Quantification of Bioaerosols in Automated Chicken Egg Production Plants

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ABSTRACT The quantity and composition of bioaerosols in a typical automated chicken egg layer management system (LMS) with a controlled internal climate (B) and without (A) were compared. The LMS-A used a fecal matter disposal system featuring a central opening in the floor through which the matter automatically dropped to an open-air lower level; the LMS-B used a conveyer belt below each hen battery set, which removed the fecal matter frequently. Bioaerosols were collected by impaction on agar. Humidity, wind velocity, temperature, and dust particle concentration were also analyzed at several locations in the LMS. The average bioaerosol concentrations (total viable aerobic bacteria) associated with the inside of LMS-A reached $\overline{\chi} = 1.1 \times 10^5$ cfu/m^3 with counts in LMS-B being $\overline{\chi} = 9.2 \times 10^4$ cfu/m^3. In both systems, the bacterial counts were significantly higher on the inside of the LMS than the outside. The LMS-A showed yeast counts of $\overline{\chi} = 6.7 \times 10^1$ cfu/m^3 with none detectable in LMS-B. Total culturable mold counts were $\overline{\chi} = 7.0 \times 10^2$ cfu/m^3, with significantly higher presumptive *Salmonella* spp. counts ($\overline{\chi} = 6.6 \times 10^1$ cfu/m^3) inside both LMS when compared with the outside. *Escherichia coli* and total culturable gram-negative counts were significantly higher in LMS-B at concentrations of $\overline{\chi} = 3.6 \times 10^1$ cfu/m^3. These counts were significantly higher compared with the outside environment. We concluded that the live birds were the major source of bioaerosols in both LMS, with the fecal matter disposal systems attributing to the difference in bioaerosol composition. Modifications to the operation protocols of both LMS to limit the contamination of eggs by bioaerosols are suggested.

(*Key words*: automated layer system, bioaerosol, chicken egg, South Africa)

2004 Poultry Science 83:1226–1231

INTRODUCTION

Modern methods for the mass production of chicken eggs are founded on advances toward increased production with minimum labor requirements. These advanced layer management systems (LMS) are usually confined structures that are densely stocked with egg-laying hens. Whereas earlier systems relied on manual labor, modern layer houses feature mechanical feeding, egg removal, and fecal waste disposal systems. Egg production and chicken health are maintained by regulating or simulating optimum environmental conditions (temperature and humidity), leaving biosecurity control strategies, breed, and health of the introduced chicken factorial in egg quality and production (Ensminger, 1992; Salatin, 1993).

In these densely populated and enclosed buildings, microorganisms originating from fecal matter or feeding material accumulate if not controlled, and are easily aerosolized. Resulting viable airborne contaminants (bioaerosols) may be solid, liquid, borne by other particles, or suspended in liquid droplets. Contaminants may contain bacteria, bacterial spores, fungi or fungal spores, antigens, toxins, viruses, plant pollens, and fecal matter. Bioaerosols are potential product contaminants and may affect employee health (Donham et al., 1986; American Conference of Governmental Industrial Hygienists, 1989).

Organisms regularly associated with bioaerosols originating from densely populated environments such as swine houses have been shown to reach levels of 10^5 to 10^7 cfu/m^3 (Clark et al., 1983; Donham et al., 1986; Cormier et al., 1990; Crook et al., 1991). The aerosolized populations include fungi at levels of up to 10^4 cfu/m^3, as well as bacteria, predominantly gram-positive species. In poultry slaughtering plants, the identified bacteria within bioaerosols followed a similar trend and were reported to be predominantly gram-positive. This group included *Bacillus* spp. and *Staphylococcus* spp., whereas the gram-negative bacteria included genera such as *Acenetobacter*, *Proteus*, and *E. coli* (Lenhart et al., 1982). According to Lenhart et al. (1982), the major source of bioaerosol within these plants was the live birds. Furthermore, the authors noted that 62% of the isolated bacilli were *E. coli*, a bacterium of fecal origin.

