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# Dust and endotoxin in laying hen dwellings

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**Abstract:** Only healthy laying hens are capable of producing an average of 280 eggs in 1 year of exploitation. Production of consume eggs takes place in intensive housing systems where the laying hens are still mainly kept in cages under controlled conditions. Technical infrastructure and hygiene quality do not guarantee a production atmosphere completely free from pollutants, including potential pathological risk factors. Laying hens and farm workers are exposed to large quantities of bioaerosols in henhouses. Bioaerosols represent a mixture of different biological particles which can, under certain circumstances, cause health and welfare problems in animals. The most important components are dust, microorganisms, and microbial constituents, such as endotoxins. Because of this complexity, numerous measurements and the application of different methods is necessary in order to assess the health effects of bioaerosols and define future research goals. Therefore, a literature review was carried out on bioaerosol composition and the amounts found in laying hen houses. This paper deals with dust and endotoxins: their sources, concentrations, and methods of determination. The wide range of particle concentrations is strongly influenced by the use of different sampling and evaluation methods—and different application and handling of the same method—as well as sampling time, season, and the type of laying henhouse. This paper recommends establishing occupational health limits on bioaerosols for animals and animal caretakers in laying hen dwellings.

**Key words:** Bioaerosol, laying hens, housing, dust, endotoxin, health, welfare

## Introduction

The term bioaerosol is defined as a mixture of biological particles that possess attributes such as sustainability, contamination, allergenity, toxicity, and pharmacological properties (1). The fragmentation of biological material produces bioaerosol, in either a liquid suspension or solid particles in a gas. Bioaerosol appears in the form of fog, clouds, smoke, steam, or as a result of rain. In cases where the aerosol appears as the suspension of mostly liquid particles, expansion depends on the size and weight of the drops. Air serves as a medium in which bioaerosol particles move from one place to another.

In animal production, bioaerosol particles originate from food, manure, litter, and, to a large extent, from the animals themselves. Because of its biological complexity, bioaerosol is described as mixed biological material. Every source mentioned produces different and mostly specific particles, such as gas, dust, or microorganisms. Their amount varies during the day, as well as over the year. Microorganisms appear in bioaerosol as whole bacterial cells, viruses,

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fungi, and spore parts; the dust appears as non-specific, organic, and inorganic (2). Chemical composition tests show that bioaerosol consists of 70% organic and 30% inorganic components (3). Regardless of their origin, dust particles are often a medium for disease agents, but they are also vectors of stable gases.

Bioaerosol, either as aggregate or single proteins, bacteria, fungi, or their parts can provoke allergic and other physiological responses in animal organisms (4). Stetzenbach et al. (5) speak of bioaerosol as clusters of biological material in the air, whose particles may cause health problems in exposed individuals. Not all particles present in bioaerosol have equal importance. Respiratory fraction attracts particular attention. These are the particles whose size ranges from 1 μm to 10 μm. Individual bacterial cells determined in bioaerosol range from 0.5 μm to 5.0 μm. These cells usually appear in aggregate formation and represent particles of larger dimensions in round, pin, or spiral form.

The list of air contaminants and their determination methods—deriving from agriculture and other human activities—can be found in sources such as the EMEP/CORINAIR *Atmospheric Emission Inventory Guidebook* (6), AP-42 US *Environmental Protection Agency* (7), or CEPMEIP *Co-ordinated European Program on Particulate Matter Emission Inventories*, *Projections and Guidance* (8), a document from The Netherlands Organisation of Science and Technology.

These documents discuss the standardisation of bioaerosol sampling methods, and the spread of bioaerosol outside housing, in terms of the impact of bioaerosols on health, in both a narrow and wide sense; agricultural activity is held responsible for 8.6% of all emissions of particles sized 10 μm (9). The same authors state that poultry, with a 57% share, and pig production, with a 32% share, are the main sources.

Microorganisms, dust, gases, and endotoxins—in their bioaerosol form—can levitate for a long time, due to particle size ($10^{-4}$ μm to $10^{2}$ μm), and therefore have epidemiological meaning. They may be the reason for the more common allergic diseases in animals. Therefore, the provision of good air quality in animal dwellings is important for the maintenance of animal health and welfare, as well as the health of the personnel caring for the animals (10).

Organic dust, biologically active components, and microorganisms are the constituents of bioaerosol in animal dwellings (11). Their source is food, in 80% to 90% of cases; litter, in 55% to 68% of cases; animals, in 2% to 12% of cases; and their faeces, in 2% to 8% of cases. A small quantity of solid particles enters the air from the outside, through ventilation systems.

