How ageing affects laying hen bone quality: a multi-approach study

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INTRODUCTION

The nanocomposite nature of bone (multiphase material formed by the arrangement of an organic matrix and a mineral phase), together with its hierarchical organization and characteristic ultrastructure, provides this mineralized tissue with exceptional mechanical properties (Reznikov et al. 2018; Tertuliano & Greer 2016). The bone scaffold is based on collagen protein molecules organized in arrays forming fibrils with alternating gap and overlap zones. These fibrils are closely associated with approximately equal volume of carbonated hydroxyapatite (cHAp) crystals (Grandfield et al. 2018) distributed as intra- and extra-fibrillar phase, roughly oriented with the c-axis parallel to the long axis of the fibrils. The balance between mineral phase and organic matrix (comprising collagenous and non-collagenous proteins, proteoglycans, lipids) assures the quality of the bone tissue and its alteration may lead to skeletal disorders. Laying hens are particularly prone to bone deterioration towards the end of the intensive egg laying cycle due to physiological stresses. Although the daily Ca mobilization to form eggshell is identified as the driving process for the bone demineralization and mineralization cycle in hens (Alfonso-Carrillo et al. 2021), it is still unclear which structural modifications cause the decline of bone quality in laying hens with age. The analysis of bone samples taken at different stages of the hen laying cycle can give insights into the structural and compositional changes related to the decrease of bone quality. To this end, tibiae from laying hens 17 (pullets) to 90-weeks-old individuals were analyzed by different techniques to characterize both the bulk composition of cortical and medullary tissues and the bone structure at microscale. The collected data reveal consistent trends that illustrate how key parameters, such as the degree of mineralization, vary with age, allowing for some initial conclusions to be drawn.

METHODOLOGY

Samples of bones from laying hens have been provided by the bio-archive of partner research centers. Tibiae of hens from different age group (17, 28, 57, 90 weeks old) have been selected for a total of 12 specimens for each age. Cortical and medullary tissue of tibiae fragments were separated for infrared spectroscopy (FTIR), thermogravimetry (TG), 2D and powder X ray diffraction (XRD) analyses. Slides of ~1x1x0.5 cm^3 of the same samples have been treated by a standardized protocol for chemical fixation (4% paraformaldehyde and 2% glutaraldehyde in cacodylate buffer solution; dehydration in a graded acetone series) and embedded in Spurr resin for Scanning Electron Microscopy (SEM) imaging and chemical composition estimates by Energy Dispersive Spectroscopy (EDS). After the extraction of bone fragments for the bulk analysis and microscopy observations, representative specimens were fixed and analyzed by X-ray computed μ-tomography (μCT).

RESULTS

The obtained results may be described as follows:
In agreement with TG data, the mineral percentage of the cortical tissue increases from the week 17 (before the start of egg-laying) to the week 57-90 (end of the laying) (Fig. 1), while at the same time water content decreases. Similar trend was observed for the medullary tissue, whose mineral/organics ratio increases notably with the age.

The bone mineral carbonation estimