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Eggshell structure, measurements, and quality-affecting factors in laying hens: a review

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ABSTRACT: Eggshell quality is one of the most significant factors affecting poultry industry; it economically influences egg production and hatchability. Eggshell consists of shell membranes and the true shell that includes mammillary layer, palisade layer, and cuticle. Measurements of eggshell quality include eggshell weight, shell percentage, breaking strength, thickness, and density. Mainly eggshell thickness and strength are affected by the time of egg components passage through the shell gland (uterus), eggshell ultra-structure (deposition of major units), and micro-structure (crystals size and orientation). Shell quality is affected by several internal and external factors. Major factors determining the quality or structure of eggshell are oviposition time, age, genotype, and housing system. Eggshell quality can be improved through optimization of genotype, housing system, and mineral nutrition.

Keywords: eggshell composition; eggshell quality; oviposition time; genotype; housing system

INTRODUCTION

The eggshell significance is related to its function to resist physical and pathogenic challenges from the external environment, such as its function as an embryonic respiratory component, in addition to providing a source of nutrients, primarily calcium, for embryo development (Hunton 2005). Moreover, eggshell quality is an important factor for egg production, for example in table eggs; shells must be strong enough to prevent failure during packing and transportation. These properties are fulfilled by the eggshell structure, because it is a highly ordered bio-ceramic complex as a consequence of controlled interactions between both mineral and organic matrix constituents. Eggshell quality plays a key role in the economics of egg production because egg breakage accounts from 8 to 10% of total egg production causing economic losses. A considerable effect on eggshell quality is associated with housing system. However, results of the effect of housing systems on eggshell quality are mixed. Inconsistent results can be explained by structural differences of the eggshell related to the interaction of housing system, genotype, age, oviposition time, and mineral nutrition. Therefore, it is important to pay attention to eggshell structure in relationship to different factors, mainly housing systems.

Eggshell quality is influenced by internal and external factors including genotype, age, oviposition time, and housing system, and also by balanced feeding with sufficient Ca, P, and trace minerals supplementation. Venglovska et al. (2014) observed beneficial effects of Mn, especially from organic sources, on eggshell quality. The importance of minerals is related to changes of the arrangement pattern of shell membrane fibres in relation to the structural composition of eggshell. Ca supplementation is key for eggshell quality, each eggshell contains up to 3 g of Ca, so the diet of hens must contain adequate amount of Ca in efficiently utilizable form (Roberts 2010).

The main objective of this review is to update and discuss the current findings related to eggshell structure, bio-mineralization process, eggshell

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properties, and measurements of quality in association with selected internal and external factors.

Eggshell structure and formation

Five hours after ovulation, the forming egg enters the red isthmus and uterus where the eggshell calcification process lasting 18–19 h takes place. During mineralization, the incomplete egg bathes in a cellular milieu (the uterine fluid) that contains ionized Ca and bicarbonate necessary for the eggshell formation. The process consists in controlled precipitation of calcium carbonate on the outer eggshell membrane fibres, and occurs in the extracellular space between the dilated shell membranes that envelope the hydrated albumen and the mucosa of uterine wall (Hincke et al. 2012; Gautron et al. 2014). The uterine fluid changes in composition during different stages of the eggshell formation and influences calcite crystal growth in different zones of the calcified shell (Nys et al. 2004).

The interest in the eggshell structure was started earlier in the 19th century by Von Nathusius who well-defined the structural polycrystalline organization. Recently, several studies on the structure of the avian eggshell have been conducted (Nys et al. 2004; Nys and Gautron 2007; Rodriguez-Navarro et al. 2007; Hincke et al. 2010, 2012; Gautron et al. 2014). Mineralized eggshell is formed of calcium carbonate (96%); the remaining components include organic matrix (2%), magnesium, phosphorus, and a variety of trace elements (Nys et al. 2004). From the inside outwards, the eggshell comprises of shell membranes and true shell that includes mammillary layer, palisade layer, vertical layer, and cuticle (Hincke et al. 2008; Gautron et al. 2014). The eggshell membranes are a fibrous structure situated between the eggshell and egg albumen. They are essential for the formation of the eggshell and also provide the shell foundation except at the blunt pole of the egg where they separate to form the air-space. The eggshell membranes are secreted and assembled during approximately one hour, resulting meshwork of interlaced fibres is composed of roughly 10% collagen and 70–75% other proteins and glycoproteins containing lysine-derived cross-links which are organized into morphologically distinct inner and outer sheets that enclose egg albumen (Hincke et al. 2012). The total thickness of these two membranes has been found at approximately 100 µm. Each of these membranes is composed of protein fibres that are arranged so as to form a semi-permeable membrane. The inner membrane remains uncalcified, while the fibres of the outer shell membrane become mineralized at discrete sites and become incorporated into the base of the eggshell (Nys et al. 2004).

