

# Research progress on bird eggshell quality defects: a review

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**ABSTRACT** The eggshell quality declined with extending of chicken laying cycles. Eggshell quality is a crucial feature that not only affects consumer preference, but also influences producers' economic profitability. The eggshell ultrastructure consists of mammillary, palisade, and vertical crystal layers. Any defect in shell structure results in a reduction in eggshell quality. Speckled, translucent, pimpled, and soft eggshells are common defects that cause significant financial losses for farmers and food security concerns for consumers. Therefore, reducing the faulty eggshells is critical for poultry production.

Defective eggshell quality has been attributed to hereditary factors and external environmental stimuli. As such, improvements can be carried out through selective breeding and environmental control of components such as temperature, moisture, and diet formula balance. In this review, the molecular mechanisms of the main eggshell quality defects (speckled, translucent, pimpled, broken, and soft-shell eggs) and the relevant improvement methods are detailed. We hope this review will serve as a useful resource for poultry production management and effectively increasing eggshell quality.

**Key words:** defective eggshell, eggshell quality, speckled egg, translucent egg, pimpled egg

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## INTRODUCTION

The production level and reproductive performance of egg-laying hens have been significantly improved after selection and breeding over the past few decades, and the breeders have set a goal of prolonging the production cycle to 100 wk and producing 500 eggs (Bain et al., 2016; Pottgiüter, 2016). However, persistency in lay can't be achieved without considering how to improve eggshell quality in longer laying cycles. With increasing hen age, poor eggshell quality, including cracks, pimples, translucency, and speckles, has become more common (Arpášová et al., 2010; Sirri et al., 2018; Ameer et al., 2021). According to Wilson et al. (1981), 4.77% of the eggs produced were thought to be unsellable, resulting in financial losses for egg producers. Improving eggshell quality while maintaining high production is a new challenge for breeders.

The eggshell serves as an effective barrier to external microorganisms and pathogens entering the egg as well as the first indicator for product selection by consumers. Eggshell formation takes place mainly in the hen's uterus (or eggshell gland) and is the longest stage of the egg-forming process, taking approximately 10 to 22 h.

Eggshell deposition consists of 3 stages: initial mineralization of the mammillary layer, rapid mineralization of the palisade layer, and terminated calcification of the vertical crystal layer. The mammillary layer originates from nucleation sites and crystals grow radially to form the bases of the palisade layer (Nys et al., 2004). The density, size, and thickness of mammillary knobs can affect palisade thickness during eggshell formation (Zhang et al., 2017b). The palisade layer generated in the second stage is a series of columnar crystals located between the mammillary and vertical crystal layers. The formation of calcium carbonate in mammillary and palisade layers is a key period in the mineralization process. The crystal arrangement is denser and more ordered in the vertical crystal layer than in the palisade layer and can be considered an extension of the palisade layer (Rodriguez-Navarro et al., 2002).

In several studies, the breadth and thickness of shell mammillary knobs as well as the thickness of the palisade layer have been demonstrated to alter eggshell mechanical characteristics (Zhang et al., 2018; Zhu et al., 2020). The state of mineralization in the uterus directly determines eggshell quality. The main causes of eggshell defects are genetic factors (Ball et al., 1973; Wolc et al., 2012; Zhang et al., 2021a) and various external environmental stimuli (Roland et al., 1975; Kursu et al., 2019; Zhang et al., 2022b), which impair uterine function and interfere with eggshell mineralization, resulting in appearance defects.

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Here, we present a concise review of eggshell defects: speckled, translucent, pimpled, broken, and soft-shelled eggs. The causes, contributing factors, and treatments of eggshell appearance are discussed in this review. Understanding the molecular basis of defective eggshell appearance is helpful in improving eggshell quality through breeding and feeding.

## SPECKLED EGGS

Speckle on eggshells is an important parameter that influences the appearance of eggs and reduces their economic value. While it is a common trait in some birds, such as the great tit, Japanese quail, magpie, and tree sparrow (Figure 1). Research on speckled eggs in chickens is still in its early stages, but is becoming increasingly important.

