Effect of Eggshell Translucency and Color on Broiler Egg Hatchability and Chick Quality and its Relationship with Other Eggshell Quality Parameters

by

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ABSTRACT

A successful hatch has a considerable economic impact on the poultry industry. The relationship between eggshell quality and hatchability has been demonstrated over the years through parameters such as thickness, eggshell weight, and specific gravity. However, increasing our knowledge of the impact of additional eggshell quality parameters on hatchability could lead to the development of novel and non-destructive metrics that could identify issues in the field and provide solutions through nutrition and/or breeder management. Therefore, we conducted two studies to explore the relationship of eggshell translucency and color lightness with hatchability, chick weight, and other egg quality parameters on broiler breeder eggs. To accomplish these objectives, eggs from YPM x Ross 708 hens older than 50 weeks were subjectively selected by their translucency score (1=low, 2=medium, 3=high) and sorted by color lightness (dark and light). In the first study, eggs were incubated to determine hatchability and chick quality; in the second study, egg quality was measured using the Egg Tester Ultimate machine for internal (HU, yolk color, albumen height) and external quality (thickness, strength, egg weight). Low translucent eggs (score of 1) and dark-colored eggs had better hatchability and chick weight results. Regarding egg quality, high translucent eggs (score of 3) and dark-colored eggs positively influenced external eggshell quality parameters, such as thickness and strength; however, only translucency was observed to impact internal quality parameters. More comparisons should be performed to observe if these results are consistent across most breeder strains.

Key words: Translucency, color, hatchability, broiler breeders, egg quality

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LIST OF ABBREVIATIONS

µm	micrometer
a*	Red/Green Value
b*	Blue/Yellow Value
BDL	Brown-egg Dwarf layers
CFU	Coliform forming units
cm	centimeter
CMV	Color Machine Vision
Cu	Cooper
DCP	Dicalcium phosphate
g	gram
HPLC	High Performance Liquid Chromatography
HSL	Hue, Saturation, and Lightness
HU	Haugh Units
ISA	Institut de Selection Animale
kg	Kilogram
L*	Lightness
MDCP	Mono-dicalcium phosphate
mg	milligram
mL	Milliliter
Mn	Manganese

NASS	National Agricultural Statistics Service
nm	nanometer
OECD	Organization for Economic Cooperation and Development
Se	Selenium
SE	Standard deviation
T	Translucency score
T1	Translucency score of 1
T2	Translucency score of 2
T3	Translucency score of 3
U.S.	United States
USDA	United States Department of Agriculture
WLL	White Leghorn Layers
Zn	Zinc

CHAPTER 1: LITERATURE REVIEW

1.1. INTRODUCTION

Poultry meat is considered an indispensable source of animal protein for human growth and development in the world; its consumption is projected to grow about 15% and account for 41% of the total animal protein consumption by 2032, according to the outlook from 2023-2032 developed by the Organization for Economic Cooperation and Development (OECD) and the Food and Agriculture Organization (FAO) of the United Nations. Some of the reasons for this increasing worldwide poultry consumption include its healthier protein balance, lower fat content, and lower price when compared to other types of meat, as well as the fact that poultry has the lowest carbon footprint of all meat, which is in line with consumer trends toward greater concern for animal welfare, sustainability, and the environment (OECD/FAO, 2023).

The U.S. is the largest producer of poultry meat in the world and the second-largest exporter after Brazil. In 2022, the poultry industry in the U.S. had total sales of $76.9 billion, representing an increase of 67% over 2021 (USDA, 2023). Currently, the poultry business vertically integrates several stages of production, beginning with primary breeders selected based on desirable genetic features. The offspring of these birds become breeders, producing birds that are directly used to generate meat (broiler chickens). The hatchery plays a central role in the poultry production chain (breeder-hatchery-broiler). At least 242 million broiler-type eggs are placed in incubators every week. Only 79.5% of those eggs successfully hatch into chicks, according to the National Agricultural Statistics Service (NASS) of the United States Department of Agriculture (USDA) (USDA-NASS, 2023). The poultry business is continuously looking for new strategies to boost the number of chicks that hatch; improving hatchability depends not only on good management at the hatcheries but also on effective management at the breeder farms. In 2008, Yassing and others reported a list of factors influencing hatchability that included genetics, health, nutrition, age of the flock, egg size, weight, and shell quality, duration and conditions of egg storage, egg sanitation, and season of the year. Each of the criteria have been the subject of extensive research, allowing for several advancements in hatcheries and breeder farms. However, there are challenges that still demand further research, such as improving eggshell quality.

The eggshell is a crucial structural component for the growth of the embryo; it provides mechanical protection, serves as a source of calcium, acts as a strong barrier to prevent external microorganisms from entering the egg, and aids in controlling the gas and water vapor flow

between the outside and the embryo (Nys et al., 2004; Messens, 2005; Cheng & Ning, 2023;). Hatching eggs require a robust shell to withstand all the manipulation before they reach the incubator, as they typically go through a collection belt, traying process, transportation to the hatchery, in-ovo vaccination, etc. The loss of eggs due to poor shell quality results in a loss in income for many hatcheries. Some factors reported to impact eggshell quality include the hen's genotype, age, oviposition time, housing system, and a diet with adequate Ca, P, and trace mineral supplementation (Ketta & Tůmová, 2016). Increasing our understanding of the relevance of eggshell quality characteristics on hatchability may create new research opportunities in the areas of nutrition and breeder management for the investigation of viable solutions. Through this study, new eggshell quality parameters that have not yet been fully explored in hatching eggs are introduced to the broiler industry and proposed as a good non-destructive indicator of hatchability.

The hen's reproductive system is divided into two distinct components: the ovary and the oviduct. The ovary is a cluster of developing yolks or ova that is fully formed in female chicks when they hatch; however, during their embryonic development, the right ovary regresses, leaving only the left ovary active, which remains relatively small until the hens reach sexual maturity. The oviduct is a tube-like organ that can reach a length of 63 to 69 cm when fully mature; all components of the egg are formed around the yolk in this section of the hen's reproductive system, and the entire process can take up to 24 hours to complete. The oviduct is composed of five morphologically and functionally distinguishable regions: the infundibulum is the site where the fertilization of the yolk occurs, and the egg white's initial layer is generated (15-30 min). The Magnum is the region where most of the egg white is produced (2-3 hours). The isthmus is the region where the inner and outer shell membranes are formed (1.5 hours). The Shell gland or uterus is the region where the egg spends the longest period while the eggshell is formed (19 – 20 hours). The vagina is the final site for egg expulsion, as the muscle of the vagina helps to turn and push the egg out when ready (Hrabia, 2021; Ottinger & Bakst, 1995).

1.1.1. Eggshell conformation

The formation of the avian eggshell is considered one of the fastest calcifying processes known in biology. It has evolved to make the egg resist physical and biological threats from the

outside environment while also meeting the metabolic and nutritional needs of the developing embryo by controlling gas and water exchange and acting as a calcium storage site (Hincke et al., 2012). It accounts for 10-11% of the egg's weight and is primarily composed of calcium carbonate, which accounts for about 95% of its conformation. It also contains 3.3-3.5% organic matrix (considering the inner and external membranes), 1.6% water, and numerous trace minerals such as copper, manganese, magnesium, and zinc (Gautron et al., 2021).

Although calcium is provided in the diet of hens, about 40% of the calcium utilized for eggshell formation comes from bone mobilization due to a mismatch between the time of feed intake (day-time) and shell production (night-time) (Nys, Bain, & Inmerseel, 2011). The eggshell is made up of four layers: the eggshell membrane, mammillary layer, palisade layer, and cuticle, which combined play a role in mechanical protection for the embryo's growth and act as a physical barrier against microbial contamination of the egg contents.

1.1.1.1 Eggshell membrane, mammillary layer, palisade layer, and cuticle.

The eggshell membrane is a double-layered membrane morphologically divided into inner and outer sheets surrounding the albumen. It is produced in the isthmus section of the oviduct and consists of a network of interconnected protein fibers arranged to form a semi-permeable structure representing about 2% of the total weight of the calcified eggshell.

The eggshell membrane is composed of 10% collagens and 70 to 75% other proteins containing lysine-derived cross-links, which is considered its major component, and 20% moisture (Yamamoto et al., 1997). Proteomic investigations have allowed the identification of hundreds of eggshell proteins; recently, nearly 500 proteins have been discovered in the eggshell membrane (Ahmed, Suso, and Hincke, 2017). The mesh structure of the shell membrane plays an important role in obstructing the invasion of microorganisms and acts as a supportive substrate or platform for the nucleation and mineralization of the eggshell (Baláz, 2014).

The eggshell calcification starts in the shell gland (uterus) approximately 5 hours after ovulation with the formation of flat disk-shaped amorphous calcium carbonate particles that nucleate over the entire outer shell membrane surface. These nucleation sites are known as mammillary bodies and become the origins of the mammillary layer, which is made up of the bases of calcified columns and knobs that penetrate the outer membrane and represent the initial stage in the production of the calcified component of the eggshell. A high-quality eggshell

should have spherical mammillary bodies that are uniform in size and distribution to provide for the greatest possible adherence to the outer membrane's fibers. Abnormalities in the mammillary layer are linked with low-quality eggshells with reduced thickness or increased porosity (Dunn et al., 2011). Some variations in structure at the level of the mammillary layer in eggshells have been described as type-B (rounded-shape and fragmented) and type-A bodies (conical rather than rounded with little contact with membrane fibers), aragonite and cubic-shaped bodies (Bain, 1992; Park et al., 2018; Fathi et al., 2007). The second stage is the formation of the palisade layer, where the calcite crystals grow perpendicular to the surface of the developing shell at a linear calcium carbonate deposition rate of 0.33 g/h (Hincke et al., 2012). This layer's columnar structure gives it a strong breaking resistance and shell thickness. The last stage of eggshell formation occurs two hours before oviposition when the mineralization is stopped, and the thin organic cuticle layer is deposited. The cuticle restricts the passage of water, gas, and bacteria through the shell's pores. (Rose-Martel and Hincke, 2015; Park and Sohn, 2018)

1.1.2 Internal egg quality

The internal content defines the egg's nutritional quality for the development of the embryo and provides biological defense through mechanisms including viscosity, pH, antimicrobial properties, immunoglobulins, etc. The internal content of the egg is mainly composed of water (74%), proteins (12%), lipids (12%), carbohydrates (<1%), vitamins and minerals (Nimalaratne and Wu, 2015). Hen’s health and egg storage conditions, such as temperature, relative humidity, and time, have been reported to affect the properties of the yolk and albumen and decrease its quality, affecting embryo development and hatchability (Brake et al., 1997). Several indicators have been continuously proposed to estimate internal egg freshness based on changes in the yolk and albumen characteristics under specific conditions (Kim et al., 2014).

The yolk accounts for about 30% of the overall egg weight, and its color is mainly determined by the hen’s nutrition and ability to absorb and deposit carotenoid components into the yolk, as the hens cannot synthesize them by themselves. The DSM's YolkFanTM has been widely used for grading yolk color; this instrument has a range of 1 to 16 and is recognized as a simple, accurate, and reliable method (DSM, 2022). The albumen represents about 60% of the egg’s weight and is composed of proteins, peptides, and amino acids, which contain

antimicrobial and natural antioxidant properties. The pH of the albumen of a freshly laid egg ranges between 5.6 to 7.5; however, as the large amount of carbon dioxide contained in the albumen is released to the environment, the pH of the albumen rises to about 9.5, affecting the structure of its proteins and viscosity. The Haugh unit (HU) value is often used to measure the quality of the inner content of the eggs. This parameter correlated the weight of the egg with the height of the central region of the thick albumen via an equation:

$$HU = 100 * \log10 (h – 1.7w0.37 + 7.6)$$

Where HU represents the Haugh unit, h represents the observed height of the albumen in millimeters, and w represents the weight of the egg in grams. The HU value ranges from 0-130 and grade the eggs as follows: AA (72 or higher), A (71-60), B (59-31), and C (30 or less) according to the USDA egg-grading manual released in 2000, where the albumen of an AA egg is firmer and thicker than the other scores (USDA, 2000)

Egg storage prior to incubation might have both detrimental as well as beneficial effects on hatchability since the change in the pH of the albumen is necessary for transport functions through the vitelline membrane. Benton and Brake (1996) suggested that the lack of a pH gradient between the yolk and the albumen may slow down vital gas diffusion processes and limit the availability of several nutrients when evaluating the embryonic development of broiler breeder eggs after the storage of 0 or 5 days. In 2021, Melo et al. observed no negative effects on hatchability after storing fertile eggs from 55-weeks Cobb 500 breeder flocks for 4 or 8 days, which agrees with Mather and Laughlin (1976), who have suggested that the adverse effects on hatchability and embryonic mortality appear after more than 7 days of storage.

1.1.3 Quality of the eggshell

Poor shell quality raises the danger of microbiological contamination, which can result in late embryonic death, yolk sac infections, or rotten eggs, which are eggs that have been contaminated by bacteria or fungi and have unpleasant odors, colors, and textures (Rezaee et al., 2021). Many parameters have been proposed to assess the quality of the eggshell: in the field, breeder farmers may use a practical judgment based on egg cleanliness and freedom from cracks or imperfections that might weaken the shell before sending eggs to the hatchery; however, a more technical judgment through factors as eggshell thickness, strength, and specific gravity have also been used extensively for research purposes (Ketta & Tůmová, 2016). Nevertheless,

new practical parameters to be used on the farm are gaining attention due to the possibility of detecting problems in a simple way. Variations in eggshell translucency and color intensity are frequently seen in farms, but there is little research into how these parameters relate to hatchability or hen's stress and health (Cheng & Ning, 2023).

1.1.3.1 Eggshell strength and thickness

Hatching eggs require a strong shell to endure all the manipulation before they reach the incubator. Some factors that have been reported to impact this parameter are flock age, egg shape, thickness, and nutrition. Roberts et al. (2012) reported a decline in shell-breaking strength with flock age, finding values of 44.1 Newtons (N) on 25-weeks-old flocks and 37.2 N >55-weeks-old flocks and similar results were reported by Perić et al. (2017). Rodriguez-Navarro et al. (2002) observed that eggs from older hens had less than half of the breaking strength than eggs from younger hens when studying brown egg-laying hens (ISA Brown) of 30 and 58 weeks old. Regarding egg shape, Essary et al. (1977) found a significant difference in shell strength between eggs with different curvatures of the large ends, where eggs with small curvatures were stronger than those with large curvatures. Similarly, Potts and Washburn found an effect of the egg's width/length ratio on breaking strength in 1983. In terms of eggshell thickness, several authors have found a strong correlation between this parameter and strength (Zhang et al., 2005; Ketta and Tůmová, 2017). Both eggshell characteristics are regarded as two of the most important indicators of eggshell quality. Regarding nutrition, the critical significance of dietary calcium, phosphorus, and vitamin D3 levels and sources for eggshell quality has been demonstrated in the literature (Światkiewicz et al., 2015). The measurement of specific gravity has been employed as an indirect measurement of the thickness of the eggshell and is probably the most common procedure employed for the assessment of the quality of the eggshell. This approach uses saline solutions with different concentrations and assesses egg flotation. Some authors have suggested that eggs with specific gravities of 1.080 or lower (less salt) are classified as thin-shelled and can compromise the hatchability and embryo mortality of eggs from broiler breeder flocks (İpek and Şahan, 2001; Bennett, 1992).

1.1.3.2 Eggshell translucency

Eggshell translucency is also commonly known as mottling, water markings, or windows. It is thought to occur due to moisture accumulation on the eggshell and uneven drying after the egg is laid (Salomon, 1991). Translucency is an eggshell quality parameter generally identified by candling in darkness that has been mostly studied for table eggs since it may affect consumer perception and bacterial penetration. Subjective approaches are commonly employed by trained and experienced operators, where eggs are usually graded based on the size and number of translucent areas. In 1932, Host, Almquist, and Lorenz proposed a grading system categorizing eggs into 1-3 levels corresponding to no translucency, medium translucency, and severe translucency, respectively. Similarly, other authors have proposed systems with different scales where eggs are graded into 1-5 levels, ranging from no translucent spots to nearly fully translucent (Shi et al., 2023). Wang et al. (2019) investigated objective approaches for categorizing eggshell translucency category using a grayscale recognition method with a camera and specific software (Photoshop, Image-Pro Plus 6.0, and ImageJ software) to identify and quantify translucent spots, their diameter, and area.

Baker and Curtis (1957) observed that translucency appears in the eggshell the same day the egg is laid. Talbot and Tyler (1978) proposed that the eggshell of a freshly laid egg is fully translucent and develops opacity as it dries. They also observed that a freshly laid egg stored in a humid environment remains wholly translucent, implying that the conditions of the environment affect the severity of the final eggshell translucency. MingKai et al. (2019) reported an effect of storage conditions on the incidence of translucent eggs, finding that eggs held at 4 °C or 90% relative humidity had the lowest incidence (~10%). Baker and Curtis (1958) found an increase in translucency on eggs laid between February and June and a reduction in July; however, Shi et al. (2023) reported contradictory results. They observed an increase in the number of translucent eggs in July and August as the temperature and humidity increased. The disparity in the results could be attributed to differences in the location where the research was conducted (New York, USA, and Zhuozhou, China), the difference in timing of each publication (65 years apart), the hen's age and breed, diet composition, etc. The age of the flock has also been shown to affect the translucency score; Roberts et al. (2012) showed that eggs from flocks during the late phases of production (55-65 weeks old) had higher translucency scores than eggs from the early stages (25-40 weeks old).

Baker and Curtis (1957) reported that the amount of translucency on the eggshell varies between hens of the same flock but remains relatively constant over a period of one month for each individual.These authors also found that translucency varies not only between individuals but also between strains when evaluating 20 strains of Single Comb White Leghorns and Barred Plymouth Rocks hens (Baker and Curtis, 1958). Zhang et al. (2021) reported that the distribution and severity of translucency also vary and are specific for each breed after examining 10 different breeds of laying hens. They also discovered that the distribution of translucent spots on the eggshell was larger towards the blunt and sharp ends than in the middle. Qu et al. (2021) revealed the heritability of translucency, which, on a scale from 0 to 1, measures how much of a trait's phenotypic variance may be attributed to genetic variation. These authors found that the single-nucleotide polymorphism heritability of translucency was between 0.18 and 0.20, which falls into the low- and very low-level heredity, after analyzing 52-week-old Dongxiang Blue-shelled and White Leghorn hens and employing a GCTA software.

1.1.3.2.1. Translucency relationship with other egg and eggshell quality parameters

Regarding the relationship of translucency with other eggshell quality parameters, Talbot and Tyler (1974) found that translucent areas had thicker shells than opaque shells. However, Wang et al. (2017) observed that translucency influenced thickness only on Brown-egg Dwarf hens but not on White Leghorn hens, suggesting that the effect of translucency on thickness may vary between breeds. On the other hand, Olkowski et al. (2015) did not find any correlation of specific gravity or shell thickness with translucency on eggs from 62-weeks-old broiler breeder hens and suggested that the parameters that determine the quality of hatching eggs are more complex than previously considered. Regarding porosity, no differences in the number of pores on the eggshell have been reported regarding translucency in laying hens (Talbot and Tyler, 1974; Wang et al., 2017). However, Ray et al. (2015) discovered that low-translucent eggs had straight pores, while high-translucent eggs had more branching pores in the eggshell.

Internal egg quality variations between high and low-translucent eggs have been reported to be non-significant by several authors. Wang et al. (2017) reported no statistical difference between translucent and opaque eggs from 56-week-old Brown-Egg Dwarf Layers in the parameters of yolk color, yolk weight, yolk ratio (yolk weight to egg weight ratio), albumen height, Haugh Units (HU), and egg shape index. Baker and Curtis (1958) determined that egg

translucency is unrelated to egg internal quality after examining eggs from single-Comb White Leghorn hens.

1.1.3.2.2. Translucency and egg ultrastructure

The eggshell ultrastructure is the product of calcium carbonate interacting with organic matrix molecules, allowing for the exchange of water and gas between the embryo and the environment while also protecting the embryo from external damage. The membrane or organic matrix serves as a platform for the start of eggshell mineralization and a barrier between the inside and the outside of the egg (Cheng and Ning, 2023). Wang et al. (2017) observed that translucent eggshells had thicker shells and thinner membranes than opaque eggshells and discovered that the membrane of translucent eggs is easier to break, allowing moisture from the interior content of the egg to penetrate the eggshell. They concluded that the eggshell membrane and the eggshells' structure serve as two criteria that reliably distinguish translucent eggs from opaque eggs. Olkowski et al. (2015) showed that eggs with different translucency scores differ in shell matrix fiber or membrane, which impacts the number of contaminated eggs during incubation. Leach et al. (1983) identified ultrastructural defects on translucent spots of the eggshells of 22-week-old White Leghorn hens. They found fewer mammillary knobs per unit of area, but those they found were larger and with irregular shapes. They suggested that the reduced number of mammillary cones may be explained by a fusion of the knobs during the early stages of shell development. Chousalkar et al. (2010) discovered that the mammillary layer of translucent eggs had altered mammillary cones alignment, resulting in the formation of long continuous grooves between the cones during the early stages of eggshell production. Similarly, Roberts et al. (2013) observed in eggs with high translucency a higher occurrence of mammillary layer variations for alignment, type-A bodies, type-B bodies, aragonite, and cubic cones but lower cap quality (relation of the size of the cap and the degree of membrane attachment). They also discovered that translucency has no effect on cuticle deposition. Bain et al. (2006) reported that translucent areas on the eggshell correspond to a network of small cracks that may extend primarily between the mammillary bodies.

1.1.3.2.3. Translucency and bacteria penetration

Translucent eggs have been reported to increase the chances of bacteria penetration. Chousalkar et al. (2010) observed a significant correlation between eggshell translucency and the penetration of Salmonella and E. coli through the eggshell at very low doses of bacterial contamination (103 CFU/mL) when evaluating commercial table eggs from laying hens. Olkowski et al. (2015) evaluated hatching eggs from 62-week-old broiler breeder hens and demonstrated that the percentage of exploding eggs due to bacteria contamination during incubation varied depending on the translucent score. They found that high translucent eggs were 3.8-fold more likely to explode during incubation than low translucent eggs and suggested that the eggshell matrix or membrane is an independent risk factor that strongly correlates with the incidence of contaminated eggs. Ray et al. (2015) observed that the number of eggshell pores and thickness did not appear to play a role in the Salmonella infection of eggs with different translucency and suggested that other unknown factors are affecting bacterial penetration. However, Shi et al. (2023) observed that translucency had no effect on the cross-shell penetration rate of E. coli of 32-weeks-old laying hens after evaluating the eggs using a method commonly employed for the assessment of cuticle quality in which bacterium penetration is confirmed using ultraviolet light after 15 minutes of egg exposure to the bacteria culture.

1.1.3.2.4. Dietary factors affecting translucency.

Translucency can be impacted by dietary factors. In 2019, MingKai et al. investigated the effect of adding additional multivitamins to the basal diet on Xinyang Black Feather layer breeders and Rhode Island Reds breeders and discovered that a 500 mg/kg vitamin complex dosage could dramatically reduce translucent egg ratio. Shi et al. (2023) observed that the addition of mono-dicalcium phosphate (MDCP) to the diet instead of dicalcium phosphate (DCP) can reduce the incidence of eggshell translucency under chronic heat stress conditions after observing lower translucent egg scores on Dwarf Pink-Shell laying hens of 36 weeks old. According to Zhao et al. (2021), the addition of 25-hydroxyvitamin D and essential oils complex to the diet can greatly reduce the incidence of translucent eggs and impact the ultrastructure of the eggshell by decreasing the width of the mammillary knob in 48-weeks-old Lohman laying hens. It was concluded by van den Brand et al. (2023) that the source of trace minerals (Zn, Mn, Cu, and Se) in the broiler breeder diet affects eggshell conductance in low translucent eggs. They

demonstrated that high translucent eggs from hens fed with inorganic trace minerals had greater weight loss than low translucent eggs, whereas this effect was not seen in eggs from hens fed with organic trace minerals.

1.1.3.3 Eggshell color

The color of the eggshell has a significant impact on an egg's visual appeal and is considered an important shell quality parameter in table eggs. Although it does not reflect the internal quality of the egg, eggshell color has a strong influence on consumer preference (Cavero et al., 2012). For many years, laying hens have been selected for color uniformity and/or intensity due to their impact on consumer acceptance. However, this parameter has not been deeply used for selection in broiler breeder eggs as its impact on hatchability has not been well established (Ingram et al., 2008). It is hypothesized that birds evolved over time to produce pigmentations for different reasons, including assisting in hiding eggs from predators, filtering harmful solar radiation for thermoregulation purposes, modifying the amount and wavelength of light transmitted through the eggshell that may affect embryonic development, protecting the embryo from trans shell microbe contamination and/or improving the strength of the shell structure (Reynolds et al., 2009). A heritability of 0.23 (in eggshell lightness) has been recently reported by Guo et al. (2020), who suggested that genetic effects could contribute to phenotypic color variation on the eggshells. The diversity of eggshell coloration is primarily attributed to the pigments protoporphyrin (rusty-brown colors) and biliverdin (blue-green colors), which are synthesized in the uterus or shell gland of the oviduct and deposited over the eggshell towards the conclusion of the egg formation (Bair et al., 1975; Wang et al., 2007).

1.1.3.3.1. Eggshell pigments

The pigments protoporphyrin, biliverdin, and zinc biliverdin chelate are responsible for the broad variance in shell color and pigment pattern. These pigments are primarily synthesized in the epithelial cells of the oviduct's shell gland, and their presence in the eggshell is determined primarily by the genotype of the hen (Hargiti et al., 2017). Wang et al. (2007) proposed that the deposition processes of the two pigments, biliverdin, and protoporphyrin, are similar since both are deposited at a higher velocity on the top layer of the eggshell near the conclusion of the egg formation. Previous research studies have suggested that the bulk of the shell pigment is

deposited in the cuticle of the shell (Sparks, 2011; Cassey et al., 2011). However, Samiullah and Roberts (2013) measured the relative proportions of pigments between the cuticle and the calcareous part of the eggshell, finding that the amount of pigment in the cuticle (13-20%) was lower than the pigmentation deposited in the eggshell's calcareous section (80-87%).

The pigment biliverdin is derived by oxidation and ring opening of tetrapyrrolic compounds of the prosthetic groups of hemoproteins, which create bile pigments such as biliverdin-IX in birds, amphibians, and fish and bilirubin in mammals (Hudson and Smith, 1975). Biliverdin is the pigment present in blue-green eggs, and it has been reported to contain antioxidant properties with antimicrobial capacities that may inhibit bacterial and viral replications in the eggshell (Morales, 2020; Kaur et al., 2003). It is hypothesized that eggs with large amounts of biliverdin in the eggshell are produced by high-quality and healthier females that can afford both, the cost of pigment deposition into the eggshell during reproduction and their demand for antioxidant compounds (Moreno and Osorno, 2003; Morales et al., 2010).

Protoporphyrin is composed of four pyrrole rings, which have been identified to be the primary eggshell pigment in brown-colored eggs (Li et al., 2013). Protoporphyrin has been reported to have a photodynamic antimicrobial activity against gram-positive bacteria, such as *Staphylococcus aureus* and *Bacillus cereus* (Ishikawa et al., 2009), and to increase eggshell strength, affecting how cracks propagate across the eggshell surface. Gosler et al. (2011) suggested that the presence of protoporphyrin speckles on the eggshell may be related to an overcompensation for shell thinning across the whole egg. Protoporphyrin, unlike biliverdin, has pro-oxidant qualities; some authors speculate that a high presence of this pigment in the eggshell could be an indicator of the hen's high antioxidant capacity to control excessive levels of pro-oxidants (Moreno and Osorno, 2003), suggesting that dark colored eggs come from healthier hens.

1.1.3.3.2 Methods of calculating eggshell color

The most common method employed for measuring shell color intensity is spectrophotometry through the use of commercial colorimeters (i.e. Hunter and Minolta meters) based on the L*a*b* color space system, and expressed as an E value, which is calculated using the formula: $E = (L2 + a2 + b2)^{½}$, where L* stands for lightness and has a range of 100 (white) to 0 (black), a* stands for greenness to redness, and b* stands for blueness to yellowness. Both of

the aforementioned values have a range of -128 to +127, and a lower E value represents a darker egg. However, other authors advise using a computer Color Machine Vision (CMV) system for an accurate measurement of the eggshell color (L*a*b*) since this method measures the color of the entire surface of the eggshell, while a conventional spectrophotometry colorimeter only measures a small section of the shell (Odabasi et al., 2006). Another approach that has been widely used is the Image Analysis Technique, which selects a rectangular area of interest in the shell and uses a computer system to calculate the average intensity of the pixels in red, green, and blue bands and utilize a formula to convert these values into Hue, Saturation, and Lightness (HSL) values (Sezer and Tekelioglu, 2009).

However, when calcium depositions mask the color of the eggshell, it is impossible to correctly determine its intensity using spectrophotometry or an imaging system. In these situations, pigment quantification is a more accurate method to determine the amount of pigment present on the eggshell. This method uses solvents to completely dissolve the eggshell, then centrifuge the solution for a period of 12 h and finally analyze the supernatant with a spectrophotometer at a wavelength of 412 nm for protoporphyrin and 670 nm for biliverdin pigments (Wang et al., 2007). Another method employed to quantify the pigment from an eggshell, uterine fluids,and parts of the shell gland (uterus) is the High-Performance Liquid Chromatography (HPLC), that can quantify the pigment from the eggshell by separating and identifying the components of a substance based on their molecular structure and composition (Liu et al., 2010); however, compared to the colorimeter described above, these last methods are more complicated and expensive.

1.1.3.3.3. Relation of eggshell color with other eggshell quality parameters and hatchability

Yang et al. (2009) studied the relationship between shell color and egg quality on brown-colored eggs. They found that the color was strongly correlated with the strength, weight, and thickness of the eggshell but not with internal egg quality characteristics (Hu value, yolk color, yolk weight, etc.). The author explained that since most of the pigment deposition occurs in the shell gland, there is no connection between shell color and internal quality. Similarly, Rosenberg and Tanaka (1950) found a strong correlation between eggshell color with breaking strength and shell thickness when evaluating eggs from White Leghorn and Rhode Island Red breeds. Hassan et al. (2013) studied the influence of eggshell color on the ultrastructure characteristics of the

shell of Japanese quail, where they found that light-colored eggshells had an unclear differentiation between mammillary and palisade layers. Similar results were found by Richards and Deeming (2001) when examining the ultrastructure of green olive and light greenish grey colored eggs from pheasants. They found that lighter eggs had no discernible demarcation between the mammillary layer and palisade layer. These authors also discovered that olive eggshells had fewer, longer, and more compact mammillary knobs than light greenish grey eggshells, as well as a lack of cuticle in light greenish gray eggshells.

Godfrey and Jaap (1949) proposed that the color of the eggshell might be used as a genetic selector to improve the quality of the shell in brown egg-laying breeders after discovering that dark-colored eggs hatched better than light-colored eggs from New Hampshire pullets. Likewise, Kumar et al. (2012) concluded in their research that dark-colored eggs from different hen breeds (Black Rock, Gramapriya, and Vanaraja) had higher fertility and hatchability rates than light-colored eggs. Moreno and Osorno (2003) hypothesized that egg coloration could also signal female antioxidant capacity and, therefore, influence hen fertility.

The color of the eggshell has been shown not only to influence the quality of the eggshell and hatchability but also the quality of the hatched chicks. Şekeroğlu and Duman (2009) found that darker-colored eggs had heavier chicks than light-colored eggs after 7 and 35 days when studying eggs from Ross-308 broiler breeders. However, other scientists have found opposite outcomes. Shafey et al. (2005) found no relationship between color intensity and thickness, eggshell weight, embryo development, or hatched chick weights when evaluating eggs from commercial meat-type breeder flocks. Baylan et al. (2017) observed that the color of the eggshell had no effect on hatchability and growth performance on Ross-308 broiler breeders but observed a higher egg yolk antibody content in dark-colored than medium and light-colored eggs. Due to the high variation in results regarding color as an indicator of hatchability or eggshell quality, some authors believe that color is not a good predictor of eggshell quality and hatchability on its own (Ingram et al., 2008).