©2004 Poultry Science Association, Inc.
Received for publication August 19, 2003.
Accepted for publication February 23, 2004.
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Abbreviation Key: LMS = layer management system; LMS-A = layer management system without controlled internal climate; LMS-B = layer management system with controlled internal climate.

Sources of bioaerosols unrelated to the chickens also occur in egg production plants. These include contaminants from waste treatment systems, building maintenance, and bacterial or fungal growth in the environment. Facility workers may be a source of bioaerosol contaminants carried to work on clothes or skin. Fecal contaminants of human origin may also be introduced into the workplace because of poor personal hygiene practices (Lutgring et al., 1997). Another major factor in the aerosolization and distribution of airborne microorganisms is the ventilation system. The ventilation system directly affects factors such as relative humidity and temperature and influences the viability of bioaerosols present, the time spent airborne, and the size of carrier droplets. All of these factors determine the bioaerosol composition and contamination levels of exposed food products (Heldman, 1974).

Given the differences in LMS facility design (controlled climate or open air) and variations in facility ventilation (filtered or nonfiltered air inlets), the effect of these factors on the concentration, distribution, and composition of bioaerosols is not completely understood when considering the quality of the eggs produced. In the South African chicken egg industry, information on the dispersion of bioaerosols in the different types of LMS is lacking, leading to considerable expenditure on facility design without the knowledge of the effect on bioaerosol dispersion. The aim of this study was to determine the level and distribution of viable airborne microorganisms in 2 commonly used systems, and to evaluate the effects of different environmental factors on the bioaerosol composition. Finally, the 2 systems are compared with respect to their ability to control the bioaerosol compositions.

MATERIALS AND METHODS

Sampling Protocol

A typical chicken egg farm with 2 types of LMS, one with a controlled internal climate (LMS-B, producing ± 20,000 eggs/d) and another with an uncontrolled internal climate (LMS-A, similar production capacity as the former) was selected for this study. Bioaerosol samples were collected 1.5 m above the floor of the parallel walkways between the high-rise battery sets of both LMS as well as from the outside environment (Figure 1) (Chang et al., 2001). Temperature, relative humidity, wind velocity, and dust particle concentration were measured at the same height as the bioaerosols using direct reading instruments (temperature: area heat stress monitor;[2] relative humidity: LMS automated humidity meter; wind velocity: anemometer;[3] airborne particle concentration: handheld aerosol monitor[4] (Chang et al. 2001)).

[2]Questemp, South Africa.
[3]Airflow Instrumentation, South Africa.
[4]PPM Enterprises, Inc., South Africa.