### Methods of Recording Egg Speckles

The first speckle measurement study was conducted by Gosler et al. (2005) who used a scoring system with multiple parameters to measure the speckle characteristics of bird eggs, including pigment intensity, speckle distribution, and speckle size. The pigment intensity score ranged from 1 (light speckle color) to 5 (dark speckle color), the speckle distribution scores ranged from 1 (more than 90% of the speckles were distributed in one place) to 5 (evenly distributed throughout the eggshell), and the speckle size score ranged from 1 (small speckle) to 3 (large speckle). All of the scores were in increments of 0.5. The same observer assessed all of the eggs. Although this method has a defined standard, it has the problem of low repeatability among observers owing to the subjective nature of determining the scores.

Martínez-de la Puente et al. (2007) estimated speckle content by calculating the percentage of speckles in the total eggshell area. The eggs were placed on a shelf in a light box with a white background. A black card was used as the background and a color chart was placed next to it. Eggs were photographed at every 90° angle using a digital camera. The light and camera focus were kept constant. Owing to the color difference between the red-brown speckles and the white calcium matrix, the measurements were adjusted according to the edge of the speckles. All photographs were analyzed by the same observer using image-editing software. The drawback of this approach is that these images cannot be used to estimate the intensity of speckle colors.

Gómez and Liñán-Cembrano (2017) provided a computing infrastructure called SpotEgg, which is a processing tool for automatically analyzing the color and speckles of eggshells. Images of the eggs were captured for analysis using this software. The requirements for this process are consistent with the equipment used to obtain images that are as clear as possible and saved by lossless compression. This method reduces the error in human estimates and significantly reduces the time spent assessing the eggs.

### The Function of Egg Speckles

As early as 1830, Newton (1896) noticed that birds in caves tended to lay white eggs, which may be because birds are more likely to find white eggs and take care of them in dark environments. Wallace (2007) later hypothesized that the color and pattern of all other types of eggs were designed to adapt to the nest's unique environment and serve as a means of evading predators. In most small passerine eggs, the blunt end of the eggshell has reddish-brown speckles (Wiens and Lack, 1969). There are many theories regarding the functions of these speckles. Most researchers believe that these speckles transmit signals. Speckles on eggshells are adaptations to the natural environment (Blanco and Bertelotti, 2002), and nests of eggs with different speckles are more conducive to avoiding predators than eggs with the same appearance (Hockey, 1982). Nest parasitism exists in birds. The eggs produced by the host are highly similar, and the host distinguishes its eggs from foreign ones by perceiving the difference in shell color and speckles. This also promotes the diversity of eggshell speckles (Karcza et al., 2003). However, this explanation does not apply to all species. To the best of our knowledge, the relative importance of predator and nest parasitism in explaining eggshell color and pattern diversity has not been definitively determined.

Other studies have supported the eggshell function hypothesis. Researchers believe that eggshell speckles are caused by changes in eggshell structure and calcium deficiencies (Gosler et al., 2005). Ding et al. (2019) demonstrated that the egg quality of tree sparrows, particularly the speckling pattern of the eggs, can be used as an indicator of metal pollution because the eggs they found in heavy metal-polluted areas (higher concentrations of Zn and Pb) had thinner shells and a more aggregated speckling distribution. Orłowski et al. (2020) found that most chemical elements tend to be higher in the speckle

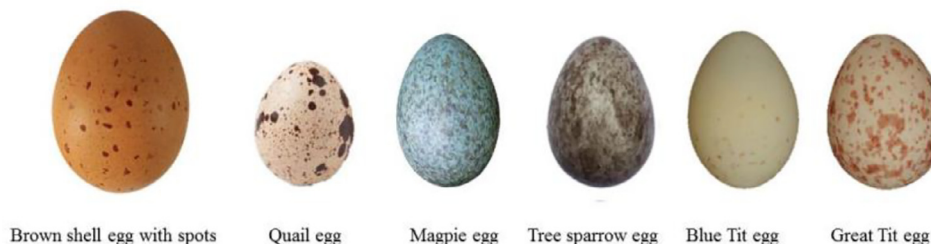


Figure 1. Speckled eggs in different birds.

region than in the background region of speckled eggs, and they speculated that the speckle regions play a functional role in the physiological deactivation of trace elements by incorporating them into the less calcified external shell layer, but do not participate in micronutrient resorption. Higham and Gosler (2006) found that speckles on eggshells of great tits affected the water loss rate of eggs during hatching, but had no effect on unincubated eggs. This is related to the water conductivity and water loss of the eggshell during incubation (Tazawa and Whittow, 2000).

