

## ORAL PRESENTATIONS

### BIOAEROSOL IN LAYING HEN HOUSE

Vučemilo, M.<sup>1</sup>, Vinković, B.<sup>2</sup>, Matković, K.<sup>1</sup> and Brezak, R.<sup>2</sup>

<sup>1</sup> Professor Marija Vučemilo, DVM, PhD – professor, Department of Animal Hygiene, Environment and Ethology, School of Veterinary Medicine, University of Zagreb, Heinzelova 55, HR-10000 Zagreb, Croatia; phone +38512390291, e-mail: vucemilo@vef.hr;

<sup>2</sup> Bara Vinković, DVM, PhD – scientific associate, Department of Zoohygiene and Livestock Technology, Croatian Veterinary Institute, Savska cesta 143, HR-10000 Zagreb, Croatia;

#### ABSTRACT

Intensive production and housing of laying hens result in a significant amount of hazardous pollutants in the air of poultry house. Under specific conditions, these pollutants can affect the health of both poultry and people who work in poultry houses. The study was carried out in winter period on a farm with a capacity of 17000 Shaver hybrid laying hens from 25<sup>th</sup> week of production. Laying hens were housed in cages, 8–10 *per* cage. Samples were collected in the morning once a week for six weeks, at 5 sites in the house. Air was sampled by use of a Merck MAS-100 (Merck KgaA, Darmstadt, Germany) device onto commercial nutrient and Sabouraud agar (Biolife, Milan, Italy). Upon incubation, microorganisms grown on the medium (bacteria and fungi) were counted and predominant species were inoculated for determination. Dust was sampled by an SKC pump (SKC Ltd., Blandford Forum, UK) on filters (Whatman International Ltd., Maidstone, UK). Temperature (t °C), relative humidity (rh %) and air velocity (w m/s) were determined by a Testo 400 (Testo Inc., Lenzkirch, Germany) device. The concentration of ammonia and carbon dioxide was determined by a Dräger-Multiwarn II (Dräger, Darmstadt, Germany) device. The measured values of study parameters were processed by Microsoft Excel and Statistica 6 software. Descriptive statistics was employed and statistical significance at 5% (p<0.05) was determined by Student's t-test. The concentration of bacteria ranged from  $1.6 \times 10^2$  to  $2.7 \times 10^3$  cfu/m<sup>3</sup>, of fungi from  $0.8 \times 10^2$  to  $6.9 \times 10^2$  cfu/m<sup>3</sup>, and of dust from 1.6 to 3.8 mg/m<sup>3</sup>. The mean level of ammonia was between 5.87 and 9.22 ppm. The predominant bacteria were from the genera *Staphylococcus* and *Streptococcus*, and fungi from the genera *Aspergillus* and *Penicillium*. The results on all microclimate parameters were in line with recommended standards. The low air count of the bacteria, fungi and dust could be attributed to the relatively low temperature recorded in the housing and its environment.

#### INTRODUCTION

Good hygiene of housing air is a major prerequisite for poultry health and productivity. In addition, poor quality of air in poultry housing can have adverse effects on the health of people working there (Stetzenbach et al., 2004). Intensive poultry production is known to be a source of numerous air pollutants including microorganisms, dust, gases, endotoxins, and offensive odor

(Takai et al., 1998; Zang, 1999). This form of contamination can be caused by inappropriate zoohygienic conditions in the housing due to inadequate or poor ventilation, overcrowding, etc. All particles present in the animal housing air, which contain microorganisms, desquamated epithelium, dried feces and other organic particles, are known under the common term of bioaerosol. In addition to the components mentioned above, bioaerosol can contain live and dead bacteria, parts of fungi, spores, mycotoxins and tannins. Bioaerosol concentrations found in the animal housing air vary depending on the animal keeping and housing conditions, age, method of feeding and feces/urine disposal, etc. Generally, air hygiene frequently presents an unsatisfactory and limiting factor of poultry productivity, health and welfare.

