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Original Article

Eggshell Quality Comparison between Blue-Shelled and White-Shelled Eggs of Brown Tsaiya Ducks

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ABSTRACT

This study aimed to compare the egg quality between the bluish-shelled (BSh) and whitish-shelled (WSh) eggs of Taiwanese Tsaiya ducks. Experiments were conducted to determine eggshell thickness and strength, heat absorption capability, and egg yolk lipid peroxidation. Our results showed that the eggshell thickness and strength of BSh eggs were higher than that of WSh eggs during both the autumn and winter seasons ($p<0.05$). The egg surface temperature in either indoors and outdoors conditions was higher in the BSh group than in the WSh group. The extent of lipid peroxidation was determined to analyze the anti-oxidative ability of egg yolk. When eggs were stored at room temperature in autumn for 0 day and 7 days, the levels of thiobarbituric acid reactive substances (TBARS) in BSh eggs (1.21±1.04 and 1.49±0.69, respectively) were lower than those of WSh eggs (3.42±1.32 and 3.74±1.86, respectively) ($p<0.05$). Moreover, TBARs in the BSh group stored at 4°C for 14 days were also lower than those of the WSh group, indicating that despite the heat absorption capability of BSh eggs being higher, the anti-oxidative ability remained sound. Then, we determined serum red blood cells, which are the original source of the anti-oxidant component biliverdin in the egg shell. The results showed that the levels of red blood cells and packed cell volume in the BSh group were higher than those of the WSh group ($p<0.05$). All in all, we suggest that BSh eggs with higher eggshell thickness and strength and antioxidant capacity may possess greater quality and shelf life.

INTRODUCTION

The Brown Tsaiya duck (*Anas platyrhynchos*) is a native breed of ducks raised in Taiwan primarily for egg production (Huang & Lin, 2011). It is one of the duck species with the highest laying capacity in the world (Tai *et al.*, 1989). According to the Agricultural Statistical Yearbook edited by the Council of Agriculture in Taiwan (2021), approximately 2,031,000 egg-laying ducks were raised and 415,520,000 eggs were laid in the year of 2020. The Tsaiya duck lays either bluish-shelled (BSh) eggs or whitish-shelled (WSh) eggs. Some egg-processing industries buy only BSh-eggs to produce Pidan (a Taiwanese word that means thousand-year egg or long-lasting egg, referring to a food appreciated by mostly elderly Taiwanese), since the whole process requires eggs with harder shell, which was commonly believed to be a strong feature of the BSh eggs. Additionally, thin-shelled duck eggs are likely to break during transportation and handling processes such as washing and machine grading. However, Wang *et al.* (1997) reported that there is no difference in shell strength between the BSh and WSh eggs, despite reports that shell strength and thickness increase as the shell color becomes darker (Ingram *et al.*, 2008; Yang *et al.*, 2009). Therefore, further investigations of the relation between eggshell strength and color remain necessary.

Taiwan is an island in a subtropical region, so the temperature is temperate-to-high all year-round, except for a few chilly days when cold fronts arrive during the winter. Therefore, fresh duck eggs cannot be stored at room temperature on a regular day for over a week (which is also true in tribes in indigenous areas). Darker color shells tend to absorb more solar radiation (Delhey, 2020) and also absorb heat more rapidly than the lighter ones when exposed to solar radiation, suggesting that egg pigmentation could play an important role in thermoregulation in cold climates (Wisocki, *et al.*, 2020). Nevertheless, dark-colored shells do not overheat, since they have a very high reflectance in the near infrared spectral region (Bakken *et al.*, 1978). Biliverdin, a common eggshell pigment, is responsible for infrared reflectance (Burley & Vadehra, 1989) and antioxidant capacity (Stocker, 2004). Given that BSh eggs contain higher levels of biliverdin (Liu, 2010), it is an open issue whether fresh BSh eggs have longer shelf life than WSh eggs. Since most egg lipids are located in the yolk and are susceptible to oxidation (Yang & Chen, 2001), and that lipid peroxidation would increase over time (Chen *et al.,* 2000), it stands to reason that shell pigmentation could affect the rate of yolk degradation. Therefore, the aim of this study was to compare the eggshell properties and egg yolk lipid peroxidation of brown Tsaiya ducks BSh and WSh eggs.

