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Bacteria Counts and Fungal Loads on Eggshells Collected from Isa-Brown Hens Raised under Different Systems

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Authors' contributions

This work was carried out in collaboration among all authors. Author MA suggested the study. Authors MA and GEO participated in the design and coordination. Authors MA, GFA and GEO carried out the field trial, while laboratory assessment was done by authors MA, GFA, GEO and JAA. Authors MA, GFA, GEO and JAA carried out statistical data analysis and interpret the results. Authors MA, GFA and GEO search for literature and prepared the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To assess bacteria and fungi loads on eggs collected from laying birds under three different rearing systems.
Study Design: Completely Randomized Design with a 3×3 factorial arrangement.
Place and Duration of Study: Field trial was done at Oluade Farms, Ilara-Mokin, while Laboratory assessment of eggs was done at Microbiology Laboratory of the Department of Animal Production and Health, Federal University of Technology, Akure. The whole study lasted for 90 days.
Methodology: Collection of eggs for assessments was done at the end of each phase and was collected three times a day for the different housing systems. The eggs collected were labelled and sealed up in transparent white polythene nylon before assessments. All data collected were subjected to analysis of variance.
Results: Highest bacteria count (43.56± 7.48×10^{-3}cfu/ml) was observed in egg collected from deep litter system, while the lowest bacteria count (13.11±7.4×10^{-3}cfu/ml) was observed in egg collected

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from the outdoor system. Highest bacteria count (75.00±12.95×10^{-3}cfu/ml) was recorded in egg collected from deep litter system in the morning while lowest bacteria count (2.67±12.95×10^{-3}cfu/ml) was recorded in egg collected from battery cage system in the evening. Highest fungi count (10.89±4.77×10^{-3}cfu/ml) was observed in egg collected from battery cage system, while lowest fungi count (2.56±4.77×10^{-3}cfu/ml) was observed in egg collected from deep litter system. *Escherichia coli* was isolated in all eggs collected from the rearing systems and collection periods except battery cage system in the morning and outdoor system in the morning. *Staphilococcus aureus* was isolated in eggs collected from all the housing systems and collection periods except in outdoor system in the afternoon. *Aspergillus niger* was isolated across all the housing systems and collection periods.

Conclusion: From the results, it shows that the different eggshells from the three rearing systems were contaminated with bacteria and fungi loads at varying levels.

Keywords: Eggshell; bacteria; fungi; hens; rearing systems.

1. INTRODUCTION

The two major animal food origins (meat and egg) are described as the most common source of food-borne infection. According to Arathy et al. [1], microbial loads of table eggs are usually evaluated before selling in countries like Canada, Japan and United State of America. This kind of practice is not common in developing countries like Nigeria due to the unavailability of facilities and skilled labour. Bacteria isolated from table eggs may also be linked to some human illness.

The public perception regarding good egg from its shell cleanliness to that of microbial level is now a concern to the consumers. Contaminations of eggs occur a few seconds after the egg is laid, through handling, processing, preparation up till consumption Indhu et al. [2]. Freshly laid eggs are generally free from microbes. Following exposure to poor environmental conditions (for example, soil, dust and dirty nesting materials etc), eggs become contaminated with different types of microbes Abdul et al. [3] and as such, the egg start to depreciate. Bialka et al. (2004) suggested that eggs are often rinsed with alkaline detergents and chlorine solutions to reduce microbial load on the shell.

According to Jehan et al. [4], the qualities of nutrients present in egg create an excellent environment for the development of different microbes. Evaluation of bacteria contamination of eggshell during production and processing was studied by Northcutt et al. [5] and in a study conducted by Jones et al. [6], he found Salmonella in 72% of the environmental samples collected from hen houses, 7.8% of eggshells before washing and 1.1% of eggshells after washing. Environmental components, parental physiology and behaviour, and their interactions are key drivers of these microbial communities Ruiz-de-Castañeda et al. [7]. Parental incubation behaviour has been found to limit bacteria growth on the eggshell and also decrease bacterial and fungal invasion of egg contents by limiting trans-shell infection when compared with eggs that are left exposed Gizzard et al. [8]. Baggott and Graeme-Cook [9] stated that environmental components such as protection against adverse conditions, nest structure, reuse of a nest, and choice of lining materials can influence bacteria loads on eggshells.

