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Evaluating phenotypic correlations between internal and external egg quality indicators of Hy-Line Brown laying hens at 35 weeks of age

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No potential conflict of interest relevant to this article was reported.

Abstract

Increased global production, and consumption of the hen egg; and the popularization of nutritional knowledge, places greater emphasis on egg quality, safety, and freshness. An understanding of the interaction between several egg quality indicators is necessary. A total of 300 freshly-laid eggs were analyzed within 24 hours of collection to investigate the phenotypic correlations between internal and external quality indicators. Eggs were analyzed for egg weight, breaking strength, shell color, albumen height, Haugh unit, yolk color, specific gravity, shell weight, shell percentage, and shell thickness at the blunt, equator, and sharp ends. Collected data was analyzed for Pearson's correlation coefficients and statistical significance was estimated at $p < 0.05$. Low positive correlations were observed between egg weight and shell weight ($r = 0.336$, $p < 0.01$). Moderate negative correlations were found between egg weight and shell percentage ($r = –0.504$, $p < 0.01$). While very strong positive correlations were found between Haugh units and albumen height ($r = 0.949$, $p < 0.01$), both parameters were not distinctly correlated to other quality indicators. Similarly, there were no significant correlations between egg yolk color and shell color to other quality indicators. While moderate positive correlations were observed between eggshell weight and shell percentage ($r = 0.631$, $p < 0.01$), both parameters were lowly correlated to the eggshell thickness and not distinctly correlated to the egg-breaking strength. High positive correlations were observed between the overall eggshell thickness and the measurements at the blunt, middle, and sharp edges ($r = 0.975$, $r = 0.965$, $r = 0.923$). Low positive correlations were observed between breaking strength and the overall, blunt edge, middle edge, and sharp edge eggshell thickness ($r = 0.465$ $r = 0.447$, $r = 0.428$, $r = 0.453$). Conclusively, no marked correlations were established between the eggshell and the internal egg quality indicators. This study contributes to understanding the relationship between shell quality and internal egg freshness, providing insights for optimizing laying hen production and egg quality assessment systems.

Keywords: Albumen, Correlation, Egg quality, Laying hen, Eggshell

INTRODUCTION

Global egg production has increased significantly over the years as the volume of egg production has

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Availability of data and material
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Authors' contributions
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Ethics approval and consent to participate
The experimental protocol and procedures were reviewed and approved by the Animal Ethics Committee of Chungnam National University (Protocol Number; 202407A-CNU-125).

improved by more than 69% from 2000 to 2021 [1]. While the avian egg is the reproductive vehicle for the domestic fowl, it also serves as an encapsulated nutrient-dense, highly digestible, and cost-efficient human food packing proteins, vitamins, micronutrients, and bioactive substances [2,3]. In line with increased global egg production and consumption, the production level and the reproductive performance of laying hens have significantly improved over the past few decades, and the current focus is on persistency in laying to 100 weeks of age and 500 eggs per production cycle [4]. Increased persistency in lay presents the challenge of reducing variability while increasing uniformity in egg quality over a longer production period. Notably, eggshell quality is of utmost importance and there is a need to reduce the significant egg breakage of up to 8% that has been reported during transport with adverse economic effects [5]. Moreover, consumer awareness and attention to egg quality and freshness has also increased with the improvement of living standards and the increased dissemination of dietary and nutritional information [6]. The diverse production, food processing, and human food provision targets present a unique challenge to poultry nutritionists, breeders, and producers and generally places a higher expectation on the overall egg quality, freshness, and safety.

Egg quality assessment determines the properties that will influence the rejection or acceptance of the egg for any intended purpose. Comprehensive egg quality assessment is focused on the three main egg components: shell, yolk, and albumen, which can be assessed through several internal and external quality indicators [7]. Several egg quality characteristics are of particular interest to consumers and producers and are generally aimed at gauging the aesthetic soundness, freshness, cleanliness, color, shape, weight, eggshell quality, internal quality, and chemical composition of eggs. A high-quality fresh table egg is elliptically shaped, with a clean, smooth, and shiny shell surface. The shell surface should bear a color that is uniform and could be either pure white or dark brown as per the breed. Eggs are usually assessed for specific quality through an overall exterior evaluation, candling, or broken-out evaluations [8]. A comprehensive analysis of whether the internal egg contents are related to the outer eggshell is needed to provide a theoretical foundation for interpreting and standardizing egg quality assessments.

