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A Strategy for Quality Poultry Egg Production
I. Eggshell Strength and Pigmentation

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양질의 계란 생산전략
I. 난각과 난각색형성

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ABSTRACT : Eggshell strength and eggshell pigmentation are described in this paper since these are needed for quality egg production. A strong eggshell is determined by the components of the shell (cuticle, true shell and membranes) as well as the proper function of the gastrointestinal tract, the shell gland, the Kidneys and the endocrine system. When the pullet reaches sexual maturity, the medullary bone must be ready for the laying hen at the peak egg shell formation. The amount of calcium in the layer diet, sources of calcium feed, the ratio of calcium and phosphorus in the layer diet, adequate levels of vitamin D and the dietary mineral (electrolyte) balance in the body fluid are important factors along with the levels of other nutrients. Biological, environmental and managerial factors such as the age of laying flock, temperature and humidity of the hen house, bird strain, disease, egg collection through transportation and others can influence the shell breakage at various stages of movement of the eggs from the producer to the consumer. The pigments present in eggshells are protoporphyrin-IX, biliverdin-IX and its zinc chelate and occasional traces of coproporphyrin-III. However, there are several causes of changes in eggshell pigmentation such as the age of hen, disease, drugs and surface defects due to abnormal post-cuticular deposits.

(Key words : eggshell strength, shell gland, endocrine system, calcium, eggshell pigments)

INTRODUCTION

There is no question that the world's population will increase in the 21st century, which means increased damand for products and increased competition as well. For agriculture then, the 21st century will mean people demanding higher quality diets. Future poultry farming also demands the production of high quality products which can be differentiated from poultry products of neighboring farms and countries. Quality egg production is one of the methods to survive in serious competition. There is likely to be an increased recognition of niche markets and the development of products such as omega eggs with altered fatty acid content to meet specific consumer demand (Blair, 1996).

Generally speaking, quality egg production involves strong eggshells, eggshell pigmentation attractive to the consumer, perfect egg quality, reduced cholesterol content, yolk pigmentation favorable for consumers and marketing, controlled egg weight and introduction of 'organic eggs'.

This paper is the first part of a series entitled "Factors to be considered for quality egg production". It will describe eggshell strength and eggshell pigmentation, which are necessary for quality egg formation.

EGGSHELL FORMATION AND STRENGTH

Cracked and broken eggshells are major source economics of losses for producers. Between the hen and the consumer's carton, about 6 to 8% of the eggs annually produced are broken or cracked. A strong eggshell allows the egg to resist dynamic and static forces encountered during production, packaging, transport and handling. The integrity or soundness of the eggshell is determined by the components of the shell (cuticle, true shell and membranes) as well as the proper function of the gastrointestinal tract, the shell gland, the

kidneys and the endocrine system.

Cracked and broken eggs may result in a reduction in the value of output, and pose a human health hazard via *Salmonella* contamination (St. Louis, 1988).

1. Measurement of Eggshell Strength

Eggshell strength has been measured using numerous techniques. Eggshell thickness has been measured directly (destructive). Specific graivity (SG) has been also used to indirectly estimate thickness (non-destructive). In addition, expensive, complicated and sophisticated measures such as quasi-static compression using an Instron Testing Instrument or Holographic Interferometry which uses a laser beam have been used.

SG is a useful measurement to differentiate in non-destructive manner between thick and thin shells. This method is based on the assumptions that all thick egg shells are strong shells and there is a simple linear relationship between SG and shell breakage. Both of these assumptions are incorrect. Thin shells are not necessarily weak shells (Potts and Washburn, 1974). Eggshell strength is dependent on many factors, including the nature of the organic matrix and crystal structure (Simons, 1971; Roland 1980b). The relationship between SG and shell breakage is curvilinear (Maurice, 1982), and not linear as assumed. Further there are numerous sources of error associated with the measurement of SG and errors up to 0.006 could occur (Voisey and Hamilton, 1976).

The latest results from scanning electron microscope technology have revealed more details on the shell strata and their individual contributions to fracture resistance (Bain, 1992). This study has lead us away from such parameters as shell thickness and into more qualitative, descriptive traits to reveal how the stresses associated with insults such as quasi-static compression lead to fractures of the shell.

The incidence of structural aberrations is also thought to vary in response to external stressors such as population density, housing systems, etc. Genetic variation may also be anticipated. The contribution of the protein matrix within the shell proper, in contrast to that in the membranes, has come under scrutiny. Although comprising less than 1 % of the total weight of the shell, these proteins are now believed to contribute in an important way to its structure and strength (Hincke et al., 1992; Krampiz, 1993). A number of protein components have also been identified and a model for their contribution to calcification of the shell has been proposed (Arias and Fernadez, 1993). The main steps in this proposal are 1) fabrication of the first organic matrix, 2) nucleation of the calcium carbonate crystals in the mammillary layer and 3) shell matrix deposition during subsequent crystal development.

The shape of the shell is another important contributor to the resistance to cracking. It has been shown that shell thickness acounted for about 56% of the variation in crushing strength, and that egg shape index explained 15 to 35% of the remaining variation (Richards and Swanson, 1965). The unique shape of the egg shell, coupled with the accepted fact that the material it contains is not homogenous, precluded the use of conventional stress analysis in describing egg shell strength (Voisey and Hunt, 1974). In another report, it was concluded that a small rounded shape was more desirable in terms of improving the strength of the egg shell (Bain, 1991).

For formation of the eggshell, the ovum passes through the various parts of the oviduct including the infundibulum, magnum, isthmus and uterus. When the egg passes into the uterus (The egg spends most of its time here, from 20 to 21 hours), a spongy or palisade layer is laid down and this layer contributes to the main strength and thickness of the shell. This palisade layer begins at the lower portion of the mammillary layer and extends within a short distance of the surface of the shell. This palisade layer is composed of crystal columns running paralle to the surface of the egg. It is of uniform consistency and appears to serve as the cement between the mammillary crystals. The thickness of the palisade layer seems to be one of the most significant factors in determining eggshell strength. Formation of this layer demands much calcium since the egg shell is made-up of 93 to 98 % calcium carbonate.

The final step of formation of the egg prior to oviposition is the deposit of the cuticle, or the thin smooth, porous, outer covering of the shell. The cuticle also has a role in maintaining the strength of the shell (El Boushy and Raterink, 1989). In the vagina, the egg is laid large end first,

revolving end on. An air cell forms after the egg is first laid, formed by cooling of the contents of the egg. These processes occur when everything is going according to plan.

2. Medullary Bone Function in Shell Formation

When the pullet reaches sexual maturity, estrogen is released from the maturing ovary, which acts in synergism with the androgens and induces the formation of medullary bone in the marrow cavity, especially in the long bones of the skeleton. The medullary bone is formed 10 day before the first egg is laid under the influence of hormones. Skeletal weight of hens increases about 20 % during this period. During the prelaying period, body weight increases by 400 to 500 g and total skeletal weight of the pullet increases by 15 to 20 g. This represent storage of an additional 4 to 5g of calcium (Scott et al., 1982; Scott, 1991).

The laying hen at peak eggshell formation cannot absorb adequate amounts of calcium from her diet. At these times she draws calcium from the specialized medullary bone. The laying hen contains approximately 20 g of calcium, most of which can be found in the skeleton. The average egg shell contains about 2.3 g of calcium. The medullary bone of the hen breaks down to provide calcium during eggshell formation and is built up for storage at other times. Different bones have the ability to provide calcium for eggshell formation to different degrees. The bones of the ribs, sternum, pelvis and spine are considered labile bones, while the skull, shank, and toe are considered nonlabile bones, and the femur, tibia and fibula are considered intermediate bones. Labile bones provide the most of calcium for eggshell formation. Medullary bone formation appears to be influenced by dietary calcium levels, since pullets that receive diets containing 3.3 % calcium from 18 to 22 weeks of age produce better medullary bone compared to pullets that received only 0.6 % calcium during this period (Hurwitz and Bar, 1971; El Boushy and Raterink, 1985). Dietary calcium (Ca) levels also had a significant effect on total medullary Ca reserves of laying hens. Previous dietary Ca levels had no significant effect upon medullary bone Ca reserves after subsequently feeding the low-Ca diet (Clunies et al., 1992a).

Formation of the egg shell takes place for the 20 hours prior to the egg being laid. This 20 hour-period is usually at night. The amount of calcium available for absorption from the intestine at night is affected by the amount and type of calcium ingested by the laying hen during the day. Laying hens usually choose to ingest more calcium near the end of the day. Large particle calcium sources persist longer in the intestinal tract than finely ground calcium sources (El Boushy and Raterink, 1985, 1989).

