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Effects of laying hens housing system on eggs microbial contamination

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ABSTRACT

Microorganisms can contaminate eggs at many stages of production, handling, preparation, and consumption. The aim of our study was the microbiological quality of the internal contents of eggs from different layer housing systems. Total bacteria, coliforms, and *Salmonella* spp. were isolated and identified by mass spectrometry. Total bacterial counts were isolated on Plate count agar for 48 hours at 30 °C, coliforms on Violet red bile lactose agar for 24 hours at 37 °C and *Salmonella* spp. on Xylose lysine deoxycholate agar for 24 hours at 37 °C. The lowest total bacterial counts were found in the cage-rearing system and the highest in the aviary-rearing system for hens housing. The number of microorganisms was evaluated on days 0. and 21. Twenty species, eighteen genera, and sixteen families were isolated from enriched cages in 0 days, while three families, three genera, and five species were isolated in 21 days, according to egg content samples. Thirteen families, sixteen genera, and twenty species were isolated from egg contents samples in the deep litter on day zero and day twenty-one, respectively, by third families, fourth genera, and seventh species. Nine families, twenty genera, and fifteen species were identified in aviaries using egg content samples on day 0, and three families, three genera, and five species on day 21. *Ralstonia pickettii* was the most isolated species among all samples.

Keywords: total count of bacteria, coliform bacteria, *Salmonella* spp., mass spectrometry, microbiota, eggs

INTRODUCTION

Food eggs should have good nutritional content as well as microbiological safety. To be profitable and accepted by consumers, eggs must be of high quality **[1]**. Consumers are now strongly interested in animal products, especially those produced in a welfare-conscious manner **[2]**, which has led to a significant increase in the proportion of hens reared in alternative farming systems to cages. There are concerns about the higher potential for bacterial contamination of table eggs due to the increase in egg production in non-cage systems. Most of the eggs produced are used as major ingredients in food products and consumed as fresh table eggs, which can pose a serious threat to food safety **[3]**, **[4]**, **[5]**. Compared to eggs from cage systems, the surface of eggs from floor and free-range systems typically contain more embryos **[6]**, **[7]**. In addition, Tomczyk et al. **[8]** demonstrated that hens housed in indoor and free-range systems produced eggshells with the highest diversity and number of microorganisms. Microbiological contamination can compromise the safety, shelf life, and quality of eggs **[9]**, **[10]**. Pathogenic microbes may be present in the eggshell microbiota, as well as microbes that cause egg spoilage. Egg consumption has been shown to contribute significantly to the incidence of occasional cases of salmonellosis among many risk factors **[11]**, **[12]**. Eggs can be contaminated with microorganisms vertically or horizontally. Vertical contamination occurs when eggs develop in the ovary or oviduct of the hen **[13]**. After egg laying, horizontal contamination occurs when bacteria break through the shell **[14]**, **[15]**. Bacteria can enter the

eggs despite having defenses such as the cuticle-covered shell, the shell membrane, and antimicrobial proteins in the white. In addition, the high pH and viscosity of albumin prevent bacteria from multiplying **[16]**. A critical factor affecting table eggs' safety is the time and circumstances during storage. Changes during storage can create an ideal environment for microbial contamination of eggs. For example, the glycolytic activity of certain bacteria can cause the breakdown of the cuticle, the essential protective layer, when the relative humidity of the eggshell surface increases.

The functional (technological) properties and antibacterial properties of egg whites are affected by various physical and chemical changes caused by storage conditions and length of storage **[17]**, **[18]**, **[19]**. The migration of water between the yolk and the white and the loss of water and carbon dioxide through the eggshell pores are the main causes of changes in egg content. The height of the white decreases with prolonged storage; nevertheless, pH and foaminess increase **[20]**. In addition, the strength of the vitreous membrane that envelops the yolk is reduced **[17]**, which promotes nutrient transfer between the white and yolk **[21]**. In addition, egg white degrades due to storage conditions and time **[18]**, **[21]**, which further reduces its antibacterial properties **[18]**. The microbiological quality, sensory properties and physicochemical properties of eggs can be significantly affected by storage conditions and length of storage.

The aim of the research was to determine the effect of different laying hen-rearing strategies on the microbiological quality of egg content and the identification of bacteria by mass spectrometry.

Scientific Hypothesis

The hypothesis of the research was to investigate the effect of different laying hen-rearing strategies on the microbiological quality of egg content and the identification of bacteria by mass spectrometry. We expected that rearing conditions would also influence microbiological abundance on the day first and after storage for 21 days.

MATERIAL AND METHODOLOGY

Samples

The laying eggs under investigation were from the Bovans Brown hybrid line, and at the start of the trial, they were grown in three different systems: 30,892 pieces in enriched cages, 11,130 pieces in deep litter, and 27,958 pieces in aviaries.

The farm uses 5ON04R cage breeding equipment—the six batteries house four-story cages. There are devices to gather eggs on the front side of the hall and a gadget to remove droppings on the back side. The cages have medicine dispensers and a central power supply with a water control gauge on each floor.

