



Impacts of Housing and Storage Environments on Physical Quality and the Potential Public Health Risks of Chicken Table Eggs

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Abstract | The freshness and safety of table eggs are important features that affect consumers' choices. A total of 210 fresh and stored table eggs were sampled from nine-layer hen flocks raised in 6 cage houses and 3-deep-litter houses. Egg measurements of external and internal quality were performed on 60 eggs, while the microbiological examination was performed on 150 eggs (every 3 eggs were examined as a composite). Additional samples from floor litter, cage swabs, feces, and ovaries were collected for bacteriological isolation. Molecular detection of genes encoding virulence factors in *Escherichia coli* and *Staphylococcus aureus* isolates were performed by PCR. Results of egg quality measurements revealed significant reductions in specific gravity, albumin index, and Haugh unit of stored eggs when compared to fresh eggs ($P < 0.05$). Storing eggs either at $> 25^{\circ}\text{C}$ or within $20 - 22^{\circ}\text{C}$ led to the deterioration of egg quality parameters but didn't affect microbial load when compared with fresh eggs ($P > 0.05$). Deep litter housing environment significantly raised the counts of aerobic bacteria ($R^2 = 0.70$, $P = 0.003$), and *Staphylococcus* spp. ($R^2 = 0.91$, $P < 0.0001$) on eggshells. Virulence genes *stx1*, *eae*, *hlyA*, and *stx2* of *E. coli* were detected in feces, ovaries, and on eggshells of composites collected from cage houses. While *Staphylococcus aureus MecA* was detected on eggshells and internal content of egg composites collected from deep litter houses. Obtained results revealed the critical role of storage temperature on egg quality parameters, as well as, the great influence of the housing environment on the microbial profile of produced eggs. Regular monitoring and corrective control measures should be set to maintain egg quality and safety at acceptable levels.

Keywords | Deep litter; Cages; Virulence genes; *Escherichia coli*; *Staphylococcus aureus*

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INTRODUCTION

Chicken eggs are the best source of cheap and high-quality protein (Farrell, 2013). For the first time in history, world egg production increased to more than 1338 billion eggs in 2015 (Conway, 2016). In intensive egg production systems laying hens are housed either in battery cages or on deep litter floor. In cage systems, eggs are physically separated from hens and manure, while in deep litter houses, eggs are in contact with litter and hens' manure contrib-

uting to an increase in shell contamination and microbial contents (Hannah et al., 2011). Hence, the type of housing could affect the microbial quality and safety of table eggs.

Egg quality focuses on the external and internal egg parameters. The external quality of eggs includes the egg weight, volume, shell characteristics, and specific gravity, while internal quality involves the albumen and the yolk indices (Şekeroğlu and Duman, 2014; Ogunwole et al., 2015). Storage of table eggs for a prolonged time results

in egg quality deterioration. Moreover, egg storage at high temperatures causes further loss to the eggs' quality (Akter et al., 2014).

Although table eggs could get contaminated with different microorganisms, a few studies focused on bacterial contamination of table eggs. Table eggs could be infected before egg-laying due to diseased hens with infected ovaries. However, many studies estimated that the greatest source of microbial contamination of eggshells occurred shortly after laying due to contact with the contaminated environment (Senbeta et al., 2015; Merino et al., 2019). Increasing the level of microbial load on the eggshell consequently increases the chance of penetration of microorganisms into internal egg content (De Reu et al., 2006; Sodagari et al., 2019). The most isolated enteric and environmental egg contaminants were *Staphylococcus* spp., *Salmonella* spp., coliforms, yeast, and mold (De Reu et al., 2008; Chaemsanit et al., 2015). Most of these microorganisms are considered public health hazards and may cause food poisoning (Adesiyun et al., 2006; Adesiyun et al., 2007). Reducing the cases of egg-borne infections requires on-farm control strategies and proper handling and storage of table eggs.

The current study aimed to evaluate the effect of storage on physical egg quality and the microbial load, as well as, determine the impact of housing systems on the microbial quality and safety of table eggs.

MATERIALS AND METHODS

LAYER HEN FLOCKS

Nine-layer hen flocks from Giza governorate, Egypt, were included in the study, during the period between April 2018 till August 2019. Out of the nine flocks, 6 of which were housed in a battery cage system, while the other three were housed on a deep-litter floor system. The raised hens were 29 to 60 weeks of age and at different stages of lay.

SAMPLING

Eggs: A total of 210 table eggs were collected using the simple random sampling method from the battery cage system (6 flocks, n= 135 eggs) and the deep litter system (3 flocks, n= 75 eggs). Both fresh (n= 105) and stored (n= 105) eggs were collected from each flock. Deep litter houses stored eggs at room temperature (> 25 °C), while cage houses stored eggs either at room temperature (> 25 °C) or at cooling temperature (20 – 22 °C). Egg quality measurements were performed on 60 eggs (30 fresh and 30 stored eggs), while the microbiological examination was performed on 150 eggs (every 3 eggs were examined as a composite).

Internal organs: Ten ovaries from 3 deep litter and 2

cage system farms were aseptically sampled during the post-mortem examination from 3 and 7 laying hens' carcasses; respectively, then transferred to the laboratory in sterile cups.

Environmental samples: For evaluating the microbial load of the egg production environment, 12 battery cages were swabbed by cotton swabs, and 3 litter samples were collected from floor houses in sterile polyethylene bags. Fecal samples from one cage system flock were collected in sterile polyethylene bags.

All samples were aseptically collected and taken to the laboratory in an icebox for further analysis.

EGG QUALITY MEASUREMENTS

Egg weight, volume, specific gravity, shape index, shell weight, and egg surface area were measured as external egg qualities (Alsaffar et al., 2013).

For testing internal egg qualities, eggs were broken and yolk weight and percentage, yolk index, albumen weight, and percentage, albumen index, and Haugh unit score were determined according to Hisasaga (2020).

MICROBIOLOGICAL EXAMINATION

Microbial count and isolation: The microbiological examination was conducted on 50 composite egg samples (Composite samples resembling three eggs each). Each composite egg sample was rinsed in 60 ml of sterile 0.1% peptone water (Lab M, 104) in sterile plastic bags and rinsed by shaking for 2 min (Al-Ajeeli, 2013). The egg rinse solution, litter samples, battery cage swabs, and fecal samples were tenth fold serially diluted with sterile peptone water up to 10⁴ for eggs and 10¹⁰ for other environmental samples. Composite samples of the internal content of eggs (the content of three eggs/composite) and ovaries were collected in sterile flasks and thoroughly mixed for further microbial isolation.

