The Influence of Organically Bound Minerals on the Process of Shell Construction

Sally E. Solomon

Senior Research Fellow

University of Glasgow

Contact Address: Vigonac, 24310, Brantome, France.

Tippett.sarah@neuf.fr

If 'the aim of biomineralisation is stability', then with reference to the hen's egg such stability is essential if the calcified package, constructed over a period of 20 hours, is to meet the demands of the developing embryo or withstand the rigours of selection, packaging and travel en route to the table. The pattern of mineralisation in which calcite, the most stable polymorph of calcium carbonate precipitates in an a-cellular milieu regulated by a variety of protein profiles, follows a specific design. The morphology of each of the six layers which comprise the 'true' shell is quite distinct but in terms of shell strength they are interdependent. Of these perhaps the mammillary (basal) layer plays the most crucial role insofar as its morphology is easily altered by a range of internal and external variables including disease and bird age.

Trace Minerals.

These are an essential ingredient in animal nutrition but over supplementation in their inorganic form has led to calls for limitation on their inclusion levels because of the environmental damage following their excretion. This has led to the development of bioplexed minerals in which the divalent trace metal is bound to a peptide or amino acid. Supplied as they are at a lower inclusion level they are less damaging to the environment and in this form their bioavailability is enhanced (Bierla *et al* 2008).

While transition metals such as zinc, iron and copper can become bioplexed, selenium, also an essential micro-element and which exists in a negatively charged form, cannot be chelated and is normally supplied as sodium selenite. When bound to a yeast, however, selenium is better absorbed than in its selenite form and so is able to rapidly assume its role as an antioxidant. The avian eggshell displays at any one time a variety of crystal modifications in its basal layer (the mammillary layer). The incidence and nature of the variations from the calcitic growth pattern are exacerbated by stress, disease, housing and diet and the impact on the role of the shell either as an embryonic chamber or table egg is compromised.
Reference has been made to the effect of supplying inorganic and organic trace minerals to breeder and laying hens in terms of improved hatchability, albumen quality and shell thickness (Rutz *et al* 2004) but less information is available on the effect of the dietary inclusion of these trace elements on the construction of the eggshell.

At the beginning of the birds reproductive life shell structure is highly variable and the basal layer presents with a variety of morphological variants. By 33 weeks of bird age and under optimal environmental conditions these variations in crystal form are reduced, only to increase again as the bird enters the final phase of egg production. This predictable rise and fall in the pattern of shell formation has strong financial implications for both the breeder and table egg and given its organic/inorganic complexity a range of dietary supplements have been used in an attempt to minimise the decline in quality at the end of lay.

This paper discusses the range of aberrant crystal forms observed in the shells of eggs from breeder and layer birds, and the changes observed in the construction of the shell following the use of organically bound minerals.

Materials and Methods

In the breeder trial (a preliminary investigation) birds were housed in one shed and divided into two groups. In the control group selenium was supplied in a commercial diet as sodium selenite (0.3ppm). In the experimental group selenium (0.3ppm) was supplied in the diet as Sel-Plex (Alltech®).

Full details of the layer trial protocol are available in Solomon and Bain (2012)

Results.

In both trials no differences were observed in the structure of the eggshells until after mid-lay. Prior to this point the mammillary layer in all shells displayed a variety of irregular crystal forms including rhombohedral calcite, spherical calcite and aragonite. These forms were not attached to the membrane fibres. In the breeder trial, following mid-lay, the shells of the birds on the experimental diet were structurally sound, the incidence of aberrant forms was reduced and the number of mammillae per unit area had increased. In the layer trial the basal layer of the shells on all treatments presented with the same range of crystal irregularities prior to 40-45 weeks of age. Following mid-lay the mammillary layer of the eggs laid by the birds on the SelPlex and Bioplex diet were characterised by the high incidence of confluence i.e. the early lateral fusion of adjacent mammillae, firmly bonded to the membrane fibres.

Discussion

In terms of its role as an embryonic chamber the shell is required to provide calcium and other trace minerals for the developing chick, a process which by thinning the shell also assists in the pipping mechanism. Its construction must also afford mechanical protection and facilitate gas exchange. The process of calcium uptake by the chick is effected through the solubilisation of the bond between the organic core of the mammillary body and its calcium reserve assembly (Terepka, 1963).

In its capacity as a table egg, toughness and protection against the ingress of microorganisms are possibly the two most important functions. There is no doubt that calcium is the dominant macro-element in the shell and that a diet deficient in calcium will manifest itself in a variety of ways ranging from skeletal compromise to shell quality issues – but a diet deficient in trace minerals can also impact on the growth and metabolism of the animal and so influence productivity.

Magnesium deficiency was shown by Leach and Gross (1982) to cause a decrease in egg production and eggshell thickness. Their investigation of shell structure concluded that the large irregular mammillary knobs observed in these shells were caused by fusion of smaller forms during the early stages of calcification. Mabe *et al* (2003) investigated the use of organic/inorganic sources of manganese, copper and zinc and demonstrated that while there was a treatment effect in the eggs of layer birds over 60 weeks of age with regard to improved breaking strength and a resistance to fracture, the effect was independent of the source of the trace elements.

In the layer trial reported here the temporal response to the inclusion of organic sources of the micro-minerals accords with the findings of Mabe *et al* (2003). There is however another temporal aspect to be considered. Shell formation is described as occurring in three phases viz. A slow phase during which the mammillary layer forms, a fast phase (palisade layer) and a terminal phase during which the vertical crystal layer and cuticle are deposited (Nys *et al* 2001). The confluent mammillae described in the results section have formed during the slow phase of mineralisation and the tight bond created between the membrane fibres and the calcite crystals may explain the observed improvement in values for fracture toughness and breaking strength (Solomon and Bain, 2012). The results of the breeder trial, which must because of the lack of replication be treated with caution, report an increased number of structurally sound mammillae after mid-lay and so possibly an increase in calcium availability for the developing chick. The commercial breeding company involved in this investigation reported an increase in hatch rate after 45 weeks of bird age. The microelements considered in this study perform a number of diverse functions and are either components of many enzymes, or are essential in their activity. The relationship between the organic and inorganic components of the shell is complex but is such that in the 'normal' shell the dominant polymorph is calcite. It is evident from this investigation and others (Solomon, 1991) however that the bird can create other less stable crystal forms and that their inclusion impinges on function.

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