The missing link in hatching egg quality

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In its role as an embryonic chamber, the bird's egg must afford physical protection against external trauma whilst satisfying the nutritional demands of the growing embryo.

The diverse pigment patterns on the surface of the eggs of many wild species also provide camouflage against predation and assist in species recognition. In every case, egg formation is a demanding process in terms of energy and nutrient requirements. So does the hatching egg fulfil its potential in terms of structure and function?

The yolk

The bulk of the yolk mass, a composite of tightly packed yolk spheres containing lipoprotein is deposited in a concentric manner 6-11 days before ovulation.

At ovulation, the largest yolk in the hierarchical arrangement of follicles within the ovary, together with its attached blastodisc moves into the upper funnel shaped portion of the infundibulum where fertilisation takes place.

Sperm which have migrated anteriorly from the vaginal host glands and located temporarily in the nutrient rich glands of the infundibulum are capable of penetrating the vitelline membrane which encapsulates the yolk. The process involves the trypsin-induced breakdown of the latter and has been shown to be enhanced by the inclusion of a variety of trace elements, in particular selenium in the diet. The energy-rich yolk comprises 21-36% lipid and 16-22% protein. Immunoglobulins, primarily IgG are also transferred during yolk accumulation.

The yellow colour of the yolk is due to the presence of carotenoids which function to protect the vulnerable embryonic tissues against damage caused by free radicals, a process also facilitated by the presence of vitamin E.

The maternal investment in egg composition is of vital importance to embryonic well being with age, diet and environment all having a role to play in the quality of the embryonic chamber and its contents.

Selective feeding can be used to manipulate the nutrient content of the egg but with varying degrees of success. Over feeding will result in obesity, excess ovarian development and an early decline in the rate of egg production.

Albumen

The multilayered egg white is produced in the longest region of the oviduct, the magnum. Complete stratification of albumen is only achieved at oviposition at which time four distinct layers can be seen. Egg white consists primarily of water (90%) and protein (10%).

As such, it provides a rich source of biologically active substances crucial to embryo protection (antibacterial proteins) and forms a mechanical buffer between the external environment and the developing chick.

Thick egg white also functions as a support for the deposition of the paired shell membranes. To understand the consequences of shell failure with respect to albumen thickness one has only to consider the effect of infectious bronchitis on the activity of the lower magnum. Watery whites are produced in the absence of the highly sulphated mucosubstances which give egg white its turgidity. In this condition they are unable to support the weight of 'normal' membrane deposition, the membranes fold and ultimately because of such physical distortion, calcium carbonate deposition is compromised giving rise to the classic 'wrinkled' shell. Egg white varies with increasing bird age, diet and environment. With age comes a natural decline in the ability of the magnum to produce the necessary proteins and under extreme conditions of stress, the magnum will break down resulting in changes in albumen viscosity.

The eggshell

The egg takes on average 24 hours to form from ovulation to oviposition. In this cyclical event 20 hours are spent forming the shell membranes and 'true' shell (the calcified portion of the shell), but is this dispropor-

Plate 1. The egg at hatching. Most of the mammillary layer has been eroded.

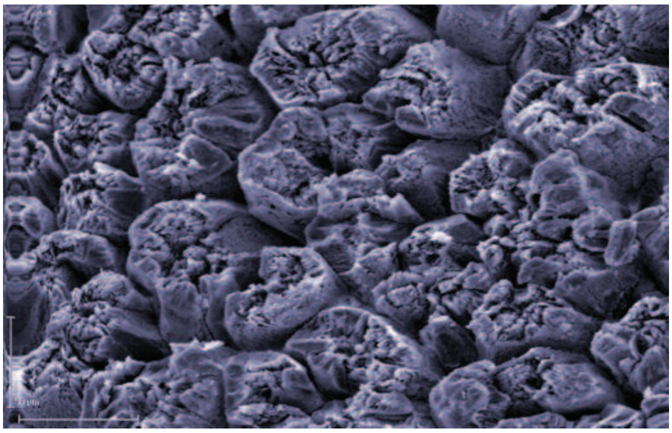
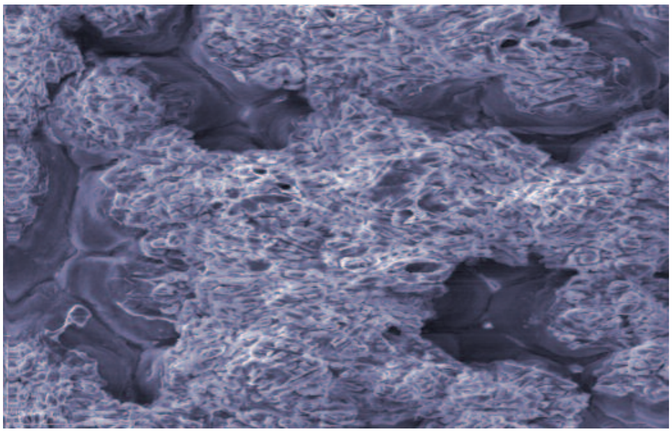


Plate 2. Many of the mammillae have fused thus altering pore distribution.



tionate rationing of time reflected in function? In recent years considerable effort has been directed towards understanding the relationship between the organic and inorganic fractions of the shell of the table egg.

This multilayered structure consists of 95% calcium carbonate plus an organic matrix (3.5%), which contains proteins, glycoproteins and proteoglycans. This matrix influences calcium carbonate deposition during all stages of shell formation, controls crystal growth and shape, and by virtue of its disposition can alter the biomechanical properties of the resulting shell.