While having distinct chemical and organic makeup, several egg components will show variations that are due to differences in the breed, age of the hens, management, hen diet, housing design, egg handling, and storage system [9]. Several egg quality parameters can be determined with a single broken-out evaluation including egg weight, breaking strength, shell color, albumen height, Haugh unit, yolk color, specific gravity, shell weight, shell percentage, yolk percentage, vitelline membrane strength, shell thickness, among others [10]. Internal egg abnormalities including the presence of meat and blood spots can also be detected. There is a need to correlate several internal and external egg quality indicators for holistic assessment and understanding of the egg produced under varied conditions of age, breed, and rearing systems. Such findings could provide insights into the interactions between internal and external eggshell quality indicators. Therefore, the current study was conducted to investigate the phenotypic correlations between external and internal egg quality characteristics in 35-week-old laying hens, to establish the implications of these correlations on the overall egg quality. It was hypothesized that external indicators (e.g., eggshell thickness) could strongly correlate with internal indicators (e.g., Haugh unit). It was reasoned that if strong correlations were found between internal and external egg quality indicators, the need for broken-out evaluations could be reduced.

MATERIALS AND METHODS

Eggs were obtained from one 35-week-old flock of 300 Hy-Line Brown laying hens raised in an enriched cage system (90 cm long by 90 cm wide by 70 cm high) within the Poultry Unit,

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Cheongyang Research Station of Chungnam National University. A total of 8 birds were housed in each of the 38 cages. At the time of egg collection, 4 mortalities had been observed, leaving 300 birds in total. The experimental protocol and procedures were reviewed and approved by the Animal Ethics Committee of Chungnam National University (Protocol Number; 202407A-CNU-125). The birds were subjected to a lighting scheme with 16 hours of continuous light and 8 hours of darkness in a windowless facility. The temperature and humidity were recorded and maintained at around 20℃–22℃ and 45%–50%, respectively. The hens were fed commercial diets that met or exceeded NRC guidelines for brown egg-laying strains.

Egg sample collection and analysis

A total of 300 freshly laid eggs were collected over two days (150 eggs per day), numbered, transported to the laboratory, and analyzed within 24 hours of collection. Abnormal eggs that were judged to be dirty, rough, misshapen, pimpled, cracked, white-banded, pale-shelled, or soft-shelled, were excluded. Eggs were analyzed for the whole egg weight, breaking strength, shell color, albumen height, Haugh unit, yolk color, specific gravity, shell weight, shell percentage, and shell thickness at the blunt, equator, and sharp ends.

The specific gravity of the eggs was determined by submerging the eggs in ascending order of a salty solution with a known specific gravity ranging between 1.065 to 1.090 [11]. Subsequently, the eggshell-breaking strength was evaluated using a texture analyzer (TA.XTplusC, Stable Micro Systems). The egg weight, shell weight, shell color, albumen height, Haugh units, and yolk color were measured using an egg multitester instrument (TSS QCM+ Range, Texture Systems) featuring a digital balance, shell color reflectometer, albumen height gauge, internal Haugh unit calculator and a yolk colorimeter. The shell color reflectometer used in the current study expresses reflectivity readings that could range from 25% to 40% for commercial brown eggs. The Haugh units were calculated automatically using the formulae:

$$\text{Haugh units (HU)} = 100 \times \text{Log}\,(AH - 1.7W^{0.37} + 7.6)$$

where AH is albumen height in mm, and W is egg weight in grams.

The thick albumen height was read at least 1 cm from the yolk as stipulated by Jones [12]. Yolk color intensity was measured against the DSM yolk color fan (1, light yellow; 15, orange). After internal egg quality analyses, the eggshells were collected, and any adhering albumen was removed using absorbent paper. The eggshells were then weighed to determine the eggshell percentage relative to the egg weight. Subsequently, a shell thickness micrometer screw gauge (Mitutoyo Digimatic MDC-MX Series, Mitutoyo) was then used to measure the shell thickness at three different locations (sharp, blunt, and equator), without the shell membranes. The mean shell thickness was obtained from the average of the sharp, equator, and blunt edges. The internal egg quality and eggshell analyses were completed within 24 hours of egg collection.

