

Research Note: L*a*b* color space for prediction of eggshell pigment content in differently colored eggs

Lingsen Zeng, Guiyun Xu, Caiyun Jiang, Junying Li, and Jiangxia Zheng¹

National Engineering Laboratory for Animal Breeding and MOA Key Laboratory of Animal Genetics and Breeding, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China

ABSTRACT Eggshell color is the most intuitive external feature that affects consumer acceptance. Different eggshell colors are mainly determined by protoporphyrin IX and biliverdin content. The selection of eggshell color by the human eye with an eggshell color fan can be roughly classified. Although more technologically advanced, a colorimeter or spectrophotometer might be of limited use when observing a variety of shell colors, such as brown, olive, green, and blue. This study aimed to provide a convenient and accurate method for predicting the content of eggshell pigments by measuring eggshell color using the Commission International de L'Eclairage (CIE) L*a*b* system.

The results showed the deepening of eggshell color in brown and blue eggs was correlated with increased eggshell pigment deposition, and eggshell color was significantly positively correlated with eggshell pigment content ($P < 0.01$). Furthermore, the adjusted R^2 of the fitted function was in the range of 0.81 to 0.89, and the Spearman rank correlation coefficient between the predicted and true values was in the range of 0.89 to 0.93. In brief, the L*a*b* method of measuring eggshell color to predict the amount of eggshell pigment deposition is convenient, effective, and promising for layer production.

Key words: eggshell color, prediction of eggshell pigment, protoporphyrin IX, biliverdin

2022 Poultry Science 101:101942
<https://doi.org/10.1016/j.psj.2022.101942>

INTRODUCTION

Eggshell quality is important for the transportation, storage, and processing of eggs. However, eggshell color is one of the most intuitive external features affecting consumer acceptance (Mertens et al., 2010). People in China, France, Great Britain, and Portugal prefer brown eggs, whereas people from the United States, Australia, Sweden, and Spain tend to choose white eggs (Odabasi et al., 2007). Consumers are concerned not only about the shades of eggshell color, but also about the uniformity of the eggshell color (Arthur and O'Sullivan, 2005). Therefore, the quality of eggshell color both affects the market and price of eggs and also affects the economic efficiency of the producer.

The eggshell color is mainly composed of pigment components, such as protoporphyrin IX, biliverdin, a small amount of zinc chelates of biliverdin, fecal porphyrins, uroporphyrins, zinc chelates of protoporphyrin

IX, and other unidentified porphyrins. Protoporphyrin IX is responsible for brown eggshells, whereas biliverdin and protoporphyrin IX result in blue eggshells (Kennedy and Vevers, 1973; With, 1974; Lang and Wells, 1987; Ito et al., 1993; Miksik et al., 1996; Mertens et al., 2010;). Protoporphyrin IX and biliverdin are synthesized in the eggshell gland and later secreted and deposited in the outermost layer of the eggshell (Lang and Wells, 1987; Wang et al., 2007). Thus, the shade of the eggshell color is directly decided by the amount of eggshell pigment deposited.

Although intense and uniform eggshell colors have been selected abroad in commercial white- and brown-shelled layers for several decades, blue eggshells have not been intensively selected (Cavero et al., 2012; Hutton, 1962). Eggshell color is mainly selected by grading using eggshell color fans, and color parameters are measured using a spectrophotometer. However, the accuracy of eggshell color fan grading is not high, and selection based on L*a*b* parameters requires a composite indicator to reflect specific differences in eggshell color, especially for eggs of similar color. The present study is the first to predict the amount of eggshell pigment using the fast and easy-to-use CIE L*a*b* system, which will help improve the genetic progress and accuracy of eggshell color selection.

© 2022 The Authors. Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Received March 6, 2022.

Accepted April 25, 2022.

¹Corresponding author: jxzheng@cau.edu.cn

MATERIALS AND METHODS

Collecting Samples

In this study, 171 brown eggs were obtained from 40-wk-old Dwarf layer chickens, 164 blue eggs were obtained from 40-wk-old Dongxiang blue-shelled chicken, and one egg was collected per chicken. All birds were kept in single cages in the National Center of Performance Testing of Poultry (Beijing, China), and the study complied with the guidelines for experimental animals established by the Animal Care and Use Committee of China Agricultural University (permit number AW32202202-1-1).