### MATERIAL AND METHODS

The study was conducted during winter period at a farm with a capacity of 17000 Shaver hybrid laying hens from 25<sup>th</sup> week of production. Laying hens were housed in cages, 8–10 *per* cage. Samples were collected in the morning once a week for six weeks, at 5 sites in the house. Air was sampled by use of a Merck MAS-100 (Merck KgaA, Darmstadt, Germany) device onto commercial nutrient and Sabouraud agar (Biolife, Milan, Italy). Upon incubation, microorganisms grown on the medium (bacteria and fungi) were counted and predominant species were inoculated for identification. Dust was sampled by an SKC pump (SKC Ltd., Blandford Forum, UK) on filters (Whatman International Ltd., Maidstone, UK). Temperature ( $t$  °C), relative humidity (rh %) and air velocity ( $w$  m/s) were determined by a Testo 400 (Testo Inc., Lenzkirch, Germany) device. The concentration of ammonia and carbon dioxide was determined by a Dräger-Multiwarn II (Dräger, Darmstadt, Germany) device. The measured values of study parameters were processed by Microsoft Excel and Statistica 6 software. Descriptive statistics was employed and statistical significance at 5% ( $p < 0.05$ ) was determined by Student's t-test (Anonymous, 1994; Petz, 2001).

### RESULTS AND DISCUSSION

Elevated bioaerosol concentration in poultry housing occurs consequentially to animal accommodation conditions (high population density, dry litter) and technology process (various manipulations). In such a setting, the air is the source and storage of various microorganisms, mostly originating from animals (80%) and their droppings. In the overall microorganism count, the genera *Staphylococcus* and *Streptococcus* account for 60% and 30%, respectively, the rest being fungi, spores and other microorganisms, however, the majority of animal housing microflora is nonpathogenic (Hartung, 1994). Many authors report on the varying bioaerosol concentration in the air of animal housing, being highest in poultry housings irrespective of poultry keeping on thick litter or in cages (Wathes, 1994; Radon et al., 2002). Otherwise, the concentration of bioaerosol depends on the number of animals, animal population density *per* area unit, type and quality of litter, ventilation, etc. (Matković et al., 2006).

Concerning gaseous air pollutants, mention should be made of ammonia produced by fecal nitrogenous organic substance decay, and of carbon dioxide. Poor ventilation of animal housing results in elevated concentrations of ammonia and carbon dioxide, which have adverse effects on the poultry health and productivity. According to Hartung (2005), the maximal allowed concentration in the air of poultry housing is 20 ppm for ammonia, 3000 ppm for carbon dioxide,

10 ppm for hydrogen sulfide, and 50 ppm for carbon monoxide. Poultry have a considerably lower tolerance to ammonia than other animals, so a concentration of 20 ppm causes irritation of the mucous membranes of the eyes and respiratory system, reduced feed intake, and occurrence of technological runts (Kristensen and Wathes, 2000).

The results obtained in the present study indicated the level of environmental air contamination with bioaerosol to be consistent with literature data, approaching the lower limit reported (Hartung, 1994; Seedorf et al., 1998; Radon et al., 2002; Hyvärinen et al., 2006). The concentration of bacteria ranged from  $1.6 \times 10^2$  to  $2.7 \times 10^3$  cfu/m<sup>3</sup> air, predominated by the genera *Staphylococcus* sp. and *Streptococcus* sp., *Escherichia coli*, *Pseudomonas* sp., *Klebsiella* sp. and *Micrococcus* sp (Table 1 and 2). The concentration of fungi ranged from  $0.8 \times 10^2$  to  $6.9 \times 10^2$  cfu/m<sup>3</sup> air, predominated by the genera *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp (Table 1 and 2). The concentration of dust during the six production weeks ranged from 1.6 to 3.8 mg/m<sup>3</sup> air (Table 1 and 2). The mean level of ammonia was between 5.87 and 9.22 ppm. The low air concentration of the microorganisms and dust could be attributed to the relatively low temperature during the study period (winter) recorded in the housing and its environment, generally characterized by lower animal activity. A higher bioaerosol concentration was only recorded in the sixth week of the study, when the values of air temperature, relative humidity and ammonia showed a slight increase. A significant differentiation in the bacterial, fungi and dust concentration was recorded between all observed weeks as demonstrated by t-test yielding statistical significance at a level of  $p < 0.05$  (Table 3).

Other microclimate indicators were generally within the allowed limits. Relative humidity in the poultry house ranged between 40% and 70%, as recommended (Whyte, 1993). Increased dust concentration may be associated with lower humidity, which has adverse effects on the poultry respiratory system.

**Table 1.** Mean levels of total bacterial count, fungi count, dust concentration and microclimate parameters in laying hen housing air

Parameter	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
<b>bacteria</b> cfu/m <sup>3</sup>	$1,6 \times 10^2$	$1,9 \times 10^2$	$1,2 \times 10^3$	$5,7 \times 10^2$	$1,1 \times 10^3$	$2,7 \times 10^3$
<b>fungi</b> cfu/m <sup>3</sup>	$0,8 \times 10^2$	$3,5 \times 10^2$	$2,8 \times 10^2$	$2,3 \times 10^2$	$6,9 \times 10^2$	$5,4 \times 10^2$
<b>dust</b> mg/m <sup>3</sup>	1,6	2,3	3,8	2,9	3,1	2,2
<b>temp.</b> °C	15,86	16,84	15,76	16,23	16,59	17,89
<b>humid.</b> %	66,04	59,92	63,29	62,20	62,19	69,55
<b>airflow</b> m/s	0,07	0,13	0,08	0,08	0,09	0,06
<b>NH<sub>3</sub></b> ppm	5,87	6,11	6,22	8,67	7,99	9,22
<b>CO<sub>2</sub></b> %	0,08	0,09	0,12	0,07	0,11	0,15

**Table 2.** Descriptive statistical analysis of bacteria, fungi, dust and microclimate factors recorded in laying hen housing air

	Week	n	arithmetic mean	minimum	maksimum	variance	SD	SE
bacteria cfu/m <sup>3</sup>	1	5	1,60 x 10 <sup>2</sup>	1,60 x 10 <sup>2</sup>	1,60 x 10 <sup>2</sup>	0,01	0,09	0,04
	2	5	1,90 x 10 <sup>2</sup>	1,90 x 10 <sup>2</sup>	1,90 x 10 <sup>2</sup>	0,00	0,01	0,00
	3	5	1,20 x 10 <sup>3</sup>	1,20 x 10 <sup>3</sup>	1,20 x 10 <sup>3</sup>	0,00	0,01	0,01
	4	5	5,70 x 10 <sup>2</sup>	5,70 x 10 <sup>2</sup>	5,70 x 10 <sup>2</sup>	0,00	0,01	0,00
	5	5	1,10 x 10 <sup>3</sup>	1,10 x 10 <sup>3</sup>	1,10 x 10 <sup>3</sup>	0,00	0,01	0,00
	6	5	2,70 x 10 <sup>3</sup>	2,70 x 10 <sup>3</sup>	2,70 x 10 <sup>3</sup>	0,00	0,01	0,00
fungi cfu/m <sup>3</sup>	1	5	8,00 x 10	8,00 x 10	8,00 x 10	0,00	0,01	0,01
	2	5	3,50 x 10 <sup>2</sup>	3,50 x 10 <sup>2</sup>	3,50 x 10 <sup>2</sup>	0,00	0,01	0,01
	3	5	2,80 x 10 <sup>2</sup>	2,80 x 10 <sup>2</sup>	2,80 x 10 <sup>2</sup>	0,00	0,01	0,01
	4	5	2,30 x 10 <sup>2</sup>	2,30 x 10 <sup>2</sup>	2,30 x 10 <sup>2</sup>	0,00	0,00	0,00
	5	5	6,90 x 10 <sup>2</sup>	6,90 x 10 <sup>2</sup>	6,90 x 10 <sup>2</sup>	0,00	0,01	0,00
	6	5	5,40 x 10 <sup>2</sup>	5,40 x 10 <sup>2</sup>	5,40 x 10 <sup>2</sup>	0,00	0,00	0,00
dust mg/m <sup>3</sup>	1	5	1,60	1,60	1,60	0,00	0,00	0,00
	2	5	2,30	2,30	2,30	0,00	0,00	0,00
	3	5	3,80	3,80	3,80	0,00	0,00	0,00
	4	5	2,90	2,90	2,90	0,00	0,00	0,00
	5	5	3,10	3,10	3,10	0,00	0,00	0,00
	6	5	2,20	2,20	2,20	0,00	0,00	0,00
microclimate	temp °C	5	16,53	15,76	17,89	0,53	0,73	0,13
	humid. %	5	63,87	59,92	69,55	10,09	3,18	0,58
	airflow m/s	5	0,09	0,06	0,13	0,00	0,02	0,00
	NH <sub>3</sub> ppm	5	7,35	5,87	9,22	1,84	1,36	0,25
	CO <sub>2</sub> %	5	0,10	0,07	0,15	0,00	0,03	0,00

