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Concentrations of airborne bacteria and fungi in a livestock building with caged laying hens

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ABSTRACT

This paper describes the concentrations of airborne bacteria and fungi in a cage housed laying hens facility, during a production year. Levels of airborne bacteria established in the air of the poultry house ranged from 1.02×10^4 CFU/m^3 measured in April, to 7.72×10^4 CFU/m^3 measured in December. Mean values of the total number of fungi ranged from 0.075×10^4 CFU/m^3 measured in September, to 8.56×10^4 CFU/m^3 measured in June. Established values of air temperature, relative humidity and air velocity were, generally, in accordance with the technology-predicted ranges. The determined number of bacteria and fungi in the air, as well as the statistically significant impact of microclimate conditions on their number in the air of the cage housed laying hens facility, are in accordance with considerations for standards set for air quality in livestock buildings and the development of reliable systems for monitoring the above factors. The aim is to create production that, besides the economic aspects, must include the protection of animals and people, as well as safety of food and environment.

Key words: air hygiene, airborne microorganisms, laying hens facility, air quality standard

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Introduction

Consumable egg production is an intensive stockbreeding activity, during which laying hens are kept mostly in cages, in precisely controlled conditions. However, the technical infrastructure and hygienic quality of the housing do not guarantee production completely free of many pollutants (HARTUNG and WATHES, 2001; HARTUNG, 2007), which are potential risk factors for pathological phenomena. The animals' reactions to contaminated air are not always identifiable and measurable. The amounts of pollutants present in the air of livestock buildings that endanger the health and affect the productivity of animals have not yet been established (SEEDORF et al., 1998b; RADON et al., 2002, VUČEMILO et al., 2007).

The quality of housing conditions is an important component of efficient production. The most prominent one is the microclimate complex, with all its biological and technological processes. The air temperature in animal housing depends on several factors. The most important are the external temperature, the heat produced by the animals, and the ventilation, which regulates this relationship (SEEDORF et al., 1998a).

HILLIGER (1990) and VUČEMILO (2008) indicate that the optimum air temperature for laying hens ranges from 15 °C to 22 °C. Temperatures beyond these limits significantly decreases laying ability and can even cause its complete cessation. The effect of humidity is closely related to air temperature. With high humidity and low temperature, animals release more heat. This can lead to their cooling, which generates suitable conditions for disease development. On the other hand, high humidity and high air temperature can lead to difficult heat release since the conductivity of air is reduced. This can result in disturbance of thermoregulation and possible overheating of the animals. The optimum relative humidity in laying hens facilities, according to ELLEN et al. (2000), is 60% to 70%. Maintaining relative humidity in this range is important for airborne microorganism control, whose survival is almost directly influenced by humidity (BICKERT, 2001). Germs, attached to dust particles in the air, have the chance to extend their survival period (ROBINE et al., 2000).

The study results obtained in practice will be compared to the recommendations of the relevant international authorities. Furthermore, they will contribute to establishing standards regarding air quality in henhouses, as well as regarding implementation system standards.

Materials and methods

The research was carried out on a farm of cage-held consumable egg-laying hens in an facility where a one-year- production cycle had started, in the central part of Croatia. The research building is 85 m long, 12 m wide and 3 m high (up to the roof). It was built of classic building elements. Ventilation is mechanical by means of 32 fans. There are 16

fans with 700 rpm and 16 with 1,400 rpm. The fans are placed along the side. The housing has a total of 180 forty-watt lighting fixtures, which are symmetrically arranged, by thirty in six rows, between the batteries along the length of the building.

The housing area was populated by 17,200 18-week-old Shaver hybrid hens. They were housed in conventional cages, sized 90 × 45 × 45 cm. The cages were assembled in five three-storey batteries, resulting in 500 cages in a battery, or a total of 2,500 cages. Approximately 7 to 10 hens were placed in every cage. The wire cage floor towards the passage was placed at an angle of 2% and ended in a slotted shape. Eggs were hand collected on cardboard trays twice a day.

Manure was removed below the lower floors in a canal, and the debris from the second and third floors of batteries was placed on a belt under the cages. Manure was then pressed by a scraper into a cross canal, from which it was loaded weekly onto a tractor using a mechanical belt, and then taken to a nearby agricultural area.