The center of the hall's slatted floor is where nipple waterers, feeders, and nest-laying nests are positioned. For bedding, dry sand that is three centimeters thick is utilized. There are nine laying hens per square meter of the hall's floor. After the chickens are taken out after the laying cycle, the droppings from the litter area and the entire hall are cleared. To prevent the collected droppings from rising above the grid after the laying cycle when the hens are removed, the grid bottom is positioned 500 mm above the floor. The laying nests are near the breast feeders. When laying chickens enter the nest, they wipe their runners on the slatted portion of the floor.

This breeding strategy can increase the number of laying hens per m2. The hall is lined with three-story aviary buildings. There is sand in the spaces beneath the structure's rows and the aisles between them. In addition to being utilized as a dust bath, the litter is used to rake laying chickens.

Chemicals

Unless otherwise noted, all chemical reagents were of analytical grade and were used exactly as given, without additional purification. All other chemicals and agars indicated were obtained from Aloquence s.r.o., Vráble, Slovakia, and were used without additional purification unless otherwise specified. All solutions, except as specified, were made using metal-free ultrapure water (also known as Milli-Q water; 18.2 MΩ cm) from a Millipore Milli-Q system located in Bedford, Massachusetts.

Animals, Plants, and Biological Materials

The study was carried out on eggs from a hybrid line of production laying hens, Bovans Brown, at the chicken farm Babičkin dvor a.s., Veľký Krtíš with number 2SK VK6-33.

Instruments

For the identification of bacteria, we used MALDI TOF-MS Biotyper (Brucker Daltonics, Bremen, Germany).

Laboratory Methods

ISO 4833-2:2013 **[22]**. Microbiology of the Food Chain. Horizontal Method for the Enumeration of Microorganisms: Colony Count at 30 °C by the Pour Plate Technique.

ISO 4832:2006 **[23]**. Microbiology of Food and Animal Feeding Stuffs. Horizontal Method for the Enumeration of Coliforms. Colony-Count Technique.

ISO 6579-1:2017 **[24]**. Microbiology of the food chain: Horizontal method for detecting, enumeration and serotyping Salmonella Part 1: Detection of *Salmonella* spp.

The identification was then completed using the MALDI TOF-MS Biotyper, according to Kačániová et al. **[25]**.

Description of the Experiment

Sample preparation: In all, 243 eggs from eight tests conducted in three separate locations (A, B, and C) were assessed between 0. and 21. days of storage in the lab. From enriched cages, on deep litter, and in aviaries, 144 pieces of eggs were used for both days of study. A constant temperature of 10 °C was maintained in the laboratory for 21 consecutive days. The number of bacteria in the egg contents was counted after egg samples were air-dried after being immersed in 75% ethanol for five minutes. After 5-10 seconds of flame exposure, the upper end of the egg was punctured using a sterile instrument.

Number of samples analyzed: 432 eggs from eight tests conducted in three locations.

Number of repeated analyses: 3.

Number of experiment replication: 3.

Design of the experiment: The entire egg's contents were mixed in a sterile polythene bag and then put on a PCA following serial dilution for an aerobic bacterial count. After the samples were serially diluted further, 100 µL of each dilution was applied to the plate count agar (PCA), Violet red bile agar with lactose (VRBL), and Xylose Lysine Deoxycholate agar (XLD) surfaces (Oxoid, Basingstoke, UK). The amount of *Salmonella* spp. (SS), coliform bacteria (CB), and total bacterial count (TBC) were all assessed. The plate diluting method was used to determine the quantitative CFU (Colony Forming Units) counts of the corresponding groups of bacteria in egg content. Plate count agar for the enumeration of the total count of bacteria was used for 48-72 h at 30 °C. Violet red bile agar with lactose for enumeration of coliform bacteria was used for 24-48 h at 37 °C, and Xylose Lysine Deoxycholate agar was used for the CFU segregation of *Salmonella* spp. For 24-48 h at 37 °C. All incubation was in aerobic conditions. Before being detected, the microbial colonies were cultured on TSA agar (Tryptone Soya Agar, Oxoid, UK) for 18 to 24 hours at 37 °C. A colony was produced using eight different bacterial strains. The identification was then completed using the MALDI TOF-MS Biotyper, according to **[25]**. A value more excellent than two was present in 1,523 isolates in total.

Statistical Analysis

The data were statistically evaluated using the Excel application. The arithmetic mean and standard deviation of the results were used for evaluation.

RESULTS AND DISCUSSION

Egg contents microbiota on day 0

Table 1 displays the total counts of microorganisms on day 0. The total number of bacteria, coliform counts, and *Salmonella* spp. were evaluated. According to our research, the only bacteria on eggs contained a total bacterial count (TCB). TCB was < 1 log CFU/ml in all hens housing system in the first experiment from 1.00 ±0.01 in enriched cages to 2.99 ±1.45 log CFU/ml in aviaries in the second experiment; in the third, from 1.30 ±0.36 in aviaries to 1.90 ±0.54 log CFU/ml in enriched cages; in the fourth, from < 1 in all hens housing systems to 2.53 ±1.65 log CFU/ml in enriched cages; in the fifth experiment, from < 1 in all housing systems to 1.30 ±0.78 log CFU/ml in enriched cages; and in the sixth experiment, from < 1 to 1.00 ±0.12 log CFU/ml in enriched cages; in a seventh experiment from < 1 in enriched cages to 1.30 ±1.05 log CFU/ml in same system; in the eighth experiment from < 1 in all systems to 1.48 ±0.67 log CFU/ml in deep litter.

