

The effect of breed and age on the gloss of chicken eggshells

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ABSTRACT The monitoring of eggshell quality is important mainly in terms of production economy. Eggshell appearance is one of the most characteristics, influencing the purchasing behavior of consumers. Besides numerous eggshell appearance quality (color, shape, etc.), gloss is an important trait to reflect the eggshell appearance. In this study, 2 experiments were conducted to investigate the effect of breed and age on the gloss of eggshells. In experiment 1, we compared the eggshell gloss of 7 chicken breeds. In experiment 2, 105 Wanan (WA) chickens were raised, and 1 egg was collected from each individual at 26, 32, 40, and 50 wks of age. Eggshell gloss, color (L^* , a^* , b^*), cuticle coverage (ΔE^*_{ab}), and thickness were measured. The results of experiment 1 showed that

the average gloss values were highly variable among different breeds, and the highest was found in WA (gloss unit [GU] = 8.12), almost 2.5 folds as many as the lowest in Rhode Island Red (GU = 3.23). Also, the eggshell gloss of the local chicken breeds was significantly higher than the highly selected lines of egg-type chicken breeds ($P < 0.001$). In experiment 2, the results showed that gloss ranged from 9.08 GU to 12.12 GU with a variation of 28.38 to 39.71%. It fluctuated with the increasing age of hens and had the peak value at 26 wk. But, the correlation analysis between eggshell gloss and other eggshell quality traits were very low (-0.07 to 0.25). This study laid a foundation for improving the uniformity and intensity of eggshell gloss for breeders.

Key words: gloss, breed, age, chicken eggshell

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INTRODUCTION

Among many quality characteristics, eggshell brightness has gradually become an important trait in the consumer's acceptability of shell eggs. For wild birds, studies showed some avian eggs looked more attractive than others because they had an extremely smooth cuticle (Igic, et al., 2015). Many studies also had shown that wild avian eggs were extremely diverse in glossy appearance (Kilner, 2006). The shininess of the surface can be measured by the gloss (Chadwick and Kentridge, 2015). Gloss represents the specular reflection ability of the materials without the influence of the color (Trezza and Krochta, 2001; Igic, et al., 2015). In the previous

study, we found that there was a strong correlation between eggshell gloss and surface roughness (Li, et al., 2019). It also showed that high roughness could reduce the gloss by increasing the diffuse scattering of light (Trezza and Krochta, 2001). The nanostructure basis of the gloss in avian eggs had been examined (Igic, et al., 2015; Li, et al., 2019). So, it is interesting to explore the gloss as a new measure of eggshell appearance along with other routine traits such as color or shape.

The eggshell quality was influenced by a wide array of factors including the age, genetics, and housing system (Jones, et al., 2002; Ketta and Tůmová, 2016). In the same way, avian eggshell gloss was influenced by several factors such as age and species (Wilson, et al., 1958; Hanley, et al., 2013; Chadwick and Kentridge, 2015; Igic, et al., 2015). All these showed that eggshell gloss was potential to be integrated into the selection index as a moderate heritable ($h^2 = 0.3-0.4$) characteristic to obtain a higher attractiveness of eggs (Icken, et al., 2013; Li, et al., 2019).

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In the current study, we examined the diversity of brightness in 7 chicken breeds and observed the dynamic change in eggshell gloss from 26 to 50 wk. The objective of this study is to investigate the effect of breed and age on the gloss of eggshells and to provide theoretical support to improve the gloss intensity of eggs.

MATERIAL AND METHODS

Ethics Statement

All of the experimental procedures were conducted in accordance with the Guidelines for Experimental Animals established by the Animal Care and Use Committee of China Agricultural University.

Sample Collection

Experiment 1: To explore the influence of long-term selection for egg production on eggshell gloss. We selected 7 different chicken breeds. Three of them were highly selected lines of egg-type chicken breeds, including Rhode Island Red (**RIR**, $n = 30$), White Leghorn (**WL**, $n = 30$), and White Leghorn \times Rhode Island White (**WR**, $n = 30$). Four were Chinese local breeds, including Dongxiang (**DX**, $n = 30$), Rugao Yellow (**RGY**, $n = 30$), Wuding chicken (**WD**, $n = 30$), and Wanan chicken (**WA**, $n = 30$), which had not selected on egg or meat production. Dongxiang, RIR, WR, and WL chickens were raised at the research station of Jiangsu Institute of Poultry Science. Wuding chicken and WA chickens were raised in the Experimental Chicken Farm of Yunnan Agricultural University. All eggs were collected at 45–50 wks of age. The eggshell gloss was measured for all samples.