All prepared samples and dilutions were subjected to microbiological examination according to (BAM 2013); for counting total aerobic bacteria (Standard Plate Count Agar, SPCA, Oxoid™ CM0463), Coliforms (Eosin Methylene Blue agar plates, EMB, Oxoid™ CM0069), Staphylococci (Baird-Parker medium supplemented with egg yolk tellurite emulsion 3.5%, Lab M™ LAB085), and total yeast and mold [Sabaroud Dextrose Agar, SDA, Oxoid™ CM0463)]. All plates were incubated at 37°C for 24-48 h, except SDA plates were kept at 25°C for 3-5 days. Furthermore, isolation and identification of *E. coli*, *S. aureus*, and *Salmonella* species were performed. Isolation of *E. coli* was carried out by streaking onto Levine's Eosin Methylene Blue plates (L-EMB), followed by incubation

Table 1: Components and amplification programs of multiplex PCR used for detection of genes encoding virulence factors.

Infectious agent	Genes encoding virulence factors	PCR components and volume (µl)	Amplification programs	Reference
<i>E. coli</i>	<i>eae, hlyA, stx1</i>	5µl Master Mix 0.5µl of each primers F&R (with total 3µl) 5µl DNA template 12µl PCR grade water	1 cycle 94°C for 5 min 35 cycles 94°C for 30 sec 62°C for 30 sec 72°C for 1 min 1 cycle 72°C for 5 min	Chandra <i>et al.</i> , 2013
	<i>stx2</i>	5µl Master Mix 1µl of primer F&R (with total 2µl) 5µl DNA template 13µl PCR grade water	1 cycle 95°C for 3 min 35 cycles 95°C for 20 sec 58°C for 40 sec 72°C for 90 sec 1 cycle 72°C for 5 min	Gannon <i>et al.</i> , 1992
	<i>mecA</i>	1X Master mix 20 pmol of each primer 5µl of DNA	1 cycle 94°C for 4min 35 cycles 94°C for 1 min, 55°C for 1 min, 72°C for 1min 1 cycle 72°C for 10 min.	Asfour and Darwish 2011
Staphylococcus aureus				

Table 2: Primer sequences, their specific targets and amplicon sizes

Primer name	Primer sequence 5'-3' (reference)	Product size	References
EAE (<i>eae</i>)	F:TCAATGCAGTTCGGTTATCAGTT R:GTAAAGTCCGTTACCCCAACCTG	482	Vidal <i>et al.</i> (2005)
Stx1 (<i>stx1</i>)	F:CGATGTTACGGTTTGTTACTGTGACAGC R:AATGCCACGCTTCCCAGAATTG	244	Müller <i>et al.</i> (2007)
HlyA (<i>hlyA</i>)	F:AGCTGCAAGTGC GGGTCTG R:TACGGGTTATGCCTGCAAGTTCAC	569	Wang <i>et al.</i> (2002)
Stx2 (<i>stx2</i>)	F:GTTTTGACCATCTTCGTCTGATTATTGAG R:AGCGTAAGGCTTCTGCTGTGAC	324	Müller <i>et al.</i> (2007)
<i>mecA</i>	F:GTGAAGATATACCAAGTGATT R:ATGCGCTATAGATTGAAAGGAT	147bp	Asfour and Darwish (2011)

Table 3: Comparison between fresh and stored eggs regarding their external and internal quality parameters (Mean±SE)

	Age of Eggs		df	t	P - value
	Fresh eggs	Stored eggs			
External characters:					
Egg weight (g)	65.44±1.52	65.35±1.11	59	0.05	0.960
Egg Volume (cm ³)	60.07±1.52	61.97±1.38	59	-0.93	0.358
Specific gravity	1.093±0.014 ^a	1.059±0.009 ^b	59	2.05	0.045
Egg width (cm)	4.50±0.04	4.45±0.03	59	1.10	0.275
Egg length (cm)	5.91±0.06	5.96±0.05	59	-0.58	0.562
Shape index (%)	76.30±0.64	74.85±0.60	59	1.65	0.105
Egg surface area (cm ²)	85.28±1.46	85.17±1.09	59	0.06	0.954
Shell weight (g)	6.85±0.12	7.06±0.10	59	-1.31	0.288
Shell ratio (%)	10.52±0.14	10.85±0.15	59	-1.56	0.680

Internal characters:					
Albumin weight (g)	40.98±0.97	40.52±0.75	58	0.38	0.707
Albumin ratio (%)	62.68±0.54	62.16±0.80	58	0.54	0.595
Albumin height (cm)	0.82±0.03 ^a	0.72±0.04 ^b	58	2.04	0.045
Albumin length (cm)	9.55±0.21 ^b	10.40±0.29 ^a	58	-2.35	0.022
Albumin width (cm)	7.88±0.15	8.46±0.27	44.83	-1.90	0.064
Albumin Ave. width (cm)	8.71±0.17	9.12±0.40	40.13	-0.95	0.346
Albumin index (%)	9.59±0.41 ^a	7.83±0.72 ^b	47.53	2.14	0.038
Haugh unit	88.68±1.56 ^a	78.40±3.67 ^b	40.43	2.58	0.014
Yolk weight (g)	17.62±0.62	17.72±0.74	58	-0.11	0.912
Yolk ratio (%)	26.80±0.57	26.08±1.19	59	0.54	0.592
Yolk height (cm)	1.59±0.05	1.46±0.06	58	1.70	0.095
Yolk width (cm)	4.46±0.08	4.62±0.13	47.64	-1.11	0.274
Yolk Index (%)	36.23±1.37	32.99±1.78	58	1.45	0.153
Yolk Albumen ratio (%)	43.09±1.33	42.69±2.43	46.41	0.14	0.888

^{a, b} Different superscripts indicate significant difference at $P < 0.05$; SE: Standard error.

at 37°C for 24–48 h. Flat colonies with dark center and green metallic luster were streaked on agar slants and kept at 35°C incubation for 18 h (Fahim et al., 2019). Biochemical identification was done as described by Da Silva et al. (2018). For isolation of *Salmonella* spp., 25 ml of the original prepared sample was aseptically transferred to 225 ml of sterile buffered peptone water and incubated at 37°C for 16–20 h. A loopful from each of the previous pre-enriched broth tubes were inoculated into a sterile tube containing 10 ml Rappaport Vassiliadis broth, then incubated at 43°C for 24 h. A loopful from each Rappaport Vassiliadis enriched tube was streaked on a dried surface of Xylose Lysin Deoxycholate agar (XLD). Inoculated plates were incubated at 37°C for 48 hours (Andrews et al., 2018). For isolation and identification of *S. aureus*, 5 typical and atypical colonies were selected from each plate, then identified according to (BAM, 2013).