Table 1 The total count of bacteria in the eggs in 0. days in log CFU/ml.

Day	Sample	1.	2.	3.	4.	5.	6.	7.	8.
0	ECA	< 1	2.98 ±0.04	1.70 ±1.23	2.11 ±1.45	1.30 ±0.78	< 1	1.00 ±0.03	< 1
0	ECB	< 1	1.48 ±0.12	1.90 ±0.54	2.53 ±1.65	< 1	1.00 ±0.12	1.30 ±1.05	< 1
0	ECC	< 1	1.00 ±0.01	1.70 ±0.34	< 1	< 1	< 1	< 1	< 1
0	DLA	< 1	1.30 ±0.17	1.95 ±0.45	< 1	< 1	1.00 ±0.07	1.00 ±0.05	< 1
0	DLB	< 1	1.70 ±1.23	1.48 ±1.07	1.00±0.07	1.00 ±0.05	1.00 ±0.09	1.30 ±0.78	< 1
0	DLC	< 1	1.00 ±0.09	1.70 ±1.03	< 1	< 1	< 1	1.00 ±0.05	1. 48 ±0.67
0	AA	< 1	2.90 ±1.23	1.30 ±0.36	< 1	< 1	< 1	1.30 ±0.45	< 1
0	AB	< 1	1.30 ±0.07	1.70 ±0.87	1.00 ±0.04	< 1	< 1	1.00 ±0.06	< 1
0	AC	< 1	2.99 ±1.45	1.48 ±1.23	1.00 ±0.08	1.00 ±0.07	< 1	1.00 ±0.07	< 1

Note: EC – enriched cages, DL – deep litter, A – aviaries.

From egg contents in enriched cages, a total of 135 isolates were found within a single day (Table 2). Egg content samples isolated 20 species, 18 genera, and 16 families. *Ralstonia picketii* is 20% of the most isolated species. *Methylobacterium fujisawaense* (7%) and *Enterobacter cloacae* (6%) were the other most isolated bacterial species.

Table 2 Isolated family, genera, and species of microorganisms of egg contents from enriched cages 0. day.

Family	Genera	Species	Number of isolates
Acidaminococcaceae	*Acidaminococcus*	*Acidaminococcus fermentans*	6
Microbacteriaceae	*Agromyces*	*Agromyces lapidis*	7
Microbacteriaceae	*Arthrobacter*	*Arthrobacter citreus*	4
Bacillaceae	*Bacillus*	*Bacillus subtilis*	5
Bacillaceae	*Bacillus*	*Bacillus subtilis* subsp. *subtilis*	6
Cryptococcaceae	*Cryptococcus*	*Cryptococcus neoformans*	6
Enterobacteriaceae	*Enterobacter*	*Enterobacter cloacae*	8
Micrococcaceae	*Glutamicibacter*	*Glutamicibacter arilaitensis*	4
Lactobacillaceae	*Lactobacillus*	*Lactobacillus delbrueckii* subsp. *delbrueckii*	6
Methylobacteriaceae	*Methylobacterium*	*Methylobacterium fujisawaense*	9
Methylobacteriaceae	*Methylobacterium*	*Methylobacterium* spp.	7
Moraxellaceae	*Moraxella*	*Moraxella catarrhalis*	7
Neisseriaceae	*Neisseria*	*Neisseria flavescens*	3
Sphingomonadaceae	*Novosphingobium*	*Novosphingobium resinovorum*	3
Pseudomonadaceae	*Pseudomonas*	*Pseudomonas mosselii*	5
Burkholderiaceae	*Ralstonia*	*Ralstonia pickettii*	27
Lactobacillaceae	*Schleiferilactobacillus*	*Schleiferilactobacillus harbinensis*	5
Sphingobacteriaceae	*Sphingobacterium*	*Sphingobacterium mizutaii*	4
Xanthomonadaceae	*Stenotrophomonas*	*Stenotrophomonas nitritireducens*	6
Zoogloeaceae	*Thauera*	*Thauera aromatica*	7
Total			**135**

Figure 1 Krona chart: Isolated species of microorganisms of egg contents from enriched cages 0. day.

In the deep litter 0. day egg contents, 148 strains in all were discovered (Table 3). A total of 13 families, 16 genera, and 20 species were separated from egg contents samples. Figure 1 shows 14% of the most isolated species was *Ralstonia pickettii*. Conversely, the other most isolated bacterial species was *Ralstonia insidiosa* (11%).

Table 3 Isolated family, genera, and species of microorganisms of egg contents from deep letter 0. day.