Statistical analysis

The results were expressed as mean values with standard deviations. Collected data was analyzed for Pearson's correlation coefficients of IBM SPSS Statistics Windows, Version 26 (IBM). Statistical significance was estimated at $p < 0.05$ and the resulting coefficients (r) were interpreted as indicated in Table 1.

Table 1. Interpretation table of the Pearson correlation coefficients[1)]

Correlation coefficient	Interpretation of r
1.00	Perfect positive/negative correlation
± 0.90 to ± 0.99	Very high positive/negative correlation
± 0.70 to ± 0.90	Considerably high positive/negative correlation
± 0.50 to ± 0.70	Moderate positive/negative correlation
± 0.30 to ± 0.50	Low positive/negative correlation
± 0.10 to ± 0.30	Very low positive/negative correlation
± 0.00 to ± 0.10	Negligible positive/negative correlation

[1)]± denotes the direction of the correlation.

RESULTS AND DISCUSSION

In line with changing global trends, greater emphasis and expectation has been placed on overall egg quality, safety, and freshness. A comprehensive understanding of whether the internal egg contents are related to the outer eggshell is needed. On this basis, phenotypic correlations between several internal and eggshell quality indicators were evaluated, and the determined Pearson's correlation coefficients (r) are presented in Table 2. The whole egg weight is an important egg grading determinant and could also influence consumer perceptions regarding the quality and nutritional content of the egg. The weight of eggs is occasionally varied and is determined by hen (genetics and age) and nutritional factors, especially dietary protein and amino acid intake and utilization [13]. Regarding the correlation between egg weight and other egg quality indicators, a low positive correlation was observed between egg weight and shell weight ($r = 0.336$, $p < 0.01$). A moderate negative correlation was found between egg weight and shell percentage ($r = -0.504$, $p < 0.01$). Percent shell is generally decreased as egg weight increases with age [9]. The current observation stresses that there is more inedible shell per unit weight with smaller eggs of lower weight.

Based on the correlation between the height of the thick albumen and egg weight, the Haugh unit is established in the literature as the 'gold standard' internal egg quality indicator based on the freshness and protein content of the egg [12]. Differences in thick albumen height which are based on varying egg weights, are usually corrected in Haugh unit calculations. As expected, a very strong positive correlation was found between the Haugh unit and albumen height ($r = 0.949$, $p < 0.01$). Furthermore, both parameters were not distinctly correlated to the egg weight and the other egg quality indicators. The current observations confirm previous reports [8,10] that Haugh unit values are dependent on albumen height, but independent of other egg quality indicators. Contained inside the vitelline membrane, the egg yolk packs water, lipids, several proteins, and carotenoids that are responsible for the yolk color [2,14]. Yolk color is an important sensory factor that defines consumer preference and perceived health benefits. It was observed that there were no significant correlations between egg yolk color and other egg quality indicators. It is well established that yolk color is known to be directly influenced by dietary intake; higher inclusion levels of xanthophyll-rich ingredients including yellow corn and corn gluten meal could improve yolk color [15].

Several parameters were evaluated for the eggshell quality evaluation including the egg-specific gravity, breaking strength, shell color, shell weight, shell percentage, and the eggshell thickness without the shell membrane at the blunt, equator, and sharp end. The numerous traits were measured for their color, structural soundness and integrity, and texture. The avian eggshell is a porous and multi-layered bioceramic composite mainly composed of 96% polymorphic calcium carbonate in the form of calcite, an organic matrix, and a variety of trace elements [16]. Balanced

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Table 2. Correlation coefficients between internal and external egg quality indicators of 35-week-old laying hens