Experiment 2: At the age of 26, 32, 40, and 50 wks, the eggs were collected from 105 WA chickens on 3 successive days to ensure 1 egg per age point. The eggshell color, gloss, cuticle coverage, and shell thickness were measured for all samples.

To illustrate the relationship between eggshell gloss and cuticle, we compared microstructure attributes of glossy and matte eggshells by scanning electron microscopy (**SEM**) at 32 wks of age in WA chicken.

All eggs were stored in a range of 4–10°C and tested within 3 D.

Measurement of Gloss, Color, Cuticle Coverage, and Thickness

For experiment 1 and 2, a commercial glossmeter (Konica Minolta CM600, Tokyo, Japan) was used to measure the gloss appearance of fresh eggs (Icken, et al., 2013; Li, et al., 2019). Data were collected with the Konica Minolta corresponding software SpectraMagic NX (Konica Minolta, Inc.). The instrument was calibrated with a white standard plate. The larger measurement value of gloss unit (GU) indicated a brighter appearance (Nadal and Thompson, 2000). The gloss of each egg was evaluated

by the mean of 3 points: the blunt end, equator, and sharp end.

Eggshell color was measured with a reflectometer (Minolta CM600) using 3 parameters: L^* , a^* , and b^* . The L^* has a maximum of 100 (white) and a minimum of 0 (black). For a^* , green is toward the negative end of the scale and red toward the positive end. For b^* , blue is toward the negative end and yellow toward the positive end of the scale. Each egg was measured at 3 points (the blunt end, equator, and sharp end), and the eggshell color was determined by the mean of the 3 points.

Cuticle Blue staining (MS Technologies Ltd., UK) was used to assess the amount and degree of the eggshell cuticle coverage. A solution of MST cuticle blue was achieved by immersion of one half of the egg for 1 min. Then, the shell was rinsed in clean water to remove excess stain and allowed to dry in air (Board and Halls, 1973; Samiullah and Roberts, 2013). The cuticle coverage (ΔE_{ab}^*) was estimated with the reflectometer (Konica Minolta CM600) by measuring the color difference (L^* , a^* , and b^*) of the eggshell before and after staining. A single score was calculated by the method as follows (Leleu, et al., 2011):

$$\Delta E_{ab}^* = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (1)$$

The eggshell thickness was measured by a micrometer gauge equipped with a digital display (Mitutoyo293-100, Kawasaki, Japan). It was determined by the mean of 3 points (the blunt end, equator, and sharp end).

SEM Imaging of the Eggshell Surface

Scanning electron microscopy (S-3400 N and SU8010, Hitachi, Japan) was used to observe the surface texture of the eggshells (Roberts, et al., 2013). Glossy (gloss = 13 GU) and matte (gloss = 3 GU) eggshells were used to observe the cuticle texture, and for each egg the piece (1 cm \times 1 cm) was taken around the equator. They were mounted on an aluminum stub and gold sputter-coated using an EIKO IB-3 (EIKO Engineering Co., Ltd., Japan) for about 15 min. Thereafter, they were viewed and photographed under the SEM.

Statistical Analysis

Statistics analysis were performed using R software (version 3.4.0, <https://cran.r-project.org/>). The data obtained were submitted to analysis of variance with the F test. Correlation coefficients between eggshell gloss and other traits were generated using Spearman's rank correlation (r).

RESULTS AND DISCUSSION

Experiment 1

The Comparison of Eggshell Gloss in Different Chicken Breeds The descriptive phenotypes of eggshell gloss of different breeds were summarized in