As protoporphyrin is closely related to heme content, some researchers have proposed the “anemia hypothesis” (De Coster et al., 2012). Researchers infected great tits with *Hemophilus*, resulting in anemia, and observed the relationship between the laying sequence and speckles. The results showed that there was a positive correlation between speckles and oviposition sequences and aggravation of anemia. In addition, compared to biliverdin, protoporphyrin in speckled eggs promotes oxidation, and an increase in pigmentation is a signal of female oxidation tolerance. Therefore, speckles appear to be related to the health status of birds (Martínez-de la Puente et al., 2007). Females with more speckled eggs should have a higher antioxidant capacity to resist toxins or more effectively remove toxins from the eggshell. However, researchers have questioned whether porphyrin is an inducer of oxidative stress and whether its accumulation can lead to liver damage. They found that females laying more speckled eggs had higher levels of HSP70 and lower concentrations of immunoglobulins, indicating that hens laying speckled eggs are in poor health (Duval et al., 2013). However, this perspective is still being debated. Studies have demonstrated that eggshell patterns reflect the quality of eggs and offspring, but not the quality of females. Chicks hatched from more pigmented eggs, which are mainly pigmented by

protoporphyrin, are more likely to attract male investment (Hargitai et al., 2016; Polacek et al., 2017).

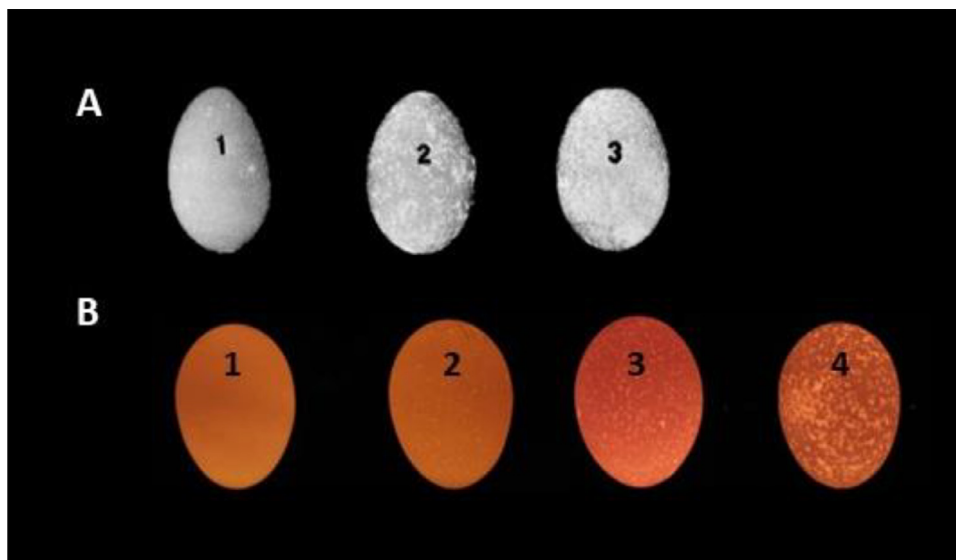
Numerous studies have been conducted to explore the function of speckled eggs in birds, exploring ideas including predator, mate selection, and anemia (Wallace, 2007; De Coster et al., 2012; Polacek et al., 2017). However, the reasons for the formation and the factors affecting speckled eggs in egg-laying chickens have not been conclusively determined.

## TRANSLUCENT EGGS

The translucency of the eggshell is a common flaw in the egg’s appearance. This trait became more noticeable when the shell is held up to a light source. Translucent eggs have been shown to have inferior breaking strength (Tyler and Geake, 1964), which may be due to the water content in the translucent areas weakening the shell. Furthermore, translucent shells increase the risk of bacterial penetration, such as *Salmonella* and *Escherichia coli*, causing food safety concerns (Chousalkar et al., 2010).