**Table 3.** t-test for dependent variables at p<0.05

Parameter		n	t	p
bacteria cfu/m <sup>3</sup>	1 week – 2 week	5	-7,40E+02	0,00
	2 week – 3 week	5	-1,52E+05	0,00
	3 week – 4 week	5	1,68E+05	0,01
	4 week – 5 week	5	-1,68E+05	0,01
	5 week – 6 week	5	-2,26E+05	0,01
fungi cfu/m <sup>3</sup>	1 week – 2 week	5	-1,10E+05	0,00
	2 week – 3 week	5	1,11E+04	0,00
	3 week – 4 week	5	8,33E+03	0,00
	4 week – 5 week	5	-1,15E+06	0,01
	5 week – 6 week	5	6,82E+04	0,00
dust mg/m <sup>3</sup>	1 week – 2 week	5	-8,14E+15	0,00
	2 week – 3 week	5	-3,06E+03	0,01
	3 week – 4 week	5	1,84E+03	0,00
	4 week – 5 week	5	-6,32E+02	0,00
	5 week – 6 week	5	2,85E+03	0,00

## CONCLUSION

In the air of housing for laying hens determined concentration of bioaerosols was in the lowest limits known from literature. Within observed six weeks of production exist significant differentiation in bioaerosol concentration that toward the end of research have significant increase.

## REFERENCES

1. Hartung, J. (1994): The effect of airborne particulates on livestock health and production. U: Ap Dewi I, Axford RFE, Mara I, Omed H. *Pollution in Livestock Systems*, CAB International, 55–69.
2. Hartung, J.(2005): Klimabedingungen. In: Siegmann, O., U. Neumann: *Kompendium der Geflügelkrankheiten*. 6., aktualisierte und erweiterte Auflage, Hannover, Schlütersche Verlagsgesellschaft mbH & Co. 55–67.
3. Hyvärinen, A., M. Roponen, P. Tiittanen, S. Laitinen, A. Nevalainen, J. Pekkanen (2006): Dust sampling methods for endotoxin – an essential, but underestimated issue. *Indoor Air*. 16, 20–27.
4. Kristensen, H.H., C.M. Wathes (2000): Ammonia and poultry welfare: a review. *Worlds Poultry Science Journal*, 56 (3) 325–345.
5. Matković, K., M. Vučemilo, B. Vinković, B. Šeol, Ž. Pavičić, A. Tofant, S. Matković (2006): Effect of microclimate on bacterial count and airborne emission from dairy barns on the environment. *Ann Agric Environ Med*. 13, 349–354.
6. Radon, K., B. Danuser, M. Iversen, E. Monso, C. Weber, J. Hartung, K.J. Donham, U. Palmgren, D. Nowak (2002): Air contaminants in different European farming environments. *Ann Agric Environ Med*. 9, 41–48.
7. Seedorf, J., J. Hartung, M. Schroder, K.H. Linkert, M.R. Holden, R.W. Sneath, J.L. Short, R.P. White, S. Pederson, H. Takai, J.O. Johnsen, J.H.M. Metz, P.W.G. Groot Koerkamp, G.H. Uenk, C.M. Wathes (1998): Concentrations and emissions of airborne endotoxins and microorganisms in livestock buildings in Northern Europe. *J. Agric. Engng. Res.* 70, 97–109.
8. Stetzenbach, L.D., M.P. Buttner, P. Cruz (2004): Detection and enumeration of airborne biocontaminants. *Current Opinion in Biotechnology*. 15, 170–174.
9. Takai, H., S. Pederson, J.O. Johnsen, J.H.M. Metz, P.W.G. Groot Koerkamp, G.H. Uenk, V.R. Phillips, M.R. Holden, R.W. Sneath, J.L. Short, R.P. White, J. Hartung, J. Seedorf, M. Schroder, K.H. Linkert, C.M. Wathes (1998): Concentrations and emissions of airborne dust in livestock buildings in Northern Europe. *Journal of Agricultural Engineering Research*. 70, 59–77.
10. Wathes, C.M. (1994): Air and surface hygiene. In: Wathes C M; Charles D R (eds): *Livestock housing*. CAB International, Wallingford, 123–148.
11. Whyte; R.T. (1993): Aerial pollutants and health of poultry farmers. *World's Poultry Science Journal* 49, 139–156.
12. Zhang, Y. (1999): Engineering control of dust in animal facilities. *Proceedings of the International Symposium on Dust Control in Animal Production Facilities*, 30 May–2 June 1999, Aarhus, Denmark. Danish Institute of Agricultural Sciences, Horsens, Denmark. 22–29.