Microbiological survey of furnished cages and aviary systems for laying hens

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Furnished cages and non-cage systems are evaluated in terms of productivity, bird welfare, health and hygiene. Data on the comparison of hygiene of egg contact - and non egg contact surfaces in these systems are limited. The aim of this study was to compare the hygiene of surfaces in a furnished cage (FC) system with an aviary (A) system, and to measure the efficacy of cleaning and disinfection in both systems.
Up to 15 critical surfaces in both systems were sampled. Aerobic plate counts (APC) and numbers of *Enterobacteriaceae* per 20 cm^2 were determined. Both experimental systems were sampled simultaneously; at the end of lay (after removal of hens), after dry cleaning followed by wet cleaning (hose), after soaping followed by wet cleaning, after disinfection and finally 7 weeks after arrival of a new flock. At the end of lay, a difference ($P < 0.001$) in geometric mean number of APC was found between both systems (FC = 10^2 and A = 10^4 CFU/20 cm^2). Counts of *Enterobacteriaceae* were significantly lower than APC counts and no significant difference ($P > 0.05$) between both systems was found (FC and A = 1 CFU/20 cm^2). After dry cleaning followed by wet cleaning, soaping followed by wet cleaning, and disinfection, no systematic significant differences were found between both systems for both bacterial variables. These findings indicate that both systems may have a comparable hygiene status after cleaning and disinfection. Dry cleaning followed by wet cleaning reduced APC (geometric means) in the aviary system from 10^4 to 10^2 CFU/20 cm^2 but had less effect in the furnished cage. In both systems, soaping followed by wet cleaning reduced the APC numbers (geometric mean) with > 0.5 log and disinfection with > 1 log; resulting in a mean contamination after disinfection with 2 CFU/20 cm^2 for both systems. The highest counts of APC (arithmetic mean) in the non-cleaned furnished cage system were found in samples from manure belts (>10^6 CFU/20 cm^2), floors (>10^4 CFU/20 cm^2), egg belts and feeders (>10^3 CFU/20 cm^2). In the non-cleaned aviary system, highest APC counts were found in samples from manure belts and floors (>10^6 CFU/20 cm^2), slatted platforms, feeders and drinkers (>10^4 CFU/20 cm^2), and finally samples from the nest bottom (>10^3 CFU/20 cm^2). Counts of *Enterobacteriaceae* were significantly lower compared to APC. Respectively 91% (FC) and 90% (A) surface samples of non-cleaned surfaces had counts <10^2 CFU *Enterobacteriaceae*/20cm^2.

Keywords: housing system; laying hens; hygiene monitoring

Introduction

Recently the housing of layers in commercial egg production has been discussed extensively, especially in Europe. The debate has focused on the barren environment and restricted available space in conventional cages and the welfare of hens housed in such cages has been questioned (Craig and Swanson 1994). Conventional cage housing for laying hens will be prohibited from 2012 in the

European Union, following EU-directive 1999/74 (Anon. 1999). From 2012 onwards, only furnished cages (FC) and non-cage systems like barn or deep litter systems and aviary (A) systems have been proposed. Furnished cages and non-cage systems are already evaluated in terms of productivity, bird welfare, health and hygiene. Data on comparison of hygiene of egg contact - and non egg contact surfaces in these systems are limited. The aim of this study was to compare the hygiene of surfaces in a furnished cage system with an aviary system, and to measure the efficacy of cleaning and disinfection in both systems.

Material and methods

Experimental housing

The furnished cages were stocked with 39 hens; 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. The nest box was positioned at one end of the cage and was lined with an Astroturf® mat. The opening to the nest 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, were supplied with a limited amount of sawdust once a day. Feed and water was available *ad libitum*, by feed pans or troughs 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 2 400 birds per hen house. Manure was dried on a manure belt and removed at least once a week.

The aviary system was divided in 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, a 1 m wide littered floor area at each side of the platform and 3 rollaway nest boxes, 240 cm long and 42 cm wide, at each side wall. The littered floor area under the nest boxes and the slatted platform was also accessible for the birds. The manure belt mounted under the slatted platforms removed the dried manure weekly. The nest boxes were lined with an Astroturf® mat and the entrance was covered by a plastic flap with two openings of 20 cm. The littered floor area contained 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 entrance of the nest boxes.

Both types of experimental housing system were arranged in one building and located side by side, separated by a wall.