1.1.3.3.4. Factors affecting color variation

Some of the main factors affecting the color variation of eggshells are related to hen age, strain, housing system, and stress. Some authors have found that the intensity of the eggshell varies among the whole laying cycle. Odabasi et al. (2007) observed that older hens produce

eggs that are lighter in color than younger hens and hypothesized that the poor pigmentation in older birds is due to their larger size with no proportional change in the amount of pigment deposited on the shell surface. However, Bi et al. (2018) found no correlation between color intensity and egg weight. Instead, they found that the color intensity was more stable after egg-laying peaks in Rhode Island Red hens. Park et al. (2017) proposed that the aging-associated drop in pigment synthesis may be related to the eggshell gland's hypofunction because of repeated oviposition, which may result in fibrosis and endometrial atrophy and can be reflected in the depigmentation of brown eggs.

Tůmová et al. (2011) studied the effect of three different housing systems (conventional cages, enriched cages, and litter) on color intensity. They reported that eggs from hens grown in cages were lighter in color than eggs from hens kept in litter. However, the authors reported that this effect differed amongst hen strains since they only found an effect in Institut de Selection Animale (ISA) Brown layers (commercial hybrids) and Moravia Black Sex Link (BSL) layers (traditional Czech hybrid) but not in Hisex Brown layers. Similarly, in 1998, Walker and Hughes demonstrated that poor eggshell color can be caused by excessive stocking densities and uncomfortable cage designs. Campo et al. (2007) suggested that the variation in oviposition time may also affect the color of the eggshell after evaluating breeds of hens laying white (Black Castellana), tinted (Buff Prat) or brown eggs (Red-barred Vasca); the authors reported that brown eggs are more likely to be laid in the morning, whereas white and tinted eggs are more likely to be laid in the afternoon.

Stressful situations may cause early or late oviposition, which can alter the pigment deposition process and result in pale or darker eggshells. It has been reported that when oviposition is delayed and the egg stays too long in the uterus, calcium deposition may mask the color of the eggshell, making it appear a little paler (Samiullah et al., 2016; Wang et al., 2007). On the other hand, when a hen experiences stress, the developing egg may be held in the shell gland longer, which results in the shell gland releasing a thin layer of calcareous material, making brown eggs look paler in color. Therefore, some authors have suggested that the color of the eggshell may be a promising tool to estimate bird health. Porphyrin levels in excreta have been proposed as a non-destructive biomarker of stress since it is a natural metabolite intermediate in the biosynthesis of a precursor of hemoglobin that can interact with contaminants in the system (Casini et al., 2001). Similarly, due to the fact that the accumulation of

protoporphyrin in the liver induces oxidative stress and results in a rapid increase in the activity of the antioxidant enzymes, the increasing amounts of protoporphyrin in the eggshell have been proposed to be related to a higher antioxidant capacity of the hen to sustain elevated levels of pro-oxidants in the liver, blood, and uterus that makes them healthier (Moreno and Osorno, 2003).

Likewise, the ingestion of certain drugs has also been reported to produce a decline in shell pigmentation. Jones et al. (1990) found a complete reduction of eggshell color in eggs from 50-week brown layers after applying a coccidiostat treatment of the sulfonamide Nicarbazin at high doses. Viral diseases that affect egg production such as Newcastle and Infectious bronchitis, also decrease the intensity of the pigmentation on the eggshell due to the damage produced in the reproductive tract of the hen (Butcher and Miles, 2018).

Due to the importance of eggshell quality in hatchability, the continue understanding of its characteristics may have a great impact for the poultry industry. This research introduces to the broiler industry new eggshell quality measures that have not yet been completely investigated in hatching eggs and suggests them as a reliable, non-destructive indicator for chick's hatchability and quality.

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CHAPTER 2: EFFECT OF TRANSLUCENCY AND EGGSHELL COLOR ON BROILER BREEDER EGG HATCHABILITY AND HATCH CHICK WEIGHT.

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2.1. ABSTRACT

A successful hatch has a considerable economic impact on all poultry companies. The aim of the current study was to describe the possible effects of shell translucency (T score) and coloration lightness (L*value) on shell thickness, hatchability, and chick weight. A total of 4320 eggs from 4 commercial Ross 708 breeder flocks (50 to 55 weeks old) were used. Eggs were selected for T score and L*value. A 3-point subjective scoring system was used for T score (1=low, 2=medium, 3=high), and an electronic colorimeter for L*value, sorting the eggs as light (avg. L*=80.7) or dark (avg. L*=76.0). Data were analyzed using the GLIMMIX procedure of SAS (V9.4), and Tukey's HSD test was performed to separate means; a significant difference was considered when $P \leq 0.05$. Results suggest that the color of the eggshell was related to the egg weight on the day of collection (P=0.0056) and at transfer (P=0.0211). In both cases, dark eggs were 0.6 g heavier than light eggs. Dark eggs had a 3.8 % increased hatchability of egg set (P=0.0481) and yielded 6 μm thicker shells (P=0.0019) compared to light eggs. Regarding translucency, egg weight at transfer was 0.8 g heavier for T score 1 eggs compared to T score 3 (P=0.0358). The translucency score of 1 had a 6.9 % higher hatchability of eggs set (P=0.0127) and 0.7 g heavier chick weight (P=0.0385) compared to T score 3. However, T score 1 eggs had shells 28 μm thinner than the T score 2 and 34 μm thinner than T score 3 (P<0.0001). An

interaction effect was observed for eggshell thickness, L*value, and T score, where eggs classified as light with T score 1 had thinner eggshells compared to those that were dark with T score 3 (P=0.0292). These results suggest that eggshell translucency and coloration lightness can be good non-invasive indicators of eggshell thickness, hatchability, and chick weight in broiler breeder flocks.

Keywords: Eggshell translucency, color, chick weight, thickness, hatchability.

2.2. INTRODUCTION

In the United States, approximately 240 million commercial broiler eggs are set in incubators every week, with an average hatchability of 80.3% (National Agricultural Statistics Service, 2022). In 2007, it was estimated that an improvement of 1% in hatchability would increase returns to more than $25 million for the hatcheries and further increase profits to the broiler production chain (Schaal and Gherian, 2007). The egg management at the hatchery, such as storage conditions, incubation temperatures, humidity, and turning frequency, is fundamental to reaching top hatchability (Reis et al., 1997; Vick et al., 1993; Elibol and Brake, 2006; Lourens et al., 2005). However, regardless of how well the egg is handled in the hatchery, eggshell quality is critical for a better chance of hatching. The eggshell regulates gas and vapor diffusion, serves as a barrier for pathogens for the survival of the developing embryo, and is used as an indicator for hatchability through parameters such as specific gravity, vapor water conductance, weight, thickness, porosity, breaking strength, elastic modulus, static and dynamic stiffness, among others (McDaniel et al., 1979; Pebbles and Brake, 1987; Liao et al, 2013; King'ori, 2011). Furthering our understanding of the importance of eggshell quality parameters on hatchability could generate new opportunities for research via nutrition, breeder management or genetics (Wilson, 1997; Emery et al., 1984; Ketta and Tůmová, 2018).

Translucency is an eggshell quality parameter that has been primarily studied for table eggs from laying hens, where its effects on bacterial penetration, eggshell structure, shell membranes, thickness, and strength have been demonstrated (Chousalkar et al., 2010; Wang et al., 2017). However, there is not much data published related to its impact on hatchability and chick quality in broiler eggs. Shell translucency is described as a mottled appearance with light-colored spots of different sizes that are easy to observe when candling eggs (Holst et al., 1932;

Baker and Curtiss, 1957). Its generation is suggested to be caused by moisture accumulation in the shell and an uneven drying after the egg is laid, leaving opaque and translucent areas (Talbot and Tyler, 1974). Factors including hen's nutrition (Gautron et al., 2021; Bouvarel, Nys, and Lescoat, 2011; Leach and Gross, 1983), breed/strain (Zhang et al., 2021; Baker and Curtiss, 1957), health, and environmental conditions (Cheng and Ning, 2023) can increase the eggshell's translucency primarily by altering and disrupting the ultrastructure and inner membrane of the eggshell.

The color of the eggshell is a physical parameter mainly attributed to the pigments protoporphyrin, biliverdin, and its zinc chelate, which are synthesized in the shell gland of the oviduct and mainly deposited over the egg towards the conclusion of eggshell formation (Wang et al., 2007). The presence or absence of a specific dye is determined by the hen's genetics, and the dye intensity may increase or decrease under different conditions due to stress, age of the hen, the health status of the flock, and environmental factors (Liu and Cheng, 2010; Odabaşi et al., 2007). However, several authors continue to doubt that color alone could serve as a reliable indicator of eggshell quality and hatchability (Ingram et al., 2008). The aim of this project is to describe the possible effects of eggshell translucency and color lightness (dark and light) on eggshell thickness, hatchability, and chick weight.

2.3. MATERIALS AND METHODS

The protocol of this experiment was previously approved by the Auburn University Institutional Animal Care and Use Committee (Reference number: 2021-3985). A total of 4320 eggs from Ross 708 breeder hens between 50 and 55 weeks of age from a commercial hatchery were used for this study. Eggs were collected over four consecutive days from different flocks each day (1080 eggs per day). Prior to being transported to the research facility, eggs were stored for four to six days in a constant environment (temperature 15°C and 70% relative humidity) in a storage room of a commercial broiler hatchery.

Translucency score, coloration lightness, thickness, and initial weight

Each day, with the use of a 500-lumen flashlight, a total of 1080 eggs were individually candled and divided into three groups according to Zinpro Corporation (Eden Prairie, MN) BlueBox^{TM} Translucency Score system (1 = low, 2 = medium, 3 = high), using a 3-point

subjective scoring system that takes into consideration the amount, size, and coverage of spot patterns or mottling areas in the eggshell (Figure 1). After scoring, eggs were sorted as light or dark, and eggshell coloration lightness (L*value) was evaluated using an electronic colorimeter (Nix Color Sensor Pro 2) to confirm an average L*=80.7 for light-colored eggs and L*=76.0 for dark-colored eggs, on a scale of 0 to 100. The evaluated eggs were placed in a total of twelve 90-egg-incubator-trays. Eggshell thickness was determined using a non-invasive ultrasound gauge (Eggshell thickness gauge by Egg-Tester). For initial egg weight, eggs were weighed before incubation as an average of the eggs per tray. Two observational units, each consisting of a tray with 90 eggs, made up the experimental unit of this study. The study used a factorial treatment design (3 eggshell translucency levels and 2 eggshell colors). Each treatment was repeated 4 times in a randomized complete block design where each day of collection was the source of variation to conform the blocks, which was considered the random factor for the statistical analysis.

Incubation

Eggs were set in four identical single-stage incubators (Nature Form, model NMC 1080) with a capacity of 1080 eggs at Auburn University Miller Research Farm in Auburn, AL. Relative humidity and temperature were maintained constant during incubation (37.7 °C and 55% relative humidity), and eggs were turned every hour.

Hatchability, transfer egg weight, weight loss percent, infertile, cracked, exploders, contaminated, unhatched and culls percent, and chick weight.

After 18 days of incubation, all eggs were candled to identify and remove eggs that appeared to be infertile or with dead embryos, cracked to confirm infertility and embryonic mortality (early, mid, and late) by visual examination, and finally counted for calculating the egg loss along with the cracked contaminated and exploder eggs. The fertile eggs placed in trays were weighted for the calculation of egg weight at transfer. Egg weight loss was estimated as follows:

$$\text{Egg weight loss (\%)} = \frac{\text{Initial egg weight (g)-Transfer egg weight(g)}}{\text{Initial egg weight (g)}} \text{x100}$$

Eggs were then transferred to hatching baskets and placed back into the same incubators at the same temperature and relative humidity. Hatchability was calculated based on the number of eggs hatched from the total of eggs set. Hatched chicks were weighed as an average of chicks per basket. Eggs that failed to hatch at day 21 were opened to visually confirm the embryonic mortality, and chicks that hatched but were weak and near death were culled and counted to calculate the percentage of unhatched + culls based on the total amount of eggs set. Hatch of fertile was calculated based on the number of chicks hatched from the total of fertile eggs transferred to the hatcher.

Statistical Analysis

Data regarding the effect of translucency and eggshell color in initial egg weight, transfer egg weight, water loss percent, hatchability percent, unhatched + culls percent, eggshell thickness, and chick weight were analyzed using the GLIMMIX procedure of SAS (V 9.4), and Tukey's HSD test was performed to separate means. A significant difference was considered when $P \leq 0.05$.

2.4. RESULTS AND DISCUSSION

Results regarding the translucency are summarized in Table 1. All the parameters analyzed were affected by translucency score except for initial egg weight, egg weight loss, and unhatched + culls percent ($P > 0.05$). This agrees with Baker and Curtiss (1957) and Wang et al. (2017), who did not observe any correlation between initial egg weight and translucency on table eggs when stored at room temperature. The percentage of egg weight loss at 18 d of incubation was similar to the values reported by Zakaria et al. (2009) when evaluating egg weight loss on flocks of 42 to 67 weeks of age but slightly lower than the values between 10 and 11.5% reported by Tona et al. (2001) and Iqbal et al. (2009). We consider that the incubation conditions used in this study may have had an impact on the results of weight loss since, as recommended by the incubator's manufacturer manual, we did not lower the temperature of the incubators following the transfer and allowed the eggs to hatch at 37.8°C. However, every treatment and repetition were carried out under similar circumstances.

Regarding the transfer egg weight ($P = 0.0358$) obtained on day 18 of incubation, results show a lower egg weight in high translucent eggs (T score 3) compared to low translucent eggs

(T score 1). Research has suggested that loss of weight during incubation could be attributed to water vapor exchange that can be influenced by eggshell porosity (Sousa de Araujo et al., 2017) and thickness (Roque and Soares, 1994). However, no differences in porosity have been reported regarding translucency in laying eggs (Talbot and Tyler, 1974; Wang et al., 2017). According to Roque and Soares (1994), thinner eggshells can lose more weight during incubation, which is contrary to our observations. We found that eggs with thicker eggshells, but high translucency may lose more weight during incubation than eggs with thin eggshells. One crucial component that allows gaseous exchange throughout the shell is the inner membrane (Kayar, Snyder, and Black, 1981). Translucent eggs have been reported to have thinner inner membranes that are more easily breakable (Wang et al., 2017), which negatively affects the flow of gases through the shell. It is suggested that even though the eggshell of those eggs is thicker, their translucency renders them more sensitive to losing weight during incubation due to a lower quality of their inner membrane.

Eggs with a translucency score of 1 had a 6.91% higher hatchability of eggs set ($P = 0.0127$) and greater chick weight ($P = 0.0385$) in comparison to eggs with a translucency score of 3. Higher hatchability can be influenced by fewer bacterial infection occurrences (Barbour et al., 1984) and a better quality of the eggshell and inner membrane, which may impact moisture regulation and gas exchange (Roque and Soares, 1994). In 2010, Chousalkar and others observed that eggs with high translucency had an increased pathogenic bacteria penetration, linking the integrity of the inner and outer eggshell membranes and the consequential shell ultrastructure to bacterial permeability. Wang et al. (2017) found that the inner membrane of high translucent eggshells was significantly thinner and had lower failure stress values, which indicates reduced toughness and elasticity. They concluded that high translucent eggs had membranes that were more easily disrupted and offered less protection to egg content.

Results of this study also suggest that translucency impacts the percentage of egg loss ($P = 0.0282$), showing a 5.79% higher egg loss in highly translucent eggs (T score 3) than low translucent eggs (T score 1). Potential causes of embryonic death in translucent eggs could be related to poor resistance to water loss and altered respiration rate of the embryo during incubation, as well as high susceptibility to bacterial contamination, as mentioned above. We also hypothesize that translucency may even be related to the nutritional and immunological

quality of the yolk or to a nutritional shortfall in the hen that could potentially affect fertility and/or embryo development. However, further studies are required to confirm this hypothesis.

Regarding eggshell thickness, it was shown that highly translucent eggshells (T score 3) had greater thickness ($P < 0.0001$). Similar results were reported by Talbot and Tyler (1974) and Wang et al. (2017); these last authors suggest that the effect of translucency on thickness varies between hens' lines, finding the same relationship between thickness and translucency only in Brown-egg Dwarf layers but not in White Leghorn lines. It is thought that size, shape, and orientation of the calcite crystals of the eggshell are responsible for its ultrastructure, breaking strength, and thickness (Nys et al. 2004). According to Liao et al. (2013), the length of the mammillary layer and the size of the mammillary cones are positively correlated with eggshell thickness. Chousalkar et al. (2010) observed that translucent eggshells have changes primarily in their mamillary layer and cones. It is suggested that the increased thickness of the highly translucent eggs is caused predominantly by alterations in their ultrastructure.

Several authors have correlated a greater eggshell thickness with better hatchability and higher egg strength (Zhang et al., 2005; Ketta and Tůmová, 2018). However, in this study, it was observed that when eggs were classified by translucency, the thinner eggshells had the highest hatchability. The differences could be attributed to potentially better uniform shell thickness over the entirety of the egg, which causes a greater strength of the eggs, as suggested by Yan et al. in 2014. These authors found that eggs with thin and uniform shells are stronger than those with thick yet fewer uniform shells. It is suggested that translucency also may affect the strength of the eggshell due to modifications in its ultrastructure (Van Toledo et al., 1982; Chousalkar et al., 2010) and weaknesses in its inner membrane (Wang et al., 2017), which make translucent eggs more susceptible to microcracking even though they have a thicker eggshell. These characteristics could affect water and vapor exchange during incubation, increase bacterial penetration, and, therefore, affect hatchability.

Regarding eggshell coloration lightness, results suggest that L* value was significantly correlated with initial egg weight ($P = 0.0056$), transfer egg weight ($P = 0.0211$), hatchability of eggs set ($P = 0.0481$), unhatched eggs + culls ($P = 0.0003$) and shell thickness ($P = 0.0019$). However, chick weight ($P = 0.4087$) and egg weight loss ($P = 0.5389$) were not affected by eggshell L*value (Table 2).

In this study, darker eggshells showed heavier initial egg weight (P = 0.0056). These results do not agree with Joseph et al. (1999) and Shafey et al. (2005), in which the authors did not find any relationship between eggshell coloration and initial egg weight when comparing different broiler breeds and ages (32, 36, and 42 weeks old). The discrepancy between their findings and ours could be attributed to the fact that these authors chose eggs of a similar size and chickens with similar body weights for their study, while we randomly selected eggs from hens between 50 and 55 weeks of age, which could have increased the variability in the egg weight in our study.

Regarding hatchability, dark-colored eggs had ~3.75% higher hatchability (P = 0.0481), which agrees with Baylan et al., who in 2017 also found a higher hatchability with darker eggshells from broiler breeder hens, as well as Kumar in 2018 who studied hatchability on brown line breeders. Darker eggshells on broiler breeders have been related to a higher maternal antibody content in the yolks (Baylan et al., 2017), lower bacteria development on the shell surface (Ishikawa et al., 2010), and higher specific gravity (Joseph et al., 1999). All these factors may explain the greater hatchability in dark-colored eggs in this experiment. On the other hand, Shafey et al. (2005) suggested that the impact of the color intensity in hatchability depends on the bird's age, where young birds (32 weeks) had better hatchability in lighter colored eggs while old birds (41 weeks) had better hatchability in dark colored eggs. The age of the hen is an important factor in eggshell color and its interaction with other shell quality parameters (Ingram et al., 2008). This could support the idea that eggshell color is only a stronger predictor of hatchability in older flocks, whereas hatchability is higher in young flocks regardless of shell color.

Dark-colored eggs also had a thicker eggshell in this study (P = 0.0019). The pigmentation of the eggshell and the calcification process are interrelated, with a significant deposit of pigment causing an increase in calcium deposition in the eggshell (Samiullah and Roberts, 2013; Lang and Wells, 1987), which may explain why darker-colored eggs are thicker. Some authors suggest that thicker eggshells have more chances of success during the incubation process (Liao et al., 2013; Narushin and Romanov, 2002) as a result of their greater resistance to physical harm and ability to withstand excessive water loss during incubation (McDaniel et al., 1979; Ketta and Tůmová, 2018). However, thickness does not affect chick weight (Yamak et al., 2015). This also agrees with our observation that dark-colored eggs had thicker eggshells and

had higher hatchability with no influence on chick weight ($P > 0.05$). In contrast, other authors (Malik et al., 2015) suggest that there is no relationship between thickness and hatchability. Such discrepancy could be attributed to the flock breed and age they used (Cobb, 64 weeks old), which was about 10 to 14 weeks older than the flock used in our study. At that late age, hatchability is at its lowest point in the entire cycle and could even be independent of shell quality measurements.

An interaction between color and translucency was observed only for eggshell thickness ($P = 0.0292$) (Table 3), where eggs classified as light-colored and with translucency score of 1 had a thinner eggshell compared to those that were dark and had a translucency score of 3. This interaction is consistent with the effect of translucency and color on thickness when evaluated independently. Thickness has been considered a very important determinant of eggshell quality. Our study suggests that thickness can be best estimated by considering the translucency and color of the eggshell.