### Measurement Methods of Translucent Eggs

A traditional scientific method for quantifying the degree of shell translucency is the grading method (Holst et al., 1932; Baker and Curtiss, 1957). Operators subjectively classify the translucent eggs into three grades, 1–3, representing, respectively, no translucency, medium translucency, and severe translucency (Holst et al., 1932) or four grades 1–4 representing, respectively, no translucency, slight translucency, medium translucency, and severe translucency (Wang et al., 2019), with both methods based on the spot size and density determined by candling in darkness (Figure 2). This method is



**Figure 2.** Measurement methods of translucent eggs. (A) Three reference samples were used for the scoring method, from left to right to be scores 1, 2, and 3 (Holst et al., 1932). (B) Four reference samples were used for the scoring method, from left to right to scores 1, 2, 3, and 4 (Wang et al., 2019).

reasonably simple and efficient, but the operator must be trained until highly consistent results are obtained to decrease the subjective error.

A new method for quantifying shell translucency is the grayscale recognition method (Wang et al., 2019). This method uses a camera and image-processing software (Photoshop, Image-Pro Plus 6.0, and ImageJ software) to recognize and quantify the shell translucency. This method improved accuracy and objectivity compared with the grading method.

The colorimetric method uses a portable spectrophotometer (CM-2600d, Konica Minolta, Inc., Tokyo, Japan) to measure the changes between the translucent and opaque areas (Wang et al., 2019). The LAB model was used for the eggshell color measurement.

### Factors Associated With Translucent Eggs

**Genetic Factors** Notably, there are significant and quantifiable differences in the degree of shell translucency, not only among individuals (Baker and Curtiss, 1957), but also among strains (Baker and Curtiss, 1958; Zhang et al., 2021a). Baker and Curtiss (1957) compared shell translucency variations between individuals of the same strain. They found a large difference in the degree of shell translucency between individuals, with the amount of translucency in the eggs remaining relatively consistent over a period of one month. Subsequently, they found significant differences in the degree of shell translucency between strains (Baker and Curtiss, 1958), which is consistent with the results of Zhang et al. (2021a), who found that significant variations of translucent eggshells between different breeds.

The differences among individuals and between strains indicate that translucency may be a heritable trait (Baker and Curtiss, 1958). Wang (2017) estimated the heritability of translucent shells using the DMU software package (version 6.0) to be 0.22, which falls into the medium- and low-level heredity categories. The low genetic correlation between translucent shells and egg quality traits suggests that translucent shells affect other shell traits at a very low level (Baker and Curtiss, 1957; Baker and Curtiss, 1958; Wang, 2017; Fu, 2019). Fu (2019) obtained similar results, in which heritability was reported to be 0.28. Wang (2017) employed a genome-wide association study to determine the genetic basis of translucent shells in brown-egg dwarf layers. They found 4 single nucleotide polymorphisms (SNPs) significantly and 170 SNPs suggestive significantly associated with translucent eggs. These loci are mainly located on chromosome 2 and 19. A recent study by Qu et al. (2021) found that 29 significant loci and 5 key genes, *RIMS2*, *SLC25A32*, *RIMBP2*, *VPS13B*, and *RGS3*, may affect the formation of translucent shells in eggs from an F2 resource population generated by crossing Dongxiang blue-shelled and white leghorn chickens. These loci are different from those reported by Wang (2017). Such robust identification of SNPs and genes provides a meaningful contribution to the understanding

of translucent shells; however, the regulatory mechanisms of these genes require further study.

**Environmental Stimulation** Stimulation from the external environment can lead to a disorder in shell gland function and changes in eggshell ultrastructure. *Mycoplasma synoviae* is known to cause respiratory diseases, synovitis, and reduced reproductive rate in poultry. Studies have found that shell quality, including egg weight, translucency, and shell reflectivity are changed by infection with *Mycoplasma synoviae* (Kursa et al., 2019; Cisneros-Tamayo et al., 2020). The mechanism by which such infection affects eggshell calcification remains unclear, although several authors have attempted to elucidate it (Dominguez-Vera et al., 2000; Santos et al., 2014).

Talbot and Tyler (1974) found that shell translucency is not permanent. Drying the shell for 24 h results in the translucent area becoming opaque. Returning the shell to a humid atmosphere causes it to become translucent again (Holst et al., 1932; Talbot and Tyler, 1974). This indicated that environmental moisture can affect translucency.

