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Bacterial eggshell contamination in conventional cages, furnished cages and aviary housing systems for laying hens

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Abstract 1. The influence of housing system on the initial bacterial contamination of the eggshell was studied. Two long-term experiments were performed.
2. Bacterial eggshell contamination, as expressed by total count of aerobic and Gram-negative bacteria, was periodically analysed for eggs from a conventional cage, a furnished cage with nest boxes containing artificial turf or grids as nest-floor material and an aviary housing system. Results were log-transformed prior to statistical analyses.
3. For both experiments no systematic differences were found between the conventional cage and furnished cage. The type of nest-floor material in the nest boxes of the furnished cages also did not systematically influence the bacterial contamination.
4. A possible seasonal influence on contamination with a decrease in the winter period (up to >0·5 log cfu/eggshell) of total count of aerobic and Gram-negative bacteria was observed in the first experiment.
5. The contamination with total aerobic flora was higher (more than 1·0 log) on eggs from the aviary housing system compared to the conventional and the furnished cage systems. For Gram-negative bacteria this was not the case.
6. During the entire period of both experiments, independent of housing system, shell contamination was not influenced by age of hens or period since placing the birds in the houses.
7. For the total count of aerobic bacteria a restricted positive correlation ($r^2 = 0{\cdot}66$) was found between the concentration of total bacteria in the air of the poultry houses and initial shell contamination.

INTRODUCTION

It is estimated that 70 to 80% of world egg production is derived from conventional caged laying hens. These cages offer the advantages of low production costs and high standards of hygiene, but due to bird welfare considerations there are calls for cages to be banned (Walker *et al*., 2001). In 1999 the European Commission passed a directive 1999/74/EC (Anon., 1999) requiring that conventional cages should not be newly installed from 2003 and must be banned from 2012 in the European Union. Alternatives such as furnished cages, aviary systems and perchery systems have been proposed. While the conventional cage provides *circa* 450 cm^2 cage area and 100 mm trough length for each hen, furnished cages provide at least 750 cm^2 per hen, a nest box, a dust bath and 15 cm perch per bird. Aviary systems provide platforms of slats at different heights, litter area on the ground and nest boxes. The perchery system also uses the vertical space of houses like the aviary system but by perches than by platforms. During a transitional period from 2003 to 2012 the usable area in conventional cages has to be increased from 450 to 550 cm^2. The alternatives for the conventional cages have been evaluated both commercially and by researchers in terms of productivity and bird welfare (Abrahamsson and Tauson, 1995; Tauson *et al*., 1999; Tauson, 2002; Wall *et al*., 2002).

Little attention has been paid to the differences in bacterial eggshell contamination although this may be important for shelf life and safety of eggs and egg products. Bacterial

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contamination of the internal egg content could be the result of the penetration of the shell by bacteria deposited on the surface of the egg after it has been laid (Harry, 1963; Quarles *et al*., 1970; Schoeni *et al*., 1995). Smith *et al*. (2000) also reported that high excreta moisture can directly increase the microbial contamination of the shell and consequently increase the risk of microbial contamination of the internal contents of ostensibly clean eggs. In early studies bacterial shell contamination has been compared in litter and wire-floor houses. Quarles *et al*. (1970) reported litter-floor houses had on average approximately 9 times more bacteria in the air, and 20 to 30 times more aerobic bacteria on the shells, than wire-floor houses. Harry (1963) reported that the shells of deep-litter eggs had on average 15 times more bacteria and a higher proportion of potential spoilage organisms than did battery eggs. Recently, Ellen *et al*. (2000) reported that dust concentrations in the air were lowest in cage systems and up to 4 or 5 times higher in other systems, such as percheries and aviaries. Important sources of dust are grain, straw, hair, excreta and soil. Micro-organisms, like bacteria, may represent only a minor percentage (<1%) of the number of airborne particles, but have a marked negative effect on the health of the livestock (Pedersen *et al*., 2000) and probably lead to higher bacterial contamination on the shell in aviary and perchery systems. Higher bacterial contamination in the air was indeed correlated with higher bacterial counts on the eggshell (De Reu *et al*., 2005). The objective of the present study was to compare bacterial eggshell contamination in conventional cages, furnished cages and aviary housing systems.