The study was undertaken to determine bacteria and fungi contents in the air of the facility, air temperature, relative humidity, airflow rate, ammonia and carbon dioxide content and light as microclimate complex components. These marked indicators were determined twice a month, during the one-year production cycle. Measuring was carried out along the two main passages in between the batteries, at the second-cage floor level, at ten positions.

Air sampling was conducted using a Merck MAS-100 device (Merck KgaA, Darmstadt, Germany). Plates with nutrient agar (Columbia agar, Biolife) were used to determine the total number of bacteria, whereas prepared plates with Sabouraud maltose agar (Biolife) were used for fungi. Petri plates with nutrient agar were placed in the thermostat with the temperature set at 37 ºC/72 h. The plates with the material sampled on Sabouraud agar were set at a temperature of 22 ºC/5 days. In totally, were analyzed 240 plates for bacteria, and 240 plates for fungi count. Grown colonies of bacteria and fungi were counted by an optical mechanical counter (Colony Counter, Testo Inc., Lenzkirch, Germany), and corrected by mathematical equation from the table attached to the Merck MAS-100 device (ANONYM., 1998).

Air temperature (T ºC), relative humidity (rh%) and air flow rate (wm/s) in the building were measured by a TESTO 400 device (Testo Inc., Lenzkirch, Germany), with a related probe. Content of ammonia (NH3 ppm) and carbon dioxide (CO_2 vol%) in the air was determined by Dräger-Multivarn II device (Dräger, Darmstadt, Germany), and light level was measured by a TESTO 0500 light meter (Testo Inc., Lenzkirch, Germany).

Statistical analysis of the results obtained was carried out by Statistica v10 computer program (StatSoft). Descriptive statistical analysis was performed to calculate the arithmetic mean, standard deviation and standard error of mean. All results were

processed according to ANOVA repeated measures. The correlation rank coefficient (r) was calculated in order to examine the connection between the results.

Results

During this research, mean values of the total number of bacteria in the poultry house air ranged from 1.02×10^4 CFU/m^3 (colony forming unit) measured in April, to 7.72×10^4 CFU/m^3 measured in December (Table 2). Mean values of the total number of fungi ranged from 0.075×10^4 CFU/m^3 measured in September, to 8.56×10^4 CFU/m^3 measured in June (Table 2). Established values of relative humidity and air velocity were generally in accordance with the technology-predicted ranges, except the air temperature from May to August, when there were considerably higher values (Table 1).

A statistically significant ($P<0.05$) difference was established for average values of bacteria and fungi for different months of the year (Table 2).

The correlation coefficient (r) indicates the relationship between individual indicators (Table 3). Statistically significant negative correlations were established ($P<0.05$) in March between air temperature, concentration of ammonia and carbon dioxide and the concentration of fungi.

In May, the number of fungi in the air was positively correlated with air temperature, relative humidity and concentration of ammonia and carbon dioxide in the air. In June, there was a significant positive correlation between air temperature and the number of fungi, while in the second half of the month, there was a significant positive correlation between air velocity and the number of bacteria.

During July the relative humidity was significantly positively correlated ($P<0.05$) with the number of bacteria, while the level of light was positively correlated to the number of fungi.

In August the relative humidity was significantly negatively correlated with ($P<0.05$) the bacteria concentration. Air temperature and the concentration of ammonia were positively correlated to the number of bacteria.

In September, air temperature was significantly ($P<0.05$) positively correlated with the number of bacteria, while airflow velocity had a negative correlation to the number of fungi. The air flow rate was significantly ($P<0.05$) negatively correlated with the number of fungi in October, and in November relative humidity was statistically significantly ($P<0.05$) positively correlated to the number of bacteria.

Table 1. Descriptive statistical analysis of measured values of airborne bacteria and fungi in a livestock building with caged laying hens, and microclimate parameters by measured terms