Cleaning and disinfection

Cleaning and disinfection occurred in 3 steps. Firstly, compartments and equipment were respectively brushed out and dry cleaned followed by wet cleaning with water (hose). Secondly, compartments and equipment were soaped with BIO-CID-S (CID Lines, Ieper, Belgium) followed by wet cleaning and finally stables and equipment were disinfected using CID 20 (CID Lines).

Sampled places

Up to 15 critical surfaces in both systems were sampled. Both experimental systems were sampled simultaneously; at the end of lay (after removal of hens), after dry followed by wet (hose) cleaning, after soaping followed by wet cleaning, after disinfection and finally 7 weeks after arrival of a new flock.

To guarantee an at random sampling of the stables, stables were divided lengthwise in 6 equal parts (A - F) and breadth wise in 3 equal parts (1 - 3) (Figure 1).

	A	**B**	**C**	**D**	**E**	**F**
1						
2						
3						

Figure 1 Division of the stable in equal parts

Sampled surfaces are listed in table 1, along with the number of samples taken of each surface and the site of sampling (A - F or 1 – 3; see figure 1) in the stable. At each sampling moment approximately 25 – 30 samples of the available surfaces were taken in each housing system (see table 1).

Table 1 Scheme of the sampled places

	Furnished cages						Aviary			
	Sample number → Sampled surface↓	1	2	3	4		1	2	3	4
1	Feeders	A*	BC	DE	F	Floor	ABC	ABC	DEF	DEF
2	Cage bottom	AB	CD	EF	-	Slatted platform	ABC	DEF	-	-
3	Cage wall	AB	CD	EF	-	Feeders	AB	CD	EF	-
4	Drinkers	AB	CD	EF	-	Drinkers	AB	CD	EF	-
5	Ceiling	1	3	-	-	Stable wall	1	3	-	-
6	Floor passage way	2	3	-	-	Ceiling	1	3	-	-
7	Air inlet	r	-	-	-	Air inlet	r	-	-	-
8	Feed hopper	r	-	-	-	Feed hopper	r	-	-	-
9	Entrance hall	r	-	-	-	Entrance hall	r	-	-	-
10	Egg belt	1	3	-	-	Floor nest box	ABC	ABC	DEF	DEF
11	Floor nest box	AB	CD	EF	-	Perch	1	3		
12	Perch	1	3	-	-	Egg belt	1	3	-	-
13	Stable wall	1	3	-	-	Manure belt	r	-	-	-
14	Manure belt	r	-	-	-	Curtain nestbox	r	-	-	-
15	Litter bath	r	-	-	-					

* = division of the stable were sample was taken (see also figure 1); r = random sampled; - = no sampling

Microbiological analyses

RODAC plates (Replicate Organism Detection And Counting) filled with Plate Count Agar (PCA, Oxoid, Hampshire, UK) and Violet Red Bile Glucose Agar (VRBGA, Oxoid) were used to sample the surfaces. Aerobic plate counts (APC) and numbers of *Enterobacteriaceae* per 20 cm^2 were determined. During 10 sec the rounded agar surface of the plate was gently pressed on the sample surface. If necessary a rolling motion, with a light uniform pressure, was used to ensure that as much surface of the agar would contact irregular surfaces. After incubation of the plates for 24h at 30°C Colony Forming Units (CFU) per RODAC plate were enumerated on the agar (range 0 – 400 CFU/cm^2). Higher counts were established by serial dilution of the agar of the RODAC plates after sampling, using quarter-strength Ringer's solution (Oxoid); followed by plating on PCA (range 10^2 – 10^8 CFU/cm^2).

Evaluation of the results and statistical analyses

Different methods were used to evaluate the results. In a first method, results of each sampled place were classified from 0 to 5. In table 2 classifications of the results for APC and *Enterobacteriaceae* are summarized. In the second method means (arithmetic and geometric) of each sampling moment or type of surface area were calculated. Statistical analysis was performed on log-transformed bacterial counts (Jarvis 1989) and significant differences were assessed using an analysis of variance (ANOVA), done in Statistica 7.0 (Statsoft Inc., Tulsa, USA).