MATERIALS AND METHODS

Housing

The different types of system were arranged in two separate identical buildings (1 and 2) with the same climate (temperature and relative air humidity), located side by side. Each building contained two adjoining hen-houses (A and B) each 6·10 m wide and 34·00 m long, separated by a wall.

Conventional cages

The conventional cage measured 50 cm × 51 cm × 43 cm (width × depth × height) with a floor slope of 7°. The 4-hen cages provided *circa* 640 cm^2 area per hen. The arrangement of conventional cages consisted of two rows of three-storey cages; housing laying hens at both sides. Each row contained 56 cages per floor at each side. In total 2688 commercial Brown layers were housed per hen-house. Food and water were available *ad libitum* by a feed trough and by nipple drinkers, manure was dried on a manure belt and removed at least once a week.

Furnished cages

Cages were of wire mesh with a floor slope of 7°, with galvanised metal partitions between cages and fully opening fronts consisting of widely spaced horizontal bars. The living area, containing 15 cm perch per hen, was 240 cm long and 110 cm deep while the nest section was 60 cm long and 55 cm deep; both sections were 53·5 cm high. The nest box was positioned at one end of the cage. The bottom of the nest boxes consisted either of wire floor or was lined with artificial turf (XPNP long, Astroturf®). The opening to the nesting area was 22 cm wide and 33 cm high. The litter baths, positioned at a height of 20 cm at the other end of the cage, contained sawdust and opened for 4·5 h in the afternoon. The cages were stocked with 39 hens; feed and water was available *ad libitum*, respectively, by a feed trough and by nipple drinkers. The furnished cages provided *circa* 750 m^2 area per hen. The commercial Brown layers were housed in two rows of three-storey cages with 10 cages per row; with *circa* 2400 birds per house. Manure was dried on a manure belt and removed at least once a week.

Aviary housing

The aviary system was divided into 4 pens, each 7·2 m long and 6·10 m wide. Each pen contained 500 commercial Brown layers. Each pen incorporated a central 2 m wide slatted platform with two levels (85 cm height between platforms), a 1 m wide littered floor area at each side of the platform and three rollaway nest boxes, 240 cm long and 42 cm wide, at each side wall. The nest boxes and the first floor slatted platform were mounted 85 cm from the ground. The littered area under the nest boxes and the slatted platform was also accessible to the birds. A manure belt mounted under the slatted platforms removed the dried manure weekly. The nest boxes were lined with artificial turf (XPNP long, Astroturf®) and the entrance was covered by a curtain made of plastic with two openings of 20 cm. Beside the slatted platform and the nest boxes alighting rails were fixed. The littered floor was covered by a thin layer of white sand. Water and food were supplied *ad libitum* from nipple drinkers and feed pans at the platform, with nipple drinkers also at the entrances of the nest boxes.

Experiments

Two experiments were performed, from August 2001 to May 2002, and from January to August 2003. Three and four designs were compared, respectively. Table 1 summarises the two experiments with their different designs. Figure 1 shows cross sections of the houses of experiment 2. In both experiments 17-week-old commercial Brown layers were transferred to the experimental buildings where they received 12 h of light per day increasing to 16 h from week 21 onwards.

Sampling

In the first experiment samples were taken at about 8-week intervals: in weeks 24, 32, 41, 50, 57 and 65; in the second experiment in weeks 33, 38, 48, 57 and 61. To produce statistically reliable results a minimum of 40 eggs from each housing system (design) were sampled (De Reu *et al.*, 2005). The eggs were picked up from the conveyor belt with the fingertips and placed in new carton filler-flats. Fingertips were disinfected between each sampling point. The eggs were taken by car, in ambient conditions, to the laboratory where they were kept for a maximum of 56 h in ambient conditions before analysing (De Reu *et al.*, 2005).