Family	Genera	Species	Number of isolates
Moraxellaceae	*Acinetobacter*	*Acinetobacter radioresistens*	6
Rhodocyclaceae	*Aromatoleum*	*Aromatoleum buckelii*	4
Enterococcaceae	*Enterococcus*	*Enterococcus faecium*	5
Micrococcaceae	*Glutamicibacter*	*Glutamicibacter arilaitensis*	5
Lactobacillaceae	*Liquorilactobacillus*	*Liquorilactobacillus nagelii*	7
Lactobacillaceae	*Lacticaseibacillus*	*Lacticaseibacillus paracasei* subsp. *paracasei*	8
Lactobacillaceae	*Latilactobacillus*	*Latilactobacillus sakei*	9
Methylobacteriaceae	*Methylobacterium*	*Methylobacterium fujisawaense*	5
Methylobacteriaceae	*Methylobacterium*	*Methylobacterium* spp.	3
Moraxellaceae	*Moraxella*	*Moraxella catarrhalis*	4
Sphingomonadaceae	*Novosphingobium*	*Novosphingobium resinovorum*	5
Pseudomonadaceae	*Pseudomonas*	*Pseudomonas balearica*	6
Burkholderiaceae	*Ralstonia*	*Ralstonia insidiosa*	15
Burkholderiaceae	*Ralstonia*	*Ralstonia pickettii*	22
Enterobacteriaceae	*Raoultella*	*Raoultella ornithinolytica*	8
Micrococcaceae	*Rothia*	*Rothia amarae*	5
Micrococcaceae	*Rothia*	*Rothia terrae*	6
Staphylococcaceae	*Staphylococcus*	*Staphylococcus haemolyticus*	7
Staphylococcaceae	*Staphylococcus*	*Staphylococcus lentus*	4
Zoogloeaceae	*Thauera*	*Thauera aromatica*	8
Total			**142**

Table 4 Isolated family, genera, and species of microorganisms of egg contents from aviaries 0. day.

Family	Genera	Species	Number of isolates
Moraxellaceae	*Acinetobacter*	*Acinetobacter calcoaceticus*	8
Moraxellaceae	*Acinetobacter*	*Acinetobacter pittii*	9
Microbacteriaceae	*Agromyces*	*Agromyces italicus*	7
Micrococcaceae	*Arthrobacter*	*Arthrobacter pyridinolis*	5
Bacillaceae	*Bacillus*	*Bacillus amyloliquefaciens* subsp. *plantarum*	6
Bacillaceae	*Bacillus*	*Bacillus cereus*	7
Bacillaceae	*Bacillus*	*Bacillus subtilis* subsp. *subtilis*	8
Debaryomycetaceae	*Candida*	*Candida krusei*	7
Debaryomycetaceae	*Candida*	*Candida utilis*	5
Enterobacteriaceae	*Enterobacter*	*Enterobacter cloacae*	7
Lactobacillaceae	*Lacticaseibacillus*	*Lacticaseibacillus paracasei* subsp. *paracasei*	8
Lactobacillaceae	*Lactobacillus*	*Lactobacillus amylovorus*	6
Lactobacillaceae	*Lactobacillus*	*Lactobacillus delbrueckii* subsp. *lactis*	6
Lactobacillaceae	*Latilactobacillus*	*Latilactobacillus sakei*	7
Lactobacillaceae	*Levilactobacillus*	*Levilactobacillus brevis*	5
Lactobacillaceae	*Ligilactobacillus*	*Ligilactobacillus agilis*	9
Bacillaceae	*Lysinibacillus*	*Lysinibacillus pakistanensis*	6
Bacillaceae	*Lysinibacillus*	*Lysinibacillus* spp.	8
Micrococcaceae	*Paeniglutamicibacter*	*Paeniglutamicibacter sulfureus*	8
Burkholderiaceae	*Paraburkholderia*	*Paraburkholderia fungorum*	8
Pseudomonadaceae	*Pseudomonas*	*Pseudomonas boreopolis*	5
Burkholderiaceae	*Ralstonia*	*Ralstonia pickettii*	9
Total			**154**

Figure 2 Krona chart: Isolated species of microorganisms of eggs content from deep litter 0. day.

A total of 154 isolates were found in the aviaries 0. day egg contents (Table 4). From egg content samples, a total of nine families, twenty genera, and fifteen species were identified. The three most isolated species were *Acinetobacter pittii*, *Ligilactobacillus agilis* and *Ralstonia pickettii*, with 6% of the total. However, *Acinetobacter calcoaceticus* and *Bacillus subtilis* subsp. *subtilis*, *Lacticaseibacillus paracasei* subsp. *paracasei*, *Lysinibacillus spp.*, *Paeniglutamicibacter sulfureus*, *Paraburkholderia fungorum* and other (5%) were the other most isolated bacterial species (Figure 2).

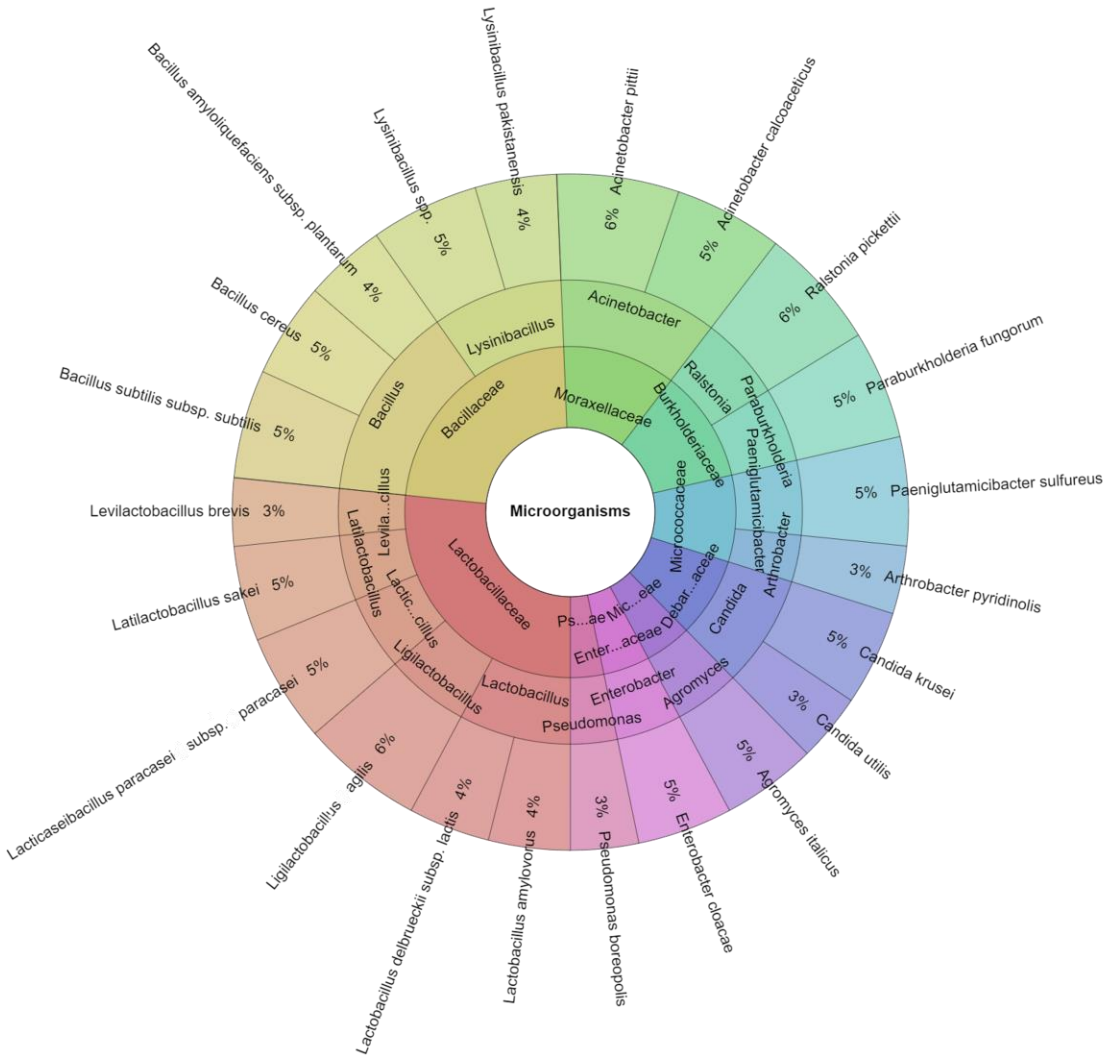

Figure 3 Krona chart: Isolated species of microorganisms of egg contents from aviaries 0. day.

Eggs content microbiota in day 21

The total number of microorganisms counted on day 21 is displayed in Table 5. The total number of bacteria, coliform bacteria, and *Salmonella* spp. were all counted in our investigation. According to our research, there were only total counts of bacteria (TCB) in eggs content. TCB in first experiment ranged from < 1 in deep litter to 2.08 ±1.34 log CFU/ml in deep litter; in second experiment from < 1 in enriched cages to 1.70 ±0.65 log CFU/ml in aviaries; in third from < 1 in enriched cages and deep litter to 2.40 ±1.23 log CFU/ml in aviaries; in fourth from < 1 in enriched cages and deep litter to 1.30 ±0.74 log CFU/ml in deep litter; in fifth from < 1 in all systems to 1.00 ±0.18 log CFU/ml in deep litter; in sixth from < 1 in deep litter to 1.60 ±0.77 log CFU/ml in aviaries; in seventh from < 1 in all systems to 1.30 ±0.77 log CFU/ml in aviaries and eight experiments were in all systems < 1.

Table 5 The total count of bacteria in egg contents in 21. day in log CFU/ml.