Specific nucleation sites on the outer surface of the outer shell membrane attract Ca salts and so initiate the formation of the mammillary layer in that region of the oviduct termed the tubular shell gland (Solomon 2010). The mammillary cones are small masses of organic matter that represent the seeding sites on which crystallization of the shell begins, these cones are penetrated by fibres of the outer eggshell membranes (Figure 1).

The mammillary cones are exclusively the main source for Ca mobilization during embryonic development (Karlsson and Lilja 2008; Chien et al. 2009). Therefore, mammillary core formation and distribution are related to the mechanical strength and respiratory quality of the eggshell (Robinson and King 1970; Koga et al. 1982). Pores formation begins at the level of the mammillary layer with the grouping of 4–5 mammillary bodies. As they grow laterally and vertically, their orientation is such that a central space is left which in functional exchange sites persists through the entire depth of the shell (Solomon 2010).

From and over the mammillary layer the palisade layer develops as the main layer of the shell, this layer comprises about 200 µm as the thickest aspect of the shell, where the calcite crystals grow with a long aspect perpendicularly to the surface. The mammillary layer is the site of a range of structural defects which can be reduced through organically bound selenium (Solomon 2009). Recently, increased chemical reactivity has been found in nano-selenium (Suchy et al. 2014). Palisade columns grow from one mammillary knob and as the calcification process proceeds, adjacent columns fuse. This layer ends at the vertical layer which has a crystalline structure of higher density than that of the palisade layer (Hincke et al. 2012).

The eggshell cuticle is an uneven organic layer covering the outer surface of the eggshell. It is composed of inner calcified and outer non-calcified water insoluble layers which are deposited directly onto the vertical crystal layer of the eggshell (Rose and Hincke 2009; Kusuda et al. 2011). Unfor-

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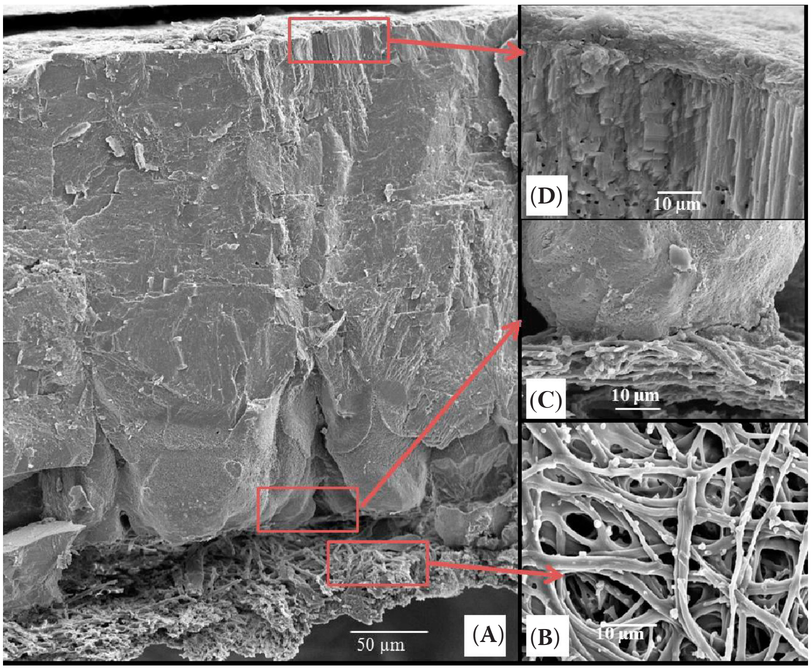

Figure 1. Scanning electron microscopy of the highly ordered structure of the chicken eggshell