However, there is a dearth of scientific information on housing systems, bacteria counts and fungal loads. Thus, this study seeks to investigate the effect of different rearing systems on the bacteria counts and fungal loads of eggs collected from Isa-Brown hens under different rearing systems.

2. METHODOLOGY

2.1 Experimental Site

The fieldwork of this study was carried out at a private farm (Oluade Farms, in Ilara-Mokin), Ifedore Local Government Ondo State, Nigeria, while the Laboratory assessment (Bacteria counts and Fungal loads) off eggs collected from the fieldwork was done at the Microbiology Laboratory of the Department of Animal Production and Health, Federal University of Technology, Akure, Nigeria. The towns are located within Latitude 7°20" N and Longitude 5°12" E, the rainfall of the humid tropics which is characterized by the hot and humid climate. The mean annual rainfall is 1800mm and the rain period is bimodal with a short break in August.

The altitude is about 323.03 m above the sea level, the mean annual humidity is less than 70% and the mean annual temperature ranges between 22-30°C Ashaolu and Adebayo, [10].

2.2 Hens Procurement

One hundred Isa-Brown hens of forty-seven (47) weeks old were procured from a reputable farm in Akure, Nigeria. On arrival, the hens were settled on the deep litter for some hours and anti-stress was administered to stabilize the hens. Later in the evening, the hens were moved to the battery cage. Feed and water were provided for two weeks and records of eggs production were taken to ascertain those hens producing at that particular time.

2.3 Hens Arrangement and Management

At the end of two weeks, ninety (90) producing hens were selected and used for the field study. The hens were divided into three (3) treatments (battery cage, deep litter and outdoor) of three (3) replicates and ten (10) hens per replicate in a Completely Randomized Design. The hens were marked using different coloured permanent markers to ascertain each group. Each hen was given 110g feed per day, while fresh/clean water was supplied throughout the feeding period which lasted for 84days. The feeding period was subdivided into three (3) phases of 28 days per phase.

2.4 Egg Collection

Collection of eggs for bacteria counts and fungi load assessments was done at the last three days of the end of each phase and was collected three times in a day for the three different rearing systems; Battery cage, Deep litter and Outdoor rearing system. Three (3) fresh eggs were collected from each of the replicates based on the collection periods. The egg collection periods were tagged morning (7:00am – 8:00am), afternoon (1:00pm – 2:00pm) and evening (4:00pm – 5:00pm). Eggs collected were labelled with the aid of a permanent marker and sealed up in transparent white polythene nylon and then taken to Microbiology Laboratory of the Department of Animal Production and Health, Federal University of Technology, Akure, Nigeria for the bacteria count and fungi load assessments.

2.5 Bacteria Counts and Fungi Load Assessments

In the Microbiology Laboratory of the Department of Animal Production and Health, Federal University of Technology, Akure, Nigeria, preparation of nutrient agar and potato dextrose agar was done. Each egg was washed into the sterile water with the aid of sterile swab sticks and then serial dilution was carried out. Two Petri dishes were prepared for each example: one for bacteria culture and the other for fungi culture. Twenty-five (25 ml) of nutrient agar was poured into the Petri dishes for bacteria culture, while another twenty-five (25 ml) of potato dextrose agar was poured into ones meant for fungi culture. One (1ml) of the samples was introduced into the agar in the plates and was rocked gently. The bacteria were cultured at a temperature of 37°C for 24 hours while the fungi were cultured at room temperature for five (5) days.

Each colony of the organism was taken from the plate with the aid of a sterile inoculating loop and then fixed on the glass slides by passing them through the flame. Staining of the slide was done by using crystal violet which was then washed with clean water; the glass slide was stained again with Lugol's iodine which acted as a mordant which was later rinsed off with 70% alcohol. The slides were rinsed with water and then allowed to dry, the slides were then stained again with Safraine and allowed to stand for 60 seconds. After washing the slide were allowed to dry and then stained with, oil and was then viewed under a microscope for identification.