In the second sampling period an Air Sampler RCS (Biotest AG, Dreieich, Germany) was used to determine total bacterial count per m^3 air in each house (1A, 1B, 2A and 2B). Strips in the air sampler contained Plate Count Agar (PCA, Oxoid, Basingstoke, Hampshire, UK). Agar strips were incubated for 3 d at 30°C. In the second experiment, temperature and atmospheric humidity were also measured.

Bacterial shell contamination

To recover bacteria from the shell the intact egg was placed in a plastic bag with 10 ml Buffered

Table 1. *Description of the experimental arrangements*

Design	Experiment 1 (August 2001 to May 2002)			Sampled cages	Design	Experiment 2 (January 2003 to August 2003)			Sampled cages
	Housing system	Nest material	Hen-house			Housing system	Nest material	Hen-house	
1	Conventional cages	Wire floor	2B	10	1	Conventional cages	Wire floor	2B	10
2	Furnished cages	Wire floor	1A	3	2	Furnished cages	Wire floor	1A/2A	4
3	Furnished cages	Artificial turf	1A	3	3	Furnished cages	Artificial turf	1A/2A	4
4	–	–	–	–	4	Aviary	Artificial turf	1B	n.a.

1A, 1B, 2A and 2B = building 1 hen-house A, building 1 hen-house B, building 2 hen-house A and building 2 hen-house B; −= no design; n.a. = not applicable.

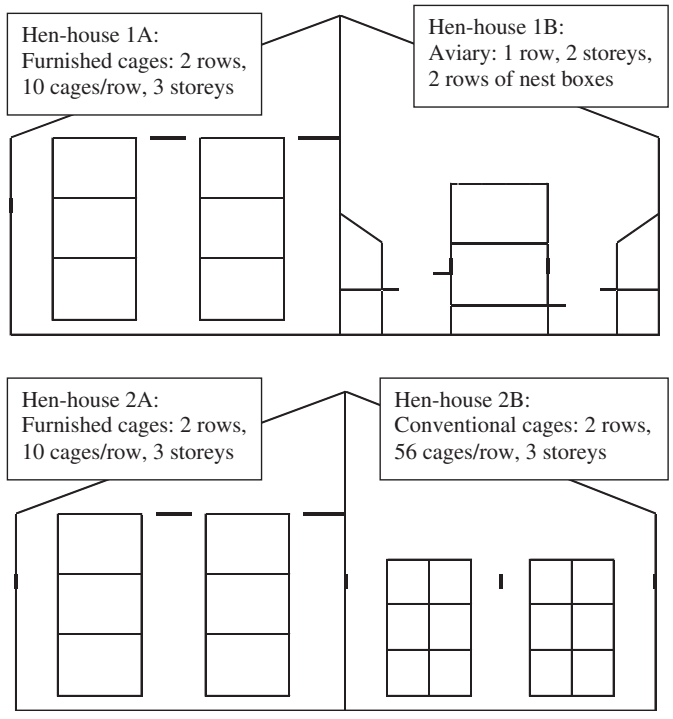

Figure 1. *Cross section of the hen-houses of experiment 2 showing the arrangements.*

Peptone Water (Oxoid) and the egg was rubbed through the bag for one minute. The diluent was plated by a spiral-plater on Nutrient Agar (Oxoid) to count the total of aerobic bacteria and on Nutrient Agar with 0·0001% crystal violet (VWR, Darmstadt, Germany) to count the total Gram-negative bacteria. Plates were incubated for 3 d at 30°C (De Reu *et al*., 2005).