MOLECULAR DETECTION OF GENES ENCODING VIRULENCE FACTORS IN *E. COLI* AND *S. AUREUS* ISOLATES

DNA was extracted from the bacterial colonies by the boiling method. Bacterial strains were grown in brain heart infusion broth at 37°C overnight. Organisms from 1.5 ml growth were pelleted by centrifugation at 1200xg for 10 min. The bacterial pellet was resuspended in 150µl of sterile distilled water. Lysis of bacteria was done by boiling in a water bath for 10 min. The lysate was centrifuged again as before, and the supernatant was used as a template for polymerase chain reaction as stated in Tables (1 and 2) (Wani et al., 2003). The PCR product was run on 1.5% agarose gel at 80 volts for 1 hour. The DNA bands were visualized using an Ultraviolet lightbox with a camera (Gel Doc 2000, BIO-RAD) and photographed. The expected size of the PCR products for virulence encoding gene of *E. coli* and *mecA* genes was estimated concerning 50 bp DNA

ladder (Jena Bioscience).

STATISTICAL ANALYSIS

Data analysis was performed using PASW Statistics Version 18.0 software (SPSS Inc., Chicago, IL, USA). Independent sample *t*-test was conducted to compare the effect of storage and housing type on physical and microbial qualities of eggs and expressed as means ± SE. One-way analysis of variance (ANOVA) was tested to compare the effect of season on physical quality of eggs and expressed as means ± SE. The statistical model formula was:

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$$

where Y_{ij} indicated the measurement of egg quality parameters. μ was the grand mean, α_i represented the season's effect, and ϵ_{ij} stated the random error.

Kruskal-Wallis *H* and Mann-Whitney *U* tests were used when data were not normally distributed. Pearson correlation (*r*) and linear regression (R^2) were used to test the association between environmental and eggshell contamination. Chi-square test for independence (χ^2) and Fisher's Exact test (*FET*) was performed to test the relation between different samples and the rate of microbial isolation. Significance was set at P -value < 0.05.

RESULTS

EGG PHYSICAL QUALITY

Results in Tables (3 and 4) showed that the specific gravity of fresh eggs was greater than that of stored eggs. This difference was significant ($t(59) = 2.05, P = 0.045$). The specific gravity of eggs stored at room temperature (> 25°C) was greater than that of eggs stored at a cooler temperature (20–22°C). This difference was significant ($t(29) = 2.37, P = 0.025$). Similarly, the albumin height of fresh eggs was

Table 4: Effect of different storage temperatures (°C) on the external and internal egg quality (Mean±SE).

	Storage condition (°C)		df	t	P - value
	Room temp. (> 25 °C)	Cooling temp.(20-22 °C)			
External characters:					
Egg weight (g)	64.41±1.37	68.05±1.45	29	-1.46	0.155
Egg Volume (cm ³)	60.30±1.51 ^b	66.75±2.62 ^a	29	-2.16	0.039
Specific gravity	1.071±0.009 ^a	1.025±0.022 ^b	29	2.37	0.025
Egg width (cm)	4.44±0.03	4.49±0.05	29	-0.79	0.438
Egg length (cm)	5.91±0.06 ^b	6.10±0.07 ^a	20.48	-2.11	0.047
Shape index (%)	75.29±0.74	73.60±0.88	29	1.24	0.226
Egg surface area (cm ²)	84.17±1.32	88.05±1.60	29	-1.59	0.123
Shell weight (g)	6.98±0.11	7.28±0.25	29	-1.30	0.203
Shell ratio (%)	10.89±0.18	10.71±0.32	29	0.51	0.613
Internal characters:					
Albumin weight (g)	40.77±0.84	39.70±1.72	28	0.60	0.556
Albumin ratio (%)	63.37±0.55	58.20±2.48	6.61	2.03	0.084
Albumin height (cm)	0.73±0.03	0.69±0.15	6.64	0.29	0.783
Albumin length (cm)	10.25±0.26	10.89±0.92	7.02	-0.67	0.526
Albumin width (cm)	8.08±0.21	9.71±0.79	6.86	-1.99	0.087
Albumin Ave. width (cm)	9.16±0.22	10.30±0.84	6.81	-1.31	0.234
Albumin index (%)	8.20±0.56	7.73±2.46	6.64	0.19	0.858
Haugh unit	83.22±2.05	73.76±9.21	6.60	1.00	0.351
Yolk weight (g)	16.66±0.66 ^b	21.20±1.85 ^a	28	-2.92	0.007
Yolk ratio (%)	25.74±0.63	30.94±2.48	6.80	-2.03	0.083
Yolk height (cm)	1.57±0.03 ^a	1.11±0.18 ^b	6.23	2.50	0.045
Yolk width (cm)	4.41±0.08	5.31±0.39	6.45	-2.26	0.062
Yolk Index (%)	35.86±0.79	23.54±6.19	6.20	1.97	0.094
Yolk Albumen ratio (%)	40.88±1.39	54.75±6.20	6.62	-2.18	0.68

^{a,b} Different superscripts indicate significant difference at $P < 0.05$; SE: Standard error.

Table 5: Effect of season on the external and internal egg quality (Mean±SE).

	Season			F _{2,58}	P-value
	Winter	Spring	Summer		
External characters:					
Egg weight (g)	64.94±2.08	64.60±1.25	68.46±1.07	1.36	0.265
Egg Volume (cm ³)	60.57±2.57 ^{ab}	59.60±1.27 ^b	66.33±1.69 ^a	3.61	0.033
Specific gravity	1.08±0.02	1.09±0.01	1.04±0.02	2.97	0.059
Egg width (cm)	4.49±0.07	4.48±0.03	4.48±0.03	0.01	0.989
Egg length (cm)	5.86±0.11 ^{ab}	5.89±0.05 ^b	6.14±0.04 ^a	3.53	0.036
Shape index (%)	76.67±1.37 ^a	76.14±0.53 ^a	72.90±0.66 ^b	5.05	0.009
Egg surface area (cm ²)	84.09±2.52	84.48±1.19	88.50±0.83	1.67	0.198
Shell weight (g)	7.04±0.16	6.90±0.10	7.10±0.19	0.56	0.573
Shell ratio (%)	10.87±0.19	10.74±0.14	10.37±0.21	1.12	0.333
Internal characters:					
Albumin weight (g)	42.61±1.50	40.87±0.78	39.23±0.89	1.21	0.304
Albumin ratio (%)	65.60±0.70 ^a	63.32±0.37 ^a	57.39±1.05 ^b	29.13	<0.0001

Albumin height (cm)	1.13±0.05 ^a	0.76±0.02 ^b	0.61±0.05 ^c	32.24	<0.0001
Albumin length (cm)	8.44±0.26 ^c	9.84±0.18 ^b	11.36±0.41 ^a	13.93	<0.0001
Albumin width (cm)	7.30±0.17 ^b	7.92±0.14 ^b	9.56±0.39 ^a	16.23	<0.0001
Albumin Ave. width (cm)	7.87±0.20 ^c	8.88±0.15 ^b	10.46±0.36 ^a	17.46	<0.0001
Albumin index (%)	14.45±0.95 ^a	8.70±0.33 ^b	6.02±0.60 ^c	33.10	<0.0001
Haugh unit	103.54±2.11 ^a	85.17±1.23 ^b	72.48±4.00 ^c	25.46	<0.0001
Yolk weight (g)	15.29±0.72 ^b	16.83±0.49 ^b	22.00±0.77 ^a	16.50	<0.0001
Yolk ratio (%)	23.54±0.74 ^b	25.94±0.41 ^b	32.13±0.99 ^a	29.03	<0.0001
Yolk height (cm)	1.79±0.04 ^a	1.62±0.02 ^a	1.04±0.08 ^b	63.82	<0.0001
Yolk width (cm)	4.06±0.09 ^b	4.40±0.06 ^b	5.30±0.17 ^a	28.46	<0.0001
Yolk Index (%)	44.19±1.60 ^a	37.03±0.53 ^b	20.40±2.16 ^c	79.33	<0.0001
Yolk Albumen ratio (%)	35.98±1.52 ^b	41.17±0.91 ^b	56.60±2.74 ^a	30.97	<0.0001

^{a,b,c} Different superscripts indicate significant difference at $P < 0.05$; SE: Standard error.

Table 6: Effect of housing and storage on the microbial count of eggshells (\log_{10} CFU/ml)

	Number of egg composites (N=50)	Incidence No. (%)	Range (\log_{10} CFU/ml)		Mean \log_{10} CFU/ml \pm SE
			Min.	Max.	
Total Aerobic Bacteria					
Deep litter	Fresh (n= 9)	9 (100%)	3.54	6.10	4.39 \pm 0.28 ^a
	Stored (n= 9)	9 (100%)	3.15	6.18	4.74 \pm 0.36 ^a
Cages	Fresh (n= 16)	16 (100%)	< 1.00	4.28	2.42 \pm 0.22 ^b
	Stored (n= 16)	16 (100%)	< 1.00	3.34	2.32 \pm 0.24 ^b
$P < 0.0001$					
Total Staphylococci					
Deep litter	Fresh	9 (100%)	2.90	6.02	4.16 \pm 0.31 ^a
	Stored	9 (100%)	3.00	6.11	4.53 \pm 0.37 ^a
Cages	Fresh	11 (68.8%)	< 1.00	3.93	1.86 \pm 0.34 ^b
	Stored	14 (87.5%)	< 1.00	3.84	2.59 \pm 0.27 ^b
$P < 0.0001$					
Total Coliform count					
Deep litter	Fresh	6 (66.7%)	< 1.00	4.60	1.93 \pm 0.56 ^{ab}
	Stored	6 (66.7%)	< 1.00	4.43	2.50 \pm 0.64 ^a
Cages	Fresh	3 (18.6%)	< 1.00	4.34	0.73 \pm 0.39 ^b
	Stored	4 (25.0%)	< 1.00	3.92	0.78 \pm 0.32 ^b
$P = 0.033$					
Total fungal count					
Deep litter	Fresh	9 (100%)	< 1.00	4.48	2.80 \pm 0.39 ^{ab}
	Stored	9 (100%)	2.00	4.13	3.15 \pm 0.23 ^a
Cages	Fresh	9 (56.2%)	< 1.00	4.60	1.59 \pm 0.41 ^{bc}
	Stored	7 (43.8%)	< 1.00	4.92	1.22 \pm 0.39 ^c
$P = 0.004$					

^{a,b,c} Different superscripts indicate significant difference at $P < 0.05$; SE: Standard error.

greater than that of stored eggs. This difference was significant ($t(58) = 2.04, P = 0.045$). The albumin length of stored eggs was greater than that of fresh eggs. This difference was significant ($t(58) = 2.35, P = 0.022$). The albu-

min index (%) of fresh eggs was greater than that of stored eggs. This difference was significant ($t(47.53) = 2.14, P = 0.038$). Haugh unit of fresh eggs was greater than that of stored eggs, this difference was significant ($t(40.43) = 2.58,$

Table 7: Effect of storage temperature on the microbial load of stored shell eggs from cage system (\log_{10} CFU/ml)

