known pathogenic bacteria. In addition to Gram-positive cocci (*Staphylococcus, Enterococcus*) and bacilli (*Bacillus*), other aerobic mesophilic bacteria include Gram-negative bacteria of the family *Enterobacteriaceae*, including *Escherichia coli, Salmonella* sp., *Shigella* sp., *Enterobacter* sp., *Proteus* sp., and *Klebsiella* sp., as well as *Pseudomonas* sp., *Acinetobacter* sp., *Flavobacterium* sp. [1–4]. The cell membranes of Gram-negative bacteria contain pro-inflammatory and allergenic lipopolysaccharide complexes, known as endoxins, which are released after the death of the bacterial cell. High endoxin concentrations are also observed in poultry houses [5]. Bioaerosols suspended in air also contain mold spores and mold fragments, mostly fungi of various genera (*Penicillium, Aspergillus, Fusarium, Mucor, Trichosporon, Alternaria, Cladosporium, Trichophyton, Epicoccum*, etc.), including pathogenic fungi that produce mycotoxins [1, 4, 6]. Pathogenic yeasts (*Candida albicans* and *Cryptococcus neoformans*) are also frequently identified in animal houses. Enzymatic and microbiological mineralization of organic compounds, processes that involve litter and fecal microbes, produces toxic gases, including ammonia and carbon dioxide (CO_2), as well as volatile toxic and aroma compounds such as cyclic hydrocarbons, aldehydes, ketones, alcohols, free fatty acids, mercaptans, esters, phenols, cyclic amines, and sulfides [7–8].

The combined effect of airborne pollutants is one of the key stressors in poultry farms. High concentrations of microorganisms, endotoxins, mycotoxins, gas, and dust exert adverse effects on the structure and protective functions of mucous membranes, in particular in the respiratory system and the conjunctiva, leading to allergic reactions, inflammations, and increasing susceptibility to infectious diseases. Numerous studies have demonstrated that excessive ammonia concentrations in hen house lower productivity [2, 9–11]. Airborne pollutants also have an adverse influence on farm employees [2]. The pollutants emitted by poultry farms have negative environmental consequences. Nitrogen compounds contaminate soil and ground waters [12], whereas gases such as CO_2, CH_4, and N_2O contribute to the greenhouse effect [13, 14]. Recent years have witnessed an increasing interest in volatile organic compounds (VOCs), which are odor-producing compounds [15, 16].

The above threats have led to the initiation of various measures to limit pollution at the source, including legal regulations (international conventions and the resulting legal acts that are binding for the signatory countries) and methods aiming to neutralize the adverse effects of pollution (dietary, production, and hygiene standards). The concentrations of biological and chemical air pollutants vary significantly between farms and livestock facilities, and they are determined not only by the animal species, but also by the housing and management system. For this reason, safe pollution thresholds are very difficult to define. Guidelines for limiting the exposure to selected chemical and physical factors have been developed in occupational medicine, but general threshold limit values (TLV) for biological compounds are very difficult to establish due to an absence of epidemiological data describing the correlations between exposure and health consequences [17]. Different organisms have varied susceptibility to toxic substances; there is a general absence of standardized measurement methods and a scarcity of source data relating to the most widespread bioaerosols, which further exacerbates the problem. Threshold values for farm buildings are even more difficult to determine due to a

higher number of limiting factors. Air pollution poses a serious health threat for animals and farm employees; therefore, new research into the type and concentrations of airborne pollutants in various housing systems is needed to effectively mitigate the problem.

This manuscript reviews the results of our previous work and other studies into quantitative and qualitative identification of microbial and gaseous contaminants in poultry houses and methods for the prevention of contamination at the source.

2. Microbial contaminations of poultry farms

The level of microbial contamination in poultry houses is one of the most important sanitary and hygienic indicators. The main sources of microorganisms in poultry houses are birds, their excrements, feed, litter, ventilation air, and even employees. Microbes carried by dust, water vapor, and secretions from the respiratory tract form bioaerosol. Birds breathe air which acts as a major vector for microorganisms. Most microbes are saprophytes, but some airborne microorganisms may be pathogenic. Pathogens that enter the respiratory system with liquid droplets and dust may cause infections. The smallest particles measuring <50 nm pose the greatest epizootic risk because they are slowly deposited and spread even at low air flow rates. The flock is constantly exposed to pathogenic bioaerosols when sick or infected birds are present in the poultry house [18].

Microbial survival is determined by temperature, humidity, and other environmental parameters. Relative humidity in poultry houses generally does not support bacterial proliferation (the 50–80% range is lethal for bacteria), and microbial contamination of air, litter, and surfaces in poultry farm buildings can be attributed mainly to high flock density and the continued presence of microbial sources. Poultry farms are significant pollutants of the external environment, and they could pose an epidemiological risk if biosecurity principles are not observed.

The microbial concentrations reported inside and outside poultry farms (**Tables 1** and **2**) differ considerably in the literature [4, 19, 20–26]. Our previous work and other studies revealed aerial contamination in the range of 3.1–6.4 $\log_{10}$ cfu/m^3 in broiler houses, 4.5–7.6 $\log_{10}$ cfu/m^3 in turkey houses, and 4.7–8.3 $\log_{10}$ cfu/m^3 in laying hen houses. Fungal concentrations in broiler, hen, and turkey houses were determined at 4.0–5.9, 3.8–5.8, and 2.7–5.5 $\log_{10}$ cfu/m^3, respectively. Outdoor concentrations were reported at 0–5.6 $\log_{10}$ cfu/m^3 for bacteria and 0–4.8 $\log_{10}$ cfu/m^3 for fungi, depending on the distance. Microbial contamination levels are influenced by various factors, including bird species, stocking density, season, ventilation system, microclimate, and litter quality.