MATERIALS AND METHODS

Experimental animals

Thirty-eight-month-old apparently healthy female Tsaiya ducks were collected from the farm in Tunghai University and used as experimental animals. The birds were categorized into the BSh and WSh egg-producing groups depending on the type of eggs they had previously been laying the most, and each group contained 15 birds that were reared in separate cages (50 × 30 × 45 cm). All birds were fed commercial laying pelleted diets (crude protein:19.5%; metabolizable energy; ME, 2810 kcal/kg), and feed and water were supplied ad libitum throughout the experimental period.

Sampling

Four experiments were conducted between September (average 30°C in autumn) and November (average 22°C in autumn). For the shell strength and thickness experiment, 30 eggs were collected from each group on September 10th and 11th, and on November 28th and 29th, totaling 120 eggs for the shell strength test. For the shell temperature test, a total of 90 eggs were collected in the period between November 15th and 18th. For the lipid peroxidation analysis, eggs were collected between September 19th and 22nd, totaling 90 eggs. On September 30th, blood samples were collected from the wing veins of each duck for an erythrocyte profile analysis.

Measurement of eggshell strength and thickness

(A) Shell breaking strength

A pull and press machine (Model HT–8116, Hung Ta Instrument Co., Ltd.) was used at the speed of 50 mm/min with the tip of the egg pointing upward to test the top force (kg) of the egg shell.

(B) Eggshell thickness

The eggshell thickness was measured using the method described by Nordskog & Farnsworth (1953). Shell pieces were collected from the equator, blunt and sharp ends of the egg. After removing the shell membrane, a thickness gage (Mitutoyo, code No. 7360) was used to measure the thickness (mm). The thickness of the eggshell was determined as the mean of the 3 values measured per egg.

Measurement of eggshell temperature

The collected eggs were further classified as washed or unwashed, and then all eggs were stored in a refrigerator at 4°C before testing. The eggs were then removed and put on a table in a laboratory room or placed outdoors under direct sunlight for 2 hours. Pictures of the eggs were taken with a thermal infrared imager (NEC AVIO, IRM-G100EXD). These images from the camera were stored on a compact flash memory card (ScanDisk®) and subsequently downloaded to a laptop computer to analyze the surface temperature of the eggshells using the Thermo data analysis system (Ching-Hsing computer-Tech Ltd.).

Measurement of lipid peroxidation

Three sample collections were performed for a total of 90 eggs, and each batch of 30 eggs was exposed to either room temperature (between 28-30°C) for 1 day, room temperature for 1 week, or 4°C for 2 weeks. After separating the egg yolk from the egg white, yolk samples were preserved under -20°C to measure the extent of lipid peroxidation. Lipid peroxidation was determined based on the amount of thiobarituric acid-reactive substances (TBARS) (Fraga *et al.*, 1988). The fluorescence of the samples was detected at an excitation wavelength of 515 nm and an emission wavelength of 555 nm in a F4500 fluorescence spectrophotometer (Hitachi, Japan), and 1, 1, 3, 3-tetramethoxypropane was used as the TBAR standard.

Analysis of blood samples

A semiautomatic electronic blood cell counter (Sysmex F-800) was used to measure the total number of red blood cells (RBC), hemoglobin (Hb), and packed cell volume (PCV) in the ducks' blood.

Statistical analysis

Data were analyzed by analysis of variance using the general linear model procedure. All statistical analyses were carried out using SAS software (SAS; Statistical Analysis System, 1996). The least square means were used to compare and estimate the differences between or among the treatments in each experiment.

RESULTS

Eggshell strength and thickness

The BSh eggs had significantly greater thickness and eggshell strength than the WSh eggs in both autumn and winter ($p<0.05$; Table 1). There was a significant difference in the thickness of the WSh group between the two seasons ($p<0.05$), but this was not found in the BSh group. There were significant positive correlation coefficients between the thickness and strength for both BSh and WSh groups ($p<0.05$).