Table 2 Classification of the results for APC and *Enterobacteriaceae*

APC		*Enterobacteriaceae*	
$CFU/20cm^2$	Classification	$CFU/20cm^2$	Classification
$\leq 10^2$	0	< 1	0
$> 10^2$ and $\leq 10^3$	1	> 1 and ≤ 10	1
$> 10^3$ and $\leq 10^4$	2	> 10 and ≤ 30	2
$> 10^4$ and $\leq 10^5$	3	> 30 and ≤ 100	3
$> 10^5$ and $\leq 10^6$	4	> 100 and ≤ 400	4
$> 10^6$	5	> 400	5

Results and discussion

Table 3 and 4 give an overview of the classification of the results for the different sampled places during different stages of housing, cleaning and disinfection. The detailed classification results for *Enterobacteriaceae* are available upon request.

Table 3 Hygienogram of total aerobic flora of furnished cages

	End of lay (hens removed)				After dry and wet cleaning				After soaping and wet cleaning				After disinfection				After 7 weeks housing			
Sample number → Sampled surface↓	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1 Feeders	4	2	1	2	2	1	0	0	2	1	1	0	0	0	0	0	3	3	4	5
2 Cage bottom	1	1	1	-	0	0	0	-	0	0	0	-	0	0	0	-	2	3	2	-
3 Cage wall	0	0	0	-	0	0	0	-	1	1	1	-	0	0	0	-	2	2	2	-
4 Drinkers	0	1	1	-	1	0	4	-	1	1	1	-	0	0	0	-	2	2	2	-
5 Ceiling	0	0	-	-	0	0	-	-	0	0	-	-	0	0	-	-	1	0	-	-
6 Floor passage way	4	1	-	-	1	0	-	-	1	0	-	-	0	0	-	-	5	4	-	-
7 Air inlet	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-
8 Feed hopper	1	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	2	-	-	-
9 Entrance hall	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-
10 Egg belt	2	1	-	-	2	5	-	-	0	0	-	-	0	0	-	-	4	3	-	-
11 Bottom nest box	3	1	1	-	-	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-
12 Perch	1	1	-	-	-	-	-	-	-	-	-	-	0	0	-	-	5	-	-	-
13 Stable wall	1	-	-	-	1	1	-	-	0	1	-	-	0	0	-	-	1	1	-	-
14 Manure belt	-	-	-	-	1	-	-	-	1	-	-	-	0	-	-	-	5	-	-	-
15 Litter bath	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-

- = no sampling

Table 4 Hygienogram of total aerobic flora of the aviary housing

	End of lay (hens removed)				After dry and wet cleaning				After soaping and wet cleaning				After disinfection				After 7 weeks housing			
Sample number → Sampled surface↓	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1 Floor	5	5	5	5	1	0	0	1	1	0	1	1	0	0	0	0	5	5	5	5
2 Slatted platform	3	1	-	-	1	4	-	-	0	1	-	-	0	0	-	-	4	3	-	-
3 Feeders	3	1	4	-	0	0	0	-	0	0	0	-	1	0	0	-	1	5	5	-
4 Drinkers	3	4	2	-	1	0	0	-	0	0	0	-	0	0	0	-	5	5	2	-
5 Stable wall	3	2	-	-	1	1	-	-	2	1	-	-	0	0	-	-	2	2	-	-
6 Ceiling	0	0	-	-	1	0	-	-	0	0	-	-	0	0	-	-	1	-	-	-
7 Air inlet	0	-	-	-	0	-	-	-	0	-	-	-	0	-	-	-	4	-	-	-
8 Feed hopper	0	-	-	-	1	-	-	-	0	-	-	-	0	-	-	-	4	-	-	-
9 Entrance hall	0	-	-	-	0	-	-	-	2	-	-	-	0	-	-	-	5	-	-	-
10 Bottom nest box	2	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11 Perch	2	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12 Egg belt	-	-	-	-	3	3	-	-	1	-	-	-	0	0	-	-	5	-	-	-
13 Manure belt	-	-	-	-	1	-	-	-	0	-	-	-	1	-	-	-	5	-	-	-
14 Curtain nestbox	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-

- = no sampling

Highest counts (arithmetic mean) of APC in the non-cleaned furnished cage system were found in samples from manure belts (>10^6 CFU/20 cm^2), floors (>10^4 CFU/20 cm^2), egg belts and feeders (>10^3 CFU/20 cm^2). In the non-cleaned aviary system, highest arithmetic mean APC counts were found in samples from manure belts and floors (>10^6 CFU/20 cm^2), slatted platforms, feeders and drinkers (>10^4 CFU/20 cm^2), and finally samples from the nest bottom (>10^3 CFU/20 cm^2). Counts of *Enterobacteriaceae* were significantly lower compared to APC. Respectively 91% (FC) and 90% (A) surface samples of non-cleaned surfaces had counts <10^2 CFU *Enterobacteriaceae*/20cm^2.