Sampling date	Descriptive statistical analyse	n	Bacteria CFU/m^3	Fungi CFU/ m^3	Temp. °C	Relative humidity %	Air velocity m/s	Ammonia ppm	Carbon dio×ide vol.%	Light l×
6 February	X	10	3.49×10^4	0.40×10^4	17.66	55.67	0.05	2.70	0.14	23.00
	s	10	8869.60	1830.60	0.66	2.30	0.03	1.64	0.01	2.00
20 February	X	10	3.36×10^4	0.29×10^4	17.13	63.24	0.06	10.40	0.10	39.00
	s	10	11979.52	1037.36	0.74	3.34	0.04	2.91	0.02	18.79
07 March	X	10	2.67×10^4	0.25×10^4	19.40	60.87	0.10	10.20	0.06	22.80
	s	10	7108.75	1476.07	1.24	4.50	0.04	4.96	0.02	11.59
20 March	X	10	1.30×10^4	0.15×10^4	14.99	61.43	0.10	4.20	0.13	27.80
	s	10	5039.23	808.36	0.68	3.93	0.05	1.23	0.01	9.67
03 April	X	10	1.33×10^4	4.40×10^4	21.94	42.33	0.11	11.30	0.05	30.70
	s	10	6141.30	84080.85	0.58	2.68	0.03	4.11	0.02	7.89
17 April	X	10	7.09×10^3	0.19×10^4	25.20	28.46	0.10	2.70	0.04	33.40
	s	10	2809.29	1080.59	0.63	1.43	0.04	4.35	0.01	6.96
08 May	X	10	1.80×10^4	1.45×10^4	30.18	41.64	0.13	1.30	0.05	22.60
	s	10	16139.19	5066.27	0.52	1.75	0.05	1.16	0.01	9.02
22 May	X	10	4.58×10^4	2.32×10^4	31.61	48.74	0.15	7.40	0.04	17.70
	s	10	99047.24	16219.0	0.50	0.67	0.04	2.67	0.01	4.90
05 June	X	10	1.50×10^4	1.64×10^5	24.84	65.95	0.20	7.90	0.03	23.60
	s	10	1867.53	106648.57	0.38	0.89	0.09	3.57	0.00	9.75
19 June	X	10	1.22×10^4	0.72×10^4	29.64	60.89	0.15	5.70	0.04	22.30
	s	10	8178.02	1893.15	2.05	2.93	0.07	1.83	0.01	5.79
10 July	X	10	3.16×10^4	0.34×10^4	18.56	73.76	0.15	0.00	0.03	14.30
	s	10	9304.10	733.03	0.38	1.09	0.10	0.00	0.00	8.17
24 July	X	10	3.64×10^4	0.14×10^4	28.46	53.32	0.09	2.90	0.04	31.80
	s	10	20368,10	361.48	0.44	2.58	0.04	1.85	0.01	4.80
07 August	X	10	2.95×10^4	0.16×10^4	25.82	56.23	0.19	3.10	0.04	37.30
	s	10	12957.73	523.24	1.14	3.01	0.13	2.42	0.01	6.55

Table 1. Descriptive statistical analysis of measured values of airborne bacteria and fungi in a livestock building with caged laying hens, and microclimate parameters by measured terms (continued)

21 August	X	10	4.58×10^4	0.41×10^4	23.89	70.24	0.17	8.30	0.04	33.80
	s	10	22163.16	1064.37	0.85	0.84	0.07	2.83	0.01	7.13
11 Sept.	X	10	1.93×10^4	1.03×10^4	18.81	78.21	0.15	7.80	0.05	23.30
	s	10	5363.87	3738.17	0.62	2.09	0.07	1.99	0.01	14.96
25 Sept.	X	10	1.54×10^4	0.46×10^4	16.77	73.39	0.17	6.40	0.06	19.10
	s	10	8920.36	1743.05	1.07	2.74	0.09	2.27	0.02	9.07
09 October	X	10	3.07×10^4	0.36×10^4	18.95	65.32	0.14	18.30	0.12	29.00
	s	10	11623.23	1178.32	0.88	2.69	0.05	4.52	0.02	16.40
23 October	X	10	2.19×10^4	0.90×10^4	14.93	61.53	0.12	10.40	0.12	24.10
	s	10	8352.70	5600.80	0.87	1.60	0.03	3.17	0.01	9.30
06November	X	10	6.01×10^4	0.76×10^4	15.61	61.89	0.10	12.20	0.13	36.70
	s	10	76831.29	4888.09	1.75	1.57,	0.04	2.82	0.02	7.70
20 November	X	10	4.11×10^4	0.55×10^4	14.25	64.19	0.06	9.10	0.14	31.00
	s	10	8768.53	2237.19	1.41	1.66	0.02	5.28	0.01	3.23
04 December	X	10	9.63×10^4	0.15×10^4	15.36	61.61	0.08	14.00	0.13	33.00
	s	10	97703.85	13702.33	0.68	2.42	0.02	3.74	0.01	4.22
18 December	X	10	5.81×10^4	0.78×10^4	11.36	56.64	0.13	3.50	0.11	11.36
	s	10	23217.20	4842.91	0.87	3.62	0.08	1.43	0.01	0.87
09 January	X	10	8.22×10^4	0.09×10^4	8.87	67.53	0.08	6.10	0.08	20.40
	s	10	80006.77	439.82	0.89	3.53	0.03	1.66	0.01	1.71
22 January	X	10	4.09×10^4	0.19×10^4	14.60	66.26	0.13	8.90	0.16	18.20
	s	10	26777.31	728,85	0.87	3.54	0.06	3.14	0.01	1.87