Table 1 – Comparisons of eggshell strength and thickness between bluish and whitish duck eggshells.

Items	Autumn		Winter			
	Bluish eggshell	Whitish eggshell	Bluish eggshell	Whitish eggshell	B	S
Strength, kg/cm^2	3.726 ± 0.723^b	3.225 ± 0.865^a	3.685 ± 0.981^b	2.966 ± 1.081^a	**	NS
Thickness, mm	0.375 ± 0.021^b	0.361 ± 0.026^{aB}	0.367 ± 0.019^b	0.342 ± 0.032^{aA}	***	
r value[1]	0.42*	0.50*	0.50*	0.73***		

$^{a-b}$ Means within the same row with different superscripts differ significantly in the same season ($p<0.05$).

$^{A-B}$ Means within the same row with different superscripts differ significantly in different season ($p<0.05$).

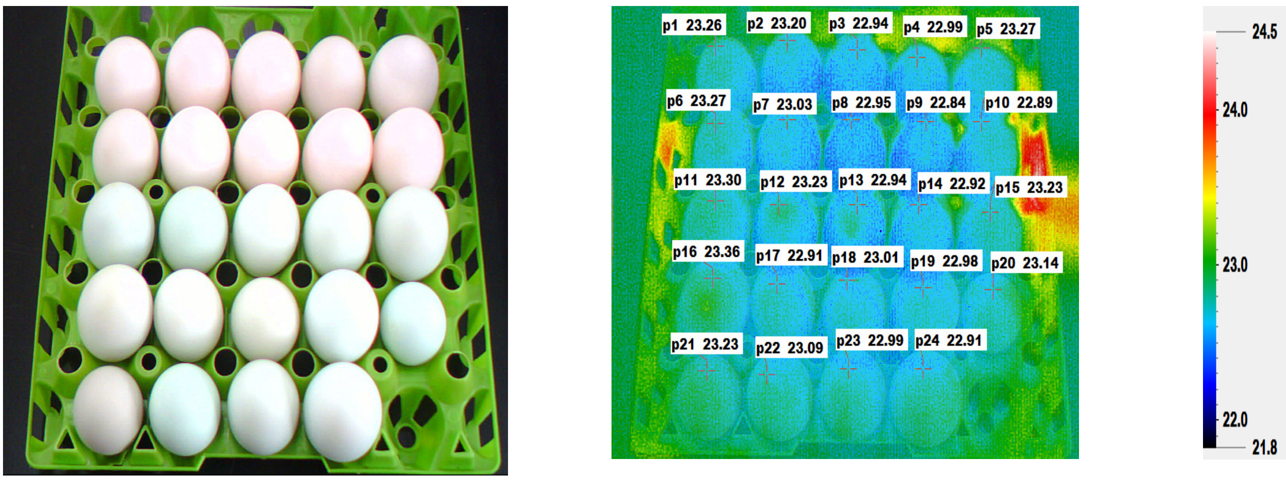
B: Breeds; S: seasons.

*$p<0.05$; ** $p<0.01$; ***$p<0.001$; NS: Not significant.

[1]Correlation between strength and thickness in eggshell.

Eggshell temperature

The surface temperature of the eggshells was measured using a thermal infrared imager (Fig.1.A) and analyzed by a Thermo data analysis system (Fig.1.B). The results showed that the washed BSh duck eggs placed indoors and outdoors had significantly higher surface temperature than the WSh ones ($p<0.05$; Table 2), with similar results being found for the non-



A B C

Figure 1 – Thermal infrared image and thermo data analysis system output to measure the surface temperature of duck eggs.

Photograph of duck eggs with thermal infrared imager. Surface temperature in duck eggs analyzed using the thermo data analysis system (e.g. p1 indicates individual egg number and its surface temperature is 23.26°C). Temperature variation expressed with different colors.

washed eggs (p<0.05). Moreover, the washed and unwashed outdoors eggs of the BSh group were 0.58°C and 0.62°C warmer than those of the WSh group, respectively.