Day	Sample	1.	2.	3.	4.	5.	6.	7.	8.
0	ECA	1.00 ±0.07	< 1	1.30 ±0.45	1.00 ±0.06	< 1	1.30 ±0.23	1.00 ±0.06	< 1
0	ECB	1.70 ±1.12	1.70 ±0.67	1.00 ±0.15	< 1	< 1	1.00 ±0.67	< 1	< 1
0	ECC	1.00 ±0.21	1.48 ±0.45	< 1	< 1	< 1	1.00 ±0.04	1.00 ±0.08	< 1
0	DLA	< 1	1.70 ±0.49	1.00 ±0.05	< 1	1.00 ±0.18	1.60 ±0.08	< 1	< 1
0	DLB	< 1	1.70 ±0.43	1.00 ±0.09	< 1	< 1	< 1	< 1	< 1
0	DLC	< 1	1.78 ±0.56	< 1	1.30 ±0.74	< 1	< 1	< 1	< 1
0	AA	2.08 ±1.34	1.00 ±0.06	2.40 ±1.23	1.00 ±0.05	< 1	1.00 ±0.12	1.30 ±0.77	< 1
0	AB	1.60 ±0.78	1.60 ±0.45	2.18 ±1.52	1.00 ±0.07	< 1	1.60 ±0.77	< 1	< 1
0	AC	1.00 ±0.98	1.70 ±0.65	1.00 ±0.07	1.00 ±0.12	< 1	1.00 ±0.08	< 1	< 1

Note: EC – enriched cages, DL – deep litter, A – aviaries.

After 21 days, 62 isolates from egg contents in enriched cages were found (Table 6). From egg content samples, 3 families, 3 genera, and 5 species were isolated. *Ralstonia picketii* was the most isolated species 40% (Figure 4). The remaining most frequently isolated bacterial species were *Lactobacillus delbrueckii* subsp. *lactis*, and *Ralstonia mannitolilytica* (16%).

Table 6 Isolated family, genera, and species of microorganisms of egg contents from enriched cages 21. day.

Family	Genera		Number of isolates
Lactobacillaceae	*Lactobacillus*	*Lactobacillus delbrueckii* subsp. *lactis*	10
Micrococcaceae	*Ralstonia*	*Ralstonia mannitolilytica*	10
Micrococcaceae	*Ralstonia*	*Ralstonia pickettii*	25
Staphylococcaceae	*Staphylococcus*	*Staphylococcus equorum* subsp. *equorum*	8
Staphylococcaceae	*Staphylococcus*	*Staphylococcus lugdunensis*	9
Total			**62**