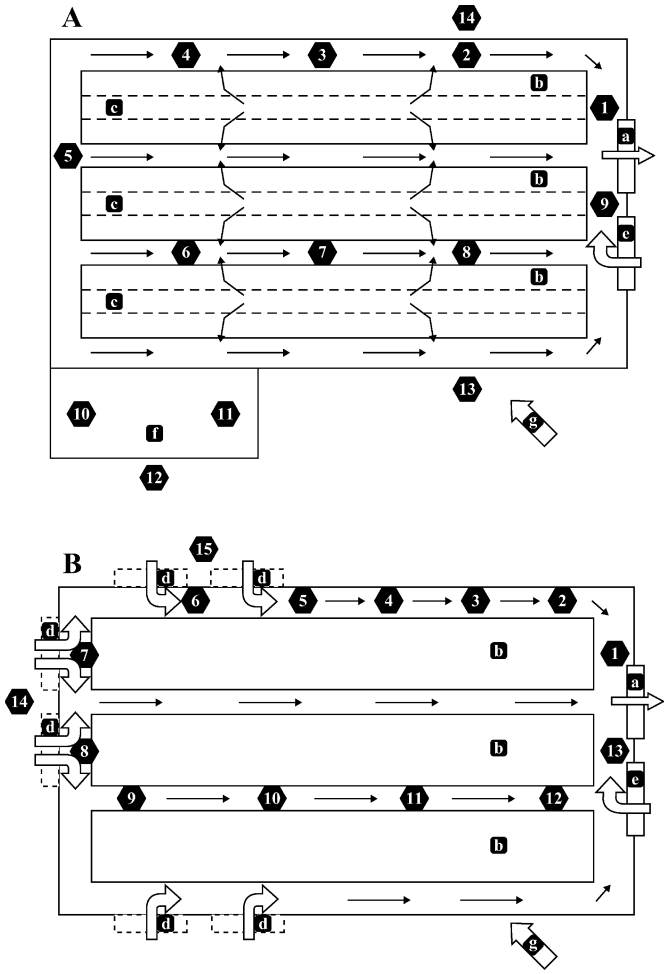

FIGURE 1. Facility design, direction of internal airflow (all arrows), extraction fans present (a-operative, e-nonoperative), high-rise hen battery sets (b), floor opening to lower fecal catchment level (c), inlet water cooled air filters (d), egg grading, quality control, packaging unit (f), external wind direction (g) of a layer management system with an uncontrolled internal climate (LMS-A), and a system with a controlled internal climate (LMS-B). All numbers represent sampling locations.

LMS Characteristics

The LMS-A (Figure 1A) was a dual level structure with several rows of high-rise hen batteries on the upper level, and was equipped with an automated egg removal system. The upper level of LMS-A was further enclosed with sheet metal roofs and walls. The fecal matter disposal system featured a central opening in the floor through which the matter automatically dropped to the open-air lower level. Accumulated fecal waste was removed bimonthly. Other than extraction fans at one end of the upper level and air vents in the roof, no ventilation or climate control systems were present. An enclosed (smooth brick wall) and mechanically ventilated egg grading, quality control, and packaging unit was situated adjacent to the LMS. One section of this unit served LMS-A (Figure 1A, (10)), and the other section (Figure 1A, (11)) served LMS-B.

The LMS-B (Figure 1B) was similar to LMS-A except that it was a single level structure enclosed with smooth

brick walls and had an automated fecal matter removal system. This system included a conveyer belt below each hen battery set, which moved the fecal matter each morning to one end of LMS-B, from where it was loaded onto a truck. A fully automated climate control system in LMS-B regulated temperature and humidity by means of water-cooled filters located in the walls at one end and extraction fans at the opposite end.

Both systems housed chickens of similar age (40 wk), and breed (Highline Silvers), and birds in both LMS were housed at the same cage density (3 birds/cage; ± 25,000 birds/LMS). Both systems used the same type of cages (4 levels in the high-rise) and biosecurity plans. Both manure removal systems (although different) are classified as dry removal systems, not flush systems.

Bioaerosol Sampling and Analysis

Microbial bioaerosols were measured by impaction on agar plates using a single stage SAS Super 90 air sampler[5] (Clark et al., 1983; Donham et al., 1986; Haglind and Rylander, 1987; Donham et al., 1989; Cormier et al., 1990; Heederik et al., 1991; Thorne et al., 1992). The air sampler was calibrated at 28.3 L/min before sampling and all removable components of the air sampler were autoclaved and disinfected with 70% ethanol between each sampling run. The air sampler operated by directly collecting airborne microbes onto 55-mm RODAC plates containing plate count agar, MacConkey agar, potato dextrose agar (pH 4), or Chromocult coliform agar, at each sampling location.[6] These media were used for the selective cultivation and enumeration of the total viable aerobic bacteria (plate count agar, Martley et al., 1970), total viable gram-negative bacteria (MacConkey agar, Atlas, 1993), total viable yeast and molds (potato dextrose agar, Beever and Bollard, 1970), total *E. coli*, and presumptive *Salmonella* spp. (Chromocult coliform agar, Kilian and Bülow, 1976; Frampton et al., 1988; Manafi and Kneifel, 1989). All evaluations were conducted in duplicate in addition to field blank samples (sampler not operating) at all sampling locations. The plates were incubated at 37°C for 48 h for Chromocult coliform agar, and at 25°C for 24 to 48 h for plate count agar, MacConkey agar, and potato dextrose agar plates (Beever and Bollard, 1970; Martley et al., 1970; Kilian and Bülow, 1976; Frampton et al., 1988; Manafi and Kneifel, 1989; Atlas, 1993). Colonies were differentiated by morphology and color, followed by enumeration using a colony counter[7] (Manafi and Kneifel, 1989). The positive-hole method was applied to the results from the air sampler for corrections of microbial coincidence (Macher, 1989).