Sample Preparation and Measurement of Indicators

Eggs were processed within one week of collection and stored at room temperature (18–20°C, 45% RH) during preservation. Each egg was numbered and weighed (**EW**). Color was measured at the blunt, middle, and sharp ends of the eggshell, using a spectrophotometer (KONICA MINOLTA CM-700d, Konica Minolta, Inc., Tokyo, Japan). The $L^*a^*b^*$ values were recorded and their means were calculated. Subsequently, the eggshell was broken and rinsed. The eggshell pieces were heat-dried in a forced-air drying oven (AC 220V \pm 10%, 50Hz \pm 2%, BGZ-140 Electric Heat forced-air drying oven, Shanghai Boxun Industry & Commerce Co., Ltd., Shanghai, China) at 75°C for 12 h. Weighed dried eggshells (**ESW**) were ground to powder in a mortar. Then, 0.25 g of the powder dissolved in 4 mL of preprepared solution (volume of methanol: volume of concentrated HCl [Vmethanol: Vconcentrated HCl] = 2:1) was added into a 15 mL centrifuge tube. The solution was kept at room temperature (18–20°C) for 12 h, dark area to extract the eggshell pigments. After centrifugation at 1,369.55 \times g for 45 min, 200 μ L of supernatant was used to measure the absorbance at 412 nm and 670 nm using an Epoch 2 microplate spectrophotometer (BioTek Instruments, Inc., VT), with 2 replicates for each sample, and the average value was calculated. The absorbance at a wavelength of 412 nm represents the content of protoporphyrin IX (Ito et al., 1993), and the absorbance at a wavelength of 670 nm represents the content of biliverdin (Zhao et al., 2006).

Developing Standard Curve to Define Sample Concentration

To prepare the standard solution, protoporphyrin IX (3.6 mg, Aladdin Reagent [Shanghai] Co., Shanghai, China) was dissolved in 6 mL of solvent (Vmethanol: Vconcentrated HCl = 2:1) added beforehand into a 15 mL centrifuge tube, vortexed, and placed in darkness for 12 h to dissolve completely. Biliverdin (0.25 mg, Aladdin Reagent) was prepared in the same manner. Then, the $2^0 \times$ protoporphyrin standard solution (1.07×10^{-3} mol/L) and $2^0 \times$ biliverdin standard

solution (6.36×10^{-5} mol/L) were prepared. Protoporphyrin standard solution (3 mL) was added to a 15 mL centrifuge tube containing 3 mL of solution (Vmethanol: Vconcentrated HCl = 2:1), vortex-oscillated to obtain $2^{-1} \times$ protoporphyrin standard solution. The rest of the solution was deduced by analogy; $2^{-2} \sim 2^{-9} \times$ protoporphyrin standard solution and $2^{-1} \sim 2^{-9} \times$ biliverdin standard solution were prepared. Then, the absorbance of the protoporphyrin standard solution (X_1) was measured at a wavelength of 412 nm, and the absorbance of biliverdin standard solution (X_2) was measured at a wavelength of 670 nm. According to the standard curve, the protoporphyrin concentration (Y_1) and biliverdin concentration (Y_2) of the sample were calculated using a linear regression equation (Wang et al., 2009).

Statistical Analysis

The quantities of true protoporphyrin IX (equation [1]) and biliverdin (equation [2]) in 1 g of egg weight (Q_p and Q_b , respectively; nmol/g) were determined as follows:

$$Q_p = (Y_1 \times 0.004L \times ESW) / (0.25g \times EW) \quad (1)$$

$$Q_b = (Y_2 \times 0.004L \times ESW) / (0.25g \times EW) \quad (2)$$

Where 0.004 L represents the 4 mL of solvent used to dissolve the rinsed eggshell, 0.25 g represents the 0.25 g rinsed eggshell; ESW represents the weight of heated-dried eggshell, and EW represents the weight of the full egg. In this experiment, blue and brown eggs were divided into different groups. Only protoporphyrin IX was detected in brown eggs, whereas both protoporphyrin IX and biliverdin were detected in blue eggs.