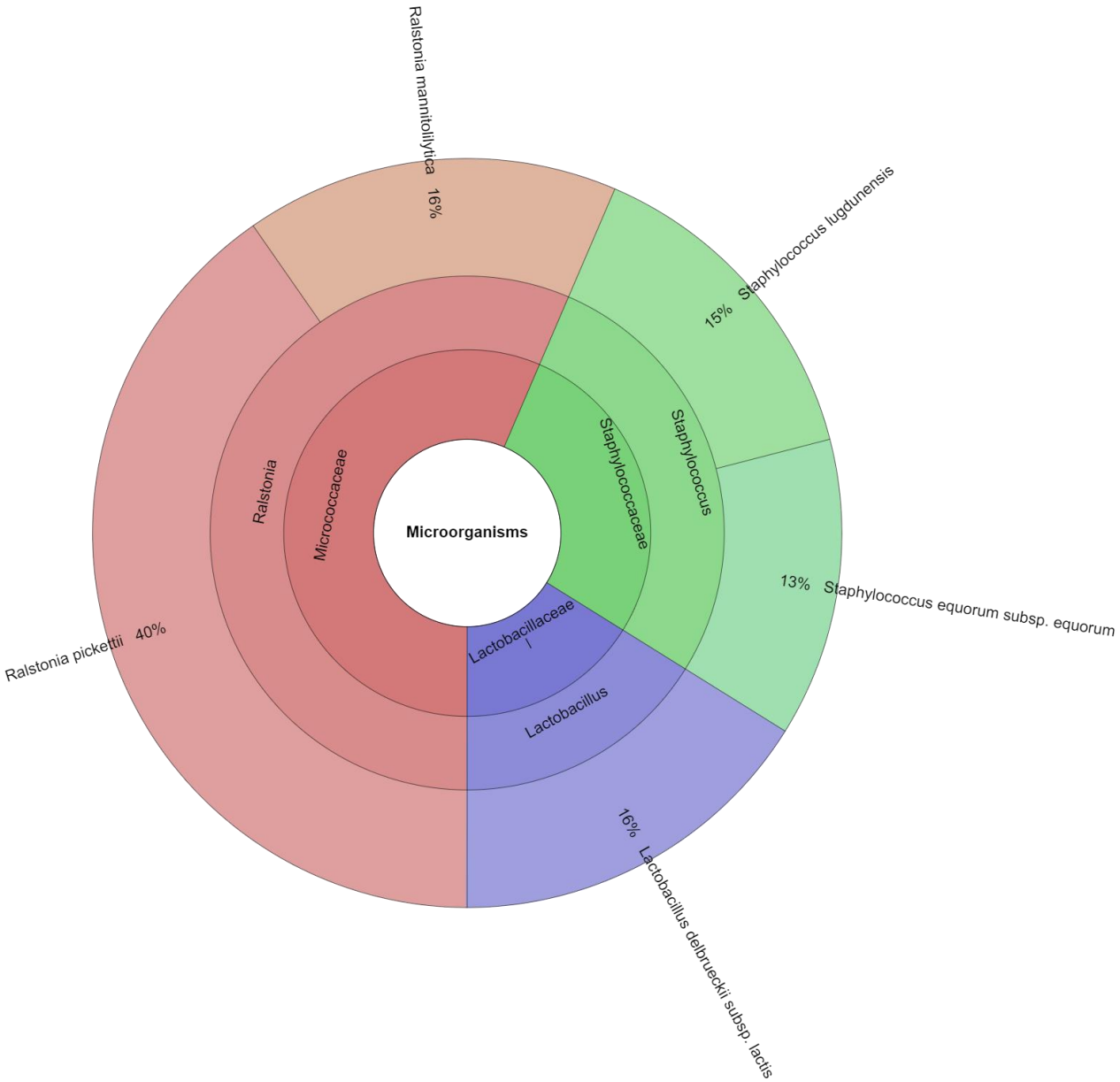

Figure 4 Krona chart: Isolated species of microorganisms of egg contents from enriched cages 21. day.

In the deep litter 21. day old egg contents, 81 isolates in total were discovered (Table 7). Third families, fourth genera, and seventh species were separated from eggs content samples. Among the most isolated species, 38% were *Ralstonia picketii* (Figure 5). Conversely, the other most isolated bacterial species were *Ralstonia mannitolilytica* (19%) and *Lactobacillus amylovorus* (12%).

Table 7 Isolated family, genera, and species of microorganisms of egg contents from deep litter 21. day.

Family	Genera	Species	Number of isolates
Debaryomycetaceae	*Candida*	*Candida glabrata*	5
Lactobacillaceae	*Lactobacillus*	*Lactobacillus amylovorus*	10
Micrococcaceae	*Ralstonia*	*Ralstonia mannitolilytica*	15
Micrococcaceae	*Ralstonia*	*Ralstonia pickettii*	31
Staphylococcaceae	*Staphylococcus*	*Staphylococcus arlettae*	5
Staphylococcaceae	*Staphylococcus*	*Staphylococcus equorum subsp. equorum*	8
Staphylococcaceae	*Staphylococcus*	*Staphylococcus xylosus*	7
Total			**81**

Figure 5 Krona chart: Isolated species of microorganisms of egg contents from deep litter 21. day.

A total of 82 isolates were found in the aviaries 21-day egg contents (Table 8). From egg content samples, a total of 3 families, 3 genera, and 5 species were identified. Ralstonia pickettii, *Ralstonia mannitolilytica*, and *Aromatoleum buckelii* accounted for 38%, 26%, and 16% of the most isolated species.

Table 8 Isolated family, genera, and species of microorganisms of egg from aviaries 21. day.

Family	Genera	Species	Number of isolates
Rhodocyclaceae	*Aromatoleum*	*Aromatoleum buckelii*	13
Micrococcaceae	*Ralstonia*	*Ralstonia insidiosa*	12
Micrococcaceae	*Ralstonia*	*Ralstonia mannitolilytica*	21
Micrococcaceae	*Ralstonia*	*Ralstonia pickettii*	31
Sphingobacteriaceae	*Sphingobacterium*	*Sphingobacterium faecium*	5
Total			**82**

Figure 6 Krona chart: Isolated species of microorganisms of egg contents from aviaries 21. day.

There are various ways that pathogenic bacteria might contaminate eggs, and there are a number of foodborne pathogens that can enter the egg and remain there for the duration of its shelf life. Considerable investigation has been conducted on the topic of bacterial contamination of table eggs **[15]**. Total viable count (TVC) on eggshells plays an important role in egg safety and product shelf life, which makes it a topic of great interest. Regulating bodies in some but not all countries suggest acceptable TVC limits for eggs or egg products **[26]**. One excellent illustration of the significant adjustments made to the agriculture industry in response to shifting societal expectations is the production of eggs. Consumer demand for the consumption of high-quality, healthful animal products that consider sustainability and animal welfare is currently quite strong. Numerous instances of significant alterations to the production process of eggs in response to societal needs may be traced back to the consideration of ethical dimensions in this industry. The primary modifications to the production system include the gradual removal of cage-housing arrangements, the requirement that no male chicks be killed, and an extension of the production period **[2]**. Our study aimed to find out how different laying hen-raising practices affected the egg contents' microbiological quality and the ability to identify germs using mass spectrometry. The laying hen strain and the housing environment may impact the microbial growth on the eggshell because of the dynamic nature of microorganisms **[27]**. This is relevant to food safety in the egg industry. Eggs from conventional cages have a lower eggshell bacterial load than eggs from alternative housing systems like aviaries, litter, free-range, or organic systems **[24]**, **[25]**, **[26]**, **[27]**, **[28]** and similar results were found in our study. There was a noticeably greater bacterial load in caged eggs, according to the few and early observations on the hygienic