Witkowska et al. [19] studied the total counts of aerobic mesophilic bacteria and fungi in fresh litter and in the air in a broiler house under changing temperature and humidity conditions, and changing physicochemical properties of litter throughout the rearing period. The total counts of aerobic mesophilic bacteria and fungi in fresh litter tended to increase during the rearing period, to reach 9 and 8 $\log_{10}$ cfu/g, respectively, in the last week. An insignificant increase in litter pH was also noted throughout the experiment, which—combined with in-

creasing excreta amounts and fermentation processes in fresh litter—could promote microbial growth. The above factors enhanced ammonia production in litter (6 mg/kg at the beginning of the experiment vs. 12 mg/kg in the last week). Despite a gradual decrease in indoor temperature accompanied by an increase in humidity, microbial air contamination did not follow the same pattern as litter contamination. Bacterial and fungal counts varied between weeks of the rearing period, most likely due to changes in dust levels and ventilation efficiency. Bacterial counts were lowest in week 3 (4.6 $\log_{10}$ cfu/m^3) and highest at the end of rearing (5.3 $\log_{10}$ cfu/m^3). Fungal counts were lowest at the beginning of the experiment (4.2 $\log_{10}$ cfu/m^3) and highest in weeks 2 and 5 (4.7 $\log_{10}$ cfu/m^3). Lawniczek-Walczyk et al. [27] observed a significant increase in the concentrations of bacterial and fungal aerosols and endotoxins in chicken houses in successive stages of production. They also reported seasonal correlations in the size of bacterial populations. The concentrations of airborne bacteria were significantly higher in summer than in winter.

Flock (no. of birds, rearing period)	Housing type (no. of buildings, ventilation system, type of bedding, season)	Total microorganisms level ($\log_{10}$ cfu/m^3) mean range (min-max)		References
A. Bacteria				
Broilers (230 400 birds, 8 weeks)	12 buildings, mechanical ventilation, sawdust or straw litter	4.8 (3.1–5.2)		Baykov and Stoyanov [20]
Broilers (5300 birds, six weeks)	One building, mechanical ventilation, sawdust or wood shaving litter, spring	5.1 (4.2–5.3)		Vučemilo et al. [4]
Broilers (350 birds, five weeks)	One building, mechanical ventilation, straw litter, winter	5.1 (5.1–5.3)		Witkowska et al. [19]
Broilers (360 birds, six weeks)	One building, mechanical ventilation, straw litter, summer and winter	Summer 5.9 (5.0–6.2)	Winter 6.0 (4.9–6.4)	Wójcik et al. [21]
Broilers (41 000 birds, six weeks)	Two buildings, mechanical ventilation, straw litter, spring/summer	– (5.1–5.7)		Lonc and Plewa [22]
Lying hens (19 500 birds)	Three buildings mechanical ventilation, straw litter, spring–autumn	7.8 (4.7–8.3)		Bródka et al. [23]
Turkeys (2000 birds)	One building, Louisiana-type, wood chips or straw litter	6.9 (4.5–7.6)		Saleh et al. [26]
B. Fungi				
Broilers (5300 birds, 6 weeks)	One building, mechanical ventilation, sawdust or wood shaving litter, spring	4.5 (4.0–4.9)		Vučemilo et al. [4]

Flock (no. of birds, rearing period)	Housing type (no. of buildings, ventilation system, type of bedding, season)	Total microorganisms level ($\log_{10}$ cfu/m³) mean range (min-max)		References
Broilers (350 birds, 5 weeks)	One building, mechanical ventilation, straw litter, winter	4.5 (4.2–4.7)		Witkowska et al. [19]
Broilers (360 birds, 6 weeks)	One building, mechanical ventilation, straw litter, summer and winter	Summer 5.3 (4.6–5.8)	Winter 5.5 (4.7–5.9)	Wójcik et al. [21]
Broilers (41 000 birds, 6 weeks)	Two buildings, mechanical ventilation, straw litter, spring/summer	– (4.6–5.0)		Lonc and Plewa [22]
Lying hens (19 500 birds, 1 year)	Three buildings, mechanical ventilation, straw litter, spring–autumn	5.3 (3.8 Spr–5.8 Aut)		Sowiak et al. [24]
Turkeys (2000 birds)	One building, Louisiana-type, wood chips or straw litter	5.0 (2.7–5.5)		Saleh et al. [26]

Table 1. Bioaerosol concentrations in poultry houses.

Type of farm (no. of birds)	Total microorganisms level ($\log_{10}$ cfu/m³) mean range (min–max)	Distance	References
A. Bacteria			
Broilers (19 200)	– (2.3–5.6)	3 km–10 m	Baykov and Stoyanov [20]
Broilers (350)	2.6 (0–2.9)	3 m	Witkowska et al. [19]
Broilers (360)	3.9 (0–4.4)	3 m	Wójcik et al. [21]
Broilers (41 000)	– (1.6–3.9)	–	Lonc and Plewa [22]
Broilers (23 000)	– (3.7–4.1)	125–10 m	Plewa-Tutaj et al. [25]
B. Fungi			
Broilers (350)	3.0 (0–3.2)	3 m	Witkowska et al. [19]
Broilers (360)	3.8 (0–4.3)	3 m	Wójcik et al. [21]
Broilers (41 000)	– (1.3–4.1)	–	Lonc and Plewa [22]
Lying hens (19 500)	4.6 (4.2–4.8)	–	Sowiak et al. [24]

Table 2. Bioaerosol concentrations around poultry houses.

Bacterial and fungal species and serotypes isolated from poultry farms are presented in **Table 3**. Numerous studies [4, 19–25] revealed that bioaerosols from poultry houses contain Gram-positive bacteria, including *Streptococcus*, *Staphylococcus*, *Micrococcus*, *Enterococcus*, *Aerococcus*, *Corynebacterium*, *Brevibacterium*, *Cellulomonas* and *Bacillus*, as well as Gram-negative bacteria, including *Escherichia*, *Enterobacter*, *Klebsiella*, *Proteus*, *Citrobacter*, *Pasteurella*, *Pantoea*,

Moraxella, and *Pseudomonas*. Witkowska et al. [19] report that in their investigation, molds (*Fusarium, Penicillium, Aspergillus*, and many others) predominated in the air and litter at the beginning of the production cycle, whereas yeast counts increased to 90–100% towards the end of the experiment, particularly in litter.

Species	Serotypes	Inside houses	Outside houses	References
A. Bacteria				
Streptococcus	*S. pyogenes*	+	–	Baykov and Stoyanov 1999 [20]; Vučemilo et al. [4]; Lonc and Plewa 2011 [22]; Bródka et al. 2012 [23]; Lawniczek-Walczyk et al. 2013 [27]; Plewa-Tutaj et al. 2014 [25]
	S. bovis	+	–	
	S. mitis	+	+	
Staphylococcus	*S. xylosus*	+	+	
	S. hyicus	+	–	
	S. saprophyticus	+	–	
	*S. aureus**	+	+	
	S. epidermidis	–	+	
	S. lentus	+	+	
	S. sciuri	+	+	
	S. chromogenes	+	–	
	S. cohnii	–	+	
Micrococcus	*M. sedentarius*	+	–	
	M. luteus	+	+	
	M. lylae	–	+	
	M. halobius	–	+	
Enterococcus	*E. faecalis*	+	–	
	E. faecium	+	–	
	sp.	–	+	
Aerococcus	*A. viridans*	+	–	
Corynebacterium	*C. xerosis*	+	+	
Brevibacterium	*sp.*	+	–	
Cellulomonas	*C. cellulans*	+	–	
Bacillus	*sp.*	+	–	
	mycoides	–	+	
Escherichia	*E. coli*	+	+	
Enterobacter	*E. sakazakii*	+	–	
	E. agglomerans	+	+	
	*E. cloacae**	+	+	
Klebsiella	*K. pneumoniae**	+	–	
Shigella	*S. boydii*	–	+	
Proteus	*P. mirabilis**	+	+	

Species	Serotypes	Inside houses	Outside houses	References
Citrobacter	*C. farmerii*	+	+	
Pasteurella	*sp.*	+	−	
Pantoea	*sp.*	+	−	
Moraxella	*sp.*	+	−	
Providencia	*sp.*	−	+	
Pseudomonas	*P. aeruginosa*	+	+	
	P. fluorescens	+	+	
	P. alcaligenes	+	−	
	P. stutzeri	+	−	
	P. chlororaphis	−	+	
Xantomonas	*X. maltophila*	−	+	
B. Fungi				
Penicillium	*P. notatum*	+	−	Vučemilo et al. 2007 [4]; Witkowska et. al. 2010 [19]; Wójcik et al. 2010 [21]; Lonc, Plewa 2011 [22]; Sowiak et al. 2012 [24]; Lawniczek−Walczyk et al. 2013 [27]
	P. expansum	+	−	
	P. olivinoviridae	+	−	
	P. claviforme	+	−	
	P. viridicatum	+	−	
	P. chrysogenum	−	+	
Aspergillus	*A. niger*	+	+	
	A. nidulans	+	+	
	A. ochraceus	+	−	
	A. oryzae	+	−	
	8A. candidus	+	−	
	*A. fumigatus**	+	−	
	A. glaucus	−	+	
	A. parasiticus	−	+	
	A. clavatus	−	+	
Fusarium	*F. oxysporum*	+	+	
	F. graminearum	+	+	
Geotrichum	*sp.*	+		
Scopulariopsis	*S. brevicaulis*	+	+	
	S. acremonium	+	+	
Alternaria	*A. alternata*	+	+	
	A. tennuissima	+	+	
Trichoderma	*T. viridae*	+	+	
Drechslera	*D. graminae*	+	+	
Mucor	*M. mucedo*	+	+	
Rhizous	*R. oryzae*	+		

Species	Serotypes	Inside houses	Outside houses	References
	R. stolonifer	+		
	R. nodosus	+		
Cladosporium	C. cladosporoides	+	+	
Candida	C. albicans	+	−	
	C. inconspicua	+	−	
	C. lambica	+	+	
	C. famata	+	−	
	C. pelliculosa	+	−	
Cryptococcus	C. laurentii	+	−	
	C. humicola	+	−	
	sp.	−	+	
Acremonium	A. strictum	+		
Trichophyton	T. mentagrophytes*	+		
Ulocladium	sp.	−	+	
Verticilium	sp.	−	+	
Scedosporium	sp.	−	+	
Mycelia	M. sterilia	−	+	
Rhodotorula	R. rubrum	−	+	

*Microorganisms classified into group 2 according to level of risk of infection [27].

Table 3. The most common microorganisms isolated from poultry farms.

Some microbial species and serotypes, such as *Staphylococcus aureus*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Trichophyton mentagrophytes*, and *Aspergillus fumigatus*, are pathogenic for animals and humans. Many bacteria and fungi are opportunists which are particularly dangerous for organisms with compromised immunity. Low hygiene standards and high levels of microbial and gaseous contamination may synergistically contribute to lower immunity and susceptibility to infections. The presence of microorganisms such as *Brevibacterium*, *Alternaria*, and *Cladosporium* in poultry houses indicates that microbes from the external environment, including soil, can spread to farm buildings. The prevalence of pathogenic microorganisms outside poultry buildings, even several kilometers away from the site, also indicates that ventilation air may contaminate the external environment.

Broiler houses are particularly infested by fungi of the genera *Penicillium*, *Aspergillus*, and *Fusarium*, which are the main fungi producing pathogenic mycotoxins such as T-2 toxin, aflatoxin, ochratoxin, and zearalenone. Other toxigenic fungi species, for example, *Alternaria*, *Cladosporium*, *Trichoderma*, *Rhizopus*, *Stachybotrys*, have also been identified in poultry buildings. Even low concentration of mycotoxins is known to cause immunosuppression, allergies, inflammation of the respiratory tract, and they may have impact on growth parameters of birds [4, 6, 19, 22, 23, 25, 27–30].

Bioaerosols also contain suspended endotoxins. These lipopolysaccharide complexes are associated with the cell membrane of Gram-negative bacteria which are released after the death of bacterial cells. High endoxin concentrations are also observed in poultry houses. Inhaled bioaerosol particles carrying endotoxins have pro-inflammatory and allergenic properties and may lead to chronic respiratory diseases in poultry [5]. The mean concentrations of endotoxins in aerosol fractions from poultry houses were determined in the range of 11.2–3406 ng/m^3 and were significantly higher than in other livestock buildings [3, 5, 27]. Seedorf et al. [31] observed the highest endotoxin concentrations in laying hen houses. Lawniczek-Walczyk et al. [27] observed that endotoxin concentrations in poultry houses increased significantly in successive stages of production. The above authors concluded that high levels of airborne microorganisms and their bioproducts could pose a serious risk of respiratory diseases. For this reason, widely accepted guidelines for hygiene evaluation need to be established in poultry farms.

In Poland, the proposed threshold limit values (TLV) are 5.0 $\log_{10}$ cfu/m^3 for bacteria and 4.7 $\log_{10}$ cfu/m^3 for fungi [27], but those limits apply to bioaerosol concentrations in employee facilities. Krzysztofik [32] recommended a limit of 5.0 $\log_{10}$ cfu/m^3 for bacteria and a more restrictive threshold value for fungi at 3.3 $\log_{10}$ cfu/m^3. The TLV for endotoxins recommended by the Polish Expert Committee for Biohazards in Indoor Environments is 200 ng/m^3 [18, 27]. There are no guidelines for poultry houses.

3. Gaseous contaminations of poultry farms

The composition of air in poultry houses significantly differs from atmospheric air. In addition to basic gaseous components (N$_2$—nitrogen, O$_2$—oxygen, Ar—argon, and CO$_2$—carbon dioxide), the air inside poultry houses also contains compounds that are not normally found in atmospheric air. Birds, their excrements, feed, and process equipment are the main sources of volatile chemical compounds in poultry houses. Ammonia and carbon dioxide are most frequently encountered in farm buildings, and they contribute to the risk of disease if present in excessive concentrations. For this reason, ammonia and carbon dioxide are regarded as the most toxic gases in poultry houses.

Carbon dioxide (CO$_2$) is a natural component of air, and its concentrations generally do not exceed 300 ppm (0.03%). Carbon dioxide is responsible for breathing control in the respiratory system. In densely stocked poultry houses, carbon dioxide concentrations are significantly higher than in atmospheric air, but they should not exceed 2000 ppm. At higher concentrations in poultry houses, CO$_2$ weakens respiratory defense mechanisms and increases susceptibility to respiratory diseases. Carbon dioxide poses a serious hazard to health and life at concentrations higher than 10 000 ppm. Carbon dioxide levels in poultry houses are a robust indicator of ventilation efficiency. Its concentrations increase rapidly in poorly ventilated buildings.

In poultry houses, ammonia (NH$_3$) is released from excreta which contain nitrogen in the form of uric acid. Ammonia is produced in the process of microbial fermentation. Ammonia production increases in conditions that support microbial proliferation, including high temperature, high humidity, high pH, and presence of organic matter. In poultry houses,

ammonia concentrations should not exceed 13 ppm for adult birds and 10 ppm for chicks. At higher concentrations, NH_3 can compromise growth, whereas exposure to more than 30 ppm of ammonia can lead to respiratory dysfunctions such as intensified mucus secretion, shallow breathing, and bronchoconstriction. High levels of ammonia can impair immunity and increase susceptibility to respiratory infections and ocular abnormalities in poultry.

Ammonia and other nitrogen compounds (NO_x) originating from poultry production contaminate soil and groundwater. Some gaseous compounds emitted by poultry farms, in particular CO_2 and NO_x, are greenhouse gases which contribute to global warming. Many volatile compounds are classified as odors, and hundreds of volatile organic compounds (VOCs) are identified in poultry houses. There are no guidelines concerning the odor detection threshold or VOCs' impact on odor formation in poultry farms due to the scarcity of simple instruments for measuring air contamination in the production process. Research studies revealed the presence of aromatic and aliphatic hydrocarbons, aldehydes, ketones, alcohols, free fatty acids, mercaptans, esters, phenols, cyclic amines, nitriles, and sulfur compounds in bird farms [7–8, 13, 15, 18, 33].

Witkowska [33] conducted qualitative and quantitative identification of gaseous contaminants on a commercial turkey farm by Fourier transform infrared spectroscopy (FTIR). It was found that ammonia and carbon dioxide were the predominant gases in turkey houses, and both were present throughout the entire growth cycle, which is consistent with the findings of other authors. The mean concentrations of carbon dioxide and ammonia were 220–2058 ppm (min = 176 ppm, max = 2460 ppm) and 4–31 ppm (min = 4 ppm, max = 58 ppm), respectively. The highest carbon dioxide concentrations (approx. 2000 ppm) were reported in farm buildings in weeks 4 and 7, and a significant decrease in 600 ppm was observed in week 10. A lessening tendency was noted until the end of the production cycle; in week 19, mean carbon dioxide concentrations in turkey houses were approximately 90% lower than at the beginning of the experiment. Average ammonia concentrations increased from 7 ppm at the beginning of the study to over 30 ppm in week 7. A significant decrease in ammonia levels was observed in subsequent weeks. The decreasing trend was sustained until the end of the rearing period, and the mean concentrations of NH_3 were 90% lower in the second half of the cycle. The decrease resulted from higher ventilation and air exchange rates in turkey houses (at the last stage of the study, and the rate of ventilation was 10-fold higher than at the initial stage). The increase in CO_2 and NH_3 levels in week 7 was related to diet modification and increased excreta moisture. Thiols, nitriles, amines, aldehydes, hydrocarbons, and other volatile organic and inorganic compounds were also identified in the air inside the buildings, but they were emitted periodically and their mean concentrations were significantly lower in comparison with CO_2 and NH_3. In contrast to the majority of other contaminants, nitrogen compounds (nitriles, amines, aldehydes) and some hydrocarbons (chloroethane, 1.3-butadiene) were present at higher concentrations in the second half of the production cycle. During the experiment, trace amounts of alcohols, organic acids, ketones, phenols, nitrogen oxides, and sulfur oxides were also detected in the air inside farm buildings. Mixtures of those compounds act as odorants even at low concentrations.

According to EU directives and Polish regulations, ammonia concentrations in broiler and laying hen houses should not exceed 20 ppm, and carbon dioxide concentrations should be limited to 3000 ppm. Gas concentrations have to be kept within safe limits in turkey, duck, and geese houses [33]. More restrictive limits have been recommended by some authors [18], and further research is needed to determine tolerable limits for different poultry species and rearing systems.

4. Hygienic methods for reducing contamination in poultry houses

The search for effective, inexpensive, and environmentally friendly methods of lowering contamination levels in poultry production has continued for many years. Ventilation systems play a key role in maintaining the optimal microclimate in poultry houses, and devices that generate negative ions remove dust and moisturize air can also be installed in farms to limit air pollution. Unfortunately, such solutions are relatively expensive, and they are not widely used in poultry production.

Witkowska and Sowińska [34] study aimed to assess the antibacterial effects of natural essential oils (peppermint oil—PO and thyme oil—TO) in broiler houses. The results of the study demonstrated that essential oil mist may improve hygiene standards in broiler farms. The mean total counts of aerobic mesophilic bacteria in the control room were significantly higher than in rooms treated with essential oils—5.8 $\log_{10}$ cfu/m^3 vs. 5.6 $\log_{10}$ cfu/m^3 (PO) and 5.5 $\log_{10}$ cfu/m^3 (TO). A similar trend was observed with regard to wall contamination—total mesophilic counts ranged from around 2.4 $\log_{10}$/100 cm^3 in rooms fogged with essential oils to 3.3 $\log_{10}$/100 cm^3 in the control room, and the statistic differences between control and experimental groups were determined. Total bacterial counts on drinker surfaces in rooms fogged with essential oils were lower than in the control room (PO–4.6 $\log_{10}$, TO–4.3 $\log_{10}$). Average drinker contamination was significantly (by 0.5 $\log_{10}$) higher in the control room than in the room fogged with thyme oil. The average total count of litter bacteria ranged from 8.9 $\log_{10}$ cfu/g in the control group to 8.2 $\log_{10}$ cfu/g in the thyme oil group, and the noted difference was statistically significant. Litter contamination was also lower in the room fogged with peppermint oil (8.5 $\log_{10}$ cfu/g), compared with the control room, but the difference was not significant. An analysis of extreme values of bacterial counts in the air on the walls and drinkers and in litter revealed that bacterial contamination levels were effectively reduced by essential oils. The average counts of bacteria of the family *Enterobacteriaceae* and mannitol-positive *Staphylococcus* in the air on wall and drinker surfaces were lower in experimental rooms than in the control room. A similar tendency was noted with respect to the counts of *Staphylococci* in litter, but no significant differences were found between groups. The counts of coliforms were lowest in the room fogged with thyme oil, and they were higher in the room treated with peppermint oil than in the control room. Both oils reduced bacterial counts, but thyme oil was more effective in eradicating *Enterobacteriaceae*, whereas peppermint oil had a higher inhibitory effect on the proliferation of *Staphylococci*.

According to Tymczyna et al. [3, 7, 35, 36], biofilters offer a relatively cheap and effective solution for poultry farms. Biofilters are containers with many partitions that house a high-

pressure fan, an air moisturizing chamber and a biofiltration chamber. The biofiltration chamber is a bed of various media, such as peat, compost, horse manure, and wheat straw. Toxic gases are partially or completely biodegraded by bacteria that occur naturally in bed media or are artificially introduced to substrates. Bacterial proliferation is influenced by the parameters of bed media, including fertility, moisture content, temperature, and pH. The cited authors demonstrated that ammonia and other toxic compounds (nitrates, nitrites, sulfates, chlorides, phosphates) present in ventilation systems can be effectively eliminated with the use of open biofilters in laying hen farms. Biological beds composed of peat, treated compost, horse manure, and wheat straw reduced ammonia concentrations by 36–89% (68.6% on average) and eliminated other harmful compounds in 66–100%.

Chmielowiec-Korzeniowska et al. [37] evaluated the effectiveness of a prototype container biofilter in eliminating organic air pollutants in a chick hatchery. The biofilter bed was composed of sallow peat (30%), fibrous peat (30%), treated compost (10%), fermented horse manure (10%), and wheat straw (20%). The tested device decreased the levels of all pollutants by 66% on average, and it was most effective in removing hexanal (95%) and toluene (76%).

The same team of researchers [3] evaluated the effectiveness of organic and organic-mineral biofilters in eliminating Gram-negative bacteria, dust, and bacterial endotoxins from exhaust air leaving a chick hatchery. All evaluated filters were effective in removing bacterial aerosols and somewhat less effective in reducing dust pollution. Endotoxins were not effectively eliminated. A biofilter with an organic-mineral bed containing 20% halloysite, 40% compost, and 40% peat was most effective in lowering contamination levels.

Numerous research studies demonstrated that aluminum silicates can be effectively used as bed media in air filtering devices. An important advantage of aluminum silicates is that they are relatively cheaper and less toxic for animals and the environment than commercially available chemical sorbents.

Opaliński et al. [38] evaluated the ability of selected aluminum silicates to absorb ammonia. The tested substrates were raw halloysite, roasted halloysite, activated halloysite, raw bentonite clay, and expanded vermiculite (EV). The experiment was conducted under strictly controlled laboratory conditions. The analyzed substrates' sorptive capacity was determined based on differences in ammonia concentrations in a stream of air before and after it passed a sorptive bed with a known volume. All evaluated sorbents lowered ammonia concentrations in air. The most effective sorbent was activated halloysite, followed by raw halloysite, roasted halloysite, and raw bentonite, whereas vermiculite was least effective in capturing ammonia.

Opaliński et al. [39] also analyzed the ability of selected aluminum silicates to eliminate noxious odors in conditions similar to those found in animal facilities. Chicken droppings were placed in fertilizer chambers, and the odor capturing abilities of raw halloysite, roasted halloysite activated halloysite, raw bentonite, roasted bentonite, and expanded vermiculite were evaluated after 24 h. Ammonia was most effectively removed (81%) by activated halloysite, whereas roasted halloysite was the least effective sorbent. In addition to ammonia, the analyzed air samples also contained 24 odorous volatile compounds, including five toxic substances. All of the tested aluminum silicates effectively decreased the concentrations of the identified com-

pounds, and their average sorptive capacity ranged from 56% for raw halloysite to 84% for roasted bentonite. Roasted bentonite reduced the levels of seven odorous compounds by more than 90% and eliminated IH-indole, dimethyl trisulfide, and pyridine in even 100%.

Organic and mineral compounds are added to litter to improve its quality [40, 41]. The objective of Korczyński et al. [42] study was to determine the effectiveness of expanded vermiculite (EV) and raw halloysite (HS) in reducing the emissions of ammonia and volatile organic compounds (VOCs) from litter in turkey houses. Mean ammonia concentrations were lower in the sectors where the analyzed sorbents were used. The average differences in NH_3 levels between the control sector and the sectors where vermiculite and halloysite were added to litter reached 15.1 and 14.6%, respectively. The highest efficacy of both sorbents was noted in the first week of the study (statistically significant differences). The application of halloysite and vermiculite decreased ammonia concentrations by 38 and 25%, respectively, compared with the control sector. Similar trends were observed in the subsequent 2 weeks, but differences in ammonia concentrations between the control sector and experimental sectors were much lower (3.4–11.4%) and statistically non-significant. A total of 15, 14, and 11 volatile organic compounds were identified in the air in the control, HS and EV sectors, respectively. Pentadecane, 1-phenylethanone, dimethyl tetrasulfide, and 4-hydroxytoluene were detected in the control sector, but they were not found in experimental sectors. Methylbenzene and 4-methyl-2-heptanone were identified in the air in the sectors where the sorbents were used, and 2-undecanone was detected in the HS sector—those compounds were not found in the control sector. Chlorobenzene was the predominant VOC in the air in all sectors. Sorbents added to litter were most effective in reducing the emissions of compounds with more complex molecular structure. VOC levels decreased by 73.4 and 83.1% following the use of halloysite and vermiculite, respectively.

Manafi et al. [43, 44] observed that high grade sodium bentonite in diet reduced the toxicity of aflatoxin and marginally ameliorated the effect of ochratoxin A and aflatoxin B_1 in broilers.

In a search for effective methods to reduce contamination levels in poultry production, various litter additives were analyzed [28, 45–47], including a microbiological preparation (Biosan-GS®) and disinfecting preparations (Lubisan®, Stalosan F®, Profistreu®). All additives contributed to a decrease in litter pH and moisture content [28, 46] thus reducing ammonia concentrations in the air and litter [45, 46], and microbial air contamination levels in poultry houses [28]. Birds kept on "optimized" litter were characterized by higher body weight gains [46] and lower culling and mortality rates, at similar feed intake levels. An analysis of internal organs (liver, spleen, kidneys, lungs, cornea) and selected blood parameters showed that the above litter additives were safe and posed no threat to bird health [45, 47].

Saponin extracts from the South American plants of Mojave yucca (*Yucca schidigera*) and soap bark tree (*Quillaja saponaria*) are added to feed and litter in poultry farms. Saponins block bacterial urease and slow down urea decomposition in the uricolytic cycle. They increase the availability of feed protein for birds and decrease the excretion of nitrogen compounds that can be converted to ammonia [48, 49].

Litter can also be disinfected with calcium compounds before animals are introduced to a farm building [50]. Many authors demonstrated that the addition of calcium oxide to poultry litter

significantly decreases bacterial counts, in particular *Salmonella* which continues to pose a serious problem for the producers and consumers of poultry meat and eggs [51, 52].

In the literature, there are no recommendations regarding the optimal doses of calcium additives in litter, but calcium compounds are popularly used in poultry farms on account of their low cost. Despite the above, calcium additives should be applied with caution because exposure to excessive calcium concentrations can irritate or burn mucosal membranes and the skin. Calcium oxide reacts with water to increase temperature, which can harm birds reared on litter. Mituniewicz [53] attempted to determine the optimal doses of calcium compounds (CaO and CaOMgO) which can be safely added to litter before birds are introduced to poultry houses. The cited author analyzed the physicochemical and microbiological parameters of litter, microclimate conditions, selected blood biochemical parameters and bird performance to conclude that a single application of 250 g CaO or CaOMgO per square meter of litter delivered the best results. Calcium compounds had a positive effect on the physicochemical parameters of litter and microbial counts. The tested additives, in particular calcium oxide, led to a significant increase in litter temperature within the safe limits. Calcium oxide also lowered the relative moisture content of litter, in particular in the last weeks of the rearing period. The combination of calcium oxide and magnesium oxide induced a greater improvement in the analyzed parameters. Calcium compounds were effective disinfectants which reduced the counts of incubated yeasts already in the third week of the experiment. Ammonia concentrations were significantly lowered in a poultry house where calcium compounds were added to litter. The results of blood serum biochemistry analyses revealed that calcium compounds did not exert a negative effect on the birds' health. Chickens reared on litter with calcium additives were characterized by higher weight gains and improved performance.

Calcium peroxide (CaO_2) is an inorganic compound and a source of oxygen. This compound is sparingly soluble in water, and hydrogen peroxide, a source of free radicals (chemical oxidation) and oxygen, is gradually released during the slow decomposition of CaO_2. The above creates a supportive environment for aerobic microorganisms [54]. Piotrowska [55] attempted to determine the optimal dose at which calcium oxide should be combined with litter to improve hygiene conditions in broiler houses and broiler performance. During a four-week laboratory experiment (without birds) involving analyses of the qualitative parameters of chicken litter and microclimate conditions in broiler houses, the cited author determined the optimal dose of CaO_2 at 2 g m^{-2} litter. The above dose was then tested under production conditions in a poultry farm. The surface temperature of the experimental litter was reduced by 1°C, and its moisture content decreased in comparison with the control litter (57.11% vs. 70.33%), which lowered the counts of aerobic mesophilic bacteria. Ammonia concentrations in the experimental poultry house did not exceed 10 ppm throughout the experiment and were lower than in the control poultry house. Aerobic mesophilic counts in air increased in both poultry houses in successive stages of production, but were lower in the house containing CaO_2 than in the control facility in weeks 3, 4, and 6. Calcium peroxide also reduced average yeast and mold counts in the experimental poultry house relative to control. The addition of CaO_2 at 2 g/m^2 litter did not compromise the birds' health and had a positive impact on performance.

In addition to technical devices and sanitary solutions, other measures are also introduced to minimize pollutant emissions from chicken houses. One of such measures relies on phytoremediation, namely the use of selected plants to accumulate and degrade polluting substances.

Sobczak et al. [56] and Domagalski et al. [57] analyzed the ability of selected greenhouse plants to reduce pollution levels in exhaust air from poultry houses. Sobczak et al. [56] demonstrated a decrease in the concentrations of carbon dioxide, ammonia, and dust when exhaust air was passed through an experimental greenhouse containing Indian shot (*Canna*) and silver grass (*Miscanthus*). Pollution levels were measured at the inlet and outlet of the greenhouse for 3 months to reveal a daily drop of 10% in CO_2 concentrations (30% during day time), a 40% drop in ammonia levels and a 14% drop in dust concentrations on average.

Domagalski et al. [57] investigated the deodorizing properties of Indian shot (*Canna × Generalis*) in a phytotron chamber for filtering exhaust air from poultry houses. The applied biofilter reduced total concentrations of odorous compounds by 20–30% and decreased ammonia levels by 29–41%.

5. Summary and conclusions

The results of the above studies demonstrate that poultry farms are significant reservoirs and emitters of microbiological and gaseous contaminants into the environment and that the type and concentrations of bioaerosols and gases produced in poultry farms are determined by various factors, including bird species, stocking density, season, time of day, stage of the production cycle, temperature, moisture content and the physicochemical parameters of litter, sampling site, ventilation efficiency, technical and process solutions, and farm management methods.