Standard curves for calculating protoporphyrin IX (equation [3]) and biliverdin (equation [4]) concentrations were fitted using R, as follows:

$$Y_1 = 7.499 \times 10^{-6} X_1 - 4.968 \times 10^{-7} \text{adjusted } R^2 = 0.9997 \quad (3)$$

$$Y_2 = 3.81 \times 10^{-5} X_2 - 1.409 \times 10^{-6} \text{adjusted } R^2 = 1 \quad (4)$$

In the equations, Y_1 and Y_2 represent the protoporphyrin IX and biliverdin concentrations of the sample, respectively; X_1 and X_2 represent the absorbance of the sample at wavelengths of 412 nm and 670 nm, respectively. Both equations were assessed using adjusted R-squared (adjusted R^2); adjusted $R^2 > 0.99$. The regression coefficients for both equations were extremely significant ($P < 0.001$).

Standard curve fitting, correlation analysis, and regression equation derivation for eggshell pigment prediction were performed using the previously established R 4.0.2 procedure (<https://www.r-project.org/>). A three-dimensional view of eggshell color was drawn using OriginPro 2021 (<https://www.originlab.com>).

RESULTS AND DISCUSSION

The correlation coefficients for eggshell color in brown and blue eggs are shown in Figure 1. EW and ESW did not correlate strongly with either eggshell color (L^* , a^* , and b^* values) or eggshell pigment amount (Q_p , Q_b ; $P > 0.05$). EW and ESW have been reported as two of the main factors affecting eggshell color (Odabasi et al., 2007), contrary to our and other researchers' correlation results (Yang et al., 2009; Bi et al., 2018). In addition, eggshell pigmentation was highly correlated with the 3 indicators of eggshell color ($P < 0.01$). Based on the results of the whole correlation, we infer that the amount of eggshell pigment deposited has a much greater effect on eggshell color than either EW or ESW. Similarly, the amount of protoporphyrin IX, as determined by Bi et al. (2018), directly determines the eggshell color. Interestingly, the correlation between biliverdin and protoporphyrin in blue eggs was 0.81, which is consistent with the correlation between eggshell pigments in different layers of the eggshell (Wang et al., 2007). Although the intensity and evenness of eggshell pigment disposition are affected by metabolic factors (Samiullah et al., 2015), predicting the amount of eggshell pigment by measuring eggshell color will offer convenience and accuracy for selecting and breeding eggshell color.

In this study, 3 equations for prediction of protoporphyrin IX (\hat{Q}_p , equation [5] and [6]) and biliverdin content (\hat{Q}_b , equation [7]) were fitted using L^* , a^* , and b^* values with interactive items using R 4.0.2, as follows:

$$\begin{aligned} Q_p &= 1.042 + 0.0137 La + 0.0763 ab \\ &\quad - 0.0014 Lab \text{ adjusted } R^2 \\ &= 0.89 \end{aligned} \quad (5)$$

$$\begin{aligned} Q_p &= -15.2355 + 0.1590 L - 2.1220 a + 1.9781 b \\ &\quad + 0.0200 La - 0.0194 Lb \\ &\quad + 0.0421 ab \text{ adjusted } R^2 \\ &= 0.81 \end{aligned} \quad (6)$$

$$\begin{aligned} Q_b &= -27.3314 + 0.2973 L - 4.1493 a + 1.5234 b \\ &\quad + 0.0396 La - 0.0150 Lb \\ &\quad + 0.0392 ab \text{ adjusted } R^2 \\ &= 0.81 \end{aligned} \quad (7)$$

We predicted the content of protoporphyrin IX and biliverdin (only in blue eggs) using regression functions; equations [5] and [6] for the prediction of protoporphyrin IX content are only applicable to brown and blue eggs, respectively, and equation [7] is suitable for predicting biliverdin content of blue eggs. The adjusted R^2 of the fitted function was between 0.81 and 0.89, which means that eggshell color can explain the magnitude of variation in eggshell pigmentation. In this experiment, the adjusted R^2 did not reach 0.9, probably because of the deposition process of not only the eggshell pigments but also other eggshell ingredients, such as calcium and phosphorus, using layer-by-layer deposition procedures. It is well known that processes of eggshell mineralization and pigmentation deposition occur in the uterus; the amounts of pigments (containing protoporphyrin IX and biliverdin) deposited in different layers of the eggshell are significantly different, with the outermost layer containing more pigments than the inner layers of the eggshell (Wang et al., 2007), and eggshell color values