Table 1. In our study, the average gloss values ranged from 3.23 GU (RIR) to 8.12 GU (WA). The gloss was found to be significantly different among 7 breeds ($P < 0.05$). Additionally, the coefficient of variation values of the gloss in 7 chicken breeds were all relatively large (22.16 to 38.64%), which reflected the absence of directional selection. Igcic et al. (2015) found that eggshell gloss varied in 4 tinamou species. The gloss of tinamou was higher than the chickens. Icken et al. (2012) reported that the eggshell shininess of Rhode Island Red lines was lower than the White Rock lines. Wilson et al. (1958) showed that there was a systematic phenomenon for the gloss to vary with the chicken parentage. These all indicated that the eggshell gloss varied greatly among different species or breeds. In the current study, chickens were divided into 2 groups; the first group was comprised of 4 Chinese local breeds (DX, RGY, WA, and WD), and the second group contained 3 egg-type chicken breeds (WL, RIR, and WR). We found that there was a significant difference in eggshell gloss between the egg-type and local chicken breeds ($P < 0.001$) (Figure 1). Highly selected lines of the egg-type breeds undergone complicated selection process, some important production traits had received most emphasis: egg weight, eggshell strength, body weight, and so on (Hocking, et al., 2003; Moula, et al., 2009). However, the intensive selection of specific trait may reduce others (Emmerson, 1997), such as larger eggs tended to have relatively less yolk (Jaffé, 1964). The selection to improve bone strength would result in less bone resorption (Mcteir, 2006), which meant more calcium was available for the keel bone and less was available for eggshell formation (Stratmann, et al., 2016). Our results showed that eggshell gloss may be influenced by intensive selection of the eggshell quality traits in the high egg production breeds for a long time. In addition, among the highly selected lines of egg-type breeds, the average eggshell gloss of WR chickens was 6.09 GU, closed to RGY's (Table 1). This may be because WR belonged to the hybrid strain. The hybrids had some advantage than their respective parental lines (Yang, et al., 1999). This also indicates the eggshell brightness of the high egg production chicken breeds still has a great potential to be enhanced.

Table 1. The comparison of eggshell gloss among different breeds of chicken.

Breed	N	Gloss (GU)			
		Mean \pm SD	CV(%)	Maximum	Minimum
RIR	30	3.23 \pm 0.88 ^c	27.17	5.33	3.00
WL	30	5.49 \pm 1.22 ^b	22.16	8.67	5.33
DX	30	5.99 \pm 1.88 ^b	31.37	10.33	3.00
WR	30	6.09 \pm 2.10 ^b	35.00	11.33	3.33
RGY	30	6.31 \pm 2.44 ^b	38.64	12.00	3.00
WD	30	6.83 \pm 1.93 ^{a,b}	28.30	10.33	3.00
WA	30	8.12 \pm 2.63 ^a	32.39	13.33	3.33

^{a-c}Traits across a column with different letters are significantly different ($P < 0.05$).

Abbreviations: CV, coefficient of variation; DX, Dongxiang; GU, gloss unit; RGY, Rugao Yellow; RIR, Rhode Island Red; WD, Wuding chicken; WA, Wanan chicken; WL, White Leghorn; WR, White Leghorn \times Rhode Island White.

Experiment 2

Effect of Age on the Eggshell Gloss Wilson et al. (1958) found that the bird eggs gradually became less glossy, usually with a great acceleration at the last egg. They thought that eggshell gloss was affected with the sequence of laying. However, the relationship between the age and the gloss had never been reported in chickens. In our study, there was a summary of the effect of age on chicken eggshell appearance in Table 2. The results showed gloss ranged from 9.08 GU to 12.12 GU with a variation of 28.38 to 39.71% during the test period. The gloss values were higher at the age of 26 wk than that of all other ages. Then, the gloss gradually decreased with age and reached the lowest at 40 wk of age, whereas a slight but a significant increase could be observed at 50 wk ($P < 0.05$). Our results showed the gloss of the eggs fluctuated with the aging of hens. This revealed that the eggshell gloss changed dynamically. It is not clear why the eggshell gloss is higher for the early lay flocks. We found that shell cuticle coverage and color also had changed with age (Table 2). Samiullah also observed an increase of eggshell reflectivity with hen age, which reflected the color of the eggshell became lighter (Samiullah, et al., 2017). This results were also consistent with previous studies that cuticle coverage and eggshell color had been found to be strongly influenced by age (Rodriguez-Navarro, et al., 2013; Bi, et al., 2018). Our results showed that eggshell gloss, like other traits, was strongly influenced by the age of laying hens, but no significant correlation was found between gloss and other traits. Table 3 reflected that the eggshell thickness ($P > 0.05$) and cuticle coverage ($P > 0.05$)