Table 2. Mean values of airborne bacteria and fungi, measured in a livestock building with caged laying hens during production yearand their statistically differentiation (P<0.05)

	Bacteria CFU/m^3	Fungi CFU/m^3
February 2007	3.43×10^4	0.34×10^{4a}
March 2007	1.99×10^{4b}	0.20×10^{4a}
April 2007	1.02×10^{4b}	2.30×10^{4a}
May 007	3.19×10^4	1.89×10^{4a}
June 2007	1.36×10^{4b}	8.56×10^{4b}
July 2007	3.40×10^4	0.24×10^{4a}
August 2007	3.77×10^4	0.28×10^{4a}
September 2007	1.74×10^{4b}	0.08×10^{4a}
October 2007	2.63×10^4	0.63×10^{4a}
November 2007	5.06×10^4	0.65×10^{4a}
December 2007	7.72×10^4	1.15×10^{4a}
January 2008	6.16×10^{4a}	0.14×10^{4a}

[a,b] bacteria and fungi values with different letter in superscript are statistically significantly different (P<0.05)

Table 3. Statistically significant (P<0.05) correlations of marked factors during production year

Month	Correlated factors	Correlation factor r (X/Y)
March	t/fungi	-0.776
	NH_3/ fungi	-0.691
	CO_2/ fungi	-0.824
May	t/fungi	0.849
	rh/fungi	0.839
	NH_3/ fungi	0.728
	CO_2/ fungi	0.754
June	t/fungi	0.644
	w/bacteria	0.865
July	rh/bacteria	0.683
	light/fungi	0.699
August	rh/bacteria	-0.641
	t/bacteria	0.730
	NH_3/bacteria	0.677
September	t/bacteria	0.691
	w/fungi	-0.746
October	w/fungi	-0.657
November	rh/bacteria	0.668

t - air temperature; NH_3 - ammonia; CO_2 - carbon dioxide; rh - relative humidity, w - air velocity

Discussion

It is difficult to determine the exact number of microorganisms present in animal dwellings. Their concentration is affected by sedimentation, aggregation, ventilation, dehydration, radiation, and other factors responsible for their viability (WILSON et al., 2002), such as the activities of the animals and the implementation of specific work procedures (ALBRECHT, 2003). In addition, dead microorganisms and their biologically active components - endotoxins are present alongside the living ones in buildings (SEEDORF et al., 1998b). The number of microorganisms determined will depend on how they are sampled, whereby it is important to mention that the number obtained refers to live microorganisms. Sustainability of microorganisms is influenced by microclimate conditions, mostly by relative humidity. If relative humidity is between 55% and 75%, then the majority of microorganisms can survive for a short time in the environment without perishing (BICKERT, 2001).

Regarding the number of microorganisms in the air of a laying hen facility, various reports can be found in the literature. Therefore, the total number of bacteria in cage-held laying hens can be 10^4 CFU/m^3 and even up to 10^8 CFU/m^3, and the total number of fungi from 10^2 CFU/m^3 to 10^9 CFU/m^3 (EDUARD, 1997; MÜLLER et al., 2004; VENTER et al., 2004). This, among other reasons, can be explained by the influence of the devices selected for air sampling, which have certain specific characteristics and technical capabilities. In addition, the levels of microbial contamination of the air that are acceptable to animals in indoor facilities have not yet been agreed upon. The method for the air sampling used in this research is in line with the research of different authors (TERZIEVA et al., 1996; EDUARD and HEEDERIK, 1998; BANHAZI et al., 2004a). The established values of basic microclimate parameters in the researched facility were mainly around the values for laying hen accommodation, as suggested and described by a number of authors in the literature, except the air temperature in the summer months (MARTHI et al., 1990; ELLEN et al., 2000; VUČEMILO, 2008). Throughout the entire production period, the number of microorganisms determined in the researched facility air was edging towards the lower limits of ranges described in the literature (EDUARD, 1997; SEEDORF et al., 1998b; SALEH et al., 2003; BAKUTIS et al., 2004; MATKOVIĆ et al., 2009).