2.6 Data Analysis

Completely Randomized Design with 3 × 3 factorial arrangement was used and all data collected were subjected to analysis of variance (ANOVA) using SPSS (2006) version 17 and the means were separated using Duncan Multiple Range Test of the same package.

3. RESULTS

3.1 Bacteria Count

Table 1 shows the total bacteria count of eggshells collected from Isa-Brown hens under three different rearing systems, collection periods and interaction between the rearing systems and collection periods. All the parameters measured were significantly ($P<0.05$) influenced by the rearing systems, collection periods and

interactions. Based on the rearing systems, highest bacteria count (43.56± 7.48×10^{-3}cfu/ml) was observed in egg collected from deep litter rearing system, while lowest bacteria count (13.11±7.4×10^{-}3cfu/ml) was observed in egg collected from outdoor rearing system. Considering the collection period, highest bacteria count (41.78±7.48×10^{-3}cfu/ml) was recorded in egg collected in the morning, while lowest bacteria count (11.67±7.48×10^{-3}cfu/ml) was recorded in egg collected in the afternoon period. For rearing systems and morning period, highest bacteria count (75.00±12.95×10^{-3}cfu/ml) and lowest bacteria count (22.67±12.95×10^{-3}cfu/ml) were recorded in eggs collected in deep litter rearing and outdoor rearing systems, respectively. For rearing systems and afternoon period, highest bacteria count (21.67±12.95×10^{-3}cfu/ml) and lowest bacteria count (3.67±12.95×10^{-3}cfu/ml) were recorded in eggs collected in deep litter rearing and outdoor rearing systems, respectively. For the rearing systems and evening period, egg collected from deep litter rearing system had the highest bacteria count (34.00±12.95×10^{-3}cfu/ml), while lowest bacteria count (2.67±12.95×10^{-3}cfu/ml) was observed in egg collected from battery cage rearing system. In general, for the interaction between rearing systems and collection periods, highest bacteria count (75.00±12.95×10^{-3}cfu/ml) was recorded in egg collected from deep litter rearing system in the morning while lowest bacteria count (2.67±12.95×10^{-3}cfu/ml) was recorded in egg collected from battery cage rearing system in the evening.

3.2 Fungi Count

The total fungi count of eggshells collected from Isa-Brown hens under three different rearing systems, collection periods and interaction is presented in Table 2. No significantly (P>0.05) difference was recorded in all the parameters measured. But numerically for the rearing systems, highest fungi count (10.89±4.77×10^{-3}cfu/ml) was observed in egg collected from battery cage rearing system, while lowest fungi count (2.56±4.77×10^{-3}cfu/ml) was observed in egg collected from deep litter rearing system. For the collection periods, the highest fungi count (10.67±4.77×10^{-3}cfu/ml) and lowest fungi count (2.67±4.77×10^{-3}cfu/ml) were recorded in egg collected in the morning and afternoon, respectively. Considering the interaction, highest fungi count (27.67±8.27×10^{-3}cfu/ml) was recorded in egg collected from battery cage rearing system in the morning, while lowest fungi count (1.67±8.27×10^{-3}cfu/ml) was recorded in egg collected from outdoor rearing system in the morning.

3.3 Isolated Bacteria

Eight (8) different bacteria isolate were identified from eggshells collected from Isa-Brown hens under three different rearing systems and collection periods as presented in Table 3. *Streptococcus faecal* was isolated in eggs collected from battery cage rearing system in the morning and afternoon, deep litter rearing system in the morning, afternoon and evening but was only observed in the evening for outdoor rearing system. *Bacillus species* was only isolated in deep litter and outdoor rearing systems both in the evening. *Escherichia coli* was isolated in all eggs collected from the rearing systems and collection periods except battery cage rearing system in the morning and outdoor rearing system in the morning. *Pseudomonas aureginosa* was observed in battery cage rearing system in the morning, afternoon and evening. It was observed only in the evening under deep litter rearing system while it was also observed in the outdoor rearing system in the afternoon and evening. *Salmonella species* were isolated in the deep litter rearing system in the morning, afternoon and evening and also in the outdoor rearing system in the afternoon and evening but was not observed in the battery cage for morning, afternoon and evening. *Staphilococcus aureus* was isolated in eggs collected from all the rearing systems and collection periods except in outdoor rearing system in the afternoon. *Micrococcus leteus* was isolated in deep litter rearing system (afternoon and evening) and the three collection periods under the outdoor rearing system. *Proteus vulgaris* was isolated only in eggs collected from deep litter rearing system in the evening.