	Egg weight	Specific gravity	Breaking Strength	Shell color	Albumen height	Haugh units	Yolk color	Shell weight	Shell percentage	Blunt end	Equator	Sharp end	Average shell thickness
Egg weight	1.000												
Specific gravity	–0.109	1.000											
Breaking strength	–0.139*	0.207**	1.000										
Shell color	0.118*	–0.061	–0.150**	1.000									
Albumen height	0.001	0.052	–0.113	–0.038	1.000								
Haugh units	–0.176**	0.076	–0.110	–0.044	0.949**	1.000							
Yolk color	0.189**	–0.100	–0.166**	–0.091	0.245**	0.212**	1.000						
Shell weight	0.336**	–0.057	0.201**	–0.088	0.062	–0.017	0.084	1.000					
Shell percentage	–0.504**	0.048	0.287**	–0.178**	0.047	0.117*	–0.089	0.631**	1.000				
Blunt end	–0.027	0.130*	0.447**	–0.148*	–0.084	–0.087	–0.130*	0.313**	0.305**	1.000			
Equator	–0.031	0.123*	0.428**	–0.148*	–0.066	–0.071	–0.108	0.308**	0.305**	0.969**	1.000		
Sharp end	–0.073	0.150**	0.453**	–0.115*	–0.068	–0.062	–0.150**	0.265**	0.298**	0.830**	0.801**	1.000	
Average shell thickness	–0.047	0.142*	0.465**	–0.144*	–0.077	–0.077	–0.137*	0.308**	0.318**	0.975**	0.965**	0.923**	1.000

*Correlation is significant at the 0.05 level.

**Correlation is highly significant at the 0.01 level.

nutrition with sufficient Ca, P and trace minerals is vital to ensure optimal eggshell quality; and could be improved by several feed additives including probiotics [10]. The shell should preferably be smooth; clean; free of cracks; and thick enough to withstand pressure and allow transportation and storage. The eggshell consists of two shell membranes, a mammillary layer, a palisade layer, a vertical crystal layer, and a cuticle [17]. The eggshell serves to not only balance the demands of protecting the internal contents of the egg from external (mechanical and micro bacterial) invasion; but also allow the exchange of water and gases during embryonic development and easy breakage from inside to allow hatching [18]. The egg-specific gravity specifically captures the quantity of the shell relative to the other egg components and is often quoted to be synonymous with the eggshell thickness and strength [19]. In the current study, very low positive correlations were observed between the egg-specific gravity and the eggshell thickness measured at the blunt, equator, and sharp end (r = 0.130, r = 0.123, r = 0.150, respectively). Contrarily, a significant association between the eggshell thickness and the specific gravity has been reported [20]. Furthermore, very low positive correlations between the egg-specific gravity and the egg-breaking strength (r = 0.207, $p < 0.01$) were observed. Taken together, no distinct correlations were observed between the egg-specific gravity with all the other internal and external egg quality indicators. Furthermore, positive correlations (r = 0.631, $p < 0.01$) were observed between the shell weight and percentage. Very low positive correlations were observed between the eggshell-breaking strength and both the shell weight (r = 0.201, $p < 0.01$), and shell percentage (r = 0.287, $p < 0.01$). Both parameters were determined to be unreliable indicators of other eggshell quality indicators including eggshell thickness, specific gravity, and breaking strength since very low positive correlations were observed.

The importance of eggshell color as an important determinant of consumer preference and its role in photoantimicrobial defense against bacteria is stated [10]. In the current study, brown eggs were analyzed, and no distinct correlations were observed between the eggshell color and the other internal and external quality indicators. It is well-accepted that shell color will largely determine the market acceptability with little or no relation to the overall egg quality. Regarding the eggshell thickness, it was observed that the correlation between the blunt end was the closest to the average eggshell thickness (r = 0.975) as compared to the mid (r = 0.965) and sharp end (r = 0.923). Contrarily, it has been previously reported that the thickness of the sharp end was numerically closest to and more representative of the average thickness [21]. For the correlation between the egg-breaking strength and shell thickness, the correlation coefficients (r) between the average eggshell thickness and the eggshell-breaking strength were positive at 0.465 ($p < 0.01$). Notably, the eggshell thickness was measured at the blunt, mid, and sharp edges to assess the possibility of varied correlation to the egg-breaking strength, as brought about by the characteristic differences in thickness from point to point across the eggshell (Table 3). In the current study, low positive correlations were observed between the eggshell breaking strength and the shell thickness at the blunt edge (r = 0.447, $p < 0.01$), equator (r = 0.428, $p < 0.01$), and sharp edge (r = 0.453, $p < 0.01$). It is evident that the coefficients obtained from the average eggshell thickness could be slightly more indicative of the egg-breaking strength. The current results underline the standard procedure of reporting eggshell thickness from the average thickness of blunt, equator, and sharp end fragments from one egg, as recorded in previous studies [10,13].