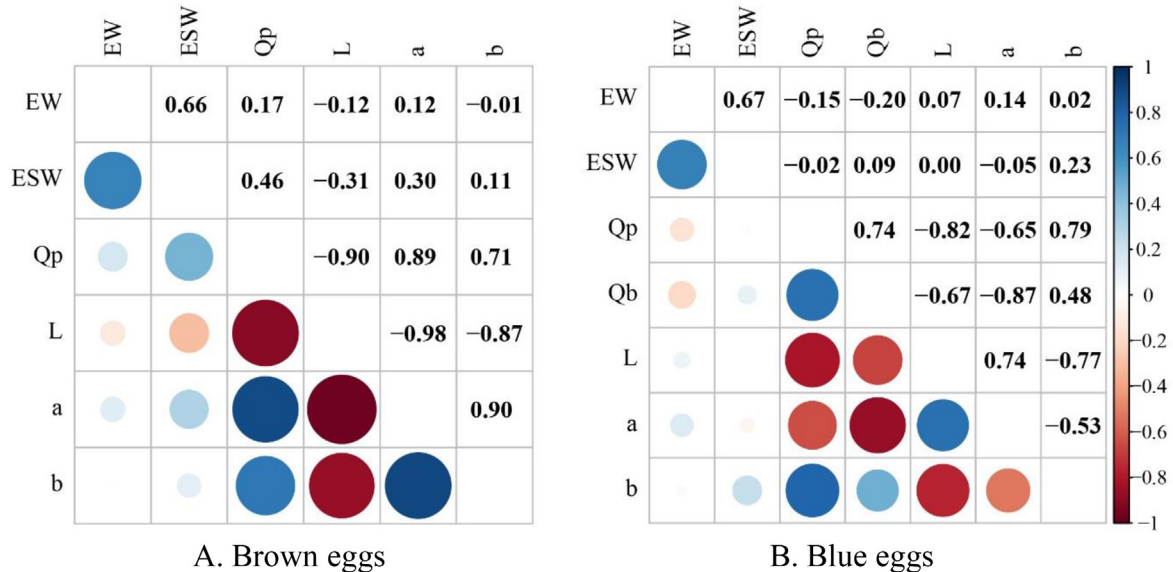


Figure 1. Eggshell color in brown eggs (A) and blue eggs (B) correlation coefficients. Color and size represent correlation, both darker color and larger size mean the greater correlation. Blue represents positive correlation and red represents negative correlation. Abbreviations: EW, egg weight; ESW, eggshell weight; Q_p , quantity of protoporphyrin; Q_b , quantity of biliverdin; L, lightness; a, redness; b, yellowness.

