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Full Length Research Paper

Study on the relationship between eggshell colors and egg quality as well as shell ultrastructure in Yangzhou chicken

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Experiments were conducted to evaluate the relation between eggshell colors and egg quality as well as shell ultrastructure in Yangzhou chicken. A total of 120 eggs on the day of lay of New Yangzhou chickens were selected. The eggshell color, shape index, shell strength, shell thickness, egg weight, albumen weight, yolk weight, shell weight, yolk color, calcium content of shell, albumen and yolk were measured. Then the ultrastructure of transect and outer shell was observed through scan electron microscopy. The results showed that the tenderness of shell strength and shell thickness reduced as the shell color became shallow, however the pores in the shell and shape index increased. There were significant correlations between shell color and shell strength, shell thickness, and shell weight (P < 0.05). There were no distinct correlations between shell color and egg weight, albumen weight, yolk weight, haugh unit, yolk color, calcium content in albumen and yolk (P > 0.05). It was concluded that some egg quality traits such as shell strength, shell thickness, shell weight and shell ultrastructure could be assessed through the shell color.

Key words: Shell color, egg quality, ultrastructure, Yangzhou chicken.

INTRODUCTION

Eggshell quality is a major concern of commercial egg producers and breeder managers. The loss of eggs due to poor shell quality results in a loss of revenue for these producers. Eggs that are damaged during routine handling and transport to retail outlets cause major financial losses to the egg industry (Hunton, 1995). Clean, intact eggshells are also required to ensure consumer satisfaction and dietary safety. Improving overall eggshell quality would have a significant economic impact on the industry.

Eggshell quality is largely based on two characteristics of the shell: its strength and its color. The mechanical properties of eggs depend on geometric variables such as the shape and thickness of the shell combined with the shell's fundamental material properties. The material properties of the shell depend on its microstructure and chemical composition, both of which vary through the shell strength (Rodriguez et al., 2002; Nys et al., 2004). In addition, Shell color is significantly correlated to shell quality (Ingram et al., 2008).

Although shell color is not an indication of internal quality of eggs (Odabasi et al., 2006), consumers in some markets throughout the world prefer brown eggs to white eggs. Shell color intensity within each country, such as Chinese, Japanese, etc, is dictated by consumer preference. Shell color, researched by many investigators, is a trait controlled by many genes. The major gene controlling shell color, is found, and then gradually applied to genetic breeding in poultry (Bartlett et al., 1996; Bitgood et al., 2000; Moreno et al., 2004; Zhang et al., 2005; Juan et al., 2006; Zhao et al., 2006).

In this experiment, the relationship between shell color and egg quality as well as shell ultrastructure was studied so as to improve egg quality and poultry breeding.

MATERIALS AND METHODS

Stock, husbandry

A total of 120 eggs on the day of lay of New Yangzhou chickens were obtained directly from the cage fronts of a brown egg-laying flock. The layers were at about 51 weeks of age, and each of them

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Table 1. The change of egg quality traits with different shell colors.

Shell color	Egg weight(g)	Albumen weight(g)	Yolk weight(g)	Shell weight(g)	Haugh unit	Yolk color
30-34.9	50.06±2.90a	26.57±2.93a	17.19±2.35a	6.30±0.51a	63.75±15.44a	7.92±1.55a
35-39.9	50.09±3.25a	27.30±3.40a	16.69±1.63a	6.16±0.58a	60.68±12.29a	7.05±1.17a
40-44.9	50.35±2.14a	27.23±2.58a	16.97±1.58a	6.15±0.57a	61.68±12.32a	7.42±1.31a
45-49.9	50.21±3.39a	27.03±3.42a	17.27±1.64a	5.91±0.46a	58.06±12.92a	7.91±1.31a
≥50	54.32±5.61b	32.12±6.34b	16.30±1.51a	5.90±0.14a	62.73±12.26a	8.25±0.50a

a, b: Means within a row with different superscript were significantly different (p<0.05).

was kept in a layer cage (48 × 42 × 45 cm). The nutrient levels of the corn-soybean based diet used to feed the hens are: 2800 kcal/kg metabolizable energy, 18% crude protein, 3.4% calcium, 0.38% available phosphorus, 0.37% methionine, 0.65% methionine + cystine and 0.78% lysine.