Statistical analysis

The bacterial counts were log-transformed prior to statistical analysis (Jarvis, 1989). Significant differences were assessed using an analysis of variance (ANOVA), done in Statistica 6·0 (Statsoft Inc., Tulsa, OK, USA). The underlying assumptions for an ANOVA were always verified: the homogeneity of variances using the Bartlett χ^2-test and the homoscedasticity of the data (meaning that the variances should be independent of the measures magnitude) using a means *vs* standard deviations plot. *Post hoc* inter factor differences were calculated using a multiple range test (Kendall and Stewart, 1968).

RESULTS AND DISCUSSION

Figure 2 shows shell contamination with total aerobic flora on the different sampling dates during experiment 1 (August 2001 to May 2002) for the three designs and two housing systems: conventional cages, furnished cages with wire-floor nest boxes and furnished cages with nest boxes lined with artificial turf (Table 1). Figure 3 shows the same data for the Gram-negative flora on the shells of the same eggs.

The results for experiment 2 (January to August 2003) are shown in Figures 4 and 5. Figure 4 shows shell contamination with total aerobic flora on the different sampling dates for the 4 designs and three housing systems; conventional cages, furnished cages with wire-floor nest boxes, furnished cages with artificial turf lined nest and an aviary housing system (Table 1 and Figure 1). Figure 5 shows the same data for the Gram-negative flora on the shell of the same eggs. Table 2 summarises the significant differences per sampling date for both experiments. More data are available upon request.

For both experiments an ANOVA showed no systematic significant differences between conventional cages and furnished cages, for either total aerobic flora or Gram-negative flora (Table 2, Figures 2 to 5). On the final sampling dates (weeks 57 and 65) of experiment 1, shell contamination with total aerobic flora was significantly higher at the 95% confidence level on the eggs from the conventional cages (Table 2 and Figure 2). Figure 2 and the ANOVA data

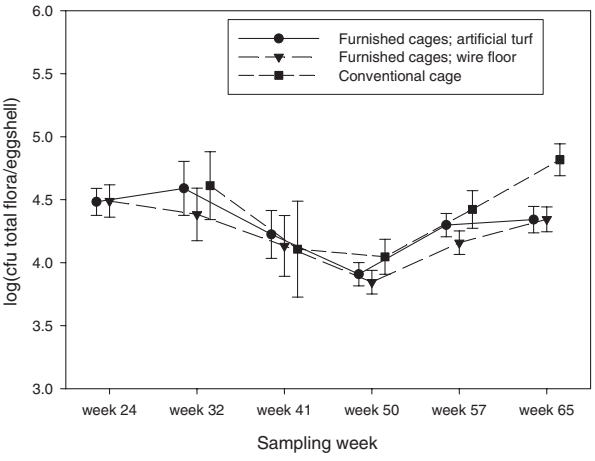

Figure 2. *Experiment 1: Eggshell contamination with total aerobic flora on different dates for the three designs including two housing systems (August 2001 to May 2002).*

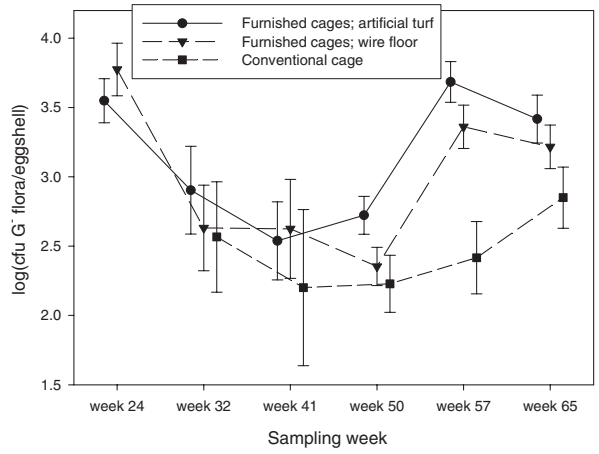

Figure 3. *Experiment 1: Eggshell contamination with Gram-negative flora on different dates for the three designs including two housing systems (August 2001 to May 2002).*