### The Formation Mechanism of Translucent Eggs

The first report of the cause of translucent eggs was when Holst et al. (1932) noticed that the shell could be covered with translucent areas of varying sizes, ranging from pinpoint to the entire shell surface. The authors showed that the uneven translucency of the eggshell is not related to the shell thickness, but mainly due to the non-uniform distribution of moisture throughout the whole eggshell. Talbot and Tyler (1974) found that translucent areas had thicker shells than opaque shells, which is contrary to the results of Holst et al. (1932) and Almquist and Burmester (1934) who reported translucent areas to be thinner than normal areas. This difference may be due to the variety of hens (Baker and Curtiss, 1957) and the different treatments of eggshell membranes.

Talbot and Tyler (1974) reported that defective eggshell ultrastructure may be responsible for the formation of shell translucency. Compared with an opaque shell, a translucent shell lacks a barrier located above the mammillary layer, which interferes with the passage of interior moisture to the exterior. These findings are in line with those of Bain et al. (2006) and Chousalkar et al. (2010), who found that translucent shells have a defect in the mammillary layer during eggshell mineralization, resulting in a thicker mammillary layer and a thinner effective layer than opaque shells. In 2013, Nie (2013) hypothesized that defective shell membranes lead to translucency. They found that the translucent shell membrane was thinner than the opaque shell. The shell membrane is a barrier preventing egg interior water from penetrating the exterior, as well as the platform for eggshell formation and mineralization initiation. The variation in eggshell structure may begin with a



variation in eggshell membrane thickness. In another study, Wang et al. (2017) found similar results, suggesting that thinner shell membranes and shells are responsible for the formation of translucent eggs.

Exploring the formation mechanism of translucent shells lays a foundation for the subsequent development of methods to reduce translucent eggs.

### Improvement Methods

The formation of translucent eggs is related to the chemical composition of eggshell membrane variation and shell structure, such as the thinned effective layer thickness and increased papillary space (Wang et al., 2017; Fu, 2019). Concerning this, it has been reported that 25-hydroxyvitamin D and essential oils complex can significantly reduce the translucent egg by increasing shell-effective thickness and decreasing mammillary thickness (Zhao et al., 2021). Diets supplemented with 1% N-carbamylglutamate have been shown to increase the effective thickness of shells and decrease the width of the mastoid gap and mammillary knobs (Ma et al., 2020). Zhang et al. (2018) found that adding manganese to the feed of 54-wk-old hens can reduce the occurrence of translucent shells by affecting the mammillary-knob density of the shell ultrastructure. L-serine has been reported to have positive effects by reducing the number of translucent eggs (Li et al., 2019). Blended oil contains a variety of oils and fats, which have rich nutritional functions, can adjust the form of biofilms, and repair damaged biofilms. Researchers have found that a diet supplemented with 1.85% blended oil can significantly reduce the degree of translucent eggs (Zhang et al., 2020). Similarly, feeding *Lactobacillus salivarius* SNK-6 can significantly reduce the translucent egg by regulating the gut microbiota in 45-wk-old Xingyang black-feathered laying hens (He et al., 2020).

### PIMPLED EGGS

Pimpled (or rough) eggs are coated with extra-calcified granules. Most pimples are unevenly distributed on the blunt end of the egg, but some also appeared on the sharp end (Figure 3). According to various studies, pimpled shell eggs have a lower breaking strength and

calcified shell thickness than normal eggs, which cause transportation problems (Garlich et al., 1975; Liu et al., 2017b). Liu et al. (2017b) conducted a study comparing pimpled eggs and normal eggs and found no differences in egg weight, shape, and shell membrane weight between normal eggs and pimpled eggs. However, other variables such as calcified shell weight, breaking strength, and overall calcified shell thickness of pimpled eggs were significantly lower than those of normal eggs. A hollow can form when the pimples are removed from the outer shell surface, affecting the shell quality and potentially causing leakage of the contents (Roland et al., 1975).