Table 2 – Comparisons of egg temperature between bluish and whitish duck eggshells under different environments.

Conditions	BSh eggs	WSh eggs
	-------------- °C --------------	
Indoors, no sunshine eggshell washing[1]	23.68±0.15 [b]	23.53±0.11 [a]
Outdoors, sunshine eggshell washing	28.91±0.28 [b]	28.33±0.51 [a]
Outdoors, sunshine, eggshell no washing	28.42±0.48 [b]	27.80±0.42 [a]

[a-b] Means within the same row with different superscripts differ significantly in the same season (p<0.05).

[1]Eggs were washed with 20°C distilled water.

Lipid peroxidation

The amount of TBARS in the egg yolk of the BSh group was significantly lower than that of the WSh group after the eggs were stored at room temperature for 1 day and 7 days (p<0.05; Table 3). Moreover, when the eggs were stored at 4°C for 14 days, the amount of TBARS in the yolk of the BSh group was also significantly lower than that of the WSh group (p<0.05).

Table 3 – Comparisons of egg yolk TBARS amount between bluish and whitish duck eggshells under different environments.

conditions	BSh eggs	WSh eggs
Storage	---------------- nmol/50μL egg yolk ----------------	
1 d, Room Temp.[1]	1.21±1.04[b]	3.42±1.32[a]
7 d, Room Temp.[1]	1.49±0.69[b]	3.74±1.86[a]
14 d, at 4°C	1.13±0.79[b]	2.20±1.08[a]

[a-b] Means within the same row with different superscripts differ significantly in the same season (p<0.05).

[1] Room Temp: 28-30°C during 1-7 days experimental period.

Analysis of blood erythrocyte

The results of erythrocyte pictures showed that ducks with bluish-shelled eggs had a higher amount of red blood cells and packed cell volume than those that produced WSh eggs (p<0.05; Table 4). There was no significant difference in the levels of hemoglobin between the two strains.

Table 4 – Comparisons of erythrocyte characteristics between the blood of ducks with bluish and whitish eggshells.

Items	BSh duck	WSh duck
Red blood cell (10^{12}/L)	2.41±0.15 [b]	2.16±0.20 [a]
Hemoglobin (g/dL)	11.40±0.579	11.17±0.976
Packed cell volume (%)	39.01±1.91[b]	35.93±2.77[a]

[a-b] Means within the same row with different superscripts differ significantly in the same season (p<0.05).

DISCUSSION

Eggshell strength and thickness

Our results showed that the eggshell strength and thickness of BSh eggs were greater than those of WSh eggs, which is consistent with the findings reported by Chen *et al.* (2022). It is known that differing bluish and whitish eggshell colors are influenced by the amount of biliverdin in the uterus fluid, and that biliverdin plays a key role in the regulation of duck eggshell colors (Liu *et al.*, 2010). The present results confirmed that the higher thickness and strength in the BSh group may be due to an increase in erythrocytes, which are the original source of biliverdin.

Duck eggs with bluish eggshells have higher eggshell thickness and eggshell strength than those with whitish eggshells in autumn and winter, suggesting a positive correlation between eggshell strength and egg color. This result is in agreement with the findings of Hu *et al.* (2002), who noted that the characteristics of eggshell color were positively correlated with both eggshell strength and eggshell thickness in ducks. Contrarily, the study by Wang *et al.* (1997) showed no difference in shell strength between bluish eggshell and whitish eggshells among Tsaiya duck eggs collected from a commercial market, which contradicts our current findings. The reason may be that the duck eggs sold in the commercial market originated from different farms and had different collection times; therefore, many factors such as the age of laying ducks and the storage conditions after egg production could contribute to the inconsistent results. Darker eggshells can enhance egg strength because pigment molecules confer resilience to the shell, and the pigments have a potential cushioning effect between the calcite crystals (Solomon, 1987; Burley & Vadehra, 1989.); moreover, egg shell color is positively correlated with both shell density and thickness (Campo & Escudero, 1984; Hu *et al.*, 2002). This viewpoint can be supported by the positive correlations between thickness and strength among the colored eggshells in this study.

Eggshell temperature

The washed eggs of the BSh group had a higher surface temperature when exposed to room temperature indoors and under sunlight outdoors than those of the WSh group, with temperature differences of 0.15 and 0.58°C, respectively. This may be explained by the fact that dark surfaces absorb a greater heat load when exposed to sunlight. These results are similar to those reported by Collier *et al.* (2003), who showed that the surface temperature of the dark hair of cows standing in an open field exposed to solar radiation was 40.7°C, while that of the white hair was 33.7°C, a huge difference of 7°C. This difference in temperature arises from the different solar absorption rate of darker and clearer shades. Gebremedhin *et al.* (2011) reported that the relation between the color of a cow's hair coat and solar absorption was of the order of 98% for black, 92% for dark red, 75% for tan, and 37% for white. Similarly, the results of our work suggest that the solar absorption of bluish eggshells is higher than that of whitish eggshells in Tsaiya ducks.

Lipid peroxidation

Our results showed that the amount of TBARS in the egg yolk of BSh eggs was lower than that of the BSh group after eggs were stored at room temperature for 7 days, suggesting that biliverdin plays a key role in anti-oxidation. Blue eggshells are rich in biliverdin (Liu *et al.*, 2010), while white eggshells contains lower levels of protoporphyrin and biliverdin (Liu *et al.*, 2010; Kennedy & Vevers,1976). A previous study also showed that the antioxidant activity of biliverdin scavenges lipid peroxyl radicals by supplying electrons (Stocker, 2004). Based on our data, a significant difference in yolk TBARS levels was observed when BSh and WSh eggs were stored at 4°C for 14 days. Under such storage conditions, both exhibited low amounts of TBARS, which is ideal for longer storage of duck eggs. The study of Hargitai *et al.* (2016) points out that eggs with darker eggshells contained lower concentrations of antioxidants in the yolk, which indicates lower yolk antioxidant levels in eggs of Eurasian Great Tits. The difference may be due to the different species and to BSh eggshell containing a high level of biliverdin, which has anti-oxidative properties, despite darker eggs having a higher capacity of absorbing solar radiation and the oxidation of yolk lipids being accelerated at high temperatures.

Hematological parameters

The total number of erythrocytes in Tsaiya ducks that lay BSh eggs is higher than that of ducks that lay WSh eggs. This result is supported to the study by Lin *et al.* (2017), in which total red blood cell counts increased with age in both Tsaiya duck strains during the laying period, though Chen *et al.* (2012) reported that there were no differences in the erythrocyte profiles of BSh and WSh strains of growing Tsaiya ducks at 12 and 14 weeks of age. One of the reasons for the discrepancies between the two strains may the biliverdin levels in BSh eggs. The blue or white color of duck eggshells depends on the concentration of biliverdin in uterine fluid (Liu *et al.*, 2010), which is one of the cleavage products of heme (Kennedy and Vevers, 1973), which in their turn are abundant molecules in red blood cells. When Tsaiya ducks laying BSh eggs start to enter the egg-laying period, it is speculated that the decomposition of red blood cells may be accelerated, leading the amount of newborn red blood cells to also increase at this time. However, this hypothesis still needs further study to be confirmed, for instance studying the increased concentration of biliverdin in the shell gland or uterine fluid, or the elevated HO-1 enzyme activity (Lin *et al.*, 2017).

All in all, based on the study of the amount of egg yolk TBARS and eggshell surface temperatures, both fresh BSh and WSh eggs should be placed under lower ambient temperatures to extend their shelf life despite of the presence or lack of the antioxidant biliverdin.

CONCLUSIONS

Eggs with bluish eggshells have higher eggshell thickness and eggshell strength than those with whitish eggshells. Moreover, the amount of egg yolk TBARS in bluish duck eggs is lower than in the white eggshell group after storage at room temperature. However, we suggest that longer shelf lives are expected for both the BSh and WSh eggs under 4°C for 14 days.

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