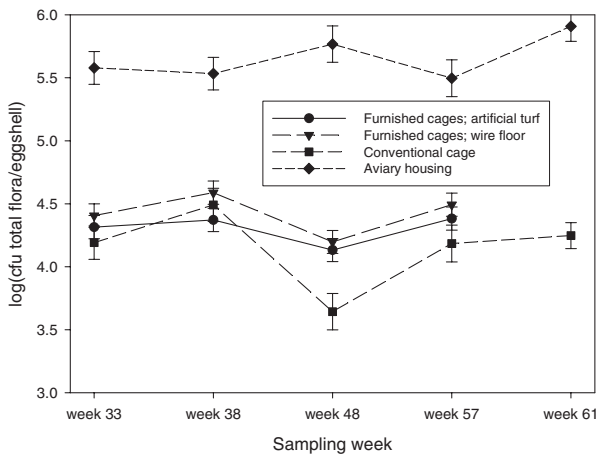

Figure 4. *Experiment 2: Eggshell contamination with total count of aerobic flora on different dates for 4 designs including three housing systems (January 2003 to August 2003).*

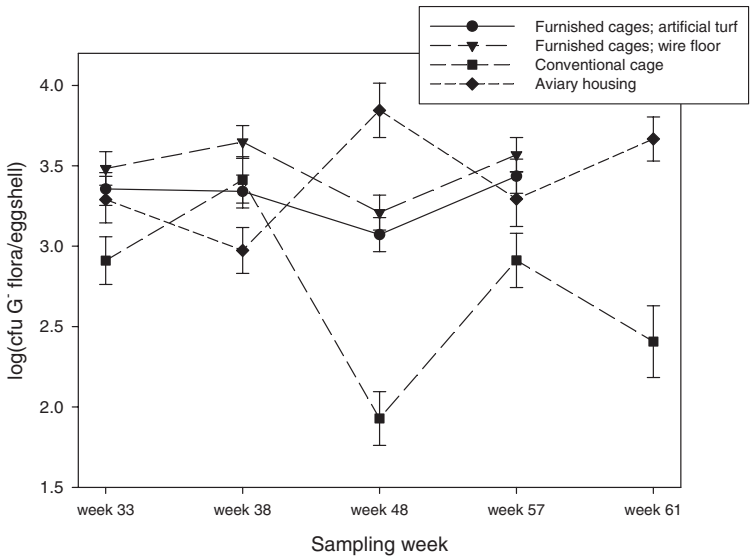

Figure 5. *Experiment 2: Eggshell contamination with Gram-negative flora on different dates for 4 designs including three housing systems (January 2003 to August 2003).*

Table 2. *Summary of the statistically significant differences per sampling date for both experiments (ANOVA)*

System	Total flora						System	Gram-negative flora					
	Week							Week					
	24	32	41	50	57	65		24	32	41	50	57	65
Experiment 1 (August 2001 to May 2002)													
Conventional cages (2B)*	–	A	A	A	A	A	Conventional cages (2B)	–	A	A	A	A	A
Furnished cages; wire floor (1A)	A	A	A	A	B	B	Furnished cages; wire floor (1A)	A	A	A	B	B	B
Furnished cages; artificial turf (1A)	A	A	A	A	B	B	Furnished cages; artificial turf (1A)	B	A	A	C	C	B
System	Total flora						System	Gram-negative flora					
	Week							Week					
		33	38	48	57	61			33	38	48	57	61
Experiment 2 (January 2003 to August 2003)													
Conventional cages (2B)*		A	A	A	A	A	Conventional cages (2B)		A	A	A	A	A
Furnished cages; wire floor (1A/2A)		C	A/C	C	C	–	Furnished cages; wire floor (1A/2A)		B	C	C	C	–
Furnished cages; artificial turf (1A/2A)		C	A/D	C	A/C	–	Furnished cages; artificial turf (1A/2A)		B	A	C	C	–
Aviary housing (1B)		B	B	B	B	B	Aviary housing (1B)		B	B	B	B	B

Systems in the same column with common letter are not significantly different. * = identification hen-house; – = no data available.