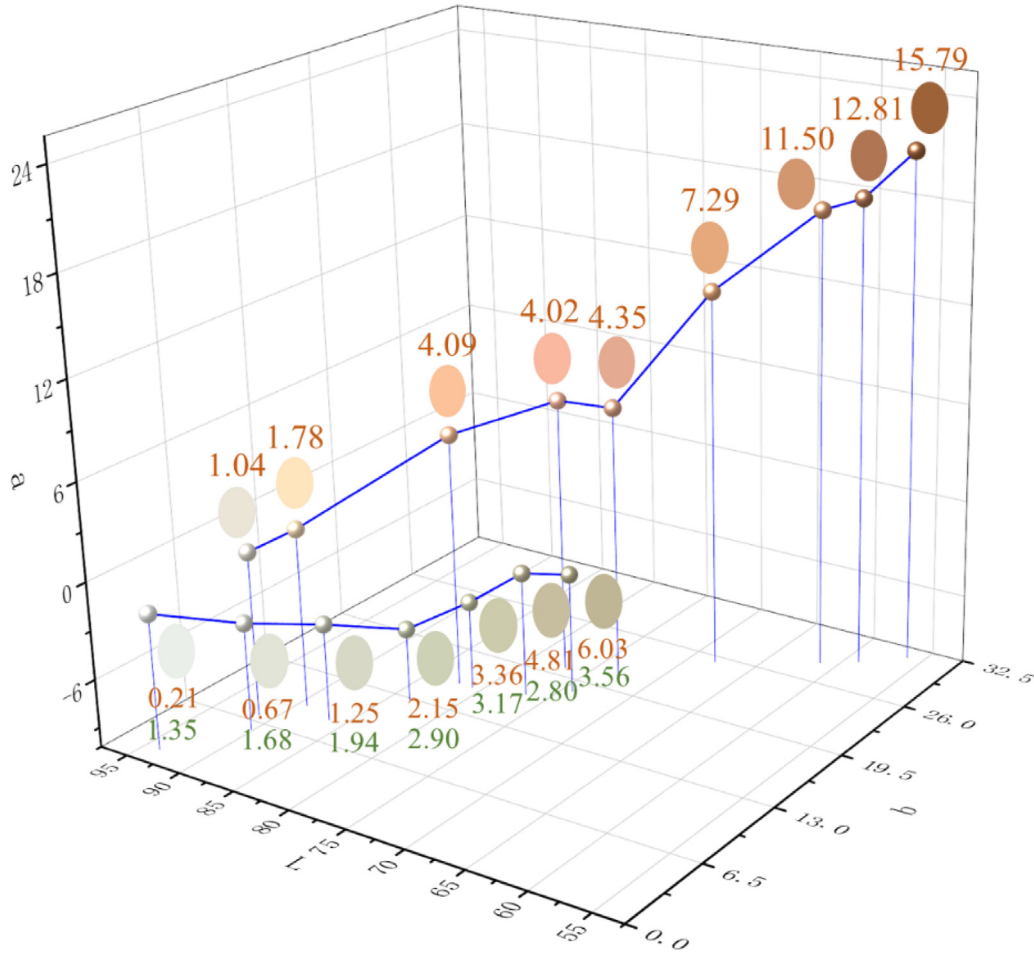


Figure 2. Variation of eggshell color in brown eggs and blue eggs with $L^*a^*b^*$ values. Brown eggshell colors got darker and predicted eggshell pigments showing an increasing trend with lower L^* value and higher a^* and b^* value. However, only the variation of a^* value in blue eggs was inconsistent. The simulated egg adjacent to each data point is the corresponding eggshell color. Brown values represent predicted protoporphyrin IX contents and green values represent predicted biliverdin contents.

are determined by the amount of pigment deposited on the eggshell surface. In addition, the Spearman rank correlation coefficients of the predicted values for the 3 fitted functions and true value were 0.89 to 0.93, which indicated high accuracy of the prediction results. However, hen-to-hen variability can also impact egg parameters. Although in the current study we used single housed hens, the correlation may have been greater if a series of eggs from individual hens were compared, thus removing the hen-to-hen variability that many researchers cannot overcome owing to communal housing of hens. [Lukanov et al. \(2015\)](#) assessed indicators describing the color characteristics, such as the eggshell color index (SCI), Hue° angle (h°), and chroma (C^*) of eggshells ([Petracci and Baéza, 2019](#)). The authors argued that SCI is only suitable for describing brown and non-pigmented eggs, C^* is dominated by the b^* value, and h° is not suitable for color analysis of eggshells because of the large variations and the possibility of obtaining similar values but with different signs in eggs with similar colors ([Petracci and Baéza, 2019](#)). Methods such as SCI based on the $L^*a^*b^*$ space are efficient but also share the above problems. In contrast, eggshell pigment experiments are accurate but inefficient. In our study,

the advantages of both methods were combined to achieve accurate and efficient determination of eggshell color.

This study predicted the eggshell pigment content for different eggshell colors based on the fitted model above, as shown in [Figure 2](#). Darker eggshell colors with a brown appearance were correlated with lower L^* values, higher a^* and b^* values, and higher amount of deposited protoporphyrin IX. Eggshell color deepening to blue was correlated with increased deposition of biliverdin and protoporphyrin IX, biliverdin content exceeding the protoporphyrin IX content, decreased L^* and a^* values, and increased b^* values. Blue-colored eggs with small amounts of red were correlated with deeper eggshell colors, lower biliverdin content compared to protoporphyrin IX content, increased protoporphyrin IX deposition, and increased a^* values. The difference in protoporphyrin IX content in blue eggs might be affected by weeks of age and blue shell breeds and their different cross patterns with white or brown eggshell breeds, such as White Leghorn or Rhode Island Red. The model in this study accurately predicted the pigment content of eggshells, which could improve eggshell color uniformity and selection accuracy.