3.4 Isolated Fungi

Aspergillus flavus was isolated in the three collection periods under outdoor rearing system. It was also isolated in battery cage and deep litter rearing systems in the morning and afternoon. *Aspergillus niger* was isolated across all the three rearing systems and collection periods. *Aspergillus fumigatus* was only isolated in battery cage rearing system in the evening. *Penicillum notatum* was isolated in battery cage rearing system in the morning, deep litter rearing system; afternoon and

evening and outdoor rearing system; morning and evening. *Neurospora crassa* was only isolated in deep litter rearing system in the evening, while *Rhizopus stolonifer* was isolated in battery cage rearing system in the evening, deep litter rearing system; morning and evening and outdoor rearing system; afternoon and evening from Table 4.

4. DISCUSSION

Most freshly laid eggs are sterile; the shells become contaminated with dust, litter droppings, prevailing environment and handling. Microbes may be found on the eggshell surface within a short time and with conducive environment may penetrate the inner content to cause spoilage

Table 1. Total bacteria count of eggshells collected from Isa-brown hens under three different rearing systems (×10^{-3}cfu/ml)

Parameters	Bacteria count
Rearing systems	
Battery cage	13.33±7.48^{b}
Deep litter	43.56±7.48^{a}
Outdoor	13.11±7.48^{b}
Collection periods	
Morning	41.78±7.48^{a}
Afternoon	11.67±7.48^{b}
Evening	16.56±7.48^{b}
Rearing systems × Collection Periods	
Battery cage × Morning	27.67±12.95^{b}
Deep litter × Morning	75.00±12.95^{a}
Outdoor × Morning	22.67±12.95^{b}
Battery cage × Afternoon	9.67±12.95^{b}
Deep litter × Afternoon	21.67±12.95^{a}
Outdoor × Afternoon	3.67±12.95^{b}
Battery cage × Evening	2.67±12.95^{b}
Deep litter × Evening	34.00±12.95^{a}
Outdoor × Evening	13.00±12.95^{b}

**Means within the same column with different superscripts are significantly different (P<0.05)*

Table 2. Total fungi count of eggshells collected from Isa-brown hens under three different rearing systems (×10^{-3}cfu/ml)

Parameters	Fungi Count
Rearing systems	
Battery cage	10.89±4.77
Deep litter	2.56±4.77
Outdoor	3.89±4.77
Collection periods	
Morning	10.67±4.77
Afternoon	2.67±4.77
Evening	4.00±4.77
Rearing systems × Collection Periods	
Battery cage × Morning	27.67±8.27
Deep litter × Morning	2.67±8.27
Outdoor × Morning	1.67±8.27
Battery cage × Afternoon	3.00±8.27
Deep litter × Afternoon	2.33±8.27
Outdoor × Afternoon	2.67±8.27
Battery cage × Evening	2.00±8.27
Deep litter × Evening	2.67±8.27
Outdoor × Evening	7.33±8.27

**Means within the same column with different superscripts are significantly different (P<0.05)*

Table 3. Isolated bacteria from eggshells collected from Isa-Brown hens under different rearing system

Bacteria Isolate	Cage (am)	Cage (noon)	Cage (pm)	Deep Litter (am)	Deep Litter (noon)	Deep Litter (pm)	Outdoor (am)	Outdoor (noon)	Outdoor (pm)
Streptoccocus faecalis	+	+	-	+	+	+	-	-	+
Bacillus species	-	-	-	-	-	+	-	-	+
Escherichia coli	-	+	+	+	+	+	-	+	+
Pseudomonas aureginosa	+	+	+	-	-	+	-	+	+
Salmonella species	-	-	-	+	+	+	-	+	+
Staphilococcus aureus	+	+	+	+	+	+	+	-	+
Micrococcus leteus	-	-	-	-	+	+	+	+	+
Proteus vulgaris	-	-	-	-	-	+	-	-	-