($P<0{\cdot}05$ week 57 and $P<0{\cdot}001$ week 65) show this difference was very significantly different only in week 65. This high value in week 65 can probably be attributed more to coincidence (a manure heap next to the conventional cage housing division) than to the type of housing system itself. At the date of sampling (week 65), manure from a period of 6 weeks before was stocked outside, next to house B of building 2, whereas on the other sampling dates manure was more regularly removed. This increase of total aerobic bacterial count in the conventional cages was not observed during experiment 2, confirming this assumption (Figure 4). In experiment 2 the differences in total aerobic flora on the shell for cage and furnished cage production were again not systematic (Table 2 and Figure 4). Only in week 48 was there a very significant difference ($P<0{\cdot}001$). In both sampling periods contamination with Gram-negative flora on shells of eggs from conventional cages was much lower for one sampling point (week 57) in experiment 1 and two sampling points (weeks 48 and 61) in experiment 2. This lower contamination was not observed on the previous or following sampling dates (Figures 3 and 5).

In both experiments there were no systematic differences in contamination with Gram-negative flora between conventional and furnished cages (Table 2, Figures 3 and 5). Both experiments also showed that accumulation of eggs in the furnished cages in an area of about 60 cm width did not necessarily increase shell contamination. Tauson (2002) reported that furnished cages increased contact between eggs and in some cases the proportion of dirty and cracked eggs. This was caused by the accumulation of the eggs on a short part of the conveyor belt next to the nest section. In our experiments only eggs laid at the nest boxes were sampled. Both experiments also showed that shell contamination was not systematically influenced by whether the nest-floor material was wire or artificial turf (Table 2, Figures 2–5). The results for total aerobic flora did not differ significantly for 8 of the 10 sampling dates and for Gram-negative flora did not differ significantly for 6 of the 10 sampling dates (Table 2). For the other dates no difference was observed. Wall and Tauson (2002) also found no significant effect of the nest-floor material on egg production or proportion of cracked or dirty eggs in furnished cages; on the other hand nest use was significantly increased where cages had nests with 100% Astroturf®, compared with 50 or 30%.

In both experiments there was no influence of the age of hens or the interval since placing the hens in the houses on shell contamination (data not shown). Comparing Figure 2 with 3 and Figure 4 with 5 shows that, regardless of housing design, a similar graphical trend was found for both total aerobic and Gram-negative flora. This suggests that the sampling date influenced the bacterial contamination; more specifically in experiment 1 the season appeared to affect shell contamination, with both total aerobic and Gram-negative flora. During the winter period, week 41 (beginning of December) and week 50 (end of January), shell contamination was lower ($P < 0{\cdot}05$) compared to the warmer periods; week 24 (August), week 32 (September) and week 65 (May) (Figures 2 and 3). Takai *et al*. (1998) also reported a seasonal influence on the dust concentration in poultry houses. Some results of Quarles *et al*. (1970) also suggested that high temperatures might influence shell contamination. However, this possible seasonal influence was not confirmed in the second one (Figures 4 and 5). During experiment 2, in the conventional cages and the aviary system, an additional sampling was performed during the heat wave period in week 61 (August 2003; outside temperature up to 40°C). Shell contamination was no higher than in the winter period; weeks 33 and 38. Similarly, Quarles *et al*. (1970) could not always confirm their supposition of the influence of the season on shell contamination.

