Effect of microclimate on the airborne dust and endotoxin concentration in a broiler house

M. Vučemilo[1], K. Matković[1], B. Vinković[2], J. Macan[3], V.M. Varnai[3], Lj. Prester[3], K. Granić[4], T. Orct[3]

[1]Department of Animal Hygiene, Environment and Ethology, School of Veterinary Medicine, University of Zagreb, Zagreb, Croatia
[2]Department of Zoohygiene and Livestock Technology, Croatian Veterinary Institute, Zagreb, Croatia
[3]Institute of Medical Research and Occupational Health, Zagreb, Croatia
[4]ZIN-LAB Laboratory for Foodstuffs of Animal Origin, Veterinary Station of Zagreb City, Zagreb, Croatia

ABSTRACT: Poultry farming is considered to be a notable source of bioaerosols. They can be a risk factor from the aspect of some diseases and for the environment. A study was conducted to assess the effect of microclimate on the level of airborne dust and endotoxins in an intensive broiler fattening facility. The content of airborne dust, endotoxins, air temperature, relative humidity, airflow velocity, ammonia and carbon dioxide were determined. The study was conducted in a poultry house accommodating 22 000 broilers of Ross-308 breed. The measured temperature in the broiler house ranged from 22.02°C to 31.05°C, relative humidity from 49.55% to 65.45%, and airflow velocity from 0.07 m/s to 0.09 m/s. The air concentration of dust ranged from 2.0 mg/m^3 at the end of fattening period to 4.9 mg/m^3 in the mid-fattening period, and endotoxins from 6.21 EU/m^3 in the second study week to 99.40 EU/m^3 at the end of fattening period. The air concentration of ammonia ranged from 5.17 ppm at the beginning to 25.49 ppm at the end of fattening period. Air concentrations of dust and endotoxins recorded in this poultry house varied during the fattening period and depended on relative humidity and temperature as demonstrated by multiple regression at the level of $P \leq 0.05$.

Keywords: broilers; dust; endotoxins; ammonia; carbon dioxide

Poultry houses are generally considered to be a major source emitting various particles to the environment, which greatly depends on the technology of animal keeping and housing. Besides other contaminants, dust and endotoxins are present in high concentrations in the air of poultry houses.

Dust concentrations in broiler facilities range from 2 to 10 mg/m^3 (Wathes et al., 1997). Investigating the mean daily airborne dust concentrations in various animal houses in England and Netherlands, Takai et al. (1998) found them to be significantly higher in poultry fattening houses than in other animal houses. They also found the airborne dust concentration in these facilities to be influenced by animal population density, animal activity, litter type, and air humidity. Hauser and Folsch (1993)

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demonstrated airborne dust to transmit various types of microorganisms which stick to its surface and can reach the lungs by inhalation. Airborne dust particles have been found to influence the immune system and to cause physiologic changes in animals, resulting in their poor gains.

According to Donham (1995), air hygiene is a major factor in industry facilities such as poultry breeding farms. Besides the animal health, airborne dust particles and endotoxins may also exert unfavourable health effects in humans working in the facilities. Various gases get adsorbed while microorganisms and endotoxins stick to airborne dust particles, thus generating bioaerosol present in the air. Gram-negative bacteria such as Enterobacteriaceae, *Klebsiella*, *Pseudomonas*, etc., are ubiquitous, i.e. omnipresent in our immediate environment. Their cell wall characteristically contains the lipid A polysaccharide chain, which is responsible for their cytotoxic action (Rylander, 2002; Madsen, 2006; Rylander, 2006). This substance is termed endotoxin, referring to natural lipopolysaccharide, a fragment of the gram-negative bacterium cell wall (Jacobs et al., 1997). This bioaerosol may reach the lungs *via* the respiratory system and cause inflammatory and allergic reactions (Pickrell et al., 1993). Consequences are nonspecific respiratory symptoms such as cough, expectoration and dyspnoea, the severity of which depends on the dose and individual susceptibility. At higher endotoxin levels, flu-like symptoms of toxic pneumonitis can be observed (Liebers et al., 2006; Rylander, 2006). A long-term exposure to endotoxins promotes irreversible changes in the lung function that can significantly reduce work ability. Several epidemiological studies have shown that the content of endotoxins in dust is related to long-term pulmonary adverse effects in exposed workers (Rylander, 2006). Asthmatic subjects and persons with other chronic respiratory diseases are more susceptible to toxic effects of endotoxins (Liebers et al., 2006).