The laying hen must consume large amounts of calcium on daily basis. When the hen is receiving insufficient amounts of calcium in the diet an overall negative calcium balance occurs and the rate of secretion of parathyroid hormone (PTH) is greatly increased. This causes the release of calcium from medullary bone which helps to maintain blood calcium levels. In acute calcium deficiencies, egg production ceases and the medullary bone is resorbed to maintain the vital functions requiring calcium (Wideman et al., 1985).

Poor bone structure of laying hens may be due to inadequate skeletal development prior to egg production, a lack of exercise and deficient nutrition during egg production. Outbreaks of poor bone structure have been associated with birds starting to lay at an early age when skeletons have been immature. Special rations for the prelaying period are desirable to provide adequate mineral formation on medullary bone (El Boushy and Raterink, 1985, 1989).

Experiments were carried out to investigate the Ca and P metabolism of hens laying thick-(THK) or thin-shelled (THN) eggs on shell-forming days (SF) and days on which shell formation does not occur (NSF) (Clunies et al., 1992b). Feed, Ca and P intake did not differ significantly between the tow groups of hens, however, feed intake and Ca retention increased significantly on SF compared with NSF days. The NHK hens retained significantly more Ca compared with the THK hens. No differences were recorded for egg production, although there were differences in egg weight and shell deformation between the two groups of hens. Increased egg weight did not account for differences in eggshell formation. Although percentage shell Ca was not significantly different, total shell Ca was different between the two groups.

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they reported that feed, Ca and P intake of hens increased significantly on SF days compared with NSF Dietary Ca levels had a significant effect on feed and Ca intake of hens. On SF days, hens retained more dietary Ca, both as a percentage and per gram Ca basis, compared with NSF days. As dietary Ca increased the percentage Ca retained decreased and per gram Ca retained increased. Dietary Ca had no effect on egg weight or egg production. Increasing dietary Ca significantly decreased shell deformation and increased shell Ca. Ca retention increased linearly as Ca intake increased, and shell weight increased quadratically. There was a diminishing response of shell weight to Ca intake at higher levels.

3. Effect of Calcium and Phosphorus on Eggshell Formation

1) Calcium Utilization by Layers

When hens receive 3.5 to 4 % calcium in all mash layins rations, they only retain about 50 % of the ingested calcium This means that a hen ingesting 3.6 g of calcium per day retains about 1.8 g, or 1800mg, of calcium during the approximately 18 hours that the feed is available. The hen, therefore, retains about 100 mg of calcium per hour. This absorption rate of 100 mg per hour is exceeded when the egg is in the uterus and is much lower when no eggshell is being deposited (Scott et al., 1982; El Boushy and Raterink, 1985).

Eggshell represents about 10 % of the weight of the egg. Egg weight continues to increase and reaches an average 62 g after 30 weeks of production (50 weeks of age) and these eggs should have a shell weighing 6.2 g (Scott et al., 1982; Scott, 1991).

Recent genetic improvement in egg production and egg size have it necessary to review the nutritional requirements of hens, not only during early laying stages, but also during the late stages. Most present-day laying strains peak at more than 90 % production and produce large eggs in less than eight weeks after the onset of egg production (El Boushy and Raterink, 1985, 1989).

Because the eggshell is almost totally calcium carbonate, and calcium represents 40 % of the calcium carbonate molecule, the hen requires 2.48 g of calcium per day to lay a large egg (62 g) with a good shell. Work at Rutgers University and Israel has indicated that hens are only able to absorb 1.88 g of calcium from a diet containing 3.75 % or more calcium as pulverized limestone. This is because hens can retain only 50 % of the ingested calcium in 3.5 to 4.0 % containing mash diets. According to these calculations a hen would need to remove 0.6 g (2.48−1.88=0.6) of calcium from her bones to make a good eggshell (Scott et al., 1982; Scott, 1991).

2) Sources and Solubility of Calcium Feed

Except for dried green meals, feedstuffs of plant origin are low in calcium. Fish meal, meat and bone scrape, bone meal, calcium phosphate supplements, limestone and oyster shells are the major feedstuffs that supply the calcium needs of layers. If the levels of calcium carbonate (limestone) and calcium phosphate are high, the diet may become unpalatable and other dietary component may be diluted. If a calcium source contains high levels of magnesium (as does dolomitic limestone), it should probably not be used in poultry diets. It was reported that crushed coral can serve as a satisfactory calcium source for laying hens (NRC, 1994).

The eggshell is composed almost entirely of calcium carbonate, and calcium represents 40 % of the calcium carbonate molecule as it was indicated before. Calcium carbonate is insoluble in water and is not absorbed from the digestive tract. Calcium absorption depends upon the degree to which calcium carbonate is dissolved by the hydrochloric acid (HCl) of the proventriculis and gizzard of the hen. This occurs according to the following reaction:

$$CaCO_3 + 2HCl = Ca^{2+} + H_2CO_3 + 2Cl^-$$

Because the acidity of the chicken's gizzard is approximately the same as 0.1 N HCl, this was used to determine relative solubility of calcium carbonate, with 100cc of 0.1 N HCl dissolving exactly 500mg of $CaCO_3$ (Scott et al., 1982).

Expermental with oyster shell show that when oyster shell contributed 50~66 % of the supplemental calcium, breaking strength was significantly improved in eggs of hens fed this diet when compared to hens receiving only pulverized limestone. Thus, 4 % oyster shell combined with 3.5 %

pulverized limestone (a total dietary calcium of about 3.5 %) would insure maximum calcium absorption throughout 24 hours each day. Particle size of the calcium carbonate needs to be large and hard enough to remain in the gizzard throughout the night. The particles should be sufficiently hard and of sufficient surface to allow the gastric activity to dissolve them at a rate that releases approximately 75 mg of calcium ion per hour into the blood (Scott et al., 1971; Summers and Leeson, 1994).

Laying hen research has shown that there was no significant differences in eggshell quality or layer performance due to switching limestone with oyster shell, lower soluble limestone to higher soluble limestone and higher soluble limestone to lower soluble limestone (Coon and Cheng, 1986). This study concluded that hens can adapt to different sources of limestone and oyster shell if a large portion of the calcium is in a large particle size and the calcium intake is adequate. Their data suggested that there is a larger difference in limestone solubility between particle size of the same limestone source than between limestone sources.

Specific gravity is in terms of "floating". The specific gravities are used to compare various samples. Water has a specific gravity of 1.0. Anything with a specific gravity less than 1.0 will float in water and those with a higher specific gravity will sink. Imagine that feed in a trough resembles flowing water, then one would expect those materials that have specific gravity values nearest to that of feed to sink more slowly through the feed than those materials with a much higher specific gravity. In one solubility study, the specific gravities of the sample feeds were carefully compared by determining the weight of 30 ml of each material (Scott, 1991). These results revealed the fallacy of supposing that the larger particles are more apt to "float" in the feeds. The results indicated that : (a) The large, hard limestone particles, which showed acid solubilities ranging from 22.5 to 42 %, had specific gravities ranging from 1.37 to 1.45 g/ml. These samples not only showed poor acid-solubilities, but also were so much heavier than the feed that they could be expected to sink through the feed as it traveled along the line. (b) The results with the granular limestone samples showed that these had specific gravities that were higher than the particular pulverized limestone used. (c) The oyster shell had the lowest specific gravity, being only slightly higher than that of the corn-soybean diet, thereby presenting a possible reason for the observation that oyster shell does not settle out during its transport along the chain feeder. Granular oyster shell also showed good solubility.

The most useful calcium supplement, to be used together with oyster shell particles, would be granular oyster shell. The only other materials to come close to oyster shell in usefulness appeared to be the pulverized limestone sample designated as 80-mesh. This sample had the highest acid solubility of all and also had a low specific gravity (Scott et al., 1982; Nahm and Chung, 1995).

High producing hens may retain the egg in the uterus for a much shorter time than do hens laying at lower rates. These high producing hens need oyster shell much more than hens that retain the egg in the shell gland over a longer period of time (Scott et al., 1982; Nahm and Chung, 1995).

3) Calcium Requirements

(1) Calcium Content of the Grower Diet

The incidence of osteoporosis (cage layer fatigue) is a problem faced by the industry during the early stages of egg production. Current commercial egg strains reach sexual maturity as early as 17 to 18 weeks. Some producers traditionally, or due to scheduling conflicts hold the birds that are already producing eggs in growing houses and on a low calcium diet up to 20 to 21 weeks of age. As a result, much of the medullary calcium storage has already been utilized for shell formation by the time of housing. During the early stages of egg production laying hens are in a negative calcium balance which cannot be alleviated by increasing the dietary levels of calcium, so the presence of adequate medullary bone is crucial for maintaining eggshell quality and bone integrity. An extra demand for calcium from the bones for shell formation during the early stages of egg production causes the bones to weaken to the extent that these birds show signs of osteoporesis (Summers and Leeson, 1984; Nahm et al., 1997).

An experiment was conducted to determine the effect of

feeding a high calcium diet for various duration in the latter part of the growing period on growth amd subsequent performance (Keshavarz, 1987a). Pullets were fed a 3.5 % calcium diet with adequate levels of available phosphorus (0.42 %) for 2 to 6 weeks prior to housing at 20 weeks of age. The bone ash and bone calcium contents were increased due to consumption of the high calcium diet for two or more weeks. Shell quality was not influenced by the calcium level fed in the growing period of this experiment. Other investigators, however, have reported favorable effects on the shell quality during the early part of the production cycle due to feeding high calcium diets in the pre-laying period (Hurwitz and Bar, 1971). These researchers recommended that growing diets should be changed to laying diets either when the secondary signs of sexual maturity (development of the comb and wattle) appear in the flock or when egg production reaches 2 to 5 %, regardless of the chronological age.

Increasing the calcium level 2 to 3 weeks prior to housing does not cause nephritis in the growing or laying periods (Keshavarz, 1987b). The incidence of nephritis (urolithiasis) in the growing or laying periods may be seen when pullets are fed a high calcium diet, particularly with low dietary phosphorus content, for long durations (10 to 14 weeks) during the growing period (Wideman et al., 1995).

(2) Calcium Content in the Layer Diet

– Calculation of calium requirement

The eggshell represents about 10 % of the weight of the egg. The eggshell is almost totally calcium carbonate and the carbonate molecule contain about 40 % calcium (Scott et al., 1982; Scott, 1991).

Step 1: The calculation of calcium content in the eggshell

For example, an egg weighing 62 g would require 2.8 g of calcium to form a good shell

Feed Ca content : 4 %
Egg weight : 62 g
Eggshell weight : 62 g × 0.1 = 6.2 g
Ca content in shell : 6.2 g × 0.4 = 2.8 g

Step 2 : The calculation of the solubility of calcium diet.

In order that a dietary intake of 4.0 g of calcium provides the needed 2.8 g of calcium for eggshell formation, the solubility of the limestone and oyster shell would need to be

2.8 ÷ 4 =70 %

Step 3 : The calculation of calcium content in the ration.

If pulverized limestone (88 % solubility) and oyster shell (45 % solubility) were used in a 50 : 50 mixture, the solubility of the calcium source would be 88 + 45 = 132 ÷ 2 = 66.5 %. The solubility at step 2 (70 %) can be used with the different ration of limestone and oyster shell instead of 50 : 50. Thus the 2.8 g of calcium needed from the diet could be achieved from 4.21 g of total calcium intake (2.8 ÷ 0.665 = 4.21 g).

In the above two example, the 2.8 g of calcium needed did not include the calcium which can be suppliedby other ingredients in the diet other than the limestone and oyster shell. In practical ration formulation, all sources of calcium must be included in the calculation.

A trial was conducted to determine the relationship of *in vitro* solubility of Ca cources (77.8 % pulverized oyster shell and 46.0 % pulverized oyster shell) with their *in vivo* utilization for bone and shell formation (Keshavarz et al., 1993). This research did not show the important relationship between solubility of the Ca sources and eggshell quality and bone formation. This trial suggested that Ca sources with a solubility of less than 46.6 % were needed to detect the Ca solubility effects on shell quality and bone formation. The results also indicated that a daily Ca intake of about 3.75 to 4 g/hen/day are required for optimum shell and bone formation. This is consistent with the 1984 NRC calcium recommendation of 3.75 g/hen/day, and suggests that the newly updated 1994 NRC recommendation of 3.25 g/hen/day may be inadequate for optimum eggshell and bone formation.

– Calcium separation and its reduction in the commercial layer diet

Extensive Ca separation takes place in various phases of the feed handing system (Keshavarz and Ackerman, 1984). This practical study showed that extensive calcium separation took place during a period of 4 to 6 days when feed was stored under normal bulk bin conditions. Calcium levels as low as 1.7 to 1.9 % were determined in feed samples during

their storage in bulk bins.

Extensive calcium separation was also found to take place along the feeding line. When the Ca level in a chain type feeding system was about 4 to 5 % in the beginning of the feeding line, the Ca level was as high as 11 to 13 % at the end of the feeding lines due to separation (Koshavarz, 1995). The pattern of separation was not similar in different farms and varied depending on the type of feeding system and the speed of movement along the feeding lines. With increases in the calcium separation, the phosphorus content remained the same and the protein content was reduced as the feed moved along the feeding lines. Calcium separation occurring on commercial farms can be a significant factor in reducing shell quality, performance, and liveability.

Laying hens can safely tolerate a diet with 6.5 % Ca when the dietary available phosphorus was adequate (0.5 %) (Keshavarz, 1987b) although production was reduced, mortility increased mainly due to visceral gout when hens were fed diets containing 6.5 % Ca and a marginal level of available phosphorus (0.2 %). This calcium: available phosphorus (AP) ratio of 32.5 : 1 is detrimental to perfom-ance and liveability. This ratio is similar to a diet containing 13 % Ca and a normal level of AP (0.4 %) which has been observed under field conditions due to Ca separation. An excess of dietary Ca interferes with the availability of other mineral such as phosphorus, magnesium, manganese and zinc. A ratio of 2 Ca to 1 nonphytate phosphorus (wt/wt) is appropriate for most poultry except for those that are laying. In laying hens, a much higher Ca level is needed to form eggshells, and ratios as high as 12 Ca to 1 nonphytate phosphorus (wt/wt) may be appropriate.

An interesting study was conducted to investigate in more detail the Ca and AP requirement to laying hens for optimum performance and egg shell quality (Keshavarz and Nakajima, 1993). The dietary treatments consisted of Ca levels from 3.5 to 5.5 % in increments of 0.5 % with constant levels of dietary AP of 0.4 % (T1-T5); a step-up Ca phase feeding regiment of 3.5, 4.5 and 5.5 % with a constant level of AP (0.4 %) (T6); a step-down AP phase feeding regimen on 0.4, 0.3 and 0.2 % with a constant level of Ca (3.5 %) (T7); a concurrent step-up Ca phase feeding and step-down AP feeding regimen with or without substitution of 50 % oyster shell for pulverized limestone (T8 and T9); and a regimen similar to T9 with a step-up cholecalciferol pahse feeding of 2,200, 4,400 and 8,800 ICU/kg (T10). These results also indicated that the tolerance for Ca in laying hens is relatively high when the AP content of the diet is adequate. The AP level can be reduced in diets with no adverse effects on hen performance. Production performance and shell quality were not influenced by these dietary treatments, except when oyster shell was added to the diet. Beneficial effects of oyster shell on eggshell quality were obtained even when the Ca content of the diet was plentiful. This suggests that the residence time of the calcium sources in the digestive system is an important factor for the imporovement of egg shell quality. About 50 % of the supplemental Ca in the laying ration should be provided in particle form with a high solubility. Another field study indicated that with the chain-type feeding system, both oyster shell and Ca chips were uniformly distributed along the feeding lines (Keshavarz et al., 1991). Oyster shell has also been shown to have a beneficial effect on shell quality under both cold and warm environmental temperatures and with a normal level of dietary Ca (Keshavarz and McCormick, 1991).

The study also proved that the reduction in shell quality with aging was not due to the hen's reduced ability to absorb and mobilize Ca. Plasma levels of Ca and P, bone Ca and absolute retention of Ca were not influenced by dietary treatments in each phase of this experiment (Keshavarz and Nakajima, 1993). Regardless of the dietary treatments, the absolute retention of Ca did not reduce with aging, and in fact it tended to increase. It is more logical to assume that reduced egg shell quality with aging is due to increased egg weight without a concomitant increase in the hen's ability to increase the absorption and retention of Ca needed for larger eggs. A step-down phosphorus regimen with aging can also be used during the laying period to reduce the feed cost and environmental pollution attributed to phosphorus.

Every effort should be made to reduce Ca separation in different phases of the feed handling systems. The extent that calcium separation is usually occurring in different phases of the feed handling systems on commercial farms can be an important factor for problems associated with performance and eggshell quality. Since different patterns of calcium

separation occur with different feeding systems, every producer needs to evaluate his situation independently and appropriate measures must be taken to overcome the problems.

The followings are a few tips for reducing calcium separation at different phases in the feed handling system (Keshavarz, 1995).

– Auger systems for feed delivery results in less calcium separation than air systems for feed delivery.

– Bulk bins should have a feed distribution system at the top and a device at the bottom to remix ingredients to prevent separation.

– Running the feeding systems at their maximum speed reduces preferential selection of ingredients by hens which may contribute to nutrient separations.

– Augers in good working condition vibrate less and may cause less separation.

– Allow the birds to clean up the feed in the trough once daily to prevent accumulation of fines in the trough which may result in excessive calcium intake by the hens.