Experiment 2 showed that shell contamination with total count of aerobic flora was more than 1 log unit higher, during the entire experiment, for eggs from the aviary system (Table 2 and Figure 4). For Gram-negative flora (Table 2 and Figure 5) no systematic differences were found between the 4 designs including the aviary system. De Reu *et al*. (2005) found that contamination with aerobic bacteria (5·8 log cfu/shell) of organic eggs, on the conveyor belt in front of the nest boxes, was also 1 log unit higher compared to eggs on the conveyor belt of a cage system. The housing system for organic eggs resembles the aviary system of our experiment. Higher contamination (De Reu *et al*., 2005) was also measured in the air of the organic house (5·6 log cfu/m^3 air) compared to the cage house (4·4 log cfu/m^3 air). In experiment 2 the influence of bacterial air contamination on shell contamination was examined and, for total aerobic bacterial count, a limited positive correlation of $r^2 = 0{\cdot}66$ was found. Figure 6 shows the bacterial air contamination for each system; the air contamination in the aviary was higher compared to the other two systems. Harry (1963) and Quarles *et al*. (1970) also reported correlations between initial shell contamination and the concentration of bacteria in the house. Quarles *et al*. (1970) reported a significant difference for air contamination between litter-floor houses (sawdust on the floor and wood shavings in the nests) and wire-floor houses (sloping wire floors and plastic rollaway nests); 3·97 and 3·03 log cfu/m^3, respectively. We obtained averages of 4·3 log cfu/m^3 for the conventional cage housing, 4·4 log cfu/m^3 for the furnished cages and >5·3 log cfu/m^3 for the aviary housing system. The concentration of

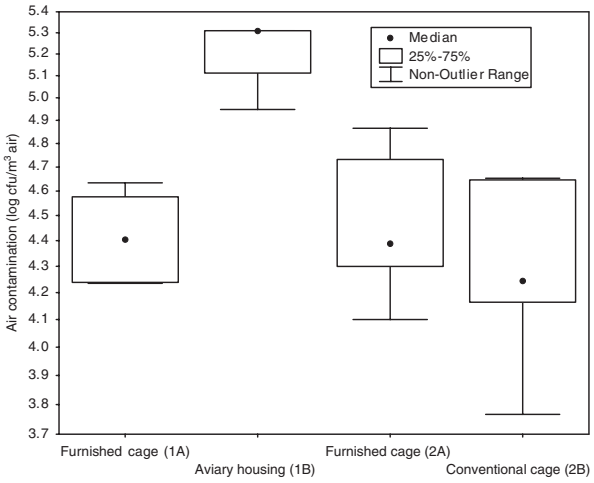

Figure 6. *Bacterial air contamination in each housing system of the second experiment.*

airborne bacteria in animal houses was also studied by Hartung and Seedorf (1999). According to their study the incidence of bacteria was highest in poultry houses (6·4 log cfu/m^3) compared to 5·1 and 4·3 log cfu/m^3 in pig and cattle sheds, respectively. Lyngtveit (1992) described the behaviour of animals affecting dust concentrations. In aviary systems the hens can move both horizontally and vertically and perform dust bathing. Their study showed significantly higher concentrations of dust in the afternoon than in the morning, owing to dust bathing behaviour. Because all our sampling was in the morning this factor could not have influenced our data. Ellen *et al*. (2000) reported a variation of the dust concentration in poultry houses from 0·02 to 81·33 mg/m^3 for inhalable dust and from 0·01 to 6·5 mg/m^3 for respirable dust. Houses with caged laying hens showed the lowest dust concentrations, less than 2 mg/m^3, while the dust concentrations in perchery and aviary systems were often 4 to 5 times higher. Other factors affecting the dust concentrations were animal category, animal activity, bedding materials and the season. Important sources of dust are the bird, excreta, food, bedding materials, floor materials and soil (Lyngtveit, 1992). Because dust contains micro-organisms like bacteria (Lyngtveit and Eduard, 1997; Pedersen *et al*., 2000) this also explains the higher air contamination with total aerobic flora that we found in the aviary systems. In contrast to the air contamination in experiment 2, no correlation was found between shell contamination and the temperature or atmospheric humidity measured in the houses (data not shown).

Further studies on the effects of housing systems on shell contamination and on the bacteriological contamination of the air are desirable for improving bacterial shell quality, food safety, health of the laying hens and the development of a healthier working environment in alternative poultry production facilities.

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