Airborne dust along with moulds and endotoxins affects epithelial cells and alveolar macrophages (Lacey, 1994). They are released to the environment upon bacterial cell wall lysis or during active cell growth. Endotoxins are more frequently found in higher concentrations in the air of animal houses characterized by breeding activities associated with high organic dust production (Olenchock, 1988). Thedell et al. (1980) found 4.5–48 μg endotoxins per gram of dust in the air of pig and poultry houses. Clark et al. (1983) reported the mean airborne endotoxin concentration of 0.12–0.31 μg/m^3 in pig and poultry houses. In their study examining the air quality in animal houses, Seedorf et al. (1998) found the concentration of airborne endotoxins to be lowest in cattle houses and highest in poultry houses, whereby daily levels exceeded nocturnal ones. According to Seedorf (2004), faeces from the litter and animal movement activities are the main factors of endotoxin emission to the environment.

The quality of poultry house air influences the health of livestock and humans working there, however, it has also been considered as a risk factor for the immediate environment (Müller and Weiser, 1987; Wathes, 1994; Wathes et al., 1998). Therefore the aim of the present study was to asses the effect of microclimate on the level of airborne dust and endotoxins in an intensive broiler breeding facility.

MATERIAL AND METHODS

The study was conducted in spring during the production period on a poultry farm with about 22 000 fattening broilers of Ross-308 breed. At the beginning of the study, the broilers were one week of age. The housing is an iron structure fitted upon concrete foundations, with thermal and damp-proof insulation between the floor and the concrete. The walls are made of brickwork with tiles on the indoor side and styrofoam on the outdoor side. The rest of the wall (2 m) is made of 8-cm wall panel. The building has 92 windows, 30 × 60 cm in size, 46 on either side. Day old chicks are housed separately according to sex. The fattening of female and male chicks takes 32 and 38 days, up to the mean weight of 1.65 kg and 2.28 kg, respectively. The size of the building is 96 × 14 × 2.7 m, accommodating 17 animals/m^2 on average. In the entrance room, 4 × 14 m in size, there is a control board for the computer guidance of technological parameters, medicator, and farmer's room. The space for animal accommodation is sized 92 × 14 m, i.e. 1 288 m^2, with 10-cm deep sawdust litter and forced pressure ventilation at 4 m^3/h/kg body mass.

Microclimate parameters were determined and air samples for airborne dust and endotoxin determination were collected three times a week during the fattening period. Microclimate measurements were performed in the middle of the facility, 0.5 m above the floor. Air temperature (°C), relative hu-

midity (%) and airflow rate (m/s) were determined with a TESTO device (Testo Inc., Germany). The concentration of ammonia and carbon dioxide was determined with a Dräger-Multiwarn II device (Dräger, Darmstadt, Germany). Dust was sampled onto filters (Whatman International Ltd., Maidstone, UK) with an SKC pump (SKC Ltd., Blandford Forum, UK). The airflow rate was 4.0 l/min. Filters were weighed before and after sampling in a controlled laboratory at air temperature of 22°C and relative humidity of 45% (± 5%). Air samples were stored at –20°C for 2–3 months until endotoxin analysis.

Endotoxin analysis

Extraction procedure of filter samples and endotoxin analysis were performed on the same day according to general recommendations (Anonymous, 1987). Sterile and pyrogen-free glassware and microplates (Greiner Labortechnik, GmbH, Germany) were used for endotoxin assay. Filter samples (including blank filters) were extracted in 5 ml of LAL non-pyrogenic water with 0.05% Tween for 2 h at room temperature and continuous shaking. Extracts were centrifuged at 3 000 rpm for 10 min. Before the endotoxin analysis, all supernatants were placed in pyrogen-free glass and left at 75°C for 20 min to avoid any possible interference. Endotoxins were analyzed using the end-point Limulus amoebocyte lysate method (LAL) previously described (Varnai et al., 2004). A commercial kit for endotoxin assay supplied by Endochrome (Charles River Endosafe, USA) included: LAL reagent (lyophilized), standard (*E. coli* control standard endotoxin), substrate-buffer solution and LAL reagent water.

The standard curve was made by reconstituting the endotoxin standard of 24 EU/ml with LAL reagent water. Multiple dilutions of filter extracts were done (1:25 to 1:200) using LAL reagent water. LAL assay was performed at 37°C. Control standard and blanks were also loaded on the microplate. Optical density was read at 405 nm on a Personal Lab microplate analyzer (Iason, Graz, Austria). Endotoxin concentrations were read from the standard curve and were proportional to colour change. As the LAL assay measures the activity of different types of airborne endotoxin, the results are expressed in Endotoxin Units per 1 m^3 (EU/m^3). Each sample was measured in duplicate. Blank filters revealed a very low level of background endotoxin. Recovery of the assay was 95%. The assay limit of detection was 0.008 EU/ml.