(**A**) cross-section of a full eggshell that reveals eggshell membranes, mammillary and palisade layers, and cuticle; (**B**) detailed focus on eggshell membranes showing the network of interlacing fibres; (**C**) cone layer section showing the insertion of mineralized cones into membrane fibres; (**D**) section showing the vertical layer and cuticle covering a mineralized eggshell (Nys et al. 2001)

tunately not all avian eggs have a cuticle layer, and the distribution of cuticle is often patchy (Bain et al. 2009; Samiullah and Roberts 2014). Cuticle deposition is important for the prevention of micro-organisms penetration, which is a frequent event in the absence of cuticle deposition. This feature is reserved by the antimicrobial substances such as lysozyme and ovotransferrin deposited in eggshell cuticle (Rose-Martel et al. 2012; Miksik et al. 2014). Additionally, Messens et al. (2007), De Reu et al. (2010), and Bain et al. (2013) observed a high correlation between the absence of cuticle and bacterial penetration across the eggshell. Cuticle is also important to create a barrier which inhibits water movement across the shell and prevents dehydration of the egg interior components (Rose-Martel et al. 2012). Cuticle deposition is affected by laying hen age (Rodriguez-Navarro et al. 2013) and housing systems (Samiullah et al. 2014).

The pores system of the avian eggshell is located at specific locations between the eggshell cones (columns, prisms) to provide gas and humidity exchange. In chicken eggs, typical pores have a funnel-shaped orifice opening at the outer shell surface at the level of the cuticle, and a single channel passing through the vertical crystal layer and the palisades region to open at the inner surface of the eggshell between neighbouring mammillae (Chien et al. 2008).

The shell structure might have a significant effect on eggshell characteristics, mainly thickness and strength. Bain (1997) suggested that the organization of the palisade columns in addition to crystals size and orientation is a major determinant of shell thickness and strength. Therefore, it is likely that changes in the thickness of the palisade layer independent of structural reorganization of the palisade columns could affect shell strength. Rodriguez-Navarro et al. (2002) revealed a correlation between eggshell strength and crystallographic texture. They concluded that about 40% of the variance in shell strength could be explained by differences in the degree of crystals orientation.

Eggshell deposition occurs in three stages coinciding with sequential secretion of organic matrix constituents in the cellular uterine fluid with the rate of calcium carbonate deposition of 0.32 g/h as the fastest known bio-mineralization event (Nys et al. 1991). The entire process lasts around 17 h in highly selected breeds as are the layers, and it is considered as the longest phase of egg formation (Nys et al. 2004). The first stage takes about 5 h and corresponds to the initiation of mineralization. The first crystals of calcite are nucleated at the sites of the organic aggregates present on the surface of outer shell membranes (Hincke et al. 2012). Distribution of these nucleation sites is under genetic control and varies among species. The second stage corresponds to the growth phase and lasts about 12 h (Gautron et al. 2014). It is an active calcification phase of forming the compact calcified palisade layer (2/3 of the total thickness

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of the shell), which extends beyond the bases of the cones and ends in the vertical crystal layer. The last stage corresponds to termination of calcification and lasts about 1.5 h (Nys et al. 2004). It is characterized by the arrest of mineralization and deposition of the organic cuticle which covers the entire surface of the egg (Hincke et al. 2010, 2012; Gautron et al. 2014).

Minerals of the eggshell are associated with the organic matrix of soluble and insoluble proteins, glycoproteins, and proteoglycans, representing about 2% by weight of the calcified eggshell, which are progressively incorporated from the uterine fluid during calcification (Hincke et al. 2010). The importance of eggshell matrix proteins consists in influencing the foundation of the eggshell and participating in antimicrobial defenses (Hincke et al. 2012; Gautron et al. 2014). Gautron et al. (2014) reported that the eggshell matrix components are divided into three groups according to their origin. The first group is composed of egg proteins originally characterized in the egg white (ovalbumin, lysozyme, and ovotransferrin). They are mainly localized in the basal parts of the shell (eggshell membranes, mammillary cone layer), but also cuticle (Hincke et al. 2010; Gautron et al. 2001, 2014), and are mainly associated with the initial phase of shell calcification in the uterine fluid. The second group consists of proteins that are widely found in various organs and biological fluids. This group includes osteopontin (a phosphorylated glycoprotein of bone, kidney and present in various body secretions and also in the core of the non-mineralized shell membrane fibres, and in the outermost part of the palisade layer of the chicken eggshell) and clusterin, a widely distributed secretory glycoprotein also present in the egg white (Brionne et al. 2014; Gautron et al. 2014). The third group is named eggshell-specific proteins because they were identified during the investigation of abundant constituents of the eggshell and uterine fluid. These components were termed as ovocleidins and ovocalyxins (Hincke et al. 2012; Gautron et al. 2014). Two possible roles for the ovocleidins and ovocalyxins have been proposed in avian reproduction: regulation of eggshell mineralization and anti-microbial defense (Gautron et al. 2001; Hincke et al. 2012).