– Time of calcium intake by layers

The time of calcium intake of layers is also important for shell formation. It has been found that the most important time for the hen to receive calcium is during the afternoon. Intake of high calcium containing feed was higher from 6 to 8 a.m., and then decreased progressively until 2 p.m.. From 2 to 4 p.m., consumption of the high calcium feed increased. Eggshell calcification continues in may hens until 8 p.m.. Most eggs are laid between 8 a.m. and 2 p.m.. After 2 p.m. another eggshell begins to form. Hens prefer a higher percentage of calcium in the feed when the eggshell is being calcified than when the calcium is being deposited in the bone (Roland and Farmer, 1984).

The beneficial effect of evening feeding of calcium on eggshell quality may be due to the following mechanism. The route calcium takes to the eggshell in morning fed bird is via the small intestine to blood to bone to egg gland and then to the eggshell. Hens fed calcium in the afternoon at the beginning of eggshell calcification can directly deposit the calcium on the egg via the blood and bypass the bone. If this mechanism is correct, an eggshell from hens fed in the morning will contain more skeletal calcium than that from hens fed in the afternoon.

– Particle size of the calcium sources

Feeding larger particles of $CaCO_3$ to laying hens in the summer improves eggshell strength, however, it was of no benefit during the winter months. When hen-sized limestone, oyster shell or puller-sized limestone composed two-thirds of the calcium content of the diet, egg shell quality, measured by specific gravity, increased during the summer months only. There also was no difference in shell strength found between oyster shell and limestone(Roland et al., 1953).

Eggshell quality was improved by the use of large particles when the diet contained 3 % or less of calcium. However, when a higher level was used, the large particles gave no response(Roland and Harms, 1972). When the diet contained 4 or 4.5 % Ca, no improvement was obtained from the use of large particles. This data indicates that the biological availability of calcium is higher from the larger particles. Producers can use a slightly lower level of calcium when larger particles are fed. Larger particles of limestone or oyster shell will not produce better egg shells if the feed contains adequate calcium.

4) The Influence of Phosphorus on Eggshell Quality

– Levels proposed for commercial practice

The paper frequently cited to support the proposition that high dietary phosphorus depresses shell strength(Taylor, 1965) does not provide sufficient evidence of the detrimental effect of 1.0 % dietary phosphrus. Shell strength does decrease by 2 % at 1.0 % phosphorus as compared to 0.46 % phosphorus. This difference was significant but of questionable value in the delineation of a nutrient window for commercial formulation. Within the range of 0.4 to 1.4 % P, changes in shell strength are on the order of 0.3 to 1 % and such changes are unlikely to result in a measurable reduction in shell breakage(Miles and Harms, 1982). The high cost of phosphorus supplements is an incentive to lower dietary phosphorus. Hence in commercial practice, there is a greater change for a phosphorus deficiency. Egg shell strength is much more sensitive to P deficiency than to an excess (Maurice, 1988).

– Physiology of phosphorus for eggshell formation

Approximately 2,100 mg of calcium in the medium weight

egg are found in an eggshell. In contrast to this, the eggshell contains only 20 mg of phosphorus while there is approximately 130 to 140 mg of phosphorus in the egg's yolk. There is only a total of approximately 160 mg in the entire egg(El Boushy and Raterink, 1985).

The amount of phosphorus consumption of hen varies during the daily feeding period. A study (Harms et al., 1965; Roland, Sr., and Harms, 1976a) reported that hens consumed a fairly high level of phosphorus from 6 to 8 a.m.. At 8 a.m., the consumption form the cup containing the high level of phosphorus increased. It started to decrease by 2. p.m. and continued to decrease until 8 p.m. (Harms et al., 1965; Roland and Harms; 1976b). This data indicated that the hen requires a higher level of phosphorus when minerals were being deposited in the bone from 8 a.m. to 2. p.m.. After eggshell calcification began, a lower phosphorus intake is required. If the levels of phosphorus were then changed in the feed in the morning and afternoon, its effect upon change eggshell quality could be determined. These hens had been receiving a diet containing 0.75 % phosphorus. One group in this study contineud to receive this diet and served as a control. All hens received this feed during the morning until 11 a.m.. From 11 a.m. to 3 p.m., the feed was removed from the troughs which allowed feed to clear from the bird's digestive tracts. At 3 p.m., one group of hens received a diet with all of the supplemental phosphorus removed: one group continued to receive the feed containing 0.75 % phosphorus and the phosphorus was increased in the third group. Result indicated that decreasing the phosphorus level in the afternoon resulted in improved eggshell quality, while increasing the phosphorus levels in the afternoon resulted in decreased eggshell quality.

Levels of phosphorus in the blood also vary. Blood phosphorus exhibits a cyclic pattern which is closely related to the egg formation cycle(Miller et al., 1979). The serum phosphorus peaked at 6 mg % approximately 30 minutes before the hen laid an egg, then dropped to 4.5 mg % within 30 minutes following oviposition. This level of 4.5 mg % was maintained for the next five hours. Then the phosphorus level began to rise gradually until the next morning 30 minutes before the next egg was laid. If the hen is placed on a phosphorus deficient diet, the serum phosphorus level will be 4.5 mg % 30 minutes before the egg is laid, and then it falls to 2.5 mg % and remains at this level for the next five hours. It then repeats in the same manner.

Calcium and phosphorus are supplied in the feed during eggshell calcification, but calcium is also withdrawn from bone. When calcium is released from the bone, phosphorus must accompany it. Bone calcium is deposited in the eggshell, along with the calcium from the feed, and increases the level of blood phosphorus. Some of this phosphorus is deposited into the developing yolk of the egg, as previously mentioned. The hen's demand for phosphorus for yolk formation is not nearly as high as the amount made available from bone and that supplied by feed. This explains the continued increase in blood phosphorus during eggshell formation. It has been suggested that if a high build-up of phosphorus in the blood can be avoided, the hen would be able to withdraw more of the calcium form the bone and improve eggshell quality. This would explain the increase in feed phosphorus levels causing decreased eggshell quality. It also may explain why eggshells are better when phosphorus is removed from the feed in the afternoon, and why eggshell quality is reduced when additional phosphorus is added to the feed in the afternoon(Maurice, 1982; Maurice 1988; Kim et al., 1995).

A marginal level of phosphorus in the diet may lead to "cage layer fatigue" (osteoporosis). This phosphorus deficiency results in high mortality, with the major portion of this mortality due to "cage fatigue" (Singsen et al., 1962). In their study, diets containing various levels of phosphorus were fed to commercial layers maintained in cages and floor pens. As the level of phosphorus in the diet increased, mortality decreased in the birds maintained in cages. Phosphorus levels in the diet did not influence mortality of hens maintained on the floor.

Minimizing phosphorus levels in also advantageous for maintaining eggshell quality, especially under heat stress conditions. Since phosphorus is very costly nutrient, high levels in the feed are usually not encountered, but limiting this nutrient in the range of 0.3 to 0.4 %, depending on flock conditions, seems ideal for maintaining egg quality(Owings et al., 1977; Leeson and Summers, 1983). Unaccountable losses in shell quality may occur occasionally, and some of these

may be related to nutrition. For example, vanadium contaminated phosphates cause unusual shell structures, and certain weed seeds, such as those of the lathyrus species, and cause disruption of the shell gland(Holder and Huntley, 1978).

5) Vitamin D and Absorption of Calcium and Phosphorus

Vitamin D has been reported not to be involved in the deposition of calcium, but it has a major role in the absorption of calcium. Phosphate absorption has also been shown to be stimulated by vitamin D. 1-alpha $25(OH)_2D_3$ is transferred to the nucleus of the intestinal cell where it interacts with chromatin. In response, specific RNAs are elaborated by the nucleus. When these are translated into specific proteins by ribosomes, this enhances calcium and phosphorus absorption. The presence of a calcium binding protein is correlated with calcium absorption. This protein does not appear to be a simple transport protein, since calcium absorption is complex and probably requires other factors besides this protein. Calcium seems to be absorbed from the intestine into the mucosal cell by an active transport system and by facilitated diffusion. These mechanisms are both vitamin D dependent(Maurice, 1988; Leeson and Summers, 1992).

When inadequate levels of vitamin D_3 or deficiencies of D_3 are present, induced calcium deficiency results quickly. In addition to the uncomplicated deficiencies of vitamin D_3, certain mycotoxins can create problems. Zearalenones, produced by *Fusarium* molds, can effectively bind vitamin D_3 and result in poor egg shell quality. Under these circumstances dosing birds with 2,000 to 25,000 IU of water soluble vitamin D_3 for three consecutive days may be advantageous(Bar and Hurwitz, 1987; Hargis, 1990).