The probability that air contaminated by bioaerosol endangers health is greater in intensive production, due to the high density of animals in confined spaces. Particles are released into the air through animal excretion, respiration, and movement in combination with air velocity. Health problems can be manifested as respiratory problems—often as respiratory stress—due to constant exposure to apathogenic bacteria, allergens, and other particles. Everything mentioned above increases the possibility of disease outbreak and consequent production losses.

Microorganisms, as a bioaerosol component, constitute only 1 specific group of potential pollutants in the areas in which animals are housed. Their presence is closely associated with the physiological processes of digestion, as well as the activities related to keeping and exploiting animals. This is usually a normal, non-harmful micro flora, combined with dust and gases. However, in special circumstances these can become pathogens and endanger animal health.

**Bioaerosol composition**

There is a large concentration of bioaerosol in the air of poultry dwellings in general. Food, litter, manure, and animals—all media suitable for growth of microorganisms—are responsible for this (2). Almost 80% of microorganisms present in the air originate from animals. Approximately 90% of them are staphylococci and streptococci. Fungi, spores, and other microorganisms account for the remaining 10% (12). Together they represent the so-called stable microflora and are mostly apathogenic in nature. Many authors have stated that the largest number of airborne microorganisms is present in poultry houses, regardless of whether the poultry is held in deep litter or cages. The research by Bakutis et al. (13), carried out in dwellings for poultry, pigs, and cows,

reached the same conclusion. The authors measured the airborne bacteria numbers, as well as the dust and endotoxin concentration, in the winter. Their reports show that the mean value of total bacteria numbers in poultry dwelings was $4.66 \times 10^5$ CFU (colony forming unit)/$m^3$, $1.23 \times 10^4$ CFU/$m^3$ of which was gram-negative. The determined endotoxin concentration was between 800 and 12,800 EU (endotoxin units)/$m^3$, i.e.- between 80 ng/$m^3$ and 1280 ng/$m^3$, while the concentration of dust was between 8.2 mg/$m^3$ and 13.6 mg/$m^3$ of air. These values were several times higher than those recorded in the dwellings for pigs and cows.

Bioaerosol concentration in dwellings for fattening poultry was high in all periods of measurement, despite the influence of seasons, Saleh et al. concluded (14). The concentration of measured microorganisms was between $0.25 \times 10^6$ CFU/$m^3$ and $115.2 \times 10^6$ CFU/$m^3$. The recorded concentration of the inhalatory fraction was between 1.3 mg/$m^3$ and 10.0 mg/$m^3$, while the respiratory was between 0.3 mg/$m^3$ and 1.5 mg/$m^3$. The same authors noted that the endotoxin concentration in both dust fractions exceeded the recommended 50 EU/$m^3$ of air by more than 200 times. Vučemilo et al. (15) measured the concentration of microorganisms in the range of values between $3.22 \times 10^3$ CFU/$m^3$ and $6.40 \times 10^7$ CFU/$m^3$ in the air of a fattening poultry dwelling.

Air quality in laying hen dwellings is often a subject of research, due to its implications for animal health and productivity. These considerations induced Heber et al. (16) to research and develop measurement methods; it was necessary to conciliate values via practical standards in order to prevent free interpretation of measurement results.

The need for standardisation was supported by the example of Donham et al. (17), who concluded that dust levels of 15 mg/$m^3$ and ammonia levels of 50 ppm were too high. They recommended levels of 2.5 mg/$m^3$ for dust and 7 ppm for ammonia in the air of animal housing, i.e. values 6 to 7 times lower. Douwes et al. (18) advocate setting standards for exposure to pollutants from the air. These authors suggest that the endotoxin concentration limit of 50 EU/$m^3$ set in the Netherlands should decrease to 20 EU/$m^3$. They also advocate the need for research and the development of new methods for determining exposure to bioaerosols and the validation of existing methods. Finally, they suggest developing new analyses for identifying and understanding of the behaviour of individual bioaerosol components under various conditions.

Dust concentration in poultry dwellings varies between 0.75 mg/$m^3$ and 8.78 mg/$m^3$ (19). This quantity directly depends on the manner of housing. A higher amount of dust and a higher number of microorganisms are found in housing with deep litter—as well as in other alternative housing (20)—than in cage-housed poultry. Venter et al. (21) recorded $1.1 \times 10^5$ CFU/$m^3$ of bacteria in dwellings with automatic manure removal and $9.2 \times 10^4$ CFU/$m^3$ of bacteria in poultry houses with weekly manure removal. The number of fungi was approximately $7.0 \times 10^2$ CFU/$m^3$ in both residences. They concluded that the bacteria and fungi number was equal in both analysed poultry houses. The greater number of gram-negative bacteria in poultry dwellings with weekly cleaning led the authors to conclude that more responsibility for the composition of microorganisms derives from the cleaning method than the overall number.