Gautron et al. (2014) reported a number of experimental observations supporting the key role of the eggshell matrix proteins in determining the fabric of the eggshell and its resulting mechanical properties. The first is related to the chicken eggshell matrix with its content of relatively specific proteins (ovocleidins and ovocalyxins), mRNA and proteins synthesized at high levels in tissues where eggshell calcification takes place, namely, the red isthmus and uterus (Gautron and Nys 2007a). The second experimental evidence is the change in the protein composition of the uterine fluid during the progressive fabrication of the eggshell. The uterine fluid of each phase of shell mineralization has a unique protein electrophoretic profile, suggesting that they play specific roles during the calcification process (Gautron et al. 1997). The nature of the interaction between matrix components and the mineral phase of the shell has been carefully investigated using *in vitro, in situ,* and genomics approaches (Gautron and Nys 2007b, Hincke et al. 2010; 2012; Gautron et al. 2014).

Eggshell quality and its measurements

The quality of the eggshell has been monitored in long term for purposes of selective breeding. Numerous parameters have been proposed to evaluate eggshell quality in order to reduce the losses of damaged eggshells. The parameters include eggshell weight, percentage, thickness, strength, and density. Eggshell quality can be measured by various methods, direct or indirect; some of these methods require destruction of the egg (Roberts 2004). Direct methods include measuring of shell breaking strength such as impact fracture force, puncture force or quasi-static compression. Indirect methods include specific gravity, non-destructive deformation. However, in commercial operations, eggs are either candled using light to detect cracks and other defects or they pass through an electronic crack detector for egg breakage detection.

For several decades, scientists spared no effort to find new effective techniques or instruments to evaluate eggshell thickness and strength to reduce the economic losses of damaged eggshells. Sun et al. (2012) introduced a new parameter called uniformity of eggshell thickness to evaluate eggshell quality. The authors defined it as the reciprocal of the coefficient of variation of eggshell thickness from multiple positions and reported that uniformity of eggshell thickness had a significant positive correlation with breaking strength which

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provided a new tool for eggshell quality evaluation. Moreover, Yan et al. (2014) studied the relationship between the uniformity of eggshell thickness and eggshell quality of Lohmann Brown eggs and reported that the uniformity of eggshell thickness is positively correlated with eggshell thickness (0.297), breaking strength (0.430), static stiffness (0.409), and fracture toughness (0.171) which might be used as an important indicator for other shell measurements in poultry breeding. Kibala et al. (2015) developed a new methodology using ultrasonic technology to record eggshell thickness at different egg latitudes. The authors observed a genetic correlation between eggshell strength and its thickness to be around 0.8, making shell thickness a selection index candidate element. Tatara et al. (2016) used dual-energy X-ray absorptiometry, quantitative computed tomography, and three-point bending test and they found positive correlation between weight, height, and width of eggs and egg mineral content and egg volume. Moreover, the mean volumetric eggshell mineral density was positively correlated with eggshell breaking strength and negatively correlated with eggshell thickness. The authors concluded that the elaborated experimental model used in the study may serve for further investigations on physiological, pharmacological, environmental, nutritional, and toxicological factors influencing egg quality not only in Japanese quails but in other bird species as well.

Cuticle estimation is an important issue regarding to its function to prevent micro-organisms penetration. The most popular method for cuticle estimation is an individual intact of an egg with a suitable stain such as MST cuticle blue stain (MS Technologies Ltd.) for 1 min and then rinsing two to three times in tap water. MST cuticle blue stain is a reliable indicator of the amount of cuticle present on an eggshell. Then the eggshell surface colour can be measured using a Konica Minolta hand-held spectrophotometer (CM-2600d) (Messens et al. 2007; Leleu et al. 2011; Roberts et al. 2013; Samiullah et al. 2014).

Factors affecting eggshell quality

Eggshell quality is influenced by a wide range of factors which combine to influence the final product. The main internal factors include e.g. time of oviposition, age, and genotype. The external factors include housing system, nutrition, microclimate, etc. All these factors are known to influence the eggshell quality characteristics, and the interactions between some of these factors could be more effective than individual factors.