In conclusion, low translucent eggs (score of 1) positively influenced hatchability and chick weight compared to high translucent eggs (score of 3), which had thicker eggshells. Regarding the impact of eggshell color, greater values of thickness and hatchability were found on dark-colored eggs. The interaction of both translucency and color lightness only impacted shell thickness. These results suggest that eggshell translucency and coloration lightness can be good non-invasive indicators of eggshell thickness, hatchability, and chick weight in breeder flocks. Further comparisons should be made to determine whether these results are common across most breeder strains.

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2.6. TABLES

Table 1. Influence of translucency in initial egg weight, final egg weight, water loss %, egg loss %, hatchability, unhatched + culls %, chick weight, and eggshell thickness

Parameter	Translucency			SE	*P value*
	T1	T2	T3		
Initial egg weight (g)	66.87	66.9	66.51	0.34	0.1962
Transfer egg weight (g)	60.74[a]	60.55[ab]	59.92[b]	0.46	0.0358
Egg weight loss (%)	9.18	9.5	9.92	0.35	0.1188
Egg loss (%)	17.53[b]	19.29[ab]	23.32[a]	2.85	0.0282
Hatchability (% eggs set)	78.14[a]	75.83[ab]	71.23[b]	3.31	0.0127
Unhatched + culls (%)	4.33	4.95	5.45	0.71	0.355
Chick weight (g)	45.79[a]	45.43[ab]	45.10[b]	0.64	0.0385
Eggshell thickness (µm)	393.77[c]	422.41[b]	427.60[a]	2.55	<.0001

[abc] Different letters represent statistically significant differences ($P \leq 0.05$) within rows.

T1 = Translucency score of 1; T2 = Translucency score of 2; T3 = Translucency score of 3; SE = Standard error.

Table 2. Influence of color in initial egg weight, final egg weight, water loss %, egg loss %, hatchability, unhatched + culls %, chick weight, and eggshell thickness

Parameter	Color		SE	*P value*
	Dark	Light		
Initial egg weight (g)	67.05[a]	66.49[b]	0.32	0.0056
Transfer egg weight (g)	60.72[a]	60.09[b]	0.44	0.0211
Egg weight loss (%)	9.44	9.62	0.29	0.5389
Egg loss (%)	19.43	20.66	2.71	0.4828

Hatchability (% eggs set)	76.94[a]	73.19[b]	0.18	0.0481
Unhatched + culls (%)	3.68[b]	6.15[a]	0.64	0.0003
Chick weight (g)	45.53	45.35	0.63	0.4087
Eggshell thickness (µm)	417.47[a]	411.72[b]	2.4	0.0019

[ab] Different letters represent statistically significant differences ($P \leq 0.05$) within rows.

SE = Standard error

Table 3. Influence of the interaction between translucency and color in initial egg weight, final egg weight, water loss %, egg loss %, hatchability, unhatched + culls %, chick weight, and eggshell thickness.

Parameter	Translucency / color						SE	P value
	T1		T2		T3			
	Dark	Light	Dark	Light	Dark	Light		
Initial egg weight (g)	67.27	66.48	67.25	66.56	66.62	66.41	0.38	0.4248
Transfer egg weight (g)	61.23	60.24	60.81	60.3	60.1	59.74	0.51	0.5856
Egg weight loss (%)	8.97	9.39	9.58	9.42	9.78	10.06	0.5	0.6872
Egg loss (%)	16.07	18.98	19.22	19.36	23.01	23.64	3.22	0.7869
Hatchability (% eggs set)	80.71	75.57	76.88	74.78	73.23	69.22	3.67	0.7917
Unhatched + culls (%)	3.22	5.45	4.05	5.85	3.76	7.14	0.9	0.577
Chick weight (g)	45.95	45.64	45.62	45.23	45.02	45.19	0.67	0.5042

Eggshell thickness (µm)	395.02[c]	392.52[c]	423.51[b]	421.30[b]	433.87[a]	421.33[b]	2.95	0.0292

[abc] Different letters represent statistically significant differences ($P \leq 0.05$) within rows.

T1 = Translucency score of 1; T2 = Translucency score of 2; T3 = Translucency score of 3; SE = Standard error.

2.7. FIGURE

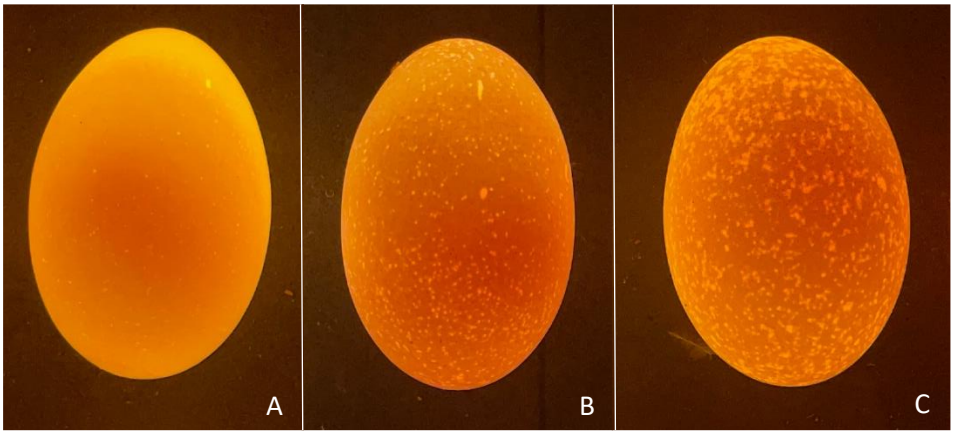

Figure 1. Grades of translucent eggshell spots. A) Eggshell translucency score of 1, B) Translucency score of 2, C) Translucency score of 3.

CHAPTER 3. RELATIONSHIP BETWEEN EGGSHELL TRANSLUCENCY AND COLOR INTENSITY WITH EGG QUALITY PARAMETERS ON BROILER EGGS.

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3.1. ABSTRACT

Eggshell quality is one of the factors that has been linked to hatchability for several years. Recently, eggshell's translucency and color intensity were reported to also impact hatchability. The aim of this study is to describe any potential relationships between eggshell translucency and color intensity with eggshell quality metrics and internal egg quality. A total of 270 eggs from Ross 708 breeder hens (52 week-old) were selected for this study based on eggshell color intensity (dark, medium, and light) and translucency level (1 =low, 2=medium, 3=high). Eggs were subjectively classified by color intensity, and the difference in L-value was confirmed using an electronic colorimeter. For the evaluation of translucency, a subjective scoring system was used based on the amount, size, and coverage of spot areas or mottling in the eggshell. The Egg Tester Ultimate was used to evaluate egg weight, albumen height, strength, shell thickness, Haugh Units (HU), and yolk color. Data were analyzed using the GLIMMIX procedure of SAS (V 9.4), and Tukey's HSD test was performed to separate means. A significant difference was considered between the means when $P \leq 0.05$. Results showed that translucency did not affect egg weight, strength, and yolk color ($P > 0.05$). Low translucent eggs (score of 1) had an eggshell 0.4 g lighter ($P=0.0017$) and 12 µm thinner ($P = 0.0207$) than high translucent eggs (score of 3). The low translucent eggs had 0.4 mm thicker albumen ($P = 0.0077$) and higher HU value ($P = 0.0173$) than medium translucent eggs (score of 2). Regarding color intensity, the difference in L-value was statistically confirmed ($P < 0.0001$): dark = 76.61, medium = 82.03, and light = 86.86. Results showed no effect on HU value and yolk color ($P>0.05$). Dark-colored eggs had 2.86 g heavier eggs ($P = 0.00022$), 0.47 g heavier eggshells ($P<0.0001$), 11 µm thicker eggshells ($P = 0.0293$), and 0.47 kg/cm^2 greater eggshell strength ($P = 0.0016$) than light colored eggs. No interaction was observed between

translucency and color intensity in any of the parameters analyzed ($P > 0.05$). It was concluded that high translucent and dark-colored eggs had the best effects on eggshell quality parameters.

Keywords: Eggshell, color intensity, translucency, eggshell quality, broiler eggs.

3.2. INTRODUCTION

Profitability in commercial hatcheries is related to egg hatchability; however, even when adequate incubation conditions are implemented, the quality of the egg plays an important role in the resultant hatch. Therefore, having the majority eggs with a standard structure and internal quality is crucial since it may influence the biological value of the eggs and affect the normal development of the embryo (Peebles et al., 2001). The egg is composed mainly of three parts: eggshell, egg white or albumen, and yolk. All three represent about 9.5%, 63%, and 27.5% of the whole egg weight, respectively (Cotterill & Geiger, 1977). The eggshell provides mechanical and biological protection for the embryo, as well as allowing gaseous exchange, and buffers temperature fluctuations. It is composed of four layers: the cuticle, mammillary, palisade, and eggshell membrane (Hincke et al., 2012). These layers work together to provide protection and serve as a source of calcium for the developing skeleton of the chick during incubation (Chien et al., 2009). Egg white or albumen provides vital nutrients and antimicrobial defense for the embryo (Carinci & Manzoli-Guidotti, 1968; Krkavcová et al., 2018). The primary components of albumen are water (88%) and protein (10.5%) (Mine, 2002). The most widely used and accepted measure of internal albumen quality is the Haugh Units (HU), which takes the height of the thick albumen and the egg weight into consideration to provide an indication of the freshness of the egg (Jones, 2012). The yolk is mainly composed of proteins (15.7-16.6%) and lipids (32-35%) (Mine, 2002). These lipids supply more than 90% of the energy required by the developing embryo (Vieira & Moran, 1998). The yolk color is mainly attributed to the presence of carotenoids and has been related to the quality of the hatched chicks (Hien et al., 2016). Both egg white and yolk contain antioxidant properties essential for the development of the embryo (Nimalaratne & Wu, 2015).

Most methods for testing egg quality are time-consuming and entail breaking the egg to evaluate its contents, which makes them unsuitable for hatch. Therefore, inferring egg quality

through alternative non-destructive approaches, such as the observation of external physical characteristics of the eggshell, could be a viable solution. The physical eggshell characteristics of translucency and color intensity have been recently linked to hatchability and shell thickness (Orellana et al., 2023). However, their relationship with other egg quality indicators of hatching eggs has not yet been thoroughly investigated. Eggshell translucency is also commonly known as windows, mottling, or water marks, which is easily observed when candling eggs. It is thought to be caused by the accumulation of moisture in the eggshell and uneven drying after the egg is laid (Solomon, 1997). The eggshell color is determined by the pigments protoporphyrin, biliverdin, and its zinc chelate; their mixtures are responsible for the various egg colors observed in nature, and their presence depends on genetics (Hargitai et al., 2017). However, variations in color intensity in the same genetic lines can be observed due to environmental conditions, stress, and age of the flock (Odabasi et al., 2007; Park et al., 2017). The purpose of this project is to evaluate the relationship of eggshell translucency and color intensity with other egg quality parameters on hatching eggs of broiler breeder hens.