**Air sampling**

In practice, the number of microorganisms in animal housing is expressed as the total number of bacteria (CFU) present in a litre of air. This number does not include dead bacteria or those that are not airborne, and it depends on the animal species and housing system (3,22).

Various methods are used for microorganism sampling in highly contaminated areas (23). Methods that are based on suction depend on aspiration, collection, and the types of substrates; the results depend largely on the methods of transport and storage of samples.

Bahhazi et al. (24) describes a number of known air sample methods and the importance of air flow. Most dust sampling devices include pumps that suck in air at high speed. Such devices also allow for the classification of particles according to their size (3,25), and their sampling methods may be based on the principle of sedimentation, impaction, leaching, filtration, or electrostatic (26).

The sedimentation method was first used by Koch in the 19th century. Plates with a nutrient substrate were exposed to the atmosphere in the animal housing at different times. Then they were incubated at 22-27 °C. This method could only roughly indicate the quantitative and qualitative composition of the air in animal housing.

The most commonly known device that works on the principle of air impaction on nutrient substrate is the Andersen impactor. This device allows particle separation by size into 6 fractions and uses a standard plastic petri plate with the desired nutrient substrate. Its widespread use allows for comparison between different studies (27).

At the impinger, air is vacuum drawn into the glass body and washed through the liquid. The content is then inoculated on the nutrient substrate. There is no danger of excessive contamination because the content can always be diluted, and the size of the flow is controlled by the opening of the glass tubes (27). There are other similar devices, ranging from the simple to those which fraction particles. Electrostatic precipitators are very effective in collecting very small particles; however, due to the adverse effects of ozone, which kills microorganisms in the sample, they are rarely used for the sampling of live microorganisms.

Samplers on the filtration principle are simple to use and inexpensive; however, they are not recommendable because the microorganisms passing through the filters may dry out (28). Therefore, they are frequently used for dust sampling in the time interval adapted to the assumed dust concentration.

The method used to determine endotoxins is the amebocyte lick Limulus test (LAL). The disadvantage of this method is that it is semiquantitative, highly variable, and can sometimes produce false negative results. As a result of their research on dust concentration in animal housing, Rosas et al. (29) concluded that the gravimetric analysis of dust from the air is a good indicator of other biological contaminants.

**Dust in the poultry dwellings air**

In a physical sense, dust includes all solid bodies in a free gas flow that settle down more slowly than established by classic laws on fall.

The dust in animal dwellings originates from several sources: food; animals, or die-out epithelium of skin, hair, feathers, and dried excrement; and bacteria, fungi, and endotoxins (30,31).

Furthermore, dust concentration is affected by environmental factors, including relative air humidity, air temperature, air flow velocity, and even lighting. Maintaining a 75% relative humidity level in the air of a broiler dwelling reduces total dust. More importantly, it reduces the respiratory fraction, according to Ellen et al. (32). The same authors decreased dust concentrations by 60% in aviaries by spraying with water containing 10% oil.

The concentration of dust in cage-housed laying hen dwellings is higher at feeding time. At night the concentration is lower, as hen activity is reduced. While exploring the mean values of daily dust amounts—especially their inhalatory fractions—in various animal dwellings, Takai et al. (19) proved statistically that weather conditions do not affect the concentration of inhalatory fractions of dust in cattle dwellings. However, seasonal variations were observed and proven in dwellings for pigs and poultry. Larsson et al. (33) researched air quality in 3 different types of poultry housing. In free-housed poultry dwellings kept on new litter, 4.8 mg/m$^3$ of dust, or 125 ng/m$^3$ of endotoxin, were recorded. In dwellings with old litter, the concentration of dust was 4.1 mg/m$^3$, and the concentration of endotoxin was 96 ng/m$^3$; in the dwellings of cage-housed poultry, 2.4 mg/m$^3$ of dust and 106 ng/m$^3$ of endotoxin were measured.

In addition to housing, the dust concentration is affected by poultry production category, the possibility of manifesting behavioural needs, and the season in which the air quality was analysed. All of these factors contribute to dust results ranging from only 0.02 mg/m$^3$ to as much as 81.33 mg/m$^3$ in poultry house air (32). Davis and Morishita (34) also established a wide range of dust levels in cage-held laying hen dwellings and associated these results with bad air. Abundant ventilation reduces the concentration of dust in animal dwellings. According to Gustaffson and von Wachenfelt (35), however, ventilation systems have limited capabilities for technical reasons. In addition, the actual effect of

ventilation depends on the activities of poultry, on the workings of the device for feeding and manure removal, and the temperature-humidity relationship.