In summary, this study used the eggshell color L^* , a^* , and b^* values to predict the amount of eggshell pigment deposition, which provides an effective and rapid method for eggshell color selection in the breeding of egg-laying hens.

ACKNOWLEDGMENTS

This study was supported by the China Agriculture Research Systems (CARS-40), the National Key Research and Development Program of China (2021YFD1200803), and the Program for Changjiang Scholars and Innovative Research Team in University (IRT_15R62).

DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could appear to influence the work reported in this paper.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.psj.2022.101942](https://doi.org/10.1016/j.psj.2022.101942).

REFERENCES

- Arthur, J. A., and N. O'Sullivan. 2005. Breeding chickens to meet egg quality needs. *Int. Hatchery Pract.* 19:7–9.
- Bi, H., Z. Liu, C. Sun, G. Li, G. Wu, F. Shi, A. Liu, and N. Yang. 2018. Brown eggshell fading with layer ageing: dynamic change in the content of protoporphyrin IX. *Poult. Sci* 97:1948–1953.
- Cavero, D., M. Schmutz, W. Icken, and Preisinger R. 2012. Attractive eggshell color as a breeding goal. 47:15-21.
- Hunton, P. 1962. Genetics of egg shell colour in a light sussex flock. *Br. Poult. Sci* 3:189–193.
- Ito, S., M. Tsudzuki, M. Komori, and M. Mizutani. 1993. Celadon: an eggshell color mutation in Japanese quail. *J. Hered* 84:145–147.
- Kennedy, G. Y., and H. G. Vevers. 1973. Eggshell pigments of the Araucano fowl. *Comp. Biochem. Physiol. B* 44:11–25.
- Lang, M. R., and J. W. Wells. 1987. A Review of eggshell pigmentation. *Worlds Poult. Sci. J.* 43:238–246.
- Lukanov, H., A. Genchev, and A. Pavlov. 2015. Colour traits of chicken eggs with different eggshell pigmentation. *Trakia J. Sci.* 13:149–158.
- Mertens, K., I. Vaesen, J. Loffel, B. Kemps, B. Kamers, C. Perianu, J. Zoons, P. Darius, E. Decuyper, J. De Baerdemaeker, and B. De Ketelaere. 2010. The transmission color value: a novel egg quality measure for recording shell color used for monitoring the stress and health status of a brown layer flock. *Poult. Sci* 89:609–617.
- Miksik, I., V. Holan, and Z. Deyl. 1996. Avian eggshell pigments and their variability. *Comp. Biochem. Physiol. B* 113:607–612.
- Odabasi, A. Z., R. D. Miles, M. O. Balaban, and K. M. Portier. 2007. Changes in brown eggshell color as the hen ages. *Poult. Sci* 86:356–363.
- Petracci, M., and E. Baéza. 2019. Harmonization of methodologies for the assessment of poultry meat quality features. *World Poult. Sci. J* 67:137–153.
- Samiullah, S., J. R. Roberts, and K. Chousalkar. 2015. Eggshell color in brown-egg laying hens - a review. *Poult. Sci* 94:2566–2575.
- Wang, X. T., X. M. Deng, C. J. Zhao, J. Y. Li, G. Y. Xu, L. S. Lian, and C. X. Wu. 2007. Study of the deposition process of eggshell pigments using an improved dissolution method. *Poult. Sci* 86:2236–2238.
- Wang, X. T., C. J. Zhao, J. Y. Li, G. Y. Xu, L. S. Lian, C. X. Wu, and X. M. Deng. 2009. Comparison of the total amount of eggshell pigments in Dongxiang brown-shelled eggs and Dongxiang blue-shelled eggs. *Poult. Sci* 88:1735–1739.
- With, T. K. 1974. Porphyrins in egg shells. *Biochem. J* 137:597–598.
- Yang, H. M., Z. Y. Wang, and J. Lu. 2009. Study on the relationship between eggshell colors and egg quality as well as shell ultrastructure in Yangzhou chicken. *Afr. J. Biotechnol* 8:2898–2902.
- Zhao, R., G. Y. Xu, Z. Z. Liu, J. Y. Li, and N. Yang. 2006. A study on eggshell pigmentation: biliverdin in blue-shelled chickens. *Poult. Sci* 85:546–549.