3.3. METHODOLOGY

A total of 270 eggs from 52-week-old Ross 708 breeder hens collected from a commercial hatchery were used for this study. Prior to collection, the eggs were stored for 3 days in a constant environment (temperature 18 °C and 70% relative humidity) in the storage room of the same commercial hatchery.

Translucency score, coloration lightness

Several eggs were subjectively sorted by eggshell color intensity (dark, medium, and light), and the (L*value) was evaluated using an electronic colorimeter (Nix Color Sensor Pro 2) to confirm an average L*=86.86 for light-colored eggs, L*=82.03 for medium-colored eggs, and L*=76.61 for dark-colored eggs, on a scale of 0 to 100. Then, 90 eggs of each group of color intensity were selected and subjectively divided into three groups based on their translucency level (1 = low, 2 = medium, 3 = high) as defined in Orellana et al. (2023). A 500-lumen flashlight was used to determine the score based on the amount, size, and coverage of spot patterns or mottling areas in the eggshell. In total, this experiment was composed of 9 treatments (3 color intensities x 3 levels of translucency).

Internal and external quality traits

Internal and external quality traits of eggs were measured within 48 hours after collection using a digital Egg Tester Ultimate ^{TM} (ORKA food technology LLC), which measured egg weight (g), eggshell strength (kg/cm^2), Haugh unit (HU), yolk color (according to the DSM Yolk color fans**),** eggshell thickness (mm), and USDA grade (AA: 72 or more; A: 71.9 – 60; B: 59.9 – 31; C: 30.9 or less), albumen height (mm). Eggshell weight (g) was measured after washing the interior of the egg membrane and drying overnight at room temperature.

Ultrastructural analysis

The eggshell of five eggs from each treatment was randomly chosen, and then one section, approximately 0.5 cm^2, was cut from the equatorial portion of the egg. This section was placed at a 90° angle on metallic stubs with conductive carbon cement to expose the transversal/cross-sectional view of the shell and membranes. Samples were then gold coated with a polaron EMS Q150R sputter coating unit and imaged using a Zeiss EVO 50 scanning electron microscope using working distances of <12 mm. The width of the inner membranes, mammillary layer, the palisade layer, and the mammillary knobs were taken. Each layer was subjectively differentiated based on its structure, and a horizontal line representing the average was used to estimate the width of each layer (figure 2).

Statistical analysis

Data including egg weight, eggshell weight, eggshell color (L-value), eggshell strength, eggshell thickness, HU value, yolk color, albumen height, inner membrane, mammillary layer, palisade layer, and cones width were analyzed using the GLIMMIX procedure of SAS (V 9.4) and Tukey's HSD test was performed to separate means. A significant difference was considered between the means when $P \leq 0.05$.

3.4. RESULTS AND DISCUSSION

Results regarding the relationship of translucency with egg quality and shell ultrastructure parameters are summarized in Table 1. In this study, no statistical difference ($P > 0.05$) was observed between translucency and egg weight, eggshell strength, eggshell color (L-

value), or yolk color. These findings are consistent with Ren et al. (2023), who found no effect of translucency on egg weight, eggshell strength, and yolk color across flock ages after evaluating late-phase Dwarf Layer-White hens of 75, 79, and 83 weeks of age after 7 days of storage. However, they discovered the opposite when evaluating Rhode Island White hens of the same age, indicating that strain has an effect on the impact of translucency in egg quality parameters. Similarly, Wang et al. (2017) found no association of translucency with egg weight and eggshell color when assessing eggs from 56-week-old Brown-Egg Dwarf layers (BDL) after one week of storage. However, they did discover a relationship between translucency and eggshell strength, where high translucent eggs had a stronger eggshell than non-translucent eggs. In our results, we only found a numerical difference that agrees with their findings in this parameter. The size of our sample was likely small (30 eggs per treatment) to allow us to detect statistical differences in eggshell strength; future investigations should increase the sample size to detect differences in this parameter.

However, eggshell weight, thickness, HU value, and albumen height were statistically affected by translucency ($P < 0.05$). The eggshell of high translucent eggs (score of 3) was 0.32 g heavier and 12 µm thicker than low translucent eggs (score of 1); this result agrees with Wang et al. (2017), who also found similar differences in both parameters among translucent and non-translucent eggs. Chousalkar et al. (2010) observed additional calcium around the mammillary cones of the shells of high translucent eggs, which may have contributed to the increased eggshell weight. Since the results of our study showed no statistically significant difference in eggshell strength, we hypothesize that the hen is adding more calcium to the shell to help with strengthening of the shell in translucent eggs to make them comparable to the non-translucent eggs; however, more research is required to fully comprehend the physiological changes that occur on the hen and cause them to lay translucent eggs.

Regarding egg internal quality, low translucent eggs (score of 1) had thicker albumen and higher HU value than eggs with a medium translucency level (score of 2), representing better interior egg quality. In contrast to our findings, Baker and Curtis (1958) and Wang et al. (2017) observed no impact of translucency on the internal quality of eggs. The quality of the albumen has been reported to decrease as egg storage time increases prior to egg placement into incubators. This is caused by an increase in pH due to the release of water and CO_2 in the environment, decreasing the height, viscosity, and HU of the egg. The quality of the eggshell and

membranes plays an important role in the rate of CO_2 release, and we propose that the properties of the eggshell membrane (Wang et al., 2017) and variations in the alignment of the layers of the eggshell (Chousalkar et al. 2010) may have a higher influence on the gas and water diffusion to the exterior than eggshell thickness when eggs are classified by translucency.

Except for eggshell thickness, our findings regarding eggshell ultrastructure (table 4) show no statistically significant differences between high- and low-translucent eggs in the parameters of the inner membrane, mammillary layer, palisade layer, and cone's width ($P > 0.05$). The inner membrane is made up of a network of fibers that envelops the albumen and is attached to the calcified mammillary knobs of the mammillary layer that extends beyond the bases of the cones and fusion forming the palisade layer (Nys et al., 2004). According to Liao et al. (2013), the length of the mammillary layer and the size of the mammillary cones are positively correlated with eggshell thickness. However, in this study, no correlation was observed between these parameters when eggs were classified by translucency. In agreement with our results, Wang et al. (2017) found no differences in the thickness of the eggshell and the mamillary layer due to translucency when evaluating eggs from pure-line White Leghorn layers (WLL) at 50 weeks of age and eggs from Brown-Egg Dwarf Layers (BDL) at 42 weeks of age. However, our findings for the mammillary layer thickness (67–76 µm) are more comparable to those found when the author analyzed white eggs from WLL (75–80 µm) than brown eggs from BDL (91–94 µm). Roberts et al. (2013) evaluated the quality of the mammillary layer based on the cap size of the cones and the degree of membrane attachment, finding a low correlation with translucency. They suggested that the ultrastructure of the eggshell may only partially explain the translucency phenomenon and that other factors may be implicated. Chousalkar et al. (2010) observed that eggs with high translucency had a good quality of the mammillary cones; however, they suggested that translucency could be more related to the alignment of the mammillary cones than to its quality. They saw that the mammillary cones of translucent eggs appear to "line up," creating long and continuous grooves between the cones. They also noticed more calcium surrounding the mammillary cones, which they believe helps translucent eggs to strengthen their shells.

Translucency has been defined as the accumulation of water on the eggshell (Solomon, 1997). Therefore, the inner membrane of the eggshell has been suggested to be important in the formation of translucency since it acts as a barrier preventing the penetration and accumulation

of the egg contents into the eggshell and serves as a platform for the initiation of the eggshell mineralization. In 2017, Wang et al. suggested that the failure stress of the inner membrane was a consistent parameter that differentiated translucent from non-translucent eggs. In terms of the thickness of the inner membrane, some authors have found thinner membranes in high-translucent eggs than in low-translucent eggs (Wang et al., 2016; Wang et al., 2017; Cheng & Ning, 2023). However, in our study, we found no difference in the thickness of the inner membrane. The discrepancy could be attributed to different breeds, ages, egg's area of sampling (blunt/sharp ends), and the tools (digital micrometer gauge) employed in their methodology, as well as the fact that in this study, a random piece from the equatorial portion of the eggshell was collected assuming that the translucency was homogenously distributed. We recommend for future research to first candle the eggshells and mark with a pencil the areas of the specific level of translucency before cutting the pieces that will be used for the evaluation of the ultrastructure.

Regarding eggshell coloration, lightness (L*value) results are summarized in Table 2. A statistical difference was observed in the parameters of egg weight, eggshell weight, eggshell thickness, and eggshell strength ($P < 0.05$), where dark-colored eggs were overall 2.86 g heavier, and their eggshells were 0.47 g heavier, 11 µm thicker, and 0.47 kg/cm^2 stronger than light-colored eggs. These results agree with our previous results regarding egg weight and shell thickness (Orellana et al., 2023), evaluating the effect of color lightness in fertile eggs from 55-week Ross 708 hens. Other scientists, however, have found different and opposite results; Shafey et al. (2005) observed that the color lightness of the shell had no effect on the parameters of egg weight, eggshell weight, and thickness in eggs from a 32-week-old meat-type breeder flock (Al-Wady Pty Limited, Riyadh, Saudi Arabia) and explained that eggshell pigment makes a very small difference in the thickness of the eggshell since the majority of the pigment is concentrated in the cuticle which is usually thin enough to not have a significant impact (Butcher and Miles, 1995). In 2007, Odabasi et al. discovered that, when examining eggs from Hy-line brown layers, the heavier the egg, the lighter-colored the eggshell was. They proposed that this might be due to a lack of a proportional increase in the pigment concentration deposited on the shell surface as the egg size increases, resulting in larger eggs with lighter-colored shells. Since the size of the egg increases as the hen ages, the decrease in eggshell pigmentation in brown-colored eggs has also been proposed to be related to fibrosis and atrophy of the hen's endometrium because of successive oviposition that affects the synthesis of protoporphyrin (Park et al., 2017; Lu et al.,

2021). The results of our study question the applicability of the previous hypotheses on white eggs from broiler breeder hens. In this study, it is suggested that eggshell color is related to improvements in health and less stress in hens, which allows them to lay heavier, thicker, stronger, and darker eggs. According to Moreno and Osorno (2003), increasing amounts of protoporphyrin in the eggshell may be related to a higher antioxidant capacity of the hen in the liver, blood, and uterus because this dye acts as a pro-oxidant in the hen's system, which stimulates the antioxidant activity of the hen.