### Causes of Pimpled Eggs

Originally, pimpled shells were thought to be caused by disease or vaccination (Van Roekel et al., 1951; Broadfoot and Smith, 1954). The avian influenza virus (AIV), a low-pathogenicity virus, is very common in domestic poultry in Asian countries (Beato et al., 2009). AIV can recognize and bind to SA  $\alpha$ -2,3 receptors, and replicate in the reproductive tract of hens (Wang et al., 2015b; Bonfante et al., 2018). Hens infected with AIV exhibit varying degrees of clinical symptoms, including increased cell apoptosis in the respiratory and digestive tracts as well as physiological changes in the reproductive tract (Bi et al., 2010; Qi et al., 2016). The oviducts of infected chickens show signs of edema, and the epithelial cells exhibit degeneration or necrosis. Immune-related proteins, such as *TLR3*, *IFN- $\beta$* , *MDA5*, and *IL-2*, show varied expression in infected oviducts (Wang et al., 2015a). Infected cells are blocked from performing specific functions, causing a decrease in egg production (Spickler et al., 2008). Newcastle disease virus (NDV) usually changes the structure of the outer shell surface and shell membrane by infecting the oviducts of laying hens (Li et al., 2017). In addition, avian infectious bronchitis virus (IBV) can infect the oviduct of birds, causing shell formation disorders, such as shell color change, soft shell, or pimpled shell formation (Broadfoot and Smith, 1954; Nii et al., 2014; Hassan et al., 2022). The expression of eggshell quality-related genes in the eggshell gland can be altered in infected chickens. For example, calbindin (CaBP)-D28K was discovered in the eggshell gland of avian species and affects  $\text{Ca}^{2+}$  transport, which is critical for the formation

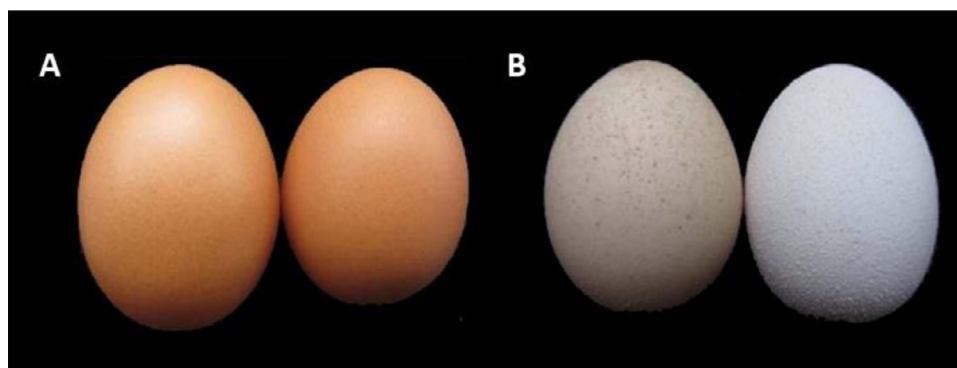


Figure 3. Pimpled and normal eggs. (A) Normal eggs. (B) Pimpled eggs (Liu et al., 2017b).

of the complete eggshell (Ebeid et al., 2012). The expression of CaBP-D28K can be disrupted in the hens' uterus infected with IBV through the action of cytotoxic cells, causing the alter shell membrane structure and deteriorate eggshell quality (Nii et al., 2014; Qi et al., 2016).

In contrast, a rough, pimped shell can also be found in pathogen-free flocks, which indicates that the pimped shell is not only caused by common infectious diseases (Ball et al., 1974). The researchers hypothesized that foreign elements adhering to the outer shell membrane prior to shell formation create a pimped shell (Ball et al., 1973). The formation of pimped eggs may also be related to the age of the laying hens. A general trend of increased incidence and severity of pimples with age has often been reported. Pimples are small and sparse at the start of the laying phase according to Ball et al. (1973), but the roughness grade increases as the laying period progresses. A similar phenomenon was observed by Swanson and Bell (1971). It has been reported that the pimpling degree is related to the dietary level of vitamin D3 (Goodson-Williams et al., 1986,1987). Too much vitamin D3 may cause soft tissue calcification, which affects the calcium metabolism of the eggshell gland. Controlling the level of vitamin D3 in the diet can effectively improve the pimpling degree of eggs in young or aged laying hens.