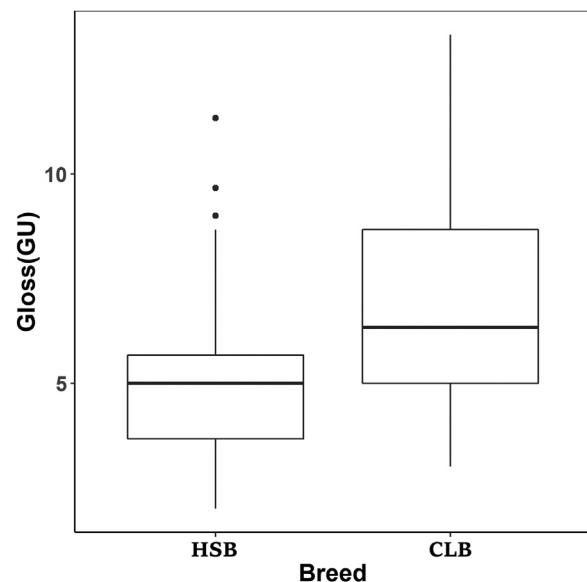


Figure 1. Boxplot of eggshell gloss between the highly selected lines of the egg-type breeds (HSB) and Chinese local breeds (CLB). Highly selected lines contained 3 chicken breeds (WL, RIR, WR). Local breeds contained 4 chicken breeds (DX, RGY, WA, and WD). Abbreviations: DX, Dongxiang; GU, gloss unit; RIR, Rhode Island Red; RGY, Rugao Yellow; WD, Wuding chicken; WA, Wanan chicken; WL, White Leghorn; WR, White Leghorn \times Rhode Island White.

Table 2. Analysis of eggshell gloss, cuticle coverage, color, and thickness at different wks of age.

Variables	Age (wk)			
	26	32	40	50
Gloss (GU)	12.12 ± 3.44 ^a	10.92 ± 3.55 ^b	9.08 ± 3.61 ^c	10.63 ± 2.37 ^b
ΔE^*_{ab}	40.86 ± 6.93 ^a	43.12 ± 6.86 ^a	38.33 ± 9.00 ^b	38.27 ± 7.73 ^b
L*	83.13 ± 5.25 ^c	85.18 ± 4.32 ^b	87.42 ± 3.51 ^a	87.72 ± 5.76 ^a
a*	4.13 ± 0.79 ^a	3.16 ± 0.59 ^b	3.00 ± 0.68 ^b	2.06 ± 0.32 ^c
b*	16.77 ± 3.84 ^a	15.75 ± 4.55 ^a	14.02 ± 3.36 ^b	14.07 ± 4.34 ^b
Thickness(um)	316.20 ± 31.51	321.66 ± 19.97	315.61 ± 31.51	314.66 ± 23.73
N	105	105	105	105

^{a-c}Traits across a row with different letters are significantly different ($P < 0.05$).
Abbreviations: ΔE^*_{ab} , cuticle coverage; GU, gloss unit.

were both weakly correlated with the gloss. The eggshell gloss was also not correlated with the color (L*, a*, and b*), regardless of the time and traits ($r = -0.08 \sim 0.25$).

The eggshell gloss was negatively correlated with the eggshell surface roughness (Li, et al., 2019). The cuticle was the last layer to be deposited on the shell before egg laying (Wilson, et al., 2017). It differed both in composition and thickness and therefore could influence the ultrastructure texture of the eggshell surface (Fecheyr-Lippens, et al., 2015). In our previous study, we observed significant differences between the high- and low-gloss eggshells by SEM and atomic force microscopy (Li et al., 2019). We also found that smoother cuticles could produce better gloss, and the gloss of eggshell decreased significantly when the cuticle was removed. Igic also found that an extremely smooth cuticle produced the glossiness (Igic, et al., 2015). These all indicated that cuticle had a strong influence on the eggshell gloss. In this study, the results showed that the gloss and cuticle coverage had similar tendency for reduction with increasing of age (Table 2). The results of SEM also showed that the glossy eggshell had smoother texture with better cuticle coverage than the matte eggshell (Supplementary Figure 1). Microelement and organic matrix of shell changed with aging of the hens (Britton, 1977; Rodriguez-Navarro, et al., 2002). The content of trace elements and matrix protein also influenced the eggshell mineralization and microstructure (Mabe, et al., 2003; Nys, et al., 2004). We speculated that the eggshell gloss might be influenced by the microstructure change of eggshell surface texture with increasing of age. However, we found that the cuticle coverage was weakly correlated

with the gloss ($P > 0.05$) (Table 3). The possible reason was that the existing cuticle staining method could not assess the cuticle coverage very well because of the unevenly distribution of the eggshell cuticle (Fecheyr-Lippens, et al., 2015). It suggested that gloss of eggshell was not only influenced by the cuticle coverage but also by other factors. However, this suggestion needs to be confirmed.