Environmental Factors

Temperature, wind velocity, and relative humidity were recorded from 1030 to 1600 h for both sampling sites using the direct reading instruments described previously. All readings were recorded in duplicate.

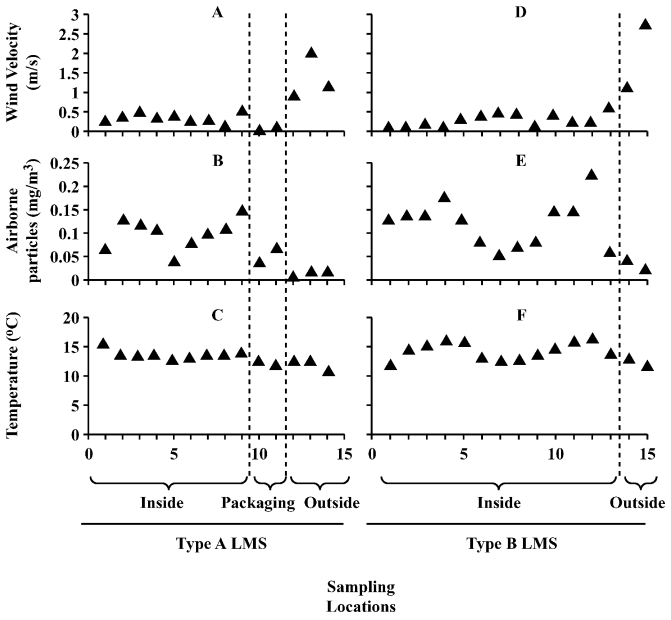

FIGURE 2. The levels of different environmental factors encountered inside and outside a layer management system with an uncontrolled inner climate (A to C) and one with a controlled inner climate (D to F).

Statistical Analysis

Data reported are the means of duplicate repetitions (2 plates) at each sampling point and were collected twice in each LMS. Minitab (version 12) software was used to test the normality of original and transformed data. For normally distributed data, ANOVA was performed to detect significant difference among means of contaminants at various measurement sites. Nonparametric analysis was carried out for nonnormally distributed data. Significance was set at $P \leq 0.05$.

RESULTS

Environmental Factors

The wind velocity, temperature, and airborne particle concentration at each sampling location are shown in Figure 2. The wind direction in LMS-A was toward the extraction fans from the lower fecal catchment level, and in LMS-B, it was toward the extraction fans from the embedded wall filters. No significant differences were noted between environmental factors of LMS-A and LMS-B: Wind velocity inside ($P = 0.197$), outside ($P = 0.322$); airborne particles inside ($P = 0.129$), outside ($P = 0.213$); temperature inside ($P = 0.057$), outside ($P = 0.291$). There was little difference in the relative humidity between the 2 types of LMS ($\overline{\chi} = 43 \pm 1.5$ for LMS-A; $\overline{\chi} = 45 \pm 0.5$ for LMS-B) and their separate effects on the bioaerosol concentration were therefore considered negligible. The wind velocity inside the LMS-A was below 0.5 m/s on average,