quality of eggs produced in the initial types of cages **[29]**, **[30]**, **[31]**, **[32]**. Eggs from backyard and free-range hens were shown to have a significant level of microbial contamination in tests conducted 30-40 years ago **[36]**. There are not many differences between cage eggs and experimental research (assuming that the eggs placed on the ground are excluded) **[33]**. However, they are often smaller at the commercial level, ranging from 0.5 to 1 log units, and tend to be larger in aviaries. Coliform numbers can occasionally be marginally lower than in conventional cages (CC). Thirteen laying houses from three EU nations were examined by De Reu et al. **[34]** with furnished cages and 7 with access to range). The amount of total aerobic bacteria found in the shells of non-cage eggs was just 0.2 log units higher than that of furnished cages (FC) eggs, which is a negligible difference in hygiene. Most of the time, the Enterobacteriaceae counts were not statistically different and almost at the detection limit (< 10 CFU). The egg contents count was relatively low (≈ 2%) in all housing systems. Still, a great deal of variation has been noted among farms that use the same technology **[38]**, **[39]**. The distinctions between NCS and cage eggs are becoming less noticeable in egg grading and packaging **[40]**. Additionally, during storage, the bacterial burden on eggshells decreases. Various elements can impact the housing system's consequences **[33]**, including layer facilities design and farming practices **[35]**. According to Rossi et al. **[37]**, it was not anticipated that the rise in non-cage systems (NCS) in the EU would substantially impact the sanitary quality of eggs. The most recent published research, which is not from the EU, corroborates the previously mentioned tendencies. When Jones et al. **[27]** studied the eggshells of hens kept in conventional cages (CC), barns, and free-range (FR) in the United States, they found that the hens in FR had greater levels of *Enterobacteriaceae*, but only in certain strains of the bird, due to their varying propensity to lay eggs on the floor. According to Parisi et al. **[38]**, there were, on average, 1.0 log CFU more *Enterobacteriaceae* in FR eggs than in CC hen eggs. According to **[31]**, FR production was the primary source of the majority of coliform isolates (62%), with *E. coli* being the predominant strain (55%), particularly in FR nest boxes (44%). Of these isolates, CC production accounted for only 15%. Samiullah et al. **[7]** compared eggs from one FR and one cage commercial farm in Australia. Although the overall bacterial burden was low in both systems, they discovered a considerably lower total microbial load in cage eggs. It is possible to counteract the tendency of increased sale contamination in NCS eggs by practicing appropriate husbandry and hygiene, collecting eggs more frequently, and, most importantly, avoiding floor lay. Additional risk factors for NCS include nest contamination, placing next boxes directly on the litter, and the buildup of eggs in egg belts **[41]**, **[42]**. However, the disparities between facilities with CC or FC might vary greatly, ranging from 5 to 15 or even 100 times larger **[38]**. The total count of bacteria (TCB) in the internal egg content in cardboard boxes was 0.00 to 1.92 log CFU/ml, while in plastic boxes, the range was 0.00 to 2.49 log CFU/ml **[43]**. In our study, the total count of bacteria ranged from < 1 to 2.99 ±1.45 CFU/ml id day 0 and from < 1 to 2.40 ±1.23 log CFU/ml. The egg white was initially thought to be infertile. On the other hand, microbes were found inside the egg white in a recent study **[44]**. Egg yolks have also been found to contain bacteria **[45]**. Past studies **[46]**, **[47]** verified the existence of bacteria in one-day-old chicks' digestive and reproductive systems. Numerous investigations have demonstrated the connection between hen stomach, fecal bacteria, and egg production and fertility **[48]**, **[49]**. In our study, the most identified bacteria were Ralstonia pickettii in all egg variants. In different study *Acinetobacter, Bacillus, Carnobacterium, Enterobacter, Herbaspirillum, Kocuria, Pseudomonas, Staphylococcus, Stenotrophomonas* genera were isolated from egg content **[43]**. After the eggshells are laid, a variety of control techniques are used to reduce the amount of bacteria present, including as cleaning and cold storage **[50]**. There are two reasons why it's critical to keep eggs refrigerated after laying. The eggs' shelf life is extended by cold storage, which also inhibits the growth of mesophilic microbes **[51]**. The average shelf life of an egg is 21-35 days, depending on the nation of origin. Egg quality and shelf life are related, and environmental factors like temperature and relative humidity can have an impact on both **[52]**. Over a 21-day period, albumin quality and egg weight dropped at storage temperatures between 20 and 22 °C but remained steady when eggs were held at 4 °C **[53]**.

CONCLUSION

Caution should be exercised when comparing the effects of housing systems, as several production factors can influence the different components of commercial egg quality. Our study aimed to determine microbial contamination of the internal contents of eggs at day 0 and during day 21 of storage at laboratory temperature. Our results showed good microbiological quality of egg contents, and the most isolated bacteria was *Ralstonia picketti*. Our results did not show the presence of *Salmonella* spp. and coliforms. The level of bacterial contamination of the egg contents depends on the housing system and is related to temperature and days of storage. According to our findings, there were more contaminated eggs at the beginning of the experiment. At the beginning of the experiment, isolated species of microorganisms also represented a larger proportion of all species. Complex processes are involved in the changes in egg microbiological quality parameters that occur during storage in various housing systems. The current study's findings highlight the significance of

microbiological quality in various housing systems for variations in egg quality during storage. Eggs placed in aviaries and cage-housing systems were found to be of a greater quality than eggs put on litter.The bacterial content of egg shells varies more in various housing systems than in cages.Richer cages and aviaries are a good alternative to traditional cages when it comes to laying hen performance and egg quality.

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