Table 4. Isolated fungi from eggshells collected from Isa-brown hens under different rearing system

Fungi Isolate	Cage (am)	Cage (noon)	Cage (pm)	Deep Litter (am)	Deep Litter (noon)	Deep Litter (pm)	Outdoor (am)	Outdoor (noon)	Outdoor (pm)
Aspergillus flavus	+	+	-	+	+	-	+	+	+
Aspergillus niger	+	+	+	+	+	+	+	+	+
Aspergillus fumigates	-	-	+	-	-	-	-	-	-
Penicillum notatum	+	-	-	-	+	+	+	-	+
Neurospora crassa	-	-	-	-	-	+	-	-	-
Rhizopus stolonifer	-	-	+	+	-	+	-	+	+

Smith et al. [11]. Lower bacteria load contamination of eggshells was observed in eggs from battery cage rearing and outdoor rearing system and this is in agreement with Englmaierova et al. [12]. The low microbial count observed in the battery cage system may be due to a better method of housing and egg collection. The least bacteria count recorded in outdoor rearing system contradicted the reports of Oviasogie et al. [13], while the highest fungi count in battery cage rearing system agrees with the report of Oviasogie et al. [13], who reported that shells of eggs collected from battery cage had the highest fungi count. The high microbial counts of outdoor chicken and deep litter eggshell samples indicate poor environment under which the eggs were laid. Numerous authors also mention that eggs from alternative housing systems are more contaminated by microorganisms on their surfaces De Reu, et al., [14,15]; Mallet et al., [16]; Huneau-Salaun et al., (2010). The higher bacteria contamination on eggs from deep litter rearing system was caused by a higher probability of egg contact with faeces or bedding materials. Englmaierova et al. [12], and the same result was reported by Singh et al. [17]. The percentage of egg in the nest/deep litter rearing system may significantly affect the bacteria load of the eggshell. *Staphylococcus spp.* dominated on the shells of table eggs and also appears to be the most dominating species in the air in the poultry houses/Pens De Reu et al. [15] and this finding was also observed in this present study. It is evident from the bacteria count in this present study that the highest bacteria count from the different eggshell samples is from deep litter system. Higher bacteria contamination of eggshells can increase the penetration of microorganism into the egg content.

The presence of *Escherichia coli* and *Pseudomonas species* which have been isolated from the eggshells with a potential to cause spoilage and enter the food chain causing infection in consumers Musgove et al. [18]. Among the common contamination organisms pathogenic to human beings are *Staphilococcus species* Osei-Sonuh et al. [19] and this is common in all the three rearing systems. Following the results of previous studies Ahmed et al. [20], Zahran [21] and Suba et al. [22], the results of this current study had similar bacteria and fungi isolated from the eggshell. Faecal and soiled laying nest may be considered as the most routes of contamination of the eggshells with microbes. The microorganism found in the faecal materials and soiled laying nest may penetrate the shell most especially when the cuticle or bloom is removed when the farmer is trying to clean the faecal or litters attached to the shell. Serious health challenges may occur as a result of this contamination most especially when consumers consumed raw or uncooked.

Jones et al. [23] found fungi contamination in eggshell in the day of egg collection. The pathogenic moulds found their way to penetrate and contaminate egg and may produce their toxin under favourable conditions. Special attention should be directed to safeguard the egg against their contamination via correct farm hygiene programmes, good handling and storage methods and examination of eggs and poultry feed Neamatallah et al. [24].

5. CONCLUSION

Conclusively, the results of this study showed that the eggshells from the three rearing systems were contaminated with bacteria and fungi at varying levels which might pose a serious risk to egg consumers if not properly handled. Meanwhile, a better shell quality (low eggshells contamination) was found in eggs collected from battery cage and outdoor rearing systems.

ETHICAL APPROVAL

The right to conduct the research granted by the Research Committee of the Department of Animal Production and Health, Federal University of Technology, Akure, Nigeria. The birds were managed following the recommendation and guidelines of the committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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