[5]International PBI, Milan, Italy.
[6]Merck NT, Gauteng, South Africa.
[7]Gerber Instruments, Effretikon, Switzerland.

other than at sampling point 9. The extraction fan located closest to sampling point 9 was inoperative at the time of sampling and acted as a window for incoming air from the outside. Little dispersion and dilution of airborne contaminants occurred by the low wind force from the lower level toward the upper level as well as throughout this system (Figure 2A). The limited dilution effect is evident from the elevated concentration of airborne particles between the high-rise battery sets (Figure 2B, sampling points 2 to 4, and 6 to 8). The lower airborne particle counts detected within the egg grading, quality control, and packaging area as well as outside indicate that chickens, fodder, and feces are the major sources of airborne particles. These results support those of Lenhart et al. (1982), who identified the live birds in poultry slaughtering plants as the major source of bioaerosols.

Similar levels of wind velocity were detected in LMS-B (Figure 2D), with the exception of sampling sites 5 to 8, which were in close proximity to the inlet filters. Samples collected among the high-rise egg batteries of LMS-B showed low wind velocities, again with little dispersion and dilution of airborne contaminants. The elevated wind velocity noted at sampling points 5 to 8 (Figure 2D), together with the lower temperatures and airborne particle concentration (Figure 2E, F), indicated a constant feed of outside air into LMS-B, because the temperature and airborne particle counts were similar to those of the outside environment (sampling points 14 and 15). Elevated airborne particle concentrations among the high-rise battery sets of the LMS again implicate the birds as the major source of bioaerosols.

Culturable Airborne Microorganisms—LMS-A

The average bioaerosol concentrations associated with LMS-A are shown in Figure 3. The total culturable bacteria ranged from 8.1×10^3 to 3.6×10^5 cfu/m^3 ($\overline{\chi} = 1.1 \times 10^5$ cfu/m^3) (Figure 3A) across sampling points 1 to 9. These concentrations were higher ($P = 0.026$) than those of the outside environment (7.0×10^2 to 3.9×10^3 cfu/m^3; $\overline{\chi} = 2.3 \times 10^3$ cfu/m^3) at sampling points 12 to 14. Culturable yeast collected from LMS-A ranged from 0 to 2.0×10^2 cfu/m^3 ($\overline{\chi} = 6.7 \times 10^1$ cfu/m^3) and did not differ from samples collected from the outside environment (Figure 3B). This was also the case with culturable molds ranging from 0 to 4.3×10^3 cfu/m^3 ($\overline{\chi} = 7.0 \times 10^2$ cfu/m^3) (Figure 3C). Presumptive *Salmonella* counts, although low, ranged from 0 to 1.0×10^2 cfu/m^3 ($\overline{\chi} = 6.6 \times 10^1$ cfu/m^3) on the inside and from 0 to 3.3×10^1 cfu/m^3 ($\overline{\chi} = 1.1 \times 10^1$ cfu/m^3) on the outside ($P = 0.006$) (Figure 3D). *E. coli* counts were only observed in one sample at a concentration of 6.6×10^1 cfu/m^3 on the inside of LMS-A with similar counts on the outside (Figure 3E). The adjacent egg grading, quality control, and packaging unit (sampling points 10 and 11) showed no evidence of *E. coli*. The total culturable gram-negative bacterial levels ranged from 0 to 6.6×10^1 cfu/m^3 ($\overline{\chi} = 1.5 \times 10^1$ cfu/m^3) inside LMS-A (Figure 3F) with similar results in the egg grading, quality control, and packaging unit, and in the outside environment.

Culturable Airborne Microorganisms—LMS-B

Figure 3 shows the average bioaerosol concentrations inside and outside LMS-B. The total culturable bacteria obtained inside LMS-B (sampling points 1 to 13) ranged from 9.0×10^2 to 1.6×10^5 cfu/m^3 ($\overline{\chi} = 9.2 \times 10^4$ cfu/m^3), and are significantly ($P = 0.0002$) higher compared with the outside environment (1.1×10^3 to 1.4×10^3 cfu/m^3; $\overline{\chi} = 1.3 \times 10^3$ cfu/m^3) at sampling points 14 to 15. No culturable yeast was detected inside LMS-B. Culturable molds ranged from 0 to 3.0×10^2 cfu/m^3 ($\overline{\chi} = 6.2 \times 10^1$ cfu/m^3), showing a significant ($P = 0.0002$) difference compared with the outside (Figure 3C). Culturable presumptive *Salmonella* concentrations ranged from 0 to 2.0×10^2 cfu/m^3 ($\overline{\chi} = 7.4 \times 10^1$ cfu/m^3) on the inside with none detectable on the outside (Figure 3D). Similarly, *E. coli* counts ranged from 0 to 1.0×10^2 cfu/m^3 ($\overline{\chi} = 3.6 \times 10^1$ cfu/m^3) on the inside, with none detectable on the outside (Figure 3E). The total culturable gram-negative bacteria levels ranged from 0 to 1.3×10^2 cfu/m^3 ($\overline{\chi} = 4.8 \times 10^1$ cfu/m^3) inside LMS-B compared with the outside environment, where no gram-negative bacteria were detected (Figure 3F).

The total culturable bacterial counts were lower, on average, in both types of LMS than have previously been reported (Lenhart et al., 1982; Clark et al., 1983; Donham et al., 1986; Cormier et al., 1990; Crook et al., 1991). The total plate counts, presented in figure 3A, showed similar results throughout the sampling spectrum with only the samples collected from the outside environment presenting lower levels. The total bacterial counts in the outside environment were significantly lower than the insides of both LMS and significant contamination of the eggs (while in the LMS) from this source is thus improbable.

Comparison Between LMS-A and LMS-B

When comparing the indoor bioaerosol composition of the 2 types of LMS, distinctive differences were noted. Aerosolized yeast was only detected in LMS-A, whereas molds were present in the bioaerosol of both LMS. Likewise, presumptive *Salmonella* counts revealed a high degree of similarity between the 2 LMS, whereas *E. coli* counts were higher in LMS-B ($P = 0.015$). This difference is probably due to the fecal waste that is scraped each morning in LMS-B, thus producing higher concentrations of aerosolized particles of fecal origin. The total culturable gram-negative bacteria supports this observation, in that LMS-B presented higher counts of these microbiota ($P = 0.011$). Relatively low counts of viable gram-negative bacteria were detected compared with the results obtained from the Chromocult coliform agar. This low sensitivity supports the report by Chang et al. (2001), who noted relatively low counts of gram-negative bacteria compared