Internal factors influencing eggshell quality. Time of oviposition plays a vital physiological role in determining eggshell characteristics, because the amount of deposited shell is a linear function of time spent in the shell gland after plumping. The distribution of oviposition times in laying hens is restricted to an 8 h period of the day with eggs being laid normally between 7:30 and 16:00 h under standard lighting conditions (Campo et al. 2007).

The oviposition time significantly affects the eggshell weight, which was higher in eggs laid before 7:45 h than in eggs laid between 7:45 h and 11:45 h (Harms 1991). Then shell weight significantly increased until 12:45 h and remained greater through the rest of the day with the exception of eggs laid between 14:45 h and 16:45 h. Tumova et al. (2009) described a declining trend in shell weight with collection time especially in Isa Brown genotype with values of 6.38 g at 6:00 h and 6.23 g at 14:00 h. On the other hand, Tumova and Ebeid (2005) and Tumova et al. (2007) indicated that eggshell weight was higher in the afternoon eggs (at 14:00 h). Therefore, it might be assumed that eggshell weight tends to increase at the terminal egg of the clutch.

Oviposition time may also affect the eggshell thickness as an important indicator for eggshell quality. Yannakopoulos et al. (1994) assumed that a higher shell quality is due to thicker shell in the afternoon eggs. These results are in agreement with the finding of Tumova and Ebeid (2005) and Tumova et al. (2007) who indicated that eggshell thickness of eggs laid in the morning is not as good as of those laid in the afternoon. On the other hand, Tumova and Ledvinka (2009) revealed a significantly higher eggshell thickness in the morning (6:00 h) decreasing with the collection time, which might be affected by genotypes used in their experiment. Moreover, eggshell quality can be affected by the content of minerals in the eggshell. Tumova et al. (2014) reported a great effect of oviposition time on shell mineral content with the highest Ca content of 352 g/kg in eggs laid at 7:30 h compared to 342 g/kg of those laid at 15:30 h. On the other hand, the P and Mg shell content increased with late oviposition time with the values of 1.20 and 3.56 g/kg respectively

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at 7:30 h and the values of 1.43 and 3.88 g/kg respectively at 15:30 h. The higher shell Ca content in early morning eggs is related to higher rates of Ca deposition in medullary bones during the dark period as it was assumed by Kebreab et al. (2009).

The eggshell characteristics might vary in different stages of laying hen age. Very young birds with immature shell glands produce shell-less eggs or eggs with a thin eggshell. The finding of Tumova and Ledvinka (2009) indicated that eggshell weight increased with hen age. The heaviest eggshells (6.67 g) were found at the age of 56–60 weeks in comparison with 5.05 g at 20–24 weeks of age. Similar findings in layers and broiler breeders were documented by Tumova et al. (2014). The increase of eggshell weight with aged hens is related to the increasing size of the egg and shell surface area.

Bozkurt and Tekerli (2009) found out a decrease in shell thickness with advancing age. On the other hand, Tumova and Ledvinka (2009) reported thicker eggshell (0.372 mm) at the age of 56–60 weeks in comparison with 20–24 weeks of age (0.354 mm).

Eggshell strength as a function of other eggshell measurements is of utmost importance for egg producers; because lower strength causes higher percentage of broken eggs increasing the economic losses. Zita et al. (2009) observed that eggshell strength was higher from the onset of lay till the end of the first phase and declined afterwards. However, Pavlik et al. (2009) indicated an eggshell breaking strength decrease with the age of birds; they ascribed it to higher plasma mineral content with aged hens. Similarly, Tumova et al. (2014) detected a decreased eggshell strength (3.33 kg/cm^2) in older hens in comparison with younger ones (3.60 kg/cm^2). In addition, hen age also affects egg specific gravity as an indicator for eggshell thickness and strength. Tumova and Gous (2012) reported a decrease in specific gravity with hen age. The differences in eggshell quality and hen age among studies can be related to genotype and experimental conditions.