The effect of dietary supplements of vitamin D metabolites on shell strength has been variable. In some experiments, a positive response was obtained with metabolites of vitamin D(McLoughlin and Soares, 1976), While others failed to detect a beneficial response (Roland, Sr. and Harms, 1976b). This discrepancy may be attributed to age differences since old hens lose their adaptive potential (Bar and Hurwitz, 1987). Exogenous 1, 25-dihyfroxy vitamin D was ineffective in altering shell strength in young and old hens (Castaldo and Maurice, 1988).

4. Effects of Other Nutrients on Eggshell Formation

1) Dietary Electrolyte Balance and Calcium Metabolism

Recently, the impact of dietary mineral(electrolyte) balance on body fluid acid-base and calcium metabolism has been researched. High dietary levels of acidogenic salt (calium salts of chloride, phosphorus and sulfate) have detrimental effects on shell quality and resulted in acidemia and increased Ca excretion (Austic, 1984). While high dietary levels of phosphorus in dibasic form is a mild acidogenic anion, high dietary levels of phosphorus in monobasic form is a strong acidogenic anion and caused serious adverse effects on performance of the hens.

2) Sodium Bicarbonate

The inclusion of sodium bicarbonate in layer feeds at levels of between 1 to 5 kg per metric ton reduced the proportion of downgrade eggs by 1 to 2 % (Imperial Chemical Industries, 1987). They concluded that refined sodium dicarbonate has three beneficial effects when added to layer feeds.

It:

a. Increased shell strength and cut downgrades
b. Optimized egg production, and
c. Improved utilization of dietary protein

Their research maintained the balance of sodium(0.14 to 0.28 %) and chloride(0.20 to 0.24 %) ions by using a non-chloride containing sodium source such as sodium bicarbonate, at a phosphorus level of 0.75 %, sodium at 0.55 % derived from sodium bicarbonate increased egg output. At a lower phosphorus content of 0.30 %, the same addition of sodium from either bicarbonate or chloride decreased egg production (Imperial Chemical Industries, 1987). Studies have also indicated that at high ambient temperatures, carbon dioxide is expired from hens and their bicaronate levels fell, resulting in poor quality shells unless sodium bicarbonate was added.

– Salt

Sodium chloride is predominantly a feed additive and the contribution form drinking water varies with the area. Egg

production was reduced by more than 10 % when NaCl in the feed exceeded 2 %, and shell strength was maintained even at 6 % NaCl in the diet for 3 weeks (Damron and Kelly, 1987). In contrast, chickens provided 1 to 2 % NaCl in the drinking water ceased laying(Heller, 1931). Layers are extremely sensitive to NaCl level in the drinking water.

A study involving a dose-response examination of NaCl concentrations between 0 and 600 ml/l indentified a significant linear increase in eggshell defects and corresponding linear decreases in various eggshell quality measurements with increasing NaCl concentrations(Balnave and Yoselewitz, 1987). However, the increased incidence of egg shell damage was not related to decreased feed intake or increased egg production or egg weight. It has also been found that administering a commercial electrolyte replacer containing 2.2 g NaCl and 4 g potassium citrate/l of drinking water for 7 days significantly increased the incidence of eggshell defects in 48-and 72-week-old laying hens(Yoselewitz and Balnave, 1989a).

Classification of the types of eggshell damage showed that, irrespective of the NaCl content of the drinking water, the major types of shell damage consisted of fine cracks, hole and star cracks. The incidence of broken and shell-less eggs increased markedly with the presence of NaCl in the drinking water (Yoselwitz and Balnave, 1989b). The poorer egg shell quality from hens receiving saline drinking water is reflected in increased damage during transport to, and during handling at, the packing station.

The impact of saline drinking water on egg shell quality is likely to be most severely felt with breeder flocks and day-old chick production. Artificially inseminated hens receiving drinking water containing 2 g/l NaCl produced significantly fewer day-old chicks than hens receiving unsupplemented water(Zhang et al., 1991). This reduction in day-old chick production was associated with reduced numbers of settable eggs and with lower hatchability of fertile eggs.

Attempts to offset the effects of saline drinking water had no, or only limited, value. In recent studies with ascorbic acid (Balnave and Zhang, 1982) and with zinc-methionine (Moreng et al., 1982) have identified two nutritional procedures for off-seting the adverse effects of saline drinking water on eggshell quality. However, these treatments are preventative rather than remedial in nature. Depending on the economics of production, the best remedy may be desalination of the drinking water (Balnave, 1993).

3) Manganese

The only trace element of concern in commercial layer diet formulation is manganese. Manganese is involved in the synthesis of components of the organic matrix (Leach, 1982), and its deficiency caused changes in the mammillary cores (Leach and Gross, 1983). Disorganized or impaired mammillary core formation causes low shell strength. Adequate manganese levels are not probided by corn-soybean meal diets and various supplements provide variable bioavailability. High levels of calcium and phosphorus reduce the availability of dietary manganese by preventing its absorption. Manganese availability can also be reduced by vitamin-mineral imbalances. Most of the B vitamins can act as metal chelators. Excess amounts of vitamins used in feed could influence manganese availability.

The manganese requirements under practical conditions to reduce eggshell breakage is considerably higher than the current NRC(1994) recommendation of 20 mg/kg. At 200 mg/kg, shell breakage was reduced 27 % in one experiment and switching hens from a low (25 mg/kg)to a high manganese diet reduced shell breakage(Whisenhunt and Maurice, 1985). Laying hens fed 0 to 6,400 mg/kg supplementary manganese showed no adverse effects. Shell breakage exhibited a significant quadratic response with minimum shell damage at 400 mg/kg(Maurice, 1982).

4) Protein and Amino Acids

Excess dietary protein levels in feed may result in increased endogenous acid production. Complete oxidation of one mole of methionine and cystine produces 2 moles of hydrogen ions. These ions are excreted as ammonia ions and acid phosphates resulting in decreased renal resorption of calcium. However, decreasing dietary protein from 18 to 15 % at 12 week intervals had no significant effect on shell surface density in four strains of hens fed a pracitical diet (Ousterhout, 1981). It is also questionable whether reducing methionine intake increases shell quality. Lysine inhibited

calcium absorption in one study, yet in another study, neither lysine nor arginine affected the absorption of calcium in the ligated loop preparations of chick intestine. It is unlikely that economically feasible variations in dietary protein and amino acids influence eggshell strength(Wasserman et al., 1957: Sallis and Holdsworth, 1962: Roland, 1980)

5. Biological, Environmental and Managerial Factors

Several factors can influence shell breakage at various stages of movement of the eggs from the producer to the consumer.

1) Late-day Feed Deprivation

Deprivation of feed in the latter part of the bird's "light" day may result in broken or cracked eggs. If hens accidentally go off their feed for even four hours, they will produce poorer eggshells the following day, but if they are not fed for 24 hours they will produce poorer shells for the next three days. Full-fed hens consume about 65 % of their feed needs in the latter part of the day, but when restricted to a 4 a.m. to noon feeding period, feed consumption quickly rose from 44 % of normal the first day to more than 70 % the second day(Arrington, 1986). This study showed that eggshell quality fell on the day following feed restriction, but began improving by the sixth day of restriction. Hens normally consume most of their daily feed intake during the second half of the day. Feed deprivation later in the day is therefore more serious since the hen cannot make up for the loss from deprivation.

2) Age of the Laying Flock

Eggshell quality is influenced by the hen's age. There have been reports of 2.7 % breakage during the first month of lay and 13.5 % in the 15th month of lay. There was no apparent effect of age on the proportion of breakage that occurred between the hen and the processing plant and the in-plant breakage. Egg size is also affected by age and this may partially explain decreases in eggshell quality after the first laying period. Egg size increases more rapidly than shell weight so there is a concomitant decrease in shell thickness and percent shell. Eggshell quality in older hens may therefore be improved by controlling egg size(Hamilton et al., 1979).

In one study, the average percentage of broken or cracked eggs was not significantly different among three young flock groups(Bell et all, 1983). They noted egg breakage did increase substantially in the youngest group while decreasing substantially in the 60 to 79 week-old group.

They also noted that the pattern in the older group of decreasing egg breakage with increasing egg weight paralleled the pattern of increasing eggshell thickness in the those eggs. Apparently, they said, increasing egg weight had a positive effect on shell thickness as the birds aged. They also reported that egg weight and shell thickness were significantly different by strain but that overall egg breakage was similar.

By shell thickness, the workers said that the average broken or cracked egg had an average thickness of 0.0330 cm, which was 5.4 % thinner than the average sound egg with a thickness of 0.0373 cm, The thinnest shelled eggs (less than 0.0305 cm) had a mean breakage rate 12.3 times that of the thickest shelled egg (greater than 0.0406 cm). The probability of the thinnest shelled egg breaking before reaching the processing plant wee more than 25 %(Bell et al., 1983). There was also a significant change in the type of breakage reported as the birds aged. Collision cracks decreased and line cracks increased as the birds aged and the shells thickened. Shell thickness can be controlled through strain selection, proper nutrient formulation and sound housing and other management programs(Bell et al., 1983).