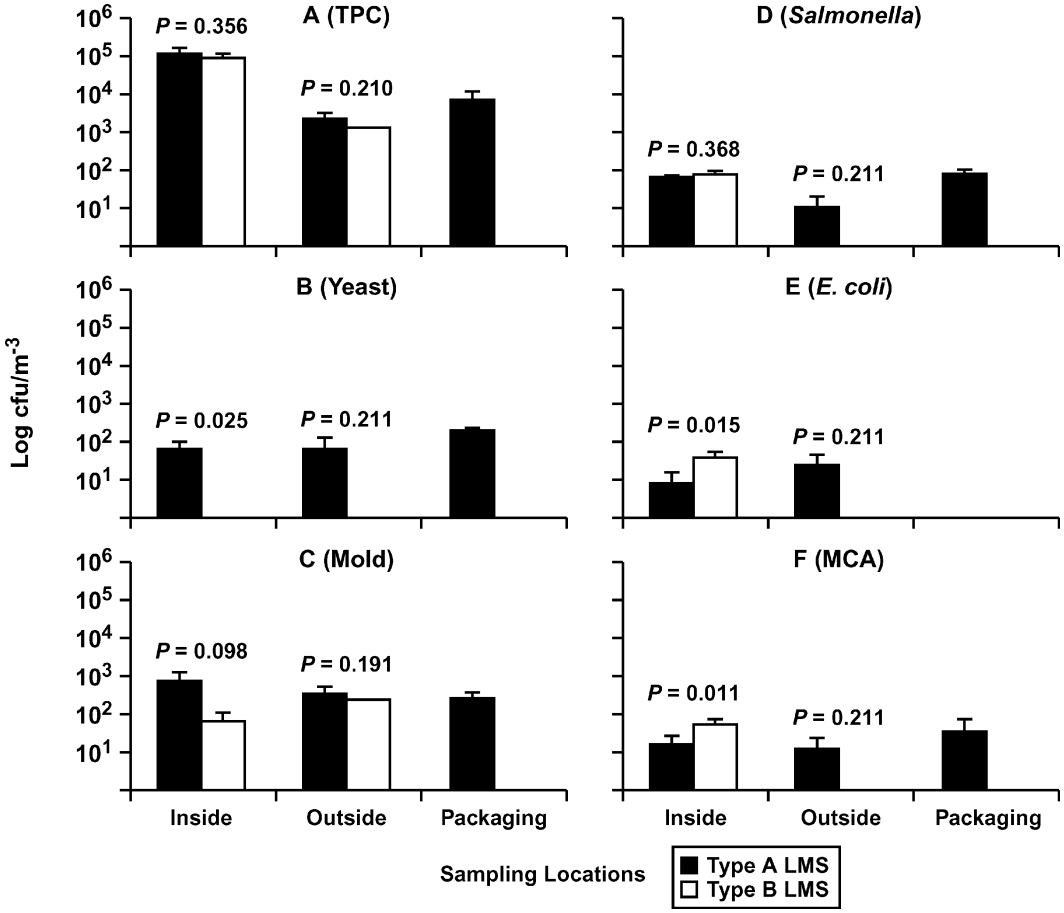

FIGURE 3. The average culturable airborne microorganism concentrations in a layer management system with an uncontrolled inner climate (LMS-A) and one with a controlled inner climate (LMS-B). Bioaerosol concentrations: A = total viable aerobic bacteria; B = total viable yeast; C = total viable molds; D = total viable presumptive *Salmonella* spp. counts; E = total viable *Escherichia coli* counts; F = total viable gram-negative bacterial counts; TPC = total plate count; MCA = MacConkey agar. Sampling locations correspond to the sampling points illustrated in Figure 1 as follows: LMS-A, inside (points 1 to 9); outside (points 12 to 14); packaging (points 10 and 11) and LMS-B, inside (points 1 to 13); outside (points 14 and 15).

with previous reports (Clark et al., 1983; Donham et al., 1986; Cormier et al., 1990; Crook et al., 1991).

In conclusion, airborne culturable bacterial levels in both types of LMS were lower than those cited in previous studies. Structural differences between the 2 types of LMS might influence the quality of the eggs produced as well as the quality of air inhaled by workers. The major benefit of an automated fecal matter disposal system, compared with the open-air lower catchment level, is the decrease in labor requirements; however, in this study it was shown to be a significant source of bioaerosols. To limit the negative effect of this system on the quality of the eggs, the LMS should be set to extract laid eggs before the fecal matter is scraped from the conveyer belts. Although the control of environmental conditions (temperature and relative humidity) are said to increase the levels of egg production (Webster and Czarick, 2000), differences noted in the concentration of bioaerosols from both systems were not due to different environmental conditions. Therefore, although an LMS with a climate control system requires considerable financial investment, which small-scale farmers in Africa often cannot afford, the same quality of bioaerosols could be achieved with systems that are technologically less advanced. The direct effect of airborne contamination on the quality of eggs produced in these types of LMS has however not been evaluated. Further research to establish the direct effect of airborne contamination on egg shelf life and quality is thus required.

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