Eggshell quality differs in individual breeds, lines, and families of the laying hens. Therefore, it is important to select an appropriate genotype and/or to improve eggshell quality through genetic selection. Among eggshell quality characteristics, differences in eggshell weight, thickness, and strength have been registered especially between white and brown eggs. Hocking et al. (2003) reported that in contrast to changes in egg weight during hens selection, eggshell weight did not change. Similarly, Singh et al. (2009) observed that eggshell weight did not differ between Lohmann White and Lohmann Brown. Both hybrids produced heavier eggshells then H&N White genotype. There are also differences in eggshell weight within brown hybrids. Tumova et al. (2011) found the heaviest eggshells in Isa Brown (6.3 g) in comparison with Hisex Brown (6.1 g) or Moravia BSL (5.5 g). Similar results were reported by Ledvinka et al. (2012). All these results correspond with findings of Hocking et al. (2003) reporting that genetic correlation for eggshell weight within commercial hybrids is 0.63.

Eggshell thickness is related to the length of eggshell formation and is more affected by genotype in comparison with eggshell weight and probably it is a more reliable indicator of eggshell quality than eggshell weight. Differences in eggshell thickness between white and brown hybrids were described by Ledvinka et al. (2000) and Leyendecker et al. (2001a). The authors found thicker shells in brown hybrids. Within brown hybrids, similar results were found both in eggshell thickness and in eggshell weight. The thinnest shells were observed in Moravia BSL (0.324 mm) in comparison with Isa Brown (0.376 mm) or Hisex Brown (0.358 mm) (Tumova et al. 2011; Ledvinka et al. 2012). Tumova et al. (2007) compared three Dominant genotypes: Plymouth Rock strain, Blue strain, and their cross. Plymouth Rock strain produced thicker eggshells in comparison with Blue strain and their cross had eggshell with the average thickness of both strains. The results correlate with Hocking et al. (2003) that selection of commercial hybrids does not change the thickness of the shell.

Eggshell weight and thickness are physical variables which correlate with the eggshell strength. Higher shell strength was revealed in white egg chicken in comparison with the brown (Ledvinka et al. 2000). Non-significant differences in shell strength were determined by Tumova et al. (2007) in variable Dominant strains. However, in experiments with brown hybrids Isa Brown, Hisex Brown, and Moravia BSL, significantly stronger shells were observed in Isa and Hisex Brown (Zita et al. 2009; Tumova et al. 2011; Ledvinka et al. 2012). The contrast results concerning eggshell strength might be related to low heritability of eggshell strength (0.24) (Zhang et al. 2005). Eggshell quality measurements have low heritability and are more affected by environmental factors; however, correlations between

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individual characteristics and eggshell strength are more important. Frank et al. (1965) indicated that differences in the physical variables like eggshell weight and thickness can explain nearly 60% of the eggshell strength variation.

Housing system as the external factor influencing eggshell quality. Housing system is one of the main external factors that influence the eggshell quality. Several studies have been performed in order to evaluate the effect of housing systems on eggshell quality parameters including conventional cages, enriched cages, litter and free-range systems. Lower number of cracked eggs have been produced in cages (Tumova and Ebeid 2005; Holt et al. 2011; Kontecka et al. 2014). Different eggshell weights have been reported in literature in connection with housing systems, e.g. Pistekova et al. (2006) detected heavier eggshells in cages (8.11 g) than on deep litter (7.71 g). Moreover, heavier eggshells in non-enriched cages in comparison with floor system and enriched cages were obtained by Lichovnikova and Zeman (2008). In contrast, Tumova et al. (2011) detected heavier eggshells on litter than in conventional cages and enriched cages. These contradictory results are presumably related to different environmental conditions among housing systems, in addition to different hen genotype used in the experiments.

Eggshell thickness also varies according to housing systems. Comparing litter, free-range, and cage housing systems, Pavlovski et al. (2001) detected thicker shells in litter eggs and thinner shells in free-range. Marked differences between cages and free-range systems in eggshell thickness were described by Leyendecker et al. (2001b) and Hidalgo et al. (2008). They found lower eggshell thickness in eggs produced in cages while free-range eggs presented the highest values. Moreover, the differences between cages and litter were reported by Ledvinka et al. (2012). The authors found thinner eggshells in cages (0.355 mm) in comparison with litter (0.358 mm). These results are in correspondence with Mostert et al. (1995) who found greater eggshell thickness in eggs from non-cage systems.