Atrophy and degeneration of the shell gland during the late laying period contribute to pimped shell development (Khogali et al., 2021), such as alterations in the lengths of primary and secondary folds. This finding is similar to that of Huntley and Holder (1978). Liu et al. (2017a) performed comparative proteomics of the eggshell matrix between normal eggs and pimped eggs, selecting 36 eggs with pimped areas on >70% of the eggshell surface and 48 eggs with normal shape and cuticle color for liquid chromatography-electrospray ionization tandem MS analysis. 25 proteins differed in expression between the 2 groups and were found to alter eggshell quality. A transcriptome study of the shell gland tissue of pimped and normal eggs was conducted by Khogali et al. (2021), who found 211 differentially expressed genes (DEGs) in the 2 groups. The DEGs are mainly related to organ morphogenesis and development. However, the detailed molecular mechanism of pimped eggs remains unclear and requires further investigation.

### Improvement Methods

Artificial molting can be induced by manipulating feeding and living conditions, which can result in a period of reproductive quiescence. It can effectively improve the shell quality and egg output of late-laying hens (Berry, 2003). During this process, the reproductive tract undergoes degeneration and regeneration. Molecular function in shell glands may be interfered by the increased lipid content in hens as they grow older. However, lipid levels can be dramatically decreased after artificial molting, and shell gland function can be remodeled at the cellular level (Heryanto et al., 1997). Artificial molting restarted the reproductive function of aged

hens by regulating the blood hormone level and the gene expression level related to antiaging and lipid metabolism (Zhang et al., 2021b,c, 2022a). Molting can increase the responsiveness of the shell gland to 25-hydroxy vitamin D3, which is beneficial for calcium absorption during shell formation (Akbari Moghaddam Kakhki et al., 2019). Many studies have shown that artificial molting can effectively improve eggshell roughness and increase egg production (Roland and Bushong, 1979; Ga et al., 2022). Hess and Britton (1988) reported similar results for Leghorn hens. Thus, artificial molting is an effective method to improve eggshell roughness.

If rough eggs are caused by a pathogen, hens can be treated with certain medications or vaccines. Live attenuated IBV vaccines are the most commonly used vaccines to prevent bronchitis. Generally, 1-day-old chickens are vaccinated with 1 to 3 serotypes of the live-attenuated IBV vaccine in the hatchery before placement on a farm. At the age of 1 to 2 months, chickens should be field-boosted at the farm to extend the duration of immunity (Jordan, 2017). Some companies and breeders typically use vaccines that combine IBV with NDV. It is also important that farms are kept clean at all times and the state of the chicken flock is continuously observed to avoid disease outbreaks.

## BROKEN EGGS

Broken (or cracked) eggs are a major cause of economic loss, as bacteria may penetrate the shell, causing a food safety problem. The external environment and genetic defect of laying hens are the main factors that cause broken eggs. First, in an unsuitable cage, eggs can roll into the egg cradle at a high speed, causing breaks in the shell. Eggs in an egg cradle may not gather in a timely manner, leading them to pile up and fall into crevices. Cracking may also occur during packing or transportation. Alternatively, a broken shell may be an inherited trait, with heritability estimated between 0.1 and 0.2 by Wolc et al. (2012), showing that it can be selected by breeders.

Creating a comfortable environment for the growth of chickens, including appropriate nutrition, light, temperature, and humidity, is necessary so that the chickens' bones and reproductive systems can reach a suitable state for production and effectively reduce the generation of broken eggs or thin shell eggs.

Nutrition is an important factor in the reduction of eggshell defects. The balance of calcium and potassium in chicken diets has been shown to have important effects on eggshell formation. Too much calcium and potassium, for example, interfere with the absorption of other nutrients in the intestine, which has a negative effect on eggshell development. Studies have demonstrated that high calcium can reduce the availability of calcium and high potassium can increase the retention of P (Lim et al., 2003). Phytase supplementary in the diet can significantly reduce the broken egg rate through increase the ileal digestibility of P (Zhai et al., 2022).

Some microelements can contribute to the improvement in egg quality. Min et al. (2018) found that feeding methionine hydroxyl analog chelated zinc to aged laying hens could promote calcium deposition and activity, thus decreasing the broken egg rate and improving shell quality. Supplementing diets with zinc improves eggshell quality via different pathways that are associated with the source of zinc and dietary levels (Zhang et al., 2017a; Han et al., 2020). Copper is a component of lysine oxidase, which is important for the formation of eggshell membranes. Numerous studies have shown that the supplementation of trace elements (manganese, zinc, and copper) in laying hen diets could increase the shell breaking strength and mass percentage of the eggshell, thus improving eggshell structure (Mabe et al., 2003; Stefanello et al., 2014; Hajjarmanesh et al., 2022).