Number of egg composites (N=16)	Incidence No. (%)	Range (\log_{10} CFU/ml)		Mean \log_{10} CFU/ml \pm SE
		Min.	Max.	
Total Aerobic Bacteria				
Room temp. (> 25 °C) (n=8)	7 (87.5%)	< 1.00	3.34	2.47 \pm 0.37 ^a
Cooling temp. (20-22 °C) (n=8)	7 (87.5%)	< 1.00	3.00	2.17 \pm 0.33 ^a
				<i>P</i> = 0.184
Total Staphylococci				
Room temp. (> 25 °C)	6 (75.0%)	< 1.00	3.23	2.10 \pm 0.47 ^a
Cooling temp. (20-22 °C)	8 (100%)	2.70	3.84	3.08 \pm 0.14 ^a
				<i>P</i> = 0.051
Total Coliform count				
Room temp. (> 25 °C)	1 (12.5%)	< 1.00	2.60	0.83 \pm 0.41 ^a
Cooling temp. (20-22 °C)	1 (12.5%)	< 1.00	3.92	0.74 \pm 0.52 ^a
				<i>P</i> = 0.700
Total fungal count				
Room temp. (> 25 °C)	9 (50.0%)	< 1.00	2.78	1.53 \pm 0.46 ^a
Cooling temp. (20-22 °C)	2 (25.0%)	< 1.00	4.92	0.90 \pm 0.64 ^a
				<i>P</i> = 0.224

^{a,b} Different superscripts indicate significant difference at *P* < 0.05 using Mann-Whitney Test; SE: Standard error.

Table 8: Effect of season on the microbial load of eggs from cage system (\log_{10} CFU/ml)

Number of egg composites (N=32)	Incidence No. (%)	Range (\log_{10} CFU/ml)		Mean \log_{10} CFU/ml \pm SE
		Min.	Max.	
Total Aerobic Bacteria				
Winter (n=8)	8 (100%)	2.30	4.28	2.86 \pm 0.22 ^a
Spring (n=12)	11 (91.7%)	< 1.00	3.34	2.34 \pm 0.24 ^a
Summer (n=12)	10 (83.3%)	< 1.00	3.32	2.06 \pm 0.30 ^a
				<i>P</i> = 0.154
Total Staphylococci				
Winter (n=8)	7 (87.5%)	< 1.00	3.93	2.67 \pm 0.42 ^a
Spring (n=12)	8 (66.7%)	< 1.00	2.78	1.69 \pm 0.36 ^b
Summer (n=12)	10 (83.3%)	< 1.00	3.84	2.47 \pm 0.36 ^a
				<i>P</i> = 0.025
Total Coliform count				
Winter (n=8)	6 (75.0%)	< 1.00	4.34	2.52 \pm 0.61 ^a
Spring (n=12)	1 (8.3%)	< 1.00	2.00	0.17 \pm 0.17 ^b
Summer (n=12)	1 (8.3%)	< 1.00	2.00	0.17 \pm 0.17 ^b
				<i>P</i> < 0.0001
Total fungal count				
Winter (n=8)	5 (62.5%)	< 1.00	4.92	2.38 \pm 0.81 ^a
Spring (n=12)	11 (91.7%)	< 1.00	3.30	2.21 \pm 0.23 ^a
Summer (n=12)	0	< 1.00	< 1.00	0.00 ^b

^{a,b} Different superscripts indicate significant difference at $P < 0.05$ using Kruskal Wallis Test; SE: Standard error.

Table 9: Microbial load of housing environment (Mean \log_{10} CFU/ml)

	Mean \log_{10} CFU/ml \pm SE				
	Deep litter sample	Cage swabs	<i>df</i>	<i>t</i>	<i>P</i> - value
Total Aerobic Bacteria	9.34 \pm 0.19 ^a	2.26 \pm 0.14 ^b	4	30.55	< 0.0001
Total Staphylococci	8.13 \pm 0.75 ^a	1.33 \pm 0.67 ^b	4	6.75	0.003
Total Coliform	6.98 \pm 0.06 ^a	1.99 \pm 0.99 ^b	4	5.04	0.037
Salmonella species	1/3 (33.3%)	6/6 (100%)		<i>FET</i>	0.083

^{a,b} Different superscripts within the same row indicate significant difference at $P < 0.05$; SE: Standard error; *FET*: Fisher's Exact Test.

$P = 0.014$).

Results of Table (5) showed that the egg volume and shape index differed significantly concerning the season of the year ($P = 0.033$ and 0.009 , respectively). Surprisingly, all internal parameters of examined eggs (except albumin weight) displayed significantly better qualities for eggs produced during the winter season compared to those laid during the summer season ($P < 0.0001$). However, yolk indices were significantly greater for eggs laid in winter than those of summer season ($P < 0.0001$).

EGG MICROBIAL QUALITY

Results of the effect of housing on total aerobic bacteria, *Staphylococcus*, coliforms, and fungal counts of eggshells produced in deep litter and battery cage housing systems were displayed in Table (6). Results showed a significant effect of housing type on the total aerobic bacterial count of eggshells, ($P < 0.0001$). Similarly, a significant effect of housing type on the total *Staphylococcus* count of eggshells was found ($P < 0.0001$). Likewise, a significant effect of housing type on the total eggshells' coliforms count was found ($P = 0.033$); Kruskal-Wallis *H* test. Additionally, there was a significant effect of housing type on eggshells' total fungal count ($P = 0.004$). Post hoc comparisons using the Tukey HSD test indicated that the mean microbial counts of eggshells produced from deep litter housing systems were significantly higher than eggshells produced from battery cage housing systems.

A Kruskal-Wallis *H* test showed that stored eggs produced from the deep-litter system showed a significantly higher coliform count on eggshell than that of fresh eggs and eggs produced from cage systems ($P = 0.033$).

Results in Table (7) demonstrated the effect of storage temperature on the eggshell microbial count of the stored eggs from the cage system. Mostly, no significant effect on total aerobic bacteria count ($P = 0.184$), *Staphylococcus* count ($P = 0.051$), coliforms count ($P = 0.700$), or fungal

count ($P = 0.224$) was observed when eggs stored in room temperature ($> 25^{\circ}\text{C}$) or in $20\text{-}22^{\circ}\text{C}$.

Table (8) displayed the relationship between the season and the microbial loads of eggshells. All eggshell contaminants reported the highest levels during the winter season. However, the significant differences were observed in *Staphylococcus* count ($P = 0.025$), coliforms count ($P < 0.0001$), and fungal count ($P < 0.0001$), while total aerobic bacteria count didn't differ significantly due to season ($P = 0.154$).