The largest, statistically significant ($P<0.05$) difference between the number of bacteria, which were at their lowest point at the time, and that of fungi, which were at their peak, was recorded in April and June (Table 1). The cause of such a large difference could be connected with the influence of the high air temperature established and low relative air humidity on the viability of bacteria (BANHAZI et al., 2004a; MATKOVIĆ et al., 2006). At the same time, the reason for such a high concentration of fungi is unclear, given that the increased percentage of moisture in the air promotes decomposition of organic raw

materials, which is a good foundation for the growth of fungi, and consequently increases the number of spores in the air (HARTUNG, 2007). In the rest of the year, mean values of bacteria and fungi ranged along the same curve, with more bacteria being recorded at all times. The values of fungi measured are approximate equivalent to the findings of WANG et al. (2007a, 2007b), who measured between 1.8×10^3 CFU/m^3 and 3.0×10^3 CFU/m^3 of air. MÜLLER (2000) states that the concentration of microorganisms in the air is influenced by the season and that their number is usually lower in the summer, which corresponds to the results of this research. One of the reasons for the lower values in the summer months is that high temperatures require increased air ventilation, and the greater speed of air flow subsequently causes frequent changes of air, thus leading to the dilution of particles present in it.

As for the surveyed parameters, in most months a statistically significant relationship ($P<0.05$) was found between air temperature and the number of fungi and bacteria, relative humidity, air flow rate and concentration of ammonia (Table 2) (ELLEN et al., 2000; SEEDORF et al., 1998a). In just a few months the number of microorganisms was connected with the concentration of carbon dioxide and light. In comparison to other seasons, there was a considerably larger number of bacteria ($P<0.05$) in the winter. The same findings were noticed by REYNOLDS et al. (1994), MÜLLER (2000) and MATKOVIĆ et al. (2009). The mean values of fungi recorded in the winter months were, in terms of statistics, significantly different from those measured during the summer months ($P<0.05$), which confirms the strong influence of the ventilation system on bioaerosol concentrations (SEEDORF et al., 1998a and b)

The determined number of bacteria and fungi in the air, as well as the statistically significant impact of microclimate conditions on their number in the air of a cage-housed laying hens facility, indicate the need for setting standards on air quality in animal dwellings and the occupational environment and for developing reliable systems for monitoring the above factors, as has also been proposed by DOUWES et al. (2003), HEBER et al. (2003) and DUQUENNE et al. (2013). The goal is to have economically viable production with the protection of animal and human health, food safety standards and less environment pollution.

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SAŽETAK

U radu je prikazana koncentracija bakterija i gljivica u zraku objekta kavezno smještenih nesilica tijekom proizvodne godine. Broj bakterija izmjerenih u zraku objekta kretao se od $1,02\times10^4$ CFU/m^3, mjereno u travnju, do $7,72\times10^4$ CFU/m^3, mjereno u prosincu. Srednje vrijednosti ukupnog broja gljivica iznosile su od $0,075\times10^4$ CFU/m^3, izmjerene u rujnu, do $8,56\times10^4$ CFU/m^3, izmjereno u lipnju. Izmjerene vrijednosti temperature zraka, relativne vlažnosti i brzine strujanja zraka bile su većinom u tehnologijom predviđenim rasponima. Utvrđeni broj bakterija i gljivica u zraku, kao i statistički značajan utjecaj mikroklimatskih uvjeta na njihov broj u zraku objekta kavezno držanih nesilica, ukazuju na potrebu za postavljanjem standarda o kakvoći zraka u nastambama za životinje te razvoj pouzdanog sustava za praćenje navedenih čimbenika. Cilj je stvoriti proizvodnju koja, osim zadovoljenja ekonomskih čimbenika, mora sadržavati i zaštitu životinja i ljudi, kao i sigurnost hrane i okoliša.

Ključne riječi: higijena zraka, mikroorganizmi, držanje nesilica, kvaliteta zraka