Major economic losses for egg producers stem from lower eggshell strength leading to eggshell breakage. Mertens et al. (2006) examined the effects of multiple housing systems (conventional cages, enriched cages, aviary, and free-range) on eggshell quality and reported that shell strength was the greatest in aviary eggs and the weakest in free-range eggs. Moreover, a study on the effect of housing system on eggshell strength conducted by Tumova et al. (2011) revealed stronger eggshells produced in cage housing system (4744 g/cm^2) compared with litter (4651 g/cm^2). Similarly, Ledvinka et al. (2012) and Englmaierova et al. (2014) found stronger shells in cages compared to litter. However, non-significant differences in shell strength between eggs from the deep litter system and cages were reported by Pistekova et al. (2006). These results could be affected either by hen genotype or different experimental conditions. However, in spite of the shell thickness was lower in eggs produced in cages, Tumova et al. (2011) and Ledvinka et al. (2012) found higher eggshell strength. The authors explained it by the ultra-structural features of the shells in cage eggs which presumably support eggshell strength. Nevertheless, it might be assumed that housing system affects eggshell microstructure resulting in different eggshell thickness and strength. The assumption is also related to the effect of housing system on pores density. A significant effect of housing system on eggshell pores density was found by Tumova et al. (2011) who observed higher pores number in cage eggs than in litter housing system.

Numerous studies have demonstrated a different incidence of cracked eggs in cage housing systems. Although Vits et al. (2005) reported stronger eggshells from birds in enriched cages compared with conventional cages, Wall et al. (2002) observed a lower percentage of broken eggs collected from hens in conventional cages compared with enriched ones. These contrast results are presumably related to Ca metabolism, because the most commonly used indicators of Ca metabolism in layers are shell quality assessment parameters (Gordon and Roland 1998). Neijat et al. (2011) indicated that enriched cages may provide better means of utilizing Ca and P than conventional cages. These results might be affected by higher feed consumption in enriched cages. Hence, giving attention to Ca and P feed content may improve the eggshell quality parameters in alternative housing systems.

Other factors contributing to the proportion of cracked eggs are cage design, egg savers, and nest floor material. Guesdon et al. (2006) explained that differences in egg breakage may be because of the influence of cage design elements, including the presence of perches (Abrahamsson and Tauson 1998), rather than specific cage effects.

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Not every genotype performs the same under a certain housing system. Therefore, the interaction between the housing system and genotype has a great effect on eggshell quality characteristics. For instance, Singh et al. (2009) recommended that the strain should be considered when using housing systems. Eggshell weight was affected by the interaction of housing and genotype in the study of Tumova et al. (2011) which was conducted on three housing systems (cages, litter, and enriched cages) and three laying hen genotypes (ISA Brown, Bovans Brown, and Moravia BSL). The authors found heavier eggshells in all genotypes on litter system than in conventional cages and enriched cages. Leyendecker et al. (2001b) studied the interaction between genotype and housing system for eggshell thickness in an experiment with Lohmann LSL and Lohmann Brown housed in conventional cages, aviaries, and under intensive free-range system. They found thicker eggshells in the intensive free-range than in conventional cages and aviaries for both lines of laying hens.

However, it might be assumed that the interaction of housing system and genotype may play a more important role in eggshell quality than each of the factors alone. Therefore, it is highly recommended to choose a genotype matching the type of housing system which might result in eggs with better eggshell characteristics.

CONCLUSION

The presented literature data show that the importance of the eggshell structure is related to its function as a highly ordered bio-ceramic complex resisting physical and pathogenic challenges from the external environment. From the inside outwards, the eggshell comprises of shell membranes and true shell that includes mammillary layer, palisade layer, vertical layer, and cuticle. This structure of the eggshell is often expressed through the eggshell weight, thickness strength, and density as the most important parameters. The eggshell quality parameters are affected by various internal and external factors. Oviposition time significantly affects eggshell weight, eggshell thickness, and eggshell mineral content with better shell parameters in eggs laid in the morning (7:00–12:00 h). A higher laying hen age has a positive effect on eggshell weight but, contrarily, a negative effect on eggshell thickness and strength causing high economic losses resulting from eggs breakage. Different genotypes of laying hens show different parameters of eggshell quality and it is important to improve eggshell quality through genetic selection to reduce eggs with inherently poor eggshell. Different eggshell quality parameters are also related to the type of housing. Cage housing systems produce eggs with lower eggshell thickness but the eggshell is much stronger, which might be related to the crystals size and orientation as the major determinants of shell thickness and strength. Eggshell quality may be improved by optimization of housing system – cages design, egg savers, and especially in alternative housing systems nest floor material, and by selecting the genotype appropriate for particular housing system, and paying attention to feed mineral balance with respect to housing and genotype.

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