Shell color, shell strength, shell thickness, albumen height, and haugh unit

Shell color, shell strength, shell thickness, albumen height and haugh unit (Haugh, 1937) were determined and recorded by a TSS QCM+ system (Technical Services and Supplies, Dunnington, York, UK). Shell color reflectometer was taken as percentage reading to 0 and 81.8% using black and white board, respectively. The micrometer electronically measures the thickness of the shell in microns with the results being entered into the test. Electronic height gauge measures albumen height. Haugh unit is automatically calculated within the system on the input of egg weight and albumen height (TSS, Dunnington, York, UK).

Egg weight, shell weight, albumen weight, and yolk weight

The unit mass weight of egg, shell, albumen and yolk of each egg was weighed with an electronic balance to the nearest 0.001 g.

Shape index

Length (L) and width (W) of eggs were measured with a digital calliper to the nearest 0.01 mm. Shape index was determined using the following equation (Anderson et al., 2004):

$$\text{Shape index} = (W/L) \times 100$$

Yolk color

Yolk color was measured using the Roche Yolk Color Fan (DSM, Basel, Switzerland).

Chemical analysis

Calcium (Ca) was determined by atomic absorption spectrophotometry according to the methods of the Association of Official Analytical Chemists (1980).

The middle shells of three different colors were selected to observe the ultrastructure of outer and transect by scan electron microscopy. All of the specimens for scanning electron microscopy were mounted on Al stubs, coated with gold Pd, and examined at a working distance of 15 mm using a Hitachi S570 scanning electron microscope (Hitachi Ltd., Tokyo, Japan) operating at 15 kv.

Statistical analysis

The data were analyzed by one-way analyses of variance and linear regression. Differences in the correlation coefficients of properties were determined, and significance tests were applied, using correlate of SPSS 13.0. Statements of significance were based on P < 0.05, unless otherwise stated.

RESULTS

Changes of weight in every part of egg, haugh unit, and yolk color in different shell colors

The egg weight was relatively weighty when shell color was 40-44.9. Egg weight appeared, decreasing tenderness when shell color was little deeper or less than this range (Table 1). However, the egg weight was heaviest when shell color was more than 50, and there were significant differences between it and other groups (P<0.05). Albumen weight declined with the shell color reducing when the shell color was 35-49.9, but it was weightier when shell color was more than 50, and there were distinct differences between it and other groups (P<0.05). The variational rule in yolk weight was not obvious, and there were no significant differences between them (P>0.05). The shell weight was bigger when shell color was deeper, but there were no marked diffe-rences between them (P>0.05). The mutative rule of Haugh unit and yolk color in different shell colors was not obvious, and there were no significant differences between them (P>0.05) (Table 1).

Changes of shell strength, shell thickness and shape index in different shell colors

Shell strength weakened when the shell color became more and more shallow. The shell strength was the hardest when shell color was 30-34.9, and there were significant differences between it and other groups (P<0.05). The change tendency in shell thickness was similar with that of shell strength. The shell thickness was the largest when the shell colors were 30-34.9, and there were significant differences between it and other groups (P <0.05). However, the shell thickness was the least when shell colors were more than 50, and there were significant diff-

Table 2. Egg shell strength, shell thickness, and shape index of different shell colors.

Shell color	Shell strength(g/cm^2)	Shell thickness(mm)	Shape index
30-34.9	4.849±0.431b	0.351±0.017c	73.10±1.81b
35-39.9	4.143±0.257a	0.326±0.021b	73.31±2.12b
40-44.9	4.127±0.293a	0.324±0.022b	73.42±1.39b
45-49.9	4.093±0.281a	0.307±0.022ab	73.42±1.95b
≥50	4.008±0.534a	0.297±0.042a	76.98±1.21a

a, b, c: Means within a row with common superscript were significantly different (p<0.05).

Table 3. The calcium content in every part of eggs with different shell colors (%).

Shell color	Eggshell	Albumen	Yolk
30-34.9	40.61±2.51	0.34±0.04	1.11±0.16
35-39.9	40.31±1.87	0.24±0.02	1.29±0.12
40-44.9	39.72±2.62	0.28±0.06	1.07±0.08
45-49.9	39.13±3.57	0.27±0.05	1.06±0.11
≥50	39.09±3.47	0.28±0.09	1.09±0.09

Table 4. Correlation coefficients between shell color and egg quality traits.

Egg quality properties	Shell color
Shape index	0.133
Shell strength	-0.262*
Shell thickness	-0.443**
Shell weight	-0.255*
Yolk weight	-0.062
Albumen weight	0.045
Haugh unit	0.191
Yolk color (DSM)	0.160

*P < 0.05; **P < 0.01.

erences between it and other groups, having shell colors less than 40 (P <0.05). The bigger the shape index was, the shallower the shell color was. It was the biggest when shell color was more than 50, and there were significant differences between it and other groups (P <0.05) (Table 2).