From the data in Table (9), an independent sample *t*-test showed that the total aerobic bacterial count of litter samples was greater than counts of cages' swabs. This difference was significant ($P < 0.0001$). As well, the *Staphylococcus* count of litter samples was greater than that of cages' swabs. This difference was significant ($P = 0.003$). Moreover, the coliforms count of litter samples was greater than that of cages' swabs. This difference was significant ($P = 0.037$). *Salmonella* spp. was isolated from 33.3% of litter samples and 100% of cage swabs ($P = 0.083$).

Results of Pearson correlation indicated that there was a strong positive association between environmental and eggshell aerobic bacterial counts ($r = 0.84$, $P = 0.001$). For each 1 \log_{10} increase in environmental bacterial count, we observed a 0.23 \log_{10} CFU increase in eggshell aerobic bacterial count ($P = 0.003$, $R^2 = 0.70$); as demonstrated in Figure (1-A). Similarly, there was a strong positive association between environmental and eggshell *Staphylococcus* counts ($r = 0.95$, $P < 0.0001$). For each 1 \log_{10} increase in environmental *Staphylococcus* count, we observed a 0.51 \log_{10} CFU increase in eggshell *Staphylococcus* count ($F(1, 8) = 78.09$, $P < 0.0001$, $R^2 = 0.91$); as demonstrated in Figure (1-B). However, there was weak association between environmental and eggshell coliform counts ($r = 0.48$, $P = 0.081$). For each 1 \log_{10} increase in environmental coliform count, we observed a 0.15 \log_{10} CFU increase in eggshell coliform count ($P = 0.162$, $R^2 = 0.23$); as demonstrated in

Table 10: Incidence of microbial isolation from egg composites

Samples	No. of samples	No. (%) of positive samples				
		Salmonella	Staphylococci spp.	Coliforms	Fungi	P-value
Ovaries	10	8 (80.0) ^a	2 (20.0) ^b	2 (20.0) ^b	2 (20.0) ^b	0.012
Deep litter	3	1/3 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	
Cages	7	7/7 (100)	2 (28.6)	2 (28.6)	2 (28.6)	
Fecal matter (cages)						
	1	1 (100)	1 (100)	1 (100)	1 (100)	
Egg composites:						
Shell						
Fresh egg	25	5 (20.0) ^b	20 (80.0) ^a	9 (36.0) ^b	18 (72.0) ^a	< 0.0001
Stored egg	25	9 (36.0) ^b	23 (92.0) ^a	10 (40.0) ^b	16 (64.0) ^b	< 0.0001
Total	50	14 (28.0) ^c	43 (86.0) ^a	19 (38.0) ^c	34 (68.0) ^b	< 0.0001
Internal content						
Fresh egg	25	2 (8.0)	3 (12.0)	1 (4.0)	2 (8.0)	0.866
Stored egg	25	1 (4.0)	4 (16.0)	0.0	0.0	0.349
Total	50	3 (6.0)	7 (14.0)	1 (2.0)	2 (4.0)	0.144 <i>FET</i>
Eggs/House type						
Deep litter	18	7 (38.8)	18 (100) ^A	12 (66.7) ^A	18 (100) ^A	
Cages	32	7 (21.9)	25 (78.1) ^B	7 (21.9) ^B	16 (50) ^B	
P-value		0.325	0.040	0.002	< 0.0001	

^{a,b} Different superscripts in the same row indicate significant difference at $P < 0.05$; ^{A,B} Different superscripts in the same column indicate significant difference at $P < 0.05$; *FET*: Fisher's Exact Test.

Table 11: Prevalence of *E. coli* and *S. aureus* virulence genes in layer farms

<i>E. coli</i>						
Samples	<i>E. coli</i> (%)	Virulent <i>E. coli</i> (%)	Virulence genes			
			Eggs	<i>stx1</i>	<i>eae</i>	<i>blyA</i>
Eggshells (cages):						
Fresh	5/16 (31.3)	3/16 (18.8)	1 st	+		+
			2 nd		+	+
			3 rd			+
Stored	4/16 (25.0)	3/16 (18.8)	1 st			+
			2 nd			+
			3 rd	+		+
Ovaries	1*/10 (10.0)	1*/10 (10.0)				+
Fecal sample (cages)	1/1 (100)	1/1 (100)		+	+	+
Cage swab	1/6 (16.7)	0/6 (0.0)				
<i>S. aureus</i>						
	<i>Staphylococcus aureus</i> (%)	Virulent <i>Staph. aureus</i> (%)	Virulence genes (<i>mecA</i>)			
Eggshells (deep litter):						
Fresh	1/9 (11.1)	1/9 (11.1)		+		
Stored	3/9 (33.3)	3/9 (33.3)		+		
Egg content (deep litter)	1/9 (11.1)	1/9 (11.1)		+		
Eggshells (cages):						
Fresh	4/16 (25.0)	0/16 (0.0)				

Stored	1/16 (6.3)	0/16 (0.0)
Fecal sample (cages)	0/1 (0.0)	0/1 (0.0)

* The positive *E. coli* ovarian sample were from hens housed in cage system.

Figure (1-C). Eventually, there was no association between environmental and eggshell fungal counts ($r = -0.06$, $P = 0.436$), ($P = 0.872$, $R^2 = 0.003$); as demonstrated in Figure (1-D).

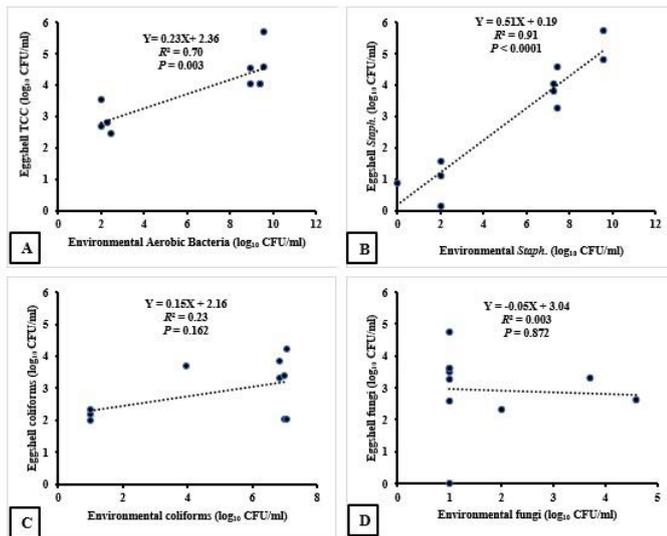


Figure 1: The association between the microbial load of the farm surface environment and the eggshell microbial contamination. R^2 : Coefficient of Determination.