3) Time of Day When Eggs Are Laid

Shell quality and breakage are also affected by the time of day that the eggs are laid. Eggs laid in the afternoon have higher specific gravities so their shells are stronger. This factor is related to the shell formation period in hours, since an eggs that stays longer in the uterus will put down a stronger shell(Hunges et al., 1986).

4) Temperature

It is known that high environmental temperatures are associated with a decrease in shell quality. The relationship between environmental temperature and shell thickness in curvilinerar when the temperature ranges from 26.5 to 35℃.

The domestic hen responds to high environmental temperatures by panting to resist rises in body temperature. This panting results in chemical changes in the blood characterized by an increase in pH. There are also decreases in the concentration of blood carbon dioxide (CO_2) and a loss of blood bicarbonate (HCO_3). The physiological state of alkaline blood (increased pH) produced during thermal panting would become life threatening to the hen if allowed to persist. Poultry have evolved compensating processes which help to reduce this rise in blood pH during panting. The prevent alkalosis the bird increases the excretion of blood HCO_3 into the urine. This action by the kidneys is an attempt to maintain the balance of the basic HCO_3 in the blood relative to the blood acid components. The maximum rate HCO_3 loss from the kidneys occurs approximately two hours after the beginning of heat stress (Summers and Lesson, 1984; Lession, 1986).

Time of day when eggs are laid, humidity, evaporative coolers, strain of bird, disease, egg collection, processing and packin, and transport also should be considered for strong eggshell formation (Nahm and Chung, 1995).

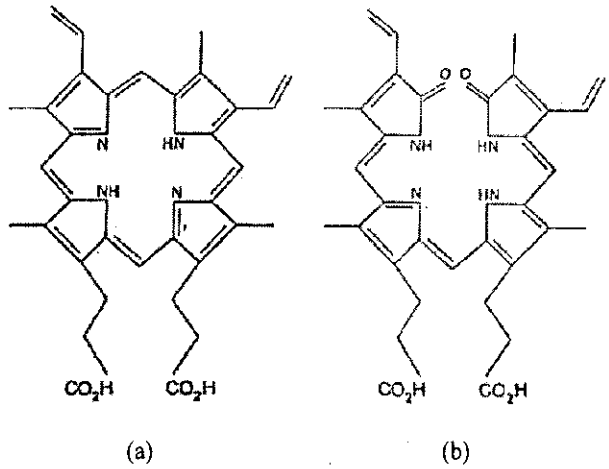
EGGSHELL PIGMENTATION

1. The Major Pigment for Eggshell Color

The pigments present in eggshell are protoporphyrin-IX, biliverdin-IX and its zinc chelate, and occasional traces of coproporphyrin-Ⅲ (Kennedy and Vevers, 1975). No pigments were found in the white eggshells fo the fulmar, imperial pigeon, dipper, roseate, cockatoo and ring- necked parakeet, other apprently white eggshells contained only protoporphyrin such as the white stork, Barbary dove, Scops owl and roller. Other shells contained both protoporphyrin and biliverdin such as those from the block-footed penguin, Humbolt's pigeon, mandarin duck and wood pegeon.

2. Eggshell Pigment Formation

Porphyrin derivatives play an important role in the biochemistry of all living systems, indeed, they have been called the pigments of life (Battersby, 1985). The porphyrin structure is found in pigments such as chlorophylls and haem. Porphyrins and their derivatives are also present in a



(a) (b)

Fig. 1. Structures of protoporphyrin-IX(a) and biliverdin-IX(b).

wide variety of other biocatalysts, e.g., cytochromes, vitamin B_{12} and prosthetic groups of enzymes whose biosynthesis was likely to be contemporary with the appearance of life on earth (Simioneseu et al., 1978). Porphyrins comprise cyclic tetrapyrrole structures and are related to the parent compound porphine. The most common form of the fifteen possible isomers among four different possible combinations of the side chains on the porphyrin nucleus is protoporphyrin-IX (Fig. 1). In porphine, the methyl, vinyl and propionic acid side chains are replaced by hydrogen atoms. All carbon and nitrogen atoms of the porphyrin ring are derived from glycine and succinic acid.

In contrast, the bile pigments are open chain tetrapyrroles. They are derived in nature by oxidative degradation and ring opening of the prosthetic groups of haemoproteins(Hudson and Smith, 1975). Biliverdin-IX(Fig. 1) is formed by the rupture of the blood pigment, hemoglobin, with the loss of the meso-carbon of the methine bridge(as carbon monoxide). In man and mammals, biliverdin is reduced to bilirubin which is catalyzed by biliberdin reductase.

This shows that the two different pigments of eggshells have different origins, despite their chemical similarities. The porphyrins are synthesized de novo in the cells in which they occur, while biliverdin is derived from erythrocytes.

3. Eggshell Pigment Distribution

In poultry, pigment is deposited during the entire process of shell formation, but the deposition rate accelerates the last

three to five hours before oviposition(Warren and Conrad, 1942;Lang and Wells, 1989). In brown eggs a significant proportion of the pigment is in the cuticle even though porphyrin is distributed in the shell membrance, shell and cuticle(Schwartz et al., 1975). The total thichness of the eggshell is approximately 375 μm. The shell membrance is the most internal layer of the shell and is made up of an inner(15 μm) and outer membrance(50 μm). The true shell is the next layer and is the thickest at 300 μm. It is made up of the cone, palisade and surface crystal layers. The cuticle is the outermost layer with a thickness of only 10 μm (Tullet, 1984). The cuticle acts as the carrier of pigment and the color of eggs is due to this colored coating. The role of the cuticle in this manner is not recognized by the poultry industry.

4. Factors for Eggshell Pigment Formation

1) Blood

The porphyrins found in eggshells are derived from erythrocytes. Which are known to synthesize these pigments (Kennedy and Vevers, 1975). There are arguments that the erythrocytes disintegrate in the mucous layer of the shell gland.

2) Shell Gland

Examination of the inner surface of the shell gland under ultra-violet light reveals a bright red fluorescence, confined to its epithelial cells(Tamura et al., 1965). The shell gland mucous epithelium secretes pigment granules at different rates depending on the area of the reproductive tract. The secretion begins at a slow rate in the magnum where the albumen is formed. The rate continues to increase in the isthmus and proximal uterus where the shell membrance formation takes place. The rate is the highest in the uterus during shell formation and peaks in the area where the cuticle is formed. The granule formation in the uterine epithelium sharply drops distal to the area of cuticle formation(Tamura and Fujii, 1966). These authors concluded that the large granules contribute to the organic matrix, while the cuticular prophyrin was derived from small pigment granules. And prophyrin concentration increases during shell formation(Baird et al., 1975). The avian shell gland is the site of biosynthesis of the egg shell porphyrins, but further research is needed(Stevens et al., 1974).

3) Causes of Shell Pigmentation Change

There are several causes of change in egg shell pigmentation.

- Age : The intensity of shell color changes as the hen ages.
- Disease : Disease, such as that caused by infectious bronchitis virus, is known to cause a marked increase in the incidence of pale-shell eggs(Cooke, 1986).
- Drugs : Sharp declines in the shell pigment can occur through ingestion of drugs, such as the coccidiostat Nicarbazin(Schwartz et al., 1975).
- Surface defects due to abnormal post-cuticular deposits (Hulan, 1988).
- The genetic effects on eggshell color

The inheritance of eggshell color in chickens has been of interest to scientist for many years. In their experiments, genetic effects, including single gene or polygene, sex-linkage, dominance, epistasis and heritability and some environmental effects such as season and egg production were investigated.

One study indicated that the inheritance of eggshell pigmentation depended on a large number of genes, each contributing a small amount of the trait(Hunton, 1962; Gowe et al., 1965). And age, hatch group and crosses each contributed significantly to the variation seen in the eggshell color(Wei et al., 1992). Distrubution comparisons indicated that two major autosomal loci affected the trait in these lines: one gene having in complete dominance controls the amount of pigment deposition; the second completely inhibits pigment deposition when homozygouse recessive. No sex-linked effects were noted. Another stucy also suggested that some major genes causing the segregation of shell color seen in brown egg flocks probably had dominance effects based on the higher variance associated with dams as compared with sires(Gowe et al., 1965).

CONCLUSION

Eggs have to compete with other foods since a wide

variety of products are becoming available to consumers. The disadvantages of egg consumption are becoming more relevant because the consumers are demanding guarantees for quality. The quality of an egg is a complex relationship between the eggshell strength, shell color, internal quality, cholesterol content, yolk color and egg size.