Calcium content in every part of eggs in different shell colors

The calcium content of shell reduced when the shell color became more and more shallow. However, there were no significant differences between the groups in different colors (P>0.05). On the other hand, the mutative rule of calcium content in albumen and yolk was not distinct, and there were no significant differences between every group (P>0.05) (Table 3).

Correlation coefficients between shell colors and egg quality traits of chicken eggs

Correlation coefficients between shell colors and egg quality traits of chicken eggs are shown in Table 4. There were significant correlations between shell colors and shell strength (-0.262), shell color and shell weight (-0.255), shell color and shell thickness (-0.443). However, there were no distinct correlations between shell color and shape index, yolk weight, albumen weight, Haugh unit, and yolk color (P>0.05) (Table 4).

Ultrastructure of different shell color

The shells having colors of 45.5, 38.5 and 30.5 were selected to observe their ultrastructure (Figure 1). Figures 1a-c were transverse shells with colors of 45.5, 38.5 and 30.5, respectively were enlarged 400 times. The transverse shells, orderly, were seen from inside to outside, which were made from film layer, cone layer, columnar layer, and surface. The thickness of shell in Figure 1a was thinner than that in Figures 1b and c. Videlicet, the thinner the thickness of the shell, the shallower the shell color was. The reason was that the cone layer and columnar layer correspondingly shortened.

Figures 1d-f were outer shells with color of 45.5, 38.5 and 30.5, respectively, which were enlarged 625 times. From these figures, the turtle-shaped cracks and many pores on the shell could be seen. The number of pores in the shell in Figure 1d was the most, but that in Figure 1f was the least. In other words, the number of pores in shallow shell was more than that in deep shell.

DISCUSSION

Correlation between shell colors and egg quality traits of chicken eggs

The color was correlated with shell strength, shell weight and shell thickness, however, it was interconnected with shape index, yolk weight, albumen weight, Haugh unit, and yolk color (Tables 1, 2 and 3). They could be observed form egg formation.

The deposition process of brown shell pigments has been reported by Baird et al. (1975). The shell is formed