EGG MICROBIAL ISOLATES

Results in Table (10) displayed that *Staphylococcus* spp. was the most predominant eggshell contaminant isolated from both fresh ($P < 0.0001$) and stored ($P < 0.0001$) egg composites. The rate of isolation of *Salmonella* was relatively low and was found in the internal content of 3/50 (6.0%) egg composites and on the shell of (28.0%) egg composites. However, a high rate of *Salmonella* infections (80%) was observed in ovarian samples collected from both floor and cage housing systems. Additionally, eggs produced in deep litter houses showed significantly higher isolation rates of *Staphylococcus* spp. ($P = 0.040$), Coliforms ($P = 0.002$), and total fungi ($P < 0.0001$) than eggs produced from cage battery houses.

MOLECULAR DETECTION OF GENES ENCODING VIRULENCE FACTORS FROM THE BACTERIAL ISOLATES

Results listed in Table (11) reported the detection of *E. coli* virulence genes (*eae*, *stx1*, *hlyA*, and *stx2*) in 8 samples out of 12 positive *E. coli* isolates. A fecal sample from caged hens showed positivity to *eae*, *stx1*, *hlyA*, and *stx2* virulence genes. Similarly, an ovary from a caged hen showed positivity to the *hlyA* virulence gene. Besides, eggs produced from cage houses revealed the presence of the *stx2* virulence gene on the eggshells of one fresh egg and two stored

eggs composites. Combined *stx1+hlyA*, *eae+hlyA*, and *stx1+stx2* virulence genes were detected in two fresh and one stored egg composites produced from cage system houses. Furthermore, the *Staphylococcus aureus* virulence gene, *mecA*, was detected in 5 strains isolated from deep litter houses out of 11 *Staphylococcus aureus* strains. Interestingly, one fresh egg composite displayed *Staphylococcus aureus* virulence gene, *mecA*, from the external eggshell and the internal egg content. As well, the *Staphylococcus aureus* virulence gene, *mecA*, was detected on the eggshell of three stored egg composites produced from deep litter houses (Table 11).

DISCUSSION

Our results revealed the impact of storage on some quality parameters of table eggs. Table eggs are stored in layer farms to be marketed. According to FAO (2003), eggs should be stored in a clean and well-ventilated environment, with constant temperature and relative humidity. Moreover, regular monitoring of the interior quality of stored eggs should be done. Also, refrigerated storage is recommended for better egg quality.

Results showed that most of the internal quality parameters of fresh eggs, especially specific gravity, albumin index, and Haugh unit, were better than stored eggs. These findings were consistent with Stadelman and Cotterill (2007) and Eke et al. (2013), who stated that the escape of moisture and gases from within the eggs via egg pores results in egg weight loss. The carbonic acid of egg white breaks down into water and carbon dioxide gas, which escapes through pores, and egg white becomes watery. This loss of carbon dioxide during egg storage changes pH to the alkaline side, which loosens the mucin fibers of albumin, causing it to lose its strength and consequently decreasing the Haugh unit of the stored egg. Moreover, the water of watery albumin is absorbed by the egg yolk to equalize the pressure inside the egg, so the yolk becomes swollen and flabby in shape, and a lower yolk index is obtained.

Results of the physical qualities of stored eggs were almost similar either in eggs stored in ambient room temperature or slightly cooler conditions (20-22°C). Grashorn (2016) reported that at 15 - 22°C, there was a rapid deterioration of egg quality, including the Haugh unit, and recommended eggs to be stored at 6°C. The same findings were reported by Ibrahim et al. (2019); the long storage at room temperature or refrigerator deteriorated egg weights, internal qualities, and Haugh unit, although storing at room

temperature revealed the lowest results. The reduction of the Haugh unit indicates the decline of lysozyme activity, which plays a vital role in protecting the egg contents from microbial contamination (Trziszka, 1994).

Regarding the effect of macroclimatic conditions on table egg qualities, we observed that eggs delivered in winter had higher freshness parameters than those laid in summer. Our findings were consistent with Gumułka et al. (2017), who reported greater egg weights, albumin%, albumin indices, Haugh units, and shell quality for eggs delivered in winter than those laid in summer. Furthermore, he stated that yolk quality was higher in summer than winter. It may be attributed to the stress of the higher temperatures in the summer season, which influence the metabolism and egg formation (Gumułka et al., 2017). Moreover, the season of the year could be a factor influencing the microbial load of table eggs as stated by Chousalkar et al. (2021), especially the degree of humidity that affects the fungal count over the eggshell.

From the microbiological point of view, stored eggs tested in this study didn't show a significant difference from fresh eggs based on microbial load. However, the microbial quality of eggs differed significantly due to the type of production housing system. Eggs produced from deep litter housing showed a higher microbial load than eggs produced from battery cage houses. De Reu et al. (2008) stated in their study that the housing system has an effect on the microbial load of eggshells and that eggs produced from non-cage systems were higher in aerobic bacterial contaminants than eggs produced from various cage systems. Additionally, Hannah et al. (2011) discussed that houses separating laying hens from manure and shaving resulted in eggs with lower bacterial counts. These findings were relevant to the results of the microbial examination of surfaces of different housing systems tested in this study. In deep litter houses, laid eggs are in contact with hens and litter, which is a mixture of wood shaving, manure, and feathers. While in battery cage houses, eggs roll on the sloped cage wire into the collection trough away from contact with hens or manure. Results of microbial examination revealed that each 1g of deep litter had more than 6 log₁₀ CFUs higher than cage wire swabs for aerobic bacteria and *Staphylococcus* counts. Similarly, Soliman et al. (2020) reported a significant difference ($P = 0.001$) in mean total bacterial counts of wood shaving litter (5.25 ± 0.00 log₁₀ CFU/ml) and battery swabs (4.14 ± 0.01 log₁₀ CFU/ml), as well as a significant difference ($P < 0.0001$) in mean total Enterobacteriaceae counts of wood shaving (3.74 ± 0.02 log₁₀ CFU/ml) and battery swabs (1.80 ± 0.01 log₁₀ CFU/ml). These findings agreed with Parisi et al. (2015), who reported that the longer the contact of eggs with hens and contaminated litter, the higher the bacterial load on eggshells compared to eggs produced from cage systems.