**Endotoxins in the air of poultry dwellings**

Endotoxin is an integral part of gram-negative bacteria cell walls and is made of thermostable lipopolysaccharide proteins and phospholipids (36). It is released into the environment after bacterial cell wall lysis, but can also be released during the active growth of bacterial cells. Gram-negative bacteria can be found in air, soil, water, and dust and is produced by industrial and agricultural activities (37,38). However, their share in the total number of microorganisms present in the air is relatively small, probably due to their sensitivity to environmental factors. For example, at a higher temperature and relative humidity, gram-negative bacteria phospholipid membranes lose thermodynamic stability and thus vitality (39).

Endotoxins cause strong inflammatory activity in the organism (38). They are composed of lipopolysaccharide, whose molecules are built from lipid A, polysaccharide nucleus, and polysaccharide chains. In all species of bacteria, lipid A is identical, and it is responsible for the toxic effects of endotoxins. Molecule strength is provided by the polysaccharide nucleus, and polysaccharide chains are created in connection with endotoxin immunogenicity. Endotoxin is resistant to heat, and normal sterilisation procedures do not destroy it; heating at 180 °C for over 4 h is recommended for this purpose.

Today, the LAL test is usual method for setting endotoxin concentration. It is a biological method, based on endotoxin ability to coagulate *Limulus polyphemus,* an arthropod from the spider family, amebocytes. The LAL test has excellent sensitivity and, more importantly, measures only biologically active endotoxin (40).

While exploring the air quality from laying hen dwellings, Zucker and Müller (41) concluded that endotoxin stays active for a long time in dust, and that its concentration does not necessarily depend on the concentration of dust and microorganisms in the housing; it can originate from other sources.

Seedorf et al. (42) explored endotoxin concentration by sampling the air in dwellings for cattle, pigs, and poultry. They measured levels from 11.8 ng/m$^3$ to 786 ng/m$^3$ of inhalatory endotoxin and between 0.6 ng/m$^3$ and 72 ng/m$^3$ of respiratory endotoxin. The determined endotoxin concentration was highest in poultry dwellings and lowest in cattle dwellings, while the day values were higher than the night values. Poultry housing dwellings, in comparison to the dwellings of other animals, have the highest amount of pollutants in the air. Radon et al. (10) support this assessment with findings of 7.01 mg of dust in 1 m$^3$ of air and mean endotoxin concentrations amounting to 257.58 ng/m$^3$. The same authors found bacteria from $5.7 \times 10^5$ CFU/m$^3$ to $1.6 \times 10^9$ CFU/m$^3$, in poultry dwellings and fungi from $1.4 \times 10^4$ CFU/m$^3$ to $1.1 \times 10^8$ CFU/m$^3$; mean ammonia concentration was 40 ppm. Average endotoxin value in the air of poultry dwellings amounted to 463.2 EU/m$^3$ air, according to measurements by Schierl et al. (43).

Kirkhorn and Schenker (44) describe the connection between exposure to organic dust and the appearance of respiratory illnesses in people who work in crop warehouses and dwellings for pigs and poultry. Laitinen et al. (45) believe that biological endotoxin activity depends on the bacteria types from which they originate. The same authors conclude that endotoxin concentrations over 25 ng/m$^3$ of air intensify respiratory disorder symptoms. Nevertheless, even lower concentrations, especially under chronic exposure, may disturb the respiratory ventilation function. This is probably the reason for the recommended limit value of 4.5 ng/m$^3$, i.e., 50 EU/m$^3$ (Dutch Expert Committee on Occupational Exposures).

In order to assess health risks it is not enough to merely measure dust amounts; endotoxin concentration must be measured as well. Inflammation of the respiratory tract occurs during exposure to air loaded with more than 100 EU/m$^3$. General symptoms appear with exposure to more than 1000 EU/m$^3$. Symptoms of toxication occur with the inhalation of air polluted with more than 2000 EU/m$^3$ (40).

A dry cough, shortness of breath, and reduced lung function occur after inhaling high concentrations of endotoxin. In addition, fever, shivering, fatigue, and headaches may appear. Long-term exposure, even to low endotoxin concentrations, may ultimately cause respiratory system problems (40).

From an epidemiologic point of view, this is justification for the proposal by the Dutch Expert Commission for Standards at Work to set a limit of 50 EU/$m^3$ for 8 h of endotoxin exposure.

## Conclusion

Numerous studies carried out on dust and endotoxin concentrations in laying hen dwellings indicate the need for developing air quality standards for animal housing. Further research on bioaerosol composition and a credible system of monitoring are also necessary in order to create production that includes animal and human welfare and food and environmental safety in addition to economic considerations.

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