Between 6 to 8 % of the eggs annually produced are broken or cracked, which causes economic losses for the producer, but may also pose a human health hazard via *Salmonella* contamination. Eggshells break due to the relationship between eggshell quality' and many biological, environmental, managerial, managerial and nutritional factors.

In many countries, the color of the eggshell is an important aspect of egg quality, so importance should be placed on the biochemical and physiological processes involved in pigment formation and deposition in and on the shell.

Other factors involved in quality egg production will be discussed in the other paper.

적 요

본 장에서는 양질의 계란생산에 필요한 난각과 난각색의 형성에 관해서 언급하였다. 강한 난각의 형성은 난각을 이루는 내적인 요인들 (큐티클층, 진각, 난각층의 조직)과 소화기, 난각형성에 관여하는 각종 체내 분비선, 신장, 호르몬 등의 적정기능에 의하여 결정된다. 병아리가 성성숙에 달하면 골수골은 산란계가 난각의 형성이 정점에 달했을 때를 대비할 수 있는 정도가 되어야 한다. 난각이 형성되는데 중요한 기능을 갖는 요인들로는 산란계 사료중의 칼슘원, 산란계사료 중의 칼슘과 인의 비율, 비타민 D의 적정수준, 체액중의 각종 무기물 균형, 기타 영양소 수준들을 들 수 있다. 또 생리적, 환경적인 요인들과 산란계의 나이나 산란계사의 온도와 습도, 산란계의 계통, 질병, 계사에서 알의 수집부터 수송에 이르기까지의 각종 관리형태 등은 계란이 생산자에서부터 소비자에 이르기까지 여러 단계에서 나타나는 각종 난각 파괴의 요인이 된다. 난각에 나타나는 색소에 관여되는 요인들로는 protoporphyrin-IX, biliverdin-IX, 아연 등이 함유된 킬레이트와 때로 적은 양이 관여하기는 하지만 coproporphyrin -III 등이 포함된다. 그러나 이러한 요인들이 난각색의 형성에 관여하는 데에는 산란계의 연령, 질병, 약품 첨가 여부, 난각 형성도 중 난각 표면의 손상 여부 등 여러가지가 관여된다.

(색인어 : 난각강도, 난각선, 호르몬조직, 칼슘, 난각샘)

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REFERENCES

Arias JJ, Fernandez MS 1993 Molecular control of avian eggshell biomineralisation. Proc 5th European Symp on the Quality of Eggs, Tours, France, pp. 116-126.

Arrington L 1986 Eggshells weakened most by late-day feed deprivation. Feedstuffs July 14 pp. 15-17, 23.

Austic RE 1984 Excess dietary chloride depress eggshell quality. Poultry Sci 63:1773-1780.

Austic RE 1996 Dietary Protein levels and the response to dietary amino acids. Cornell Nutr Conf Report pp. 168 -181.

Bain ME 1991 Future consideration. In: Eggshell Quality, Wolfe Publication Ltd., London. Chapter 8.

Bain ME 1992 Eggshell strength: a relationship between the mechanism and the ultrastructural organisation of the mammillary layer. Br Poultry Sci 33:303-319.

Baird T, Solomon SE, Tedstone DR 1975 Localisation and charaterisation of eggshell porphyrin in several avian species. Br Poultry Sci 16:201-208.

Balnave D, Yoselewitz I 1987 The relation between sodium chloride concentration in drinking water and eggshell damage. Br J Nutrition 58:503-509.

Balnave D 1993 Influence of saline drinking water on eggshell quality and formation. World's Poultry Sci J 49 :109-119

Balnave D, Zhang D 1992 Responses in eggshell quality from dietary ascorbic acid supplementation of hens receiving saline drinking water. Aust J Agric Res 43:1259-1264.

Bar A, Hurwitz S 1987 Vitamin D metabolism and calbincin (calcium-binding protein)in aged laying hens. J Nutr 117:1775-1779.

Battersby AR 1985 Biosynthesis of the pigments of life. Proc Royal Soc B225:1.

Bell D, Ernst R, Adams C, Johnson G 1983 Management programs which emphasize strong eggshells and careful handling can keep this breakage at a minimum. Progress in Poultry pp. 23-26.

Blair R 1996 Future of feeding poultry to vary by region, Feedstuffs, Dec 16. pp. 17-20.

Castaldo DJ, Maurice DV 1988 The role of calcitriol in eggshell strength. Poultry Sci 67:65-71.

Clunies M, Emslie J, Leeson S 1992a Effect of dietary calcium level on medullary bone calcium reserves and shell weight of leghorn hens. Poultry Sci 71:1348-1356.

Clunies M, Parks D, Leeson S 1992b Calcium and phosphorus metabolism and eggshell thickness in laying hens producing thick or thin shells. Poultry Sci 71: 490-498.

Cooke JKA 1986 Pale shelled eggs can be caused by IB virus. Misset International Poultry 2:38-48.

Coon C, Cheng TK 1986 Effect of limestone solubility on layer performance and shell quality. Feedstuffs Oct 3 pp. 12-14.

Damron BL, Kelly LS 1987 Short-term exposure of laying hens to high dietary sodium chloride levels. Poultry Sci 66:825-831.

El Boushy AR, Raterink R 1985 Eggshell strength: The causes of egg breakage in relation to nutrition, management and environment. Feedstuffs, Aug 12 pp. 7-9.

EI Boushy AR, Raterink R 1989 Various aspects of egg yolk pigmentation explored. Feedstuffs, Jan 30 pp. 41-48. Cited in Netke(1976).

Gowe RS, Budde HW, McGann PJ 1965 On measuring eggshell color in poultry breeding and selection programs. Poultry Sci 44:264-270.

Hamilton RMG, Hollands KG, Viosey PW, Grunder AA 1979 Relation ship between eggshell quality and shell breakage and factor that affect shell breakage in the field. World's Poultry Sci J 35:177-185.

Hargis PS 1990 Dietary modification of fatty acid composition of shell eggs. Proceedings of fat and cholesterol reduced foods. Mar 22-24 New Orleans LA IBC USA. Inc., 8 pleasant St., Bldg. D. South Natick, Mass. 01760.

Harms RH, Darmron BL, Waldroup PW 1965 Influence of high phosphorous in caged layer diets. Poultry Sci 44: 1249-1254.

Heller VG 1931 Oklahoma's water supply. Rep Oklahoma A&M College Agric Expt Sta pp. 181-183.

Hincke MT, Bernard AM, Lee ER, Tsang CPW, Narbaitz R 1992 Soluble protein constituents of the domestic fowl's eggshell. Br Poultry Sci 33:505-516.

Holder DP, Huntley DM 1978 Influence of added manganese, magnesium, zinc and calcium level on eggshell quality. Poultry Sci 57:1629-1639.

Hudson MF, Smith KM 1975 Bile pigments. Chem Soc Rev 4:363-399.

Huges BO, Gilbert AB, Brown MF 1986 Categorisation and causes of abnormal eggshells: relationship with stress. Br Poultry Sci 27:325-337.

Hulan HW 1988 Redfish meal oil raises fatty acid level of poultry meat. Feedstuffs April 2 pp. 13-15.

Hurwirz S, Bar A 1971 The effect of pre-laying mineral nutrition on the development performance and mineral metabolism of pullets. Poultry Sci 50:1044-1050.

Hunton P 1962 Genetics of eggshell color in a light Sussex flock. Br Poultry Sci 3:189-193.

Imperical Chemical industries(ICI) 1987 Sodium bicarbonate boosts egg production shell strength. Feedstuffs, Aug. 10. pp.16-18. Cited in Choi and Han, 1987.

Kennedy GY, Vevers HG 1975 A survey of avian eggshell pigments. Comp Biochem Physiol 558:117-123.

Keshavarz K 1987a Interaction between calcium and phosphorus in laying hens. Nutr Rep Int 36:9-16.

Keshavarz K 1987b Influence of feeding a high calcium diet for various durations in prelaying period on growth and subsequent performance of white Leghorn pullets. Poultry Sci 66:1576-1583.

Keshavarz K 1995 An overview of calcium and phosphorus nutrition of growing pullets and laying hens. Proc 1995 Cornell Nutr. Conf Oct 24-26 pp. 161-170.

Keshavarz K, Ackerman SE 1984 On farm survey finds calcium formation problems. Feedstuffs 56:18-20.

Keshavarz K, Ackerman SE, Park KS 1991 How uniformly are the particulated sources of calcium distributed along the feeding line? Feedstuffs, Oct 6 pp. 12-14.

Keshavarz K, McCormick CC 1991 Effect of sodium aluminosilicate, oyster shell and their combinations on acid-base balance and eggshell quality. Poultry Sci 70: 313-319.