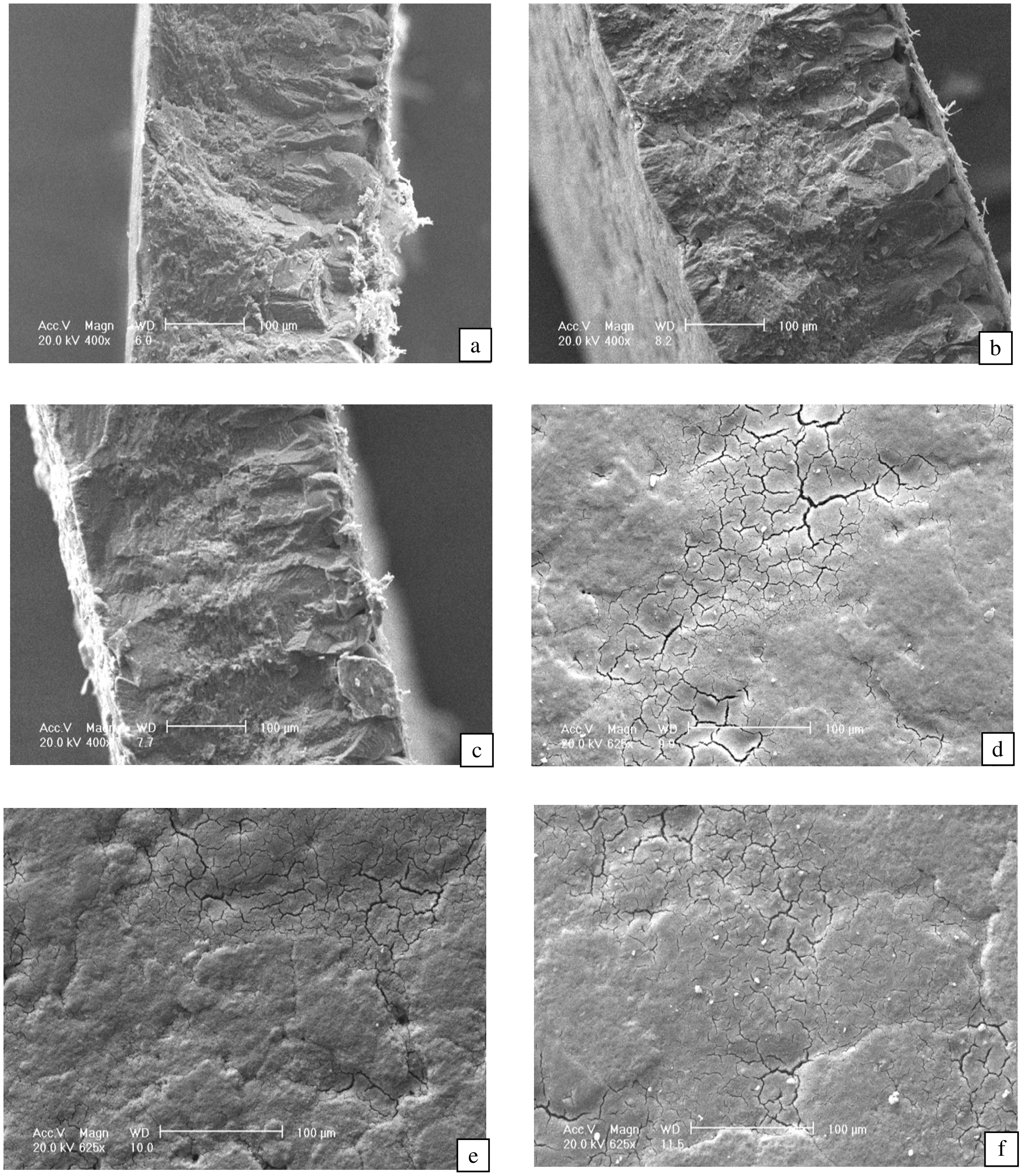

Figure 1. a. Transverse shell of color 45.5 × 400; b. transverse shell of color 38.5 × 400; c. transverse shell of color 30.5 × 400; d. outer shell of color 45.5× 625; e. outer shell of color 38.5 × 625; f. outer shell of color 30.5 × 625.

during passage of the egg through the oviduct, with the various layers of the shell assembled sequentially as the egg passes through the successive sectors of the oviduct. After fertilization of the ovum in the infundibulum and secretion of albumen in the magnum, they deposit on the surface of egg. Different parts of eggs are formed in different places. Egg enters the isthmus 2 to 3 h after ovulation. In the isthmus, the granular cells secrete various components of the shell membranes such as collagen type X (Arias et al., 1991, 1997). Most of the calcium deposition in the eggshell occurs in the shell gland (Creger et al., 1976; Stemberger et al., 1977). During the latter egg formation, there is no connection between shell color and albumen weight, yolk weight, such that these factors were not distinct (Table 1). However, the shell weight and shape index reduced, while shell strength, shell thickness increased, with the shell color shallower (Tables 2 and 3).

Shell color formation

Brown shells get their color from a substance called protoporphyrin that comes from the breaking down of hemoglobin in blood. This pigment is deposited on the surface of an otherwise white egg as the egg is formed, and can be rubbed off with sandpaper or dissolved off with vinegar. Deposited on a white shell, the egg appears brown; on a blue shell, it appears green. Zhao et al. (2006) found that biliverdin should be synthesized in shell gland and then deposited onto the shell of chickens. Wang et al. (2007) inferred that both biliverdin and protoporphyrin in shell were synthesized in shell gland and then were deposited to shell simultaneously, and the deposition rate of them both reached the maximal level at the end of shell formation. This study found that shell colors of New Yangzhou chickens were mainly 30 - 50, shell color of few eggs were less than 30 or more than 50.

Shell color significantly affected shell strength, shell weight, and shell thickness. The deep color shell may be due to the long duration of shell formation. This long time makes pigment and calcium deposit more and more, and that not only makes shell thicker and stronger, but also gets shell deeper. The shell color was found to also be highly significantly correlated to shell thickness (Ingram et al, 2008). On the contrary, the short formation time makes the shell shallower, and the number of stomata more.

Changes of shell color

The shell color is usually affected by the season of lay, nutrition, diseases, all kinds of stimulus, and drug, and so on. The phenomena that hens lay eggs with shallow color shell indicate that the lay performance reduces. So, when shell colors were more than 50, the shell strength, shell thickness diminished, whereas shape index and pores in shell increased (Table 2; Figure 1).

In addition, the shell color decreases as hen ages. This is directly attributed to an increase in egg size without an accompanying increase in pigment deposition onto the surface of the shell. As a result, more shell surface is covered with a given amount of pigment as the hen ages and lays larger eggs (Odabasi et al., 2007). So the reason should be found out why the shell color changes to shallow.

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