Eggshell breaking strength is inherently related to shell quality and generally denotes the ability of eggshell to withstand externally applied force without cracking or breaking. Any approach that aims to improve the quality and safety of eggs is of limited application unless it can safeguard the inner contents of the egg through an improved mechanical breaking strength. The eggshell thickness as well as the egg shape, size, and curvature determine the structural properties of the eggshell-breaking strength [22,23]. Eggshell-breaking strength is also known to be affected

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Table 3. Descriptive statistics of the internal and external egg quality indicators of 35-week-old laying hens

	N	Minimum	Maximum	Mean	SD	CV (%)
Internal egg quality characteristics						
Albumen height (mm)	300	4.100	10.100	7.647	1.608	21.027
Haugh units	300	52.400	109.800	86.971	9.743	11.202
Yolk color	300	6.000	10.000	8.790	0.664	7.552
External egg quality characteristics						
Egg weight (g)	300	37.520	87.190	57.228	6.083	10.630
Egg specific gravity	300	1.060	1.130	1.076	0.070	6.481
Egg breaking strength (kgf/cm^2)	300	2.110	7.550	5.278	0.844	15.996
Shell color	300	17.000	32.000	23.433	2.958	12.622
Shell weight (g)	300	5.190	10.260	7.233	0.792	10.949
Shell percentage (%)	300	8.000	17.510	12.718	1.491	11.721
Eggshell thickness without membrane (mm)						
Blunt end	300	0.210	0.438	0.336	0.033	9.815
Equator	300	0.230	0.456	0.356	0.033	9.214
Sharp end	300	0.227	0.470	0.363	0.036	9.812
Average shell thickness	300	0.227	0.444	0.352	0.032	9.177

N, sample size; SD, standard deviation; CV, coefficient of variation.

by several material factors including the organic and inorganic components of the cuticle, shell membrane, and organic matrix [22]. In the current study, the specific gravity, shell weight, and shell percentage were judged to be unreliable indicators of eggshell-breaking resistance. Furthermore, as previously stated, low positive correlations were observed between the eggshell-breaking strength and the shell thickness. Considering the interplay of the structural and material properties affecting the eggshell-breaking strength, the current results confirm that thicker eggshells does not necessarily translate to higher eggshell-breaking strength, as has been corroborated elsewhere [8]. As outlined by Hincke et al. [16], further focus should be placed on other factors that could directly affect the mechanical breaking resistance property of the eggshell including the mineral components of the eggshell, mineral density, and the spatial architectural arrangement of the ultrastructure (the extent and disposition of major structural eggshell units) and microstructure or texture (the size of crystals and mammillary cones, their shape and crystallographic orientation).

Conclusively, no distinct correlations were observed between appearance traits (yolk and eggshell color) to other internal and external egg quality indicators. Marginal correlations were observed between shell thickness to other eggshell quality parameters including shell breaking strength, shell weight, and shell percentage. Significant correlations were observed between shell percentage and shell weight; shell percentage and egg weight; and Haugh unit and albumen height. Taken together, the present study revealed that no significant correlations exist between eggshell quality and the internal egg contents, suggesting that internal and external quality indicators can be considered independently. The need to develop simple, inexpensive, high-throughput, and non-invasive methods that can easily detect internal egg quality is stressed. This study contributes to understanding the relationship between shell quality and internal egg freshness, providing insights for optimizing egg production and quality assessment systems. The current findings are specific to the Hy-Line Brown hens at 35 weeks of age under enriched cage conditions. Additional research involving different breeds, rearing systems, and broader age ranges is recommended.

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