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GUINEA FOWL EGGSHELL STRUCTURAL ORGANIZATION AND PARTICULAR ORGANIC MATRIX PROTEIN PATTERNS TO DECIPHER ITS EXCEPTIONAL BIOMECHANICAL PROPERTIES

Nathalie Le Roy¹, Lucie Combes-Soia², Aurélien Brionne¹, Valérie Labas^{2,3}, Alejandro B. Rodriguez-Navarro⁴, Yves Nys¹ and Joël Gautron¹

¹BOA, INRA, Université de Tours, 37380 Nouzilly, France

²UMR PRC, INRA 85, CNRS 7247, Université de Tours, IFCE, 37380 Nouzilly, France

³CIRE, Pôle d'Analyse et d'Imagerie des Biomolécules, INRA, CHRU de Tours, Université de Tours, 37380 Nouzilly, France.

⁴Departamento de Mineralogia y Petrología, Universidad de Granada, 18071 Granada, Spain

The Guinea fowl (*Numidameleagris*) presents a highly resistant eggshell compared to the other birds. We will describe in this study the particular ultrastructure of the Guinea fowl shell that confers exceptional mechanical properties and how changes in organic matrix components control the development of this structure. The inner part of the shell is similar to other birds, but an additional change in the size and orientation of crystals (switch) is observed at about 1/3 of the calcified layer. Large columnar calcite units break into smaller crystal units with varying crystallographic orientations forming a microstructure with an intricate interlacing of calcite crystals.

We recently reported the Guinea fowl shell structural organization from the micro to the Angstrom, which underlined that this particular shell is a bilayer structure. Organic matrix is suspected to firstly induce the initial microstructure shift and then the secondary nucleation events resulting in smaller crystals with increasing misorientations. Consequently, the change of the intra-crystalline organic matter level during the crystal switch was also investigated. A proteomic survey allowed us to identify and characterize 149 proteins in Guinea fowl shell. These proteins were quantified at five calcification stages corresponding to the first events of mineral deposition, the growth of calcite units just prior the shift of crystal orientation, then to the period of the deposition of newly formed crystalline shape and to later stage when the growth of the newly formed crystals is stabilized after the microstructure shift and the secondary nucleation events. We have observed 61 matrix proteins only present in the shift period and potentially responsible of the change of mineral in the shell. Amongst them are calcium binding proteins (NPNT-X1, CALBP1, Protein S100-A6, ANXA1 and 2, CDH2...), core proteins of proteoglycans (TSKU, GPC4...), and other proteins regulating the activity of proteins driving the mineralization (SSP1, OC-116GDF6...).

These proteins interact with mineral to produce changes in crystal size and orientation and consequently the new shell structure and its resulting mechanical properties. Data obtained will allow the determination of biological markers that will be used for the genomic selection of chicken layers with improved mechanical shell mechanical properties. Additionally, they provided a list of organic products that will be tested as additives for material and ceramics.