Chickens absorb nutrients from the gut to maintain their overall metabolism. Probiotics can effectively regulate the structure and quantity of the intestinal flora and increase the absorption and utilization of nutrients by the body. Supplementing the diet with *Bacillus subtilis* significantly reduced unmarketable eggs, increase eggshell strength and improve egg quality through improved gut microflora balance and absorption efficiency (Abdelqader et al., 2013; Guo et al., 2017). Yang et al. (2020) observed that a combination of *Bacillus licheniformis* and *Bacillus subtilis* in an aging laying hen diet can significantly reduce the soft broken egg rate and malformed egg rate, and improve the small intestinal morphology. *Pediococcus acidilactici* has been found to have the potential function of decreasing broken eggs and shell-less eggs and improving shell quality in the early laying period (Mikulski et al., 2012). This may be due to the production of metabolites, such as short-chain fatty acids, which can modulate the gut pH and improve the absorption of mineral nutrients, thus improving eggshell quality.

## SOFT-SHELLED EGGS

Soft-shelled (or shell-less) eggs are usually more likely to occur in the first 10 mo of production and most frequently during the late laying period (Wilson et al., 1981), or may be laid by young chickens with immature reproductive traits. The formation of soft shells is related to the time of egg laying. Soft-shelled eggs are usually expelled during the formation of mammillary knobs or more advanced stages (Koga et al., 1982). Soft-shelled eggs can be induced by stress (Wolford and Tanaka, 2019), diseases (Broadfoot and Smith, 1954), hormones (Hester et al., 1991), and dietary nutrients (Narbaitz et al., 1987). However, the organ weight of chickens producing soft-shelled eggs is the same as that of normal chickens, particularly for the thyroid gland, adrenal gland, ovary, oviduct, and uterus (Wilson et al., 1981).

Phosphate has been reported to prevent the precipitation of calcium carbonate. The eggs entering the eggshell gland could be prematurely expelled after injection of

the orthophosphate solution into the uterus of laying hens (Ogasawara et al., 1975). It has been suggested that the lack of calcium utilization in the eggshell glands of laying hens leads to the formation of soft-shell eggs. Hens laying soft-shelled eggs have a higher plasma phosphate concentration than those laying normal-shelled eggs (Hester et al., 1980). While hens were stressed, their plasma corticosterone concentrations become higher than that of normal hens (Klingensmith et al., 1984). Other hormones, such as prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ), are involved in the premature expulsion of soft-shelled eggs (Hester et al., 1991). Klingensmith et al. (1988) employed gas chromatography to identify the amino acid composition of shell membranes of normal and soft-shelled eggs. Tryptophane and ornithine were identified as shell membrane components, whereas no significant differences in amino acid concentrations were found between the 2 kinds of shell eggs.

Scientists have conducted numerous studies about how to alleviate the formation of soft-shelled eggs. Some nutritional strategies have helped reduce the rate of soft and broken shells.

## CONCLUSIONS

This review evaluates the current literature related to common eggshell defects. Substantial evidence obtained from scientific literature supports the importance of eggshell quality with respect to poultry production. Numerous studies have revealed that flaws in chicken breeds and stimulation from the outside environment, such as temperature, moisture, stress, and sickness, are the primary causes of defective shell production. Several studies have been conducted to rectify these flaws, such as improving cages, storage conditions, and dietary nutrition. In addition, 2 new types of faulty eggshell quality are described in this review: speckled eggs and transparent eggs. These 2 defective characteristics also affect customer consumption owing to the outer appearance of eggs. As the egg production cycle of chickens extends, the stability of eggshell quality must be investigated. In addition to common eggshell quality defects, several newly defective eggshell traits also need to be improved. This review identifies factors for producers to improve eggshell quality defects as well as for researchers looking to conduct a more in-depth investigation of eggshell quality faults.

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## DISCLOSURES

No conflict of interest exists in the submission of this manuscript, and the manuscript is approved by all authors for publication.



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