The measured parameter values were processed by Microsoft Excel and Statistica 6 software. The analysis included non-parametric statistics for non-normal data at a statistical significance of 5% ($P \leq 0.05$ by use of multiple regression) (Anonymous, 1994; Petz, 2001).

RESULTS AND DISCUSSION

Mean levels of airborne dust and endotoxins, and microclimate parameters in a poultry house during 5-week fattening period are shown in Table 1. The air concentration of dust ranged from 2.0 mg/m^3 at the end of fattening period to 4.9 mg/m^3 in mid-fattening period, endotoxins from 6.21 EU/m^3 in the second study week to 99.40 EU/m^3 at the end of fattening period. The measured values of air temperature were between 22.02°C in the fifth fattening week to 31.05°C at the beginning, relative humidity between 49.55% in the first week and 65.45% in the fourth study week and airflow rate from 0.07 to 0.09 m/s. The air concentration of ammonia ranged from 5.17 ppm at the beginning to 25.49 ppm at the end of fattening period. The level of carbon dioxide

Table 1. Mean levels of airborne dust and endotoxins, and microclimate parameters in poultry house during the 5-week fattening period

Parameter	Week 1	Week 2	Week 3	Week 4	Week 5
Dust (mg/m^3)	3.60	2.71	4.90	2.60	2.00
Endotoxins (EU/m^3)	54.00	6.21	85.26	82.16	99.40
Temperature (°C)	31.05	27.34	26.04	24.17	22.02
Relative humidity (%)	49.55	52.48	59.23	65.45	60.18
Airflow rate (m/s)	0.07	0.09	0.08	0.07	0.09
Ammonia (ppm)	5.17	8.34	16.22	18.87	25.49
Carbon dioxide (%)	0.32	0.30	0.28	0.21	0.32

Table 2. Multiple regression for non-normal data at $P \leq 0.05$

$n = 3$		Dust				
		temperature	relative humidity	air flow rate	ammonia	carbon dioxide
Week 1	$t(1)$	9.37	15.30	3.23	2.23	–0.48
	P-level	0.07	0.04*	0.19	0.27	0.72
Week 2	$t(1)$	12.24	22.96	0.61	4.13	–0.45
	P-level	0.05*	0.03*	0.65	0.15	0.73
Week 3	$t(1)$	6.71	14.52	–0.56	4.40	–0.51
	P-level	0.09	0.04*	0.68	0.14	0.70
Week 4	$t(1)$	16.11	92.39	0.04	7.81	2.02
	P-level	0.04*	0.01*	0.97	0.08	2.02
Week 5	$t(1)$	12.11	34.11	–0.53	15.27	–0.39
	P-level	0.05*	0.02*	0.69	0.04*	0.76

n = number of weekly measurements; t = test coefficient; *statistically significantly different at $P \leq 0.05$

was between 0.21% in the fourth week and 0.32% in the first and fifth week of study.

Recommended microclimate levels in the broiler facility for temperature are from 18 to 20°C; relative humidity from 60 to 75%, airflow rate from 0.15 to 0.20 m/s, ammonia concentration to 20 ppm, and carbon dioxide to 3 500 ppm (Siegmann and Neumann, 2005). The results showed that relative humidity and airflow rate were at the lowest or below recommended levels, temperature exceeded the values from the mid-fattening period. The increase in ammonia concentration in the fifth week could be attributed to animal age and higher content of excrements in the litter. The level of carbon dioxide was within the recommended limits.

The composition of air pollution and its effect on animal health and productivity have been investigated in numerous studies conducted on poultry farms. Vučemilo et al. (2005, 2007) found the concentration of airborne bioaerosol in a poultry house to increase with animal age. A study in 69 poultry stockmen showed 20% of them to be exposed to a dust concentration 2.5 times exceeding the maximal recommended level of 10 mg/m^3 regulated by the respective by-law (Whyte et al., 1993). In Switzerland, Radon et al. (2002) investigated 36 poultry farms and found a mean dust concentration of 7.01 mg/m^3, posing a risk of asthma symptoms for poultry stockmen. The level of airborne endotoxins ranged from 19 to 1 634 ng/ m^3 (190 –16 340 EU/m^3), mean 258 g/m^3 (2 580 EU/m^3). Similar studies and results were reported by Seedorf et al. (1998), Takai et al. (1998) and Hyvärinen et al. (2006). In poultry facilities Wathes et al. (1998) recommend a dust amount of 3.4 mg/m^3 air.

The concentration, size distribution and chemical composition of airborne dust particles in animal houses depend on the type of feed, method of feeding, litter quality, and animal species and their accommodation. The airborne dust concentration rises significantly with the use of dry and bulky or concentrated feed and with the use of litter (Seedorf et al., 1998; Takai et al., 1998). In addition, the distribution and size of dust particles are greatly influenced by air temperature, humidity and content of gaseous pollutants such as ammonia, carbon dioxide, hydrogen sulphide and hydrocarbons.

In our study the dust concentration was mostly influenced by relative humidity and temperature. Multiple regression showed a statistical significance ($P \leq 0.05$) between the dust concentration and relative humidity for all five fattening weeks, as well as dust concentration and temperature (Table 2). Ammonia also influenced the dust concentration in the fifth week which was the higher noticed. The highest airborne dust concentration in the poultry house was recorded in the third study week, which can be related with the higher activity of broilers and enough space for such activity. The lowest concentration of dust was at the end of fattening, which can be related to their mass and low activity. High air humidity may precipitate the rate of dust particle sedimentation, whereas low humidity results in a high airborne dust concentration,

Table 3. Multiple regression for non-normal data at $P \leq 0.05$

$n = 3$		Endotoxins					
		dust	temperature	relative humidity	air flow rate	ammonia	carbon dioxide
Week 1	$t(1)$	55.29	1.24	1.64	–3.16	–0.47	0.58
	P-level	0.01*	0.43	0.35	0.20	0.72	0.66
Week 2	$t(1)$	2.07	4.50	9.18	3.21	0.97	0.63
	P-level	0.29	0.14	0.07	0.19	0.51	0.64
Week 3	$t(1)$	19.50	4.22	5.58	0.58	3.82	–3.15
	P-level	0.03*	0.15	0.11	0.67	0.16	0.20
Week 3	$t(1)$	107.72	0.92	1.61	–0.58	0.13	3.17
	P-level	0.01*	0.53	0.35	0.67	0.92	0.19
Week 5	$t(1)$	175.75	0.83	1.28	0.58	–0.28	0.58
	P-level	0.00*	0.56	0.42	0.67	0.83	0.66

n = number of weekly measurements; t = test coefficient; *statistically significantly different at $P \leq 0.05$

and thus also in an increased air concentration of endotoxins (Hristov, 2002).

The measured levels of airborne endotoxins were below the levels reported in the literature, with a marked decrease in the second week, which could not be associated with the macroclimatic parameters influencing the gram-negative bacterial count. At the end of the study, the airborne endotoxin concentration was 99.40 EU/m^3, i.e. twice higher than that recorded in the first week. Multiple regression showed that the concentration of endotoxins depends on dust concentration with the exception of the second study week (Table 3). During all fattening periods (except the second week) the endotoxin concentration exceeded proposed Dutch endotoxin thresholds of 50 EU/m^3 (Anonymous, 1998).

In general, for the interpretation of our results a small number of sampling sites and method of sampling must be taken into account. In some above-mentioned studies dust and endotoxin samples were collected by a personal sampler while we used a stationary sampler. But even in studies using stationary samplers the recorded results of dust and endotoxin concentrations varied greatly, but they were usually higher than the results we obtained.

CONCLUSION

The results indicate that the concentration of airborne dust during the fattening period is significantly influenced by temperature and relative humidity as well as by a higher concentration of ammonia. The concentration of endotoxins is influenced by a dust level in the air. The measured concentrations indicate that there is still a potential risk for both animal's and humans' health and animal performance. The highest airborne dust concentration of 4.9 mg/m^3 was recorded in the mid-fattening period to 2.0 mg/m^3 towards the end of fattening period. Throughout the study period, the concentration of airborne endotoxins was below the levels reported in the literature; however, the twofold initial concentration was measured in the last week of fattening period (99.40 EU/m^3).

The study was carried out as a follow-up of previous studies of airborne pollutants on poultry farms in order to contribute to the identification of the level and composition of airborne bioaerosols and to find practical methods for their reduction.

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Corresponding Author

Prof. dr. sc. Marija Vučemilo, Department of Animal Hygiene, Environment and Ethology, School of Veterinary Medicine, University of Zagreb, Zagreb, Croatia
e-mail: marija.vucemilo@vef.hr