Results of the current study revealed that the predominant contaminant of eggshells and internal egg content was *Staphylococcus* spp. (86% and 14%, respectively), followed by total fungi (68% and 4%, respectively), then coliforms (38% and 2%, respectively). These frequencies agreed with EL-Kholy et al. (2020) who found *Staphylococcus* spp. in the internal content of 13% of examined eggs. However, *Salmonella* spp. was isolated in a relatively low frequency (28% and 6%, respectively), which agreed with what was reported by Musgrove et al. (2008). Though egg contamination with *Salmonella* is a critical public health risk, limited research studies focused on other bacterial contaminants that threaten public health, as noted by De Reu et al. (2008).

Bacterial contamination of eggs can occur via vertical or horizontal routes (European Food Safety Authority, EFSA, 2005). Hens have a common cloacal opening for intestinal, urinary, and reproductive tracts, which could contribute to eggshell contamination while the egg moves along this route. Furthermore, minor defects in the eggshell may provide access to bacteria into the internal egg contents (De Reu et al., 2006). Eggs could act as a vehicle for pathogens in the food chain, including extraintestinal pathogenic *Escherichia coli* (ExPEC) (Mitchell et al., 2015). However, the potential of eggs as sources of Diarrheagenic *E. coli* (DEC) has been investigated to a much less extent (Chousalkar et al., 2010).

Escherichia coli (*E. coli*) is a normal gut flora of birds and humans, and some *E. coli* strains have acquired virulence genes that enable them to induce diarrhea and other associated illnesses (Wani et al., 2004). The most important group of the zoonotic *E. coli* are the Diarrhoeagenic class that includes Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), Enterotoxigenic *E. coli* (ETEC), and Shiga toxin-producing *E. coli* (STEC) (Murase et al., 2012). Shiga toxin-producing *E. coli* (STEC) are known to produce Shiga toxins (*Stx1*, *Stx2*) and are recognized as the causative agent for life-threatening conditions in humans, such as hemorrhagic colitis and/or hemolytic-uremic syndrome (HUS). While Enterohemorrhagic *E. coli* (EHEC) produces the plasmid-encoded Enterohaemolysin (*hlyA*). Hence, strains that carry both *Stx* and *hlyA* virulence genes are potentially considered more dangerous to humans than those with only one virulence gene (Rasheed et al., 2014).

In this study, the findings confirmed the presence of different encoding virulence genes in *E. coli* strains isolated from different samples at a rate of 66.6% (8 out of 12). Markedly, most of the *E. coli* strains, isolated from eggshells of eggs produced from cage housing systems, encoded for virulence genes (6 out of 8), followed by an ovarian sample (1 out of 8) and a fecal sample (1

out of 8). The most predominant virulence gene among the positive *E. coli* isolates was the *stx* gene. This result agreed with (Morran et al., 2013), who found that 53% of the *E. coli* strains isolated from chickens' lesions contained *stx* gene sequences, with the majority containing the *stx1* allele, but no *eae* or *E-blyA* virulence genes were detected in *stx*-positive strains. In our study, the virulence genes *stx1*, *eae*, *blyA*, and *stx2* were identified from a fecal sample from the same flocks raised in cage systems, which suggests the possibility of fecal contamination of the corresponding eggshells. Similarly, AL-Ashmawy (2013) in her study reported the detection of the *stx2* virulence gene in 37/39 of the *E. coli* strains isolated from table eggs. On the other hand, the results of the current study differed from a study done in Egypt by (Galal et al., 2013), who detected both *stx1* and *stx2* genes with the *eaeA* gene in 2/19 (10.52%) of egg samples. Furthermore, AL Ashmawy, (2013) reported either *stx1* or *stx2* with *eaeA* gene in 3/19 (15.78%) of egg samples.

Staphylococcus aureus MecA virulence gene conferred resistance to methicillin and other β -lactam antibiotics, by altering the penicillin-binding protein located within the cell wall. This alteration renders antibiotics that act by interfering with bacterial cell wall synthesis (Sexton et al., 2006). Uncontrolled use of antibiotics in livestock production may be responsible for the emergence of antibiotic resistance in *S. aureus* strains, which may cause infections to human handlers (Price et al., 2012). The results revealed that *Staphylococcus aureus* was detected in 11 fresh and stored egg composites collected from deep litter houses. Out of the 11 *Staphylococcus aureus* isolates, 45% (5/11) were positive for the *mecA* virulence gene (MRSA, methicillin-resistant *S. aureus*). These results agreed with (Eid et al., 2015), who reported that four isolates out of the 11 tested for Staphylococci were positive for the *mecA* gene. However, (Pyzik et al., 2014) detected the *mecA* gene in two *S. aureus*-like strains isolated from table eggs. According to the guidelines of Egyptian Organization for Standardization and Quality Control, table egg content should be free from *Staphylococcus aureus* (Nil) (EL-Kholy et al., 2020).

Collectively, these results suggest that housing type does influence the microbial quality of table eggs. Specifically, our results suggest that eggs produced from cage houses have better microbial quality than eggs produced from deep litter houses. Moreover, our finding could be evidence for the probability of the transfer of the antimicrobial resistance from food-producing animals to humans, either directly via the food chain or via the close contact between infected animals and humans.

Eggs are considered a valuable nutritive source; however, they could be a potential source of illness and food poisoning to humans. The environment in which the eggs are produced, handled, and stored does influence the quality and safety of table eggs. Eggs produced from the cage housing system were much cleaner than those produced from the deep litter housing system. *E. coli* and *Staphylococcus aureus* strains encoding virulence genes of public health importance could contaminate the external or internal content of eggs, inducing illness in consumers. Regular monitoring of internal and external quality parameters of table eggs should be implemented. Storage of egg till marketing should be in low temperatures to save the eggs from deterioration and microbial contamination.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

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AUTHORS CONTRIBUTION

All authors shared in the aim of the work and designing of the study; Mahmoud A.M. Khalf: Farm visits, Collection of egg and environmental samples and technical data; Karima M. Fahim and Elshaimaa Ismael: Egg quality measurements and microbiological analysis; Sara Nader: Molecular analysis of bacterial isolates; Elshaimaa Ismael: Data analysis and interpretation. All authors shared in writing and revising the manuscript.

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