At the end of lay, a significant difference in arithmetic mean APC was found between both systems (FC = 10^4 and A = 10^7 CFU/20 cm^2). Counts of *Enterobacteriaceae* were significantly lower than APC counts and no significant difference between both systems was found (FC < 1 and A = 2 CFU/20 cm^2). After dry followed by wet cleaning, soaping followed by wet cleaning, and disinfection, no significant differences were found between both systems for both bacterial variables. Dry cleaning reduced APC (arithmetic mean) in the aviary housing from 10^7 to 10^4 CFU/20 cm^2 but did not have an effect in the furnished cages. In both systems, soaping followed by wet cleaning reduced the APC numbers with 2 log and disinfection with another 2 log; resulting in an arithmetic mean contamination after disinfection of respectively 4 (FC) and 9 (A) CFU/20 cm^2.

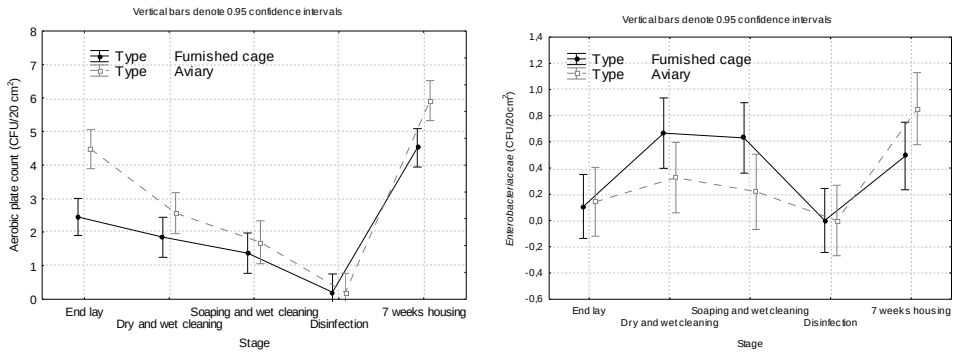

Figure 2 Evolution of the geometric mean surface contamination

Figure 2 and table 5 show the evolution of the geometric mean surface contamination taking into account all sampled surfaces of one sampling moment. At the end of lay (no hens) and after 7 weeks housing of the hens the sampled surfaces of the aviary housing were significantly ($P < 0.001$) more contaminated with APC. After dry followed by wet cleaning, soaping followed by wet cleaning, and disinfection, no systematic significant differences were found between both systems for both bacterial variables. The limited difference ($P < 0.05$) in contamination with *Enterobacteriaceae* after soaping followed by wet cleaning is not relevant from a microbiological point of view.

Table 5 Evolution of the geometric mean surface contamination

Housing type	Sampling moment				
	End lay (no hens)	After dry and wet cleaning	After soaping followed by wet cleaning	After disinfection	After 7 weeks housing
	Geometric mean APC (CFU/20cm^2)				
Furnished cage	$2{,}8x10^{2\ AAA}$	$7{,}0x10^{1}$	$2{,}4x10^{1}$	$1{,}6x10^{0}$	$3{,}3x10^{4\ BBB}$
Aviary	$3{,}0x10^{4\ AAA}$	$3{,}7x10^{2}$	$5{,}0x10^{1}$	$1{,}6x10^{0}$	$8{,}4x10^{5\ BBB}$
	Geometric mean *Enterobacteriaceae* (CFU/20cm^2)				
Furnished cage	$1{,}3x10^{0}$	$4{,}7x10^{0}$	$4{,}3x10^{0\ A}$	<1	$3{,}1x10^{0}$
Aviary	$1{,}4x10^{0}$	$2{,}1x10^{0}$	$1{,}7x10^{0\ A}$	<1	$7{,}1x10^{0}$

Values in the same column with common letters are significant different; A = $P < 0.05$; AAA, BBB = $P < 0.001$

The obtained results demonstrate that despite the higher contamination of the surfaces of the aviary housing at the end of lay (no hens) and after 7 weeks housed, both systems my have a similar hygiene status after cleaning and disinfection.

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