A microbiological analysis of bird facilities revealed that threshold concentrations of airborne bacteria and fungi recommended in the literature [27, 32] are often exceeded in practice. In cited studies, the lowest bacterial concentrations in a broiler house were determined at 3.1 $\log_{10}$ cfu/m³; however, in the most cases, minimum value approximated the safe threshold for poultry houses (5.0 $\log_{10}$ cfu/m³) already at the beginning of the production cycle. The highest concentrations of airborne bacteria were determined in hen houses at 8.3 $\log_{10}$ cfu/m³. The proposed safe threshold for fungal concentrations of 3.3 $\log_{10}$ cfu/m³ for poultry was also most often exceeded at the beginning of the production cycle, and fungal concentrations ranged from 2.7 (turkeys) to 5.9 $\log_{10}$ cfu/m³ (broilers).

Poultry litter was an even more abundant source of microorganisms. In our study, the highest bacterial concentrations in hen house litter reached 9–10 $\log_{10}$ cfu/g, and the highest fungal concentrations reached 8 $\log_{10}$ cfu/g, but the above results cannot be compared with reference values due to an absence of normative threshold levels in the literature.

An IR spectroscopy analysis of chemical air pollution in a commercial turkey farm supported the determination of the type and concentrations of inorganic compounds (ammonia, carbon dioxide, nitric oxide, and phosphine) and VOC (sulfur and nitrogen compounds: nitriles,

amines, and aldehydes; hydrocarbons: methane, dichloromethane, chloromethane, bromomethane, 1,3-butadiene). In most studies, volatile compounds in farm buildings are identified by gas chromatography. This method is characterized by high precision, but it is rarely used in practice because analyses have to be performed under laboratory conditions. A comparison of our findings with the results of chromatographic analyses indicates that FTIR is a practical method for evaluating gas contamination in field conditions because analyses can be conducted *in situ* with the use of a portable device, which eliminates the problems associated with sample collection and transport. IR spectroscopy supports the identification of aroma compounds even at very low concentrations.

Selected volatile compounds, which were also determined in our study of hen houses (e.g., ethanethiol, methanethiol, acrylonitrile), can be harmful at very low concentrations at the limit of detection of measuring devices equipped with electrochemical sensors for selective detection [58]. Due to analytical constraints, only general threshold limit values (TLV) have been determined for carbon dioxide, ammonia, and hydrogen sulfide at 1800–3000, 10–30, and 5–10 ppm, respectively, in housing facilities for juvenile and adult animals [18]. The relevant legal regulations set TLVs for NH_3, CO_2 and H_2S for calves and pigs, and NH_3 and CO_2 for chickens at 3000, 20, and 5 ppm, respectively. The regulations addressing other animal species, including turkeys, merely state that VOC concentrations should be kept at a safe level [59].

The growing number of protests staged by local communities against odor-producing animal farms, in particular animal production facilities situated inside the protective zone surrounding residential districts, has attracted researchers' attention to the odor-producing qualities of approximately 300 identified volatile compounds. The detection limit of many gases, including mercaptans, amines, sulfur compounds, and phenol derivatives, can be very low. Measures that effectively limit the production of odorous gas mixtures at the source require the identification of the highest number of components, even at very low concentrations. Analyses of trace amounts of toxic compounds are often burdened with error; therefore, the higher the number of replications and standardized measuring techniques, the greater the effectiveness of the proposed protective measures.

The EU climate and energy package places the Member States under the obligation to reduce their greenhouse gas emissions. Animal farms contribute to an increase in atmospheric concentrations of CO_2, CH_4, and NO_x. Farm emissions are determined based on standard formulas and computer simulations that do not account for hygiene standards. This approach could lead to unjust prosecution of farmers who take active steps to reduce pollution at the source. For this reason, gas concentrations and actual emissions from farm buildings characterized by different hygiene levels should be determined to effectively reduce atmospheric concentrations of pollutants.

In our study, the attempts to limit the concentrations of harmful gases and microbiological pollutants in farm buildings generated positive results. Total bacterial counts, including *Enterobacteriaceae* and *Staphylococcus* counts, were reduced in hen houses sprayed with essential oil solutions. Despite promising initial results, further analyses are needed to determine the effectiveness of essential oils in practice. Essential oils can be ineffective in small concentrations, and they can pose a health threat when applied excessively. The main advantage of

essential oils is that they are effective antimicrobials, and microorganisms, which can acquire resistance to chemical substances, have not been found to develop a resistance to essential oils. The results of our study demonstrate that essential oil sprays could deliver even more satisfactory results in hen houses because their efficacy is determined by microbial species. The application of adsorbents also delivered promising results. Vermiculite and halloysite reduced VOC concentrations by 83 and 73%, respectively. Ammonia adsorption was determined at 15%. Despite the above, adsorbent efficiency was reduced over time as the volume of poultry droppings increased. The findings of other authors also indicate the positive results of biofilters, different additives to the litter and even phytoremediation in pollutants reduction in poultry houses.

Reliable criteria for evaluating poultry exposure to biological and chemical pollutants and the relevant reference values should be developed to maintain high poultry welfare standards in farms. For such criteria to be acceptable, they have to be carefully balanced to ensure that they deliver the highest level of poultry welfare and are achievable in practice with the involvement of the available methods.

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