In this study, we also found that the eggshell gloss value was correlated with 2 adjacent time periods (Figure 2). To be specific, during the egg laying period, the correlation coefficients of gloss were 0.60 ($P < 0.001$), 0.61 ($P < 0.001$), and 0.37 ($P < 0.001$) between the 2 adjacent wks, meanwhile the correlations were moderate between 26 and 40 wk ($r = 0.38$, $P < 0.001$), 26 and 50 wk ($r = 0.42$, $P < 0.001$), and 40 and 50 wk ($r = 0.44$, $P < 0.001$). These indicated that eggshell gloss was a relatively stable trait, even though it had never been selected in chicken.

CONCLUSIONS

In conclusion, our study found that the gloss of eggshell had significant difference among different breeds. Local breeds had definitely advantage than the highly selected lines of egg-type breeds. Meanwhile, we also described the dynamic change of eggshell gloss with the increasing age of hens. It is the first time to explore the effect of age and breed on chicken eggshell gloss which providing theoretical support to use gloss as a new selection trait in chicken breeding programs.

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Table 3. Correlations between eggshell gloss and cuticle coverage, color, and thickness at the same week of age.

Age (wk)	ΔE^*_{ab}	L*	a*	b*	Thickness(um)
26	-0.07	0.21*	-0.25*	0.25	-0.15
32	0.10	0.18	-0.20	0.22	-0.08
40	0.12	0.19	-0.17	0.20	-0.07
50	0.19	0.06	-0.08	0.13	-0.03

ΔE^*_{ab} , cuticle coverage.

*Significant linkage at $P < 0.05$.

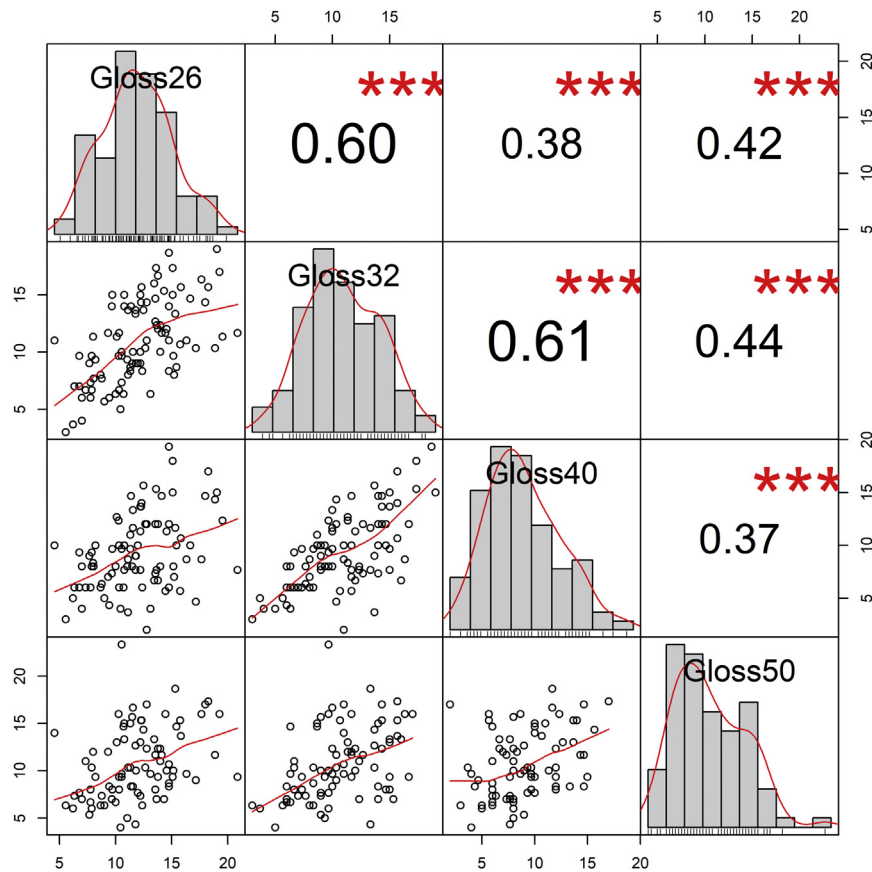


Figure 2. The correlation matrix plot of eggshell gloss among different wks of age. Gloss26, Gloss32, Gloss40, and Gloss50 represent eggshell gloss at different wks of age. On the diagonal are the univariate distributions, plotted as histograms and kernel density plots. On the right of the diagonal are the phenotypic pair-wise correlations, with red stars indicating significance levels (***) $P < 0.001$). On the left side of the diagonal is the scatter-plot matrix, with LOESS smoothers in red to illustrate the underlying relationship. The correlation matrix plot of eggshell gloss between different wks of age was analyzed with R package corrplot.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at <http://doi.org/10.1016/j.psj.2020.01.010>.

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