Keshavarz K, Nakajima S 1993 Re-evaluation of calcium and phosphorus requirement of laying hens for optimum performance and eggshell quality. Poultry Sci 72:144-149.

Keshavarz K, Scott ML, Blanchard J 1993 The effect of solubility and particle size of calcium sources on shell quality and bone mineralization. J Appl Poult Res 2: 259-264.

Kim DJ, Myang WJ, Nahm KH, Ko YD, Chang MB 1995 A Textbook of Feed Resources(Korean edition). Hyang Mun Pub Co Seoul Korea pp. 324-354.

Krampits G 1993 Molecular mechanisms of eggshell formation. Proceedings of 5th European Symposium on the Quality of Eggs. Tours France pp. 99-109.

Kuit AR 1984 Egg's interior quality. Feedstuffs, Sept 24 pp. 14-16.

Lang MR, Wells JW 1989 A review of eggshell pigmentation. World's Poultry Sci J 43:238-246.

Leach RM 1982 Biochemistry of the organic matrix of the eggshell. Poultry Sci 61:2040-2049.

Leach RM, gross JR 1983 The effect of manganese deficiency upon the ultrastructure of the eggshell. Poultry Sci 62:499-517.

Leesons S 1986 Nutritional considerations of poultry during heat stress. World's Poultry Sci J 42:69-76.

Leeson S, Summers JD 1983 Performance of laying hens allowed self-selection of various nutrients. Nutr Rep Int 27:837-844.

Leeson S, Summers JD 1992 Commercial Poultry Production. Guelf University Press, ON. Canada.(1st Ed).

Maurice DV 1982 Dietary manganese and eggshell quality. Proc. Maryland Nutr Conf pp.90-96.

Maurice DV 1988 Diet and eggshell strength. 49th Minnesota Nutr Conf Sept 19 pp. 212-223.

McLoughlin CP, Soares JP Jr1976 A study of the effects of 25-hydroxycholecalciferol and calcium source on eggshell quality. Poultry Sci 55:1400-1409.

Miles RD, Harms RH 1982 Relationship between egg specific gravity and plasma phosphorus from hens fed different dietary calcium, phosphorus and sodium levels. Poultry Sci 61:175-181.

Miller ER, Harms RH, Wilson HR 1979 Cyclic changes in serum phosphorus of laying hen. Poultry Sci 56:586-593.

Moreng RE Balnave D, Zhang D 1992 Dietary zinc methionine effect on eggshell quality of hens drinking saline water. Poultry Sci 71:1163-1167.

Nahm KH, Chung KK, Go YD, Kim YK 1997 Animal Nutrition (Korean edition). Yu Han Pub Co.

Nahm KH, Chung SB 1995 A Textbook of Chicken production (English edition). MunDang Pub Co Seoul Korea.

National Research Council 1994 Nutrient Requirements of Poultry. 9th Revised Ed. National Academy Press Washington DC.

Ousterhout LE 1981 The effects of phased feeding protein and calcium on egg weight and shell quality with four strains of white Leghorn hens. Poultry Sci 60:1036-1042.

Owings WJ, Sell JJ, Balloun SL 1977 Dietary phosphorus needs of laying hens. Poultry Sci 50:2056-2062.

Potts PL, Washburn KW 1974 Shell evaluation of white and brown egg strains by deformation, breaking strength, shell thickness and specific gravity. Poultry Sci 53:1123 -1129.

Richards JF, Swanson MH 1965 The relationship of egg shape to shell strength. Poultry Sci 44:1555-1558.

Roland DA Sr 1980b Eggshell quality. II. Effect of dietary manipulations of protein, amino acids, energy and calcium in young hens on egg weight, shell werght, shell quality and egg production. Poultry Sci 59:2047-2052.

Roland DA Sr, Farmer M 1984 Importance of time of calcium intake with emphasis on broilers breeders. World's Poultry Sci J 40:255-260.

Roland DA Sr, Harms RH 1972 Calcium metabolism in the laying hen. Poultry Sci 52:369-378.

Roland DA Sr, Harms RH 1976a The influence of feeding diets containing different levels of phosphorus. Poultry Sci 55:637-642.

Roland DA Sr, Harms RH 1976b The lack of response of 25-hydroxy vitamin D_3 on eggshell quality or other criteria on laying hens. Poultry Sci 55:1983-1989.

Roland DA Sr, Sloan DR, Harms RH 1953 Effects of various levels of calcium with and without pellet -size limestone on shell quality. Poultry Sci 53:662-670.

Sallis JD, Holdworth ES 1962 Influence of vitamin D_3 on calcium absorption in the chick. Am J Physiol 203:497-503.

Schwartz S, Stephenson BD, Sarkar DH, Bracho MA 1975 Red, white and blue eggs as models of prophyrin and heme metabolism, Ann NY Acad Sci 244:570-588.

Scott M 1991 How can calcium be supplied to high-producing hens? Feedstuffs Sept 23 pp. 16-17.

Scott ML, Hull SJ, Mullenhoff PA 1971 The calcium requirement of laying hens and effects of dietary oyster shell upon eggshell quality. Poultry Sci 50:1055-1063.

Scott ML, Nesheim MC, Young RJ 1982 Nutrition of the Chicken. 3rd ed Ithaca NY ML Scott.

Simionescu CI, Mora R, Simionescu BC 1978 Porphyrins and the evolution of biosystems. Bioelectrochem Bioenergetics 5:1-17.

Simons PCM 1971 Ultrastructure of the hen egg shell and it physiological interpretation. Agriculture center, Pulication and Documentation, Wageningen, The Netherlands.

Singsen EP, Spandorf AH, Matterson LD, Sorafin JA, Tlustochwicz JJ, 1962. Phosphorus in the nutrition of adult hens. Poultry Sci 41:1401-1410.

St. Louis ME, Morse DL, Potter ME, Guzewich JJ, Blake PA 1988 The emergency of grade A eggs as a major source of *Salmonella enteritidis* infections. J Am Med Assoc 259:2103.

Stevens EV, Miller LK, Weinsteins S, Kappas A 1974 Biosynthesis of 5-aminolevulinic acid and prophbilinogen in the domestic fowl. Comp Biochem Physiol 478: 779-789.

Summers JD, Leeson S 1984 Poultry Nutrition Handbook. The University of Guelph ON Canada.

Tamura T, Fujii S 1966 Histological observations on the quail oviduct, on the secretions in the mucous epithelium of the uterus. J Facult Fisheries Anim Husbandry Hiroshima Univ 6:357-371.

Tamura T, Fujii S, Kunisake H, Yamane M1965 Histological observations on the quail oviduct: with reference to pigment in the uterus. J Facult Fisheries Anim Husbandry Hiroshima Univ 6:37-57.

Taylor TG 1965 Dietary phosphorus and eggshell thickness in the domestic fowl. Br Poultry Sci 6:70-79

Tullett SG 1984 The porosity of avian eggshells. Comp Biochem Physiol 78A: 5013. Voisey PW and RMG Hamilton 1976 Notes on the measurement of egg specific gravity to estimate egg shell quality. Report 7322-598. Eng Res Service Agri Ottawa Canada.

Voisey PW, Hunt JR 1974 Measurement of eggshell strength. J Texture Studies 5:135-182.

Warren DC, Conard RM 1942 Time of pigment deposition in brown shelled hen eggs and in turkey eggs. Poultry Sci 21:515-520.

Wasserman RH, Comar CL, Schooley JC, Lengemann FW 1957 Interrelated effects of L-lysine and other dietary factors on the gastrointestinal absorption of calcium 45 in the rat and chick. J Nutr 62:367-370.

Wei R, Bitgood JJ, Dentine MR 1992 Inheritance of tinted eggshell colors in white shell stocks. Poultry Sci 71:406-418.

Whisenhunt JE, Maurice DV 1985 Effect of dietary manganese and phosphorus on the strength of avian eggshell. Nutr Rep Int 31:757-762.

Wideman RF Jr, Closser JA, Roush WB, Cowen BS 1985 Urolithiasis in pullets and laying hens: Pole of Dietary calcium and phosphorus. Poultry Sci 64:2300-2311.

Wideman RF Jr, Colsser JA Roush AWB, Cowen BS 1985 Urolithiasis in pullets and laying hens: Role of dietary calcium and phosphorus. Poultry Sci 64:2300.

Yoselewitz I, Balnave D 1989a Electrolyte replacer and eggshell quality. In: Recent Advances in Animal Nutrition in Australia 1989. (Ed. Farrell, D. J.). NSW Australia pp. 1A.

Yoselewitz I, Balnave D 1989b Classification of eggshell damage resulting from the use of normal and saline drinking water. Proc Austr Poultry Sci Symp p. 90.

Zhang D, Moreng RE, Balnave D 1991 Reproductive performance of artificially inseminated hens receiving saline drinking water. Poultry Sci 70:776-779.