Both physical and ultrastructural analyses of the shell demonstrate its variability under the duress of disease and environmental and nutritional stress.

In terms of its configuration and total thickness the shell represents the main barrier to bacterial transfer, although each layer has a specific role to play in the process.

At the external surface of the shell, the cuticle, a protein/carbohydrate-rich layer, is classically described as 'the first physical and chemical barrier against bacterial invasion', partially blocking the gas exchange pores which open to the environment at this level.

However, cuticle deposition in today's eggs appears to be the exception rather than the rule with most eggs, particularly the hatching egg, only having a patchy external covering. The fact that eggs with such a poor cuticle hatch, is due in large measure to the controlled and hygienic conditions within the incubator.

The question then arises as to whether the possession of an uneven outer covering significantly influences gas exchange and water loss and, if so, is the hatchling compromised in any way by having an incompletely formed egg?

The vertical crystal layer of the shell lies immediately beneath the cuticle and at this level the organic matrix proteins are vertically orientated. In the honeycombed palisade layer which lies beneath the vertical crystal layer and which forms the bulk of the 'true' shell (about 200μm), the organic matrix is arranged perpendicularly. This change in the orientation of the matrix proteins throughout the thickness of the shell assists in strengthening the end product.

Each layer of the shell supports a variety of protein types, some classified as 'egg white proteins' and others as 'shell specific proteins'. Ovocleidin-17 is one such shell specific protein identified both within the palisade layer and the basal or nucleation layer of the shell, the mammillary layer.

The complexity of the design, that has been formed in order to maximise the functional properties of the shell, has now been revealed at nanostructural level.

Response to change

The design and topography of the mammillary layer exerts a profound effect on the performance of the shell under load and as a consequence considerable effort has been expended on understanding its response to changes in a variety of physiological and environmental factors. This effort however, has been directed almost entirely towards the table egg.

Likewise feeds formulated to alter the nutritional value of table egg contents are commonplace and the omega3 egg, the cholesterol-reduced egg, and in many countries, the selenium-enriched egg sit regularly on the supermarket shelf besides other dietary staples. Since the table egg is a commodity whose contents are judged by its external appearance, colour, texture and size, the condition of content must also come into the list of selection criteria. Technology has thus been developed to understand the mechanisms of shell breakage and the aetiology of shell and content defects in response to the ever increasing demands of the supermarket. No such critical attention has been paid to the hatching egg!

During the incubation process the shell thins and there is an acidic breakdown of the bond between the shell membranes and the shell, as well as the removal of the bulk of the mammillary layer (Plate 1).

This process not only provides the chick with a source of calcium and a variety of other essential trace elements but also facilitates pipping. In terms of its basic structure, the calcified aspect of the breeder eggshell is no different from its layer counterpart. It is multilayered, matrix proteins permeate its thickness and it is composed of calcium carbonate in its calcitic form – so wherein lies the difference?

The answer is to be found in the mammillary layer. Of the 12 structural imperfections commonly found at this level in the shells of the layer bird, only 5-6 will regularly occur in the foundation layer of the hatching egg (Plate 2).

These changes in structure reflect simultaneous variations in the fluid environment of the shell forming region of the oviduct. The degree of incidence and the morphology of each variant will be reflected in the values recorded for traditional measures of quality such as shell thickness, deformation and egg weight.

In a recent investigation, an improvement in hatch rate at 54 weeks of age has been correlated with a significant increase in the number of 'normal' nucleation points in the shells of birds fed on a diet containing 0.3ppm Sel-Plex (Alltech Inc) compared with 0.3ppm sodium selenite for a period of 30 weeks.

Results showed 33 nucleation points per unit area in the experimental group (Plate 3) compared with 24 in the control group (Plate 4). The shells also displayed a reduction in the incidence of morphological variants in the mammillary layer at 54 weeks of age.

In the absence of shell structure data, it might be supposed that the increase in hatchability in this trial was due in large measure to the enhancement of the antioxidant capacity of different embryonic tissues, which is still a valid consideration.

But, shell structure improved, calcium availability from discrete nucleation sites increased and the changes in pore distribution associated with the structural changes will have influenced both gas exchange and water conductance – is this now a more favourable environment for embryonic growth?

Given its crucial and diverse roles, the scant attention given to the eggshell of the breeder bird is unacceptable – somewhat akin to buying the 'ideal' home but failing to ask whether or not the roof leaks! ■

Plate 3. There are 33 nucleation points per unit area in the experimental group.

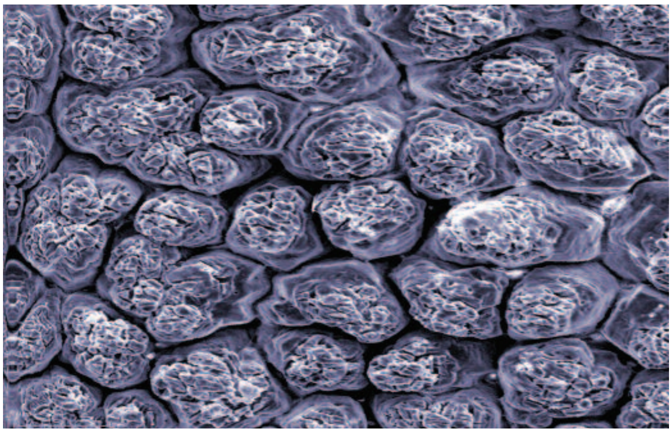


Plate 4. There are 24 nucleation points per unit area in the control group (Plates 1-4, Sally Solomon, 2008).