Regarding the relation of color lightness with internal egg quality parameters, only albumen height showed a statistical difference, where dark-colored eggshells had 0.44 mm thicker albumen than light-colored eggs. However, we attribute this effect to the heavier weight of the dark-colored eggs shown in this study, which may indicate a higher amount of albumen in the egg that increased its thickness measurement. The HU value has been frequently utilized to compensate for the effect of egg weight and provide a more accurate evaluation of internal quality. This measurement is a logarithmic function of the height of albumen corrected by egg weight (Eisen et al., 1962). In this study, no effect of color lightness was observed in the HU value ($P > 0.05$). However, there was a trend ($P=0.056$) for dark-colored eggs to have a higher HU than medium or light-colored eggs. As stated, this measurement (HU) is a function of albumen height and egg weight; in this study, the dark-colored eggs had a higher albumen height and weight than the other two-color grades. In contrast, Aygün (2014) observed a positive correlation of color lightness with the HU value of eggs from 60-week-old H&N Brown Nick hens, where the lower the L* value (darker eggshells), the lower the HU value. The difference between these results could be related to the different breed, age, and egg storage conditions utilized in their study (1 day at 20 °C and no relative humidity specification) against our study (3 days at 18 °C and 70% relative humidity). Williams (1992) proposed that bird age and storage conditions have the largest impact on albumen quality, while other factors, such as nutrition, environmental, or heat stress, have little to no direct effect. Our findings imply that the color lightness of the eggshell does not directly influence HU value. We suggest that this could be explained by the fact that albumen and pigment secretion occur in separate sectors of the oviduct, with the albumen secreted in the magnum and the pigment secreted in the shell gland (uterus) and deposited over the eggshell near the end of egg formation, with no influence of one on the

other (Ottinger and Bakst, 1995). However, we do not discard that darker eggshells may indicate better overall bird health, indirectly affecting albumen height and HU score.

Regarding the effect of color lightness in the eggshell ultrastructure, only the thickness of the eggshell and the palisade layer between dark- and light-colored eggs showed differences ($P < 0.05$) with dark-colored eggshells having higher values than light-colored eggs. The findings on thickness concur with those of Yang et al. (2009) and Aygün (2014), who discovered that the thickness and strength of the eggshell increased as the egg became darker. The palisade layer makes up over 70% of the eggshell's total thickness, and reductions in this layer have been related to the weakening of the eggshell. (Rodríguez-Navarro et al., 2002; Ahmed et al., 2005; Park & Sohn, 2018). Therefore, the thicker palisade layer that was observed in this study may be considered as being the cause of the stronger and thicker eggshell in dark-colored eggs.

No interaction between eggshell translucency and color lightness was observed (Table 6) in any of the parameters analyzed. These findings suggest that translucency and color lightness should be investigated separately since both may yield conflicting conclusions. We propose that the application of one or the other criterion as a non-destructive egg quality predictor may rely on how frequently eggshells with high translucency or darker/lighter characteristics are found in the field.

In conclusion, high translucent and dark-colored eggs positively influenced external eggshell quality parameters; however, only translucency was observed to impact internal quality parameters. These results suggest that eggshell translucency and color intensity can be good non-invasive indicators of eggshell thickness, HU value, strength, and eggshell weight in broiler breeder eggs from Ross 708 hens. Further comparisons should be made to determine whether these results are common across other breeder strains.

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3.6. TABLES

Table 4. Influence of translucency in egg quality and ultrastructure parameters.

Parameter	Translucency			SE	*P value*
	T1	T2	T3		
External egg quality					
Egg weight (g)	66.62	66.21	66.83	0.480	0.6498
Eggshell weight (g)	5.42[b]	5.55[ab]	5.74[a]	0.063	0.0017
Eggshell thickness (µm)	331.30[b]	335.50[ab]	343.30[a]	3.10	0.0207
Eggshell strength (kg/cm^2)	3.72	3.83	3.98	0.105	0.2117
Eggshell color (L*value)	81.90	82.22	81.40	0.307	0.1630
Internal egg quality					
HU value	67.43[a]	64.01[b]	65.01[ab]	0.860	0.0173
Yolk color	7.52	7.48	7.68	0.08	0.1647
Albumen height (mm)	5.23[a]	4.85[b]	5.00[ab]	0.870	0.0077
Eggshell ultrastructure					
Inner membrane (µm)	49.95	53.49	49.57	2.35	0.4394
Mammillary layer (µm)	67.39	74.00	76.04	3.25	0.1530
Palisade layer (µm)	242.40	249.09	258.2	4.68	0.0682
Cones width (µm)	64.82	61.30	65.94	2.33	0.3468
Egg shell thickness (µm)	359.78[b]	376.50[ab]	383.69[a]	6.42	0.0382

[abc] Different letters represent statistically significant differences ($P \leq 0.05$) within rows.

T1 = Translucency score of 1; T2 = Translucency score of 2; T3 = Translucency score of 3; SE = Standard error.

Table 5. Influence of color intensity in egg quality and ultrastructure parameters.

Parameter	Color			SE	*P value*
	Dark	Medium	Light		
External egg quality					
Egg weight (g)	68.07[a]	66.37[b]	65.21[b]	0.481	0.0002
Eggshell weight (g)	5.80[a]	5.57[ab]	5.33[b]	0.063	<0.0001
Eggshell thickness (µm)	343.20[a]	335.20[ab]	341.7[b]	3.12	0.0293
Eggshell strength (kg/cm^2)	4.15[a]	3.69[b]	3.68[b]	0.105	0.0016
Eggshell color (L*value)	76.62[b]	82.03[ab]	86.86[a]	0.307	<0.0001
Internal egg quality					
HU value	67.10	65.24	64.19	0.860	0.0566
Yolk color	7.60	7.64	7.43	0.08	0.1290
Albumen height (mm)	5.27[a]	4.98[ab]	4.83[b]	0.12	0.0024
Eggshell ultrastructure					
Inner membrane (µm)	54.18	46.47	52.36	2.35	0.0655
Mammillary layer (µm)	77.33	72.83	67.27	3.24	0.1025
Palisade layer (µm)	256.67[a]	252.95[ab]	240.01[b]	4.68	0.0411
Cones width (µm)	67.87	62.86	61.33	2.33	0.1200
Egg shell thickness (µm)	388.25[a]	372.21[ab]	359.53[b]	6.61	0.0131

[ab] Different letters represent statistically significant differences ($P \leq 0.05$) within rows.

SE = Standard error

Table 6. Influence of the interaction between translucency and color in egg quality and ultrastructure parameters.

Parameter	Translucency / color									SE	*P value*
	T1			T2			T3				
	Dark	Medium	Light	Dark	Medium	Light	Dark	Medium	Light		
External egg quality											
Egg weight (g)	69.01	66.10	64.74	67.55	65.86	65.21	67.65	67016	65.67	0.83	0.5540
Eggshell weight (g)	5.70	5.44	5.11	5.75	5.50	5.41	5.96	5.79	5.47	0.11	0.7156
Eggshell thickness (µm)	336.90	327.70	329.30	337.30	336.00	333.20	355.30	342.00	332.5	5.39	0.4223
Eggshell strength (kg/cm2)	3.98	3.63	3.55	4.00	3.75	3.73	4.48	3.70	3.77	0.18	0.5980
shell color (L*value)	76.71	81.80	87.18	76.59	82.94	87.14	76.56	81.37	86.27	0.53	0.5649
Internal egg quality											

HU value	69.11	67.04	66.13	65.64	63.32	63.07	66.55	65.36	63.38	1.49	0.9856
Yolk color	7.45	7.63	7.47	7.63	7.40	7.40	7.73	7.90	7.41	0.14	0.2874
Albumen height (mm)	5.52	5.15	5.02	5.08	4.77	4.69	5.19	5.01	4.79	0.15	0.9649
Eggshell ultrastructure											
Inner membrane (µm)	54.23	43.91	51.71	53.89	50.91	55.68	54.41	44.61	49.68	4.06	0.8727
Mammillary layer (µm)	74.49	65.78	61.90	73.86	77.32	70.83	83.63	75.40	69.10	5.62	0.7642
Palisade layer (µm)	249.88	243.68	233.64	253.86	251.72	241.48	266.26	263.44	244.90	8.11	0.9741
Cones width (µm)	68.30	60.82	65.35	65.61	59.21	59.06	69.71	68.53	59.57	3.99	0.5754
Egg shell thickness (µm)	378.88	353.20	347.26	381.60	379.96	368.00	404.26	383.46	363.34	11.34	0.7212

[abc] Different letters represent statistically significant differences ($P \leq 0.05$) within rows.

T1 = Translucency score of 1; T2 = Translucency score of 2; T3 = Translucency score of 3; SE = Standard error.

3.7. FIGURE

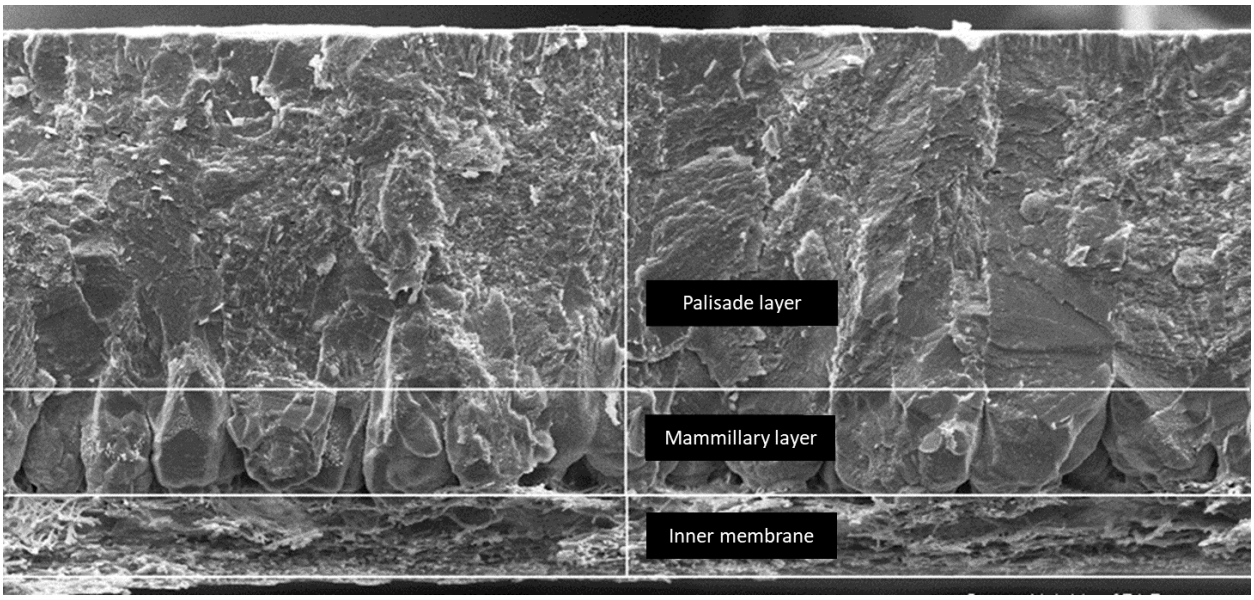

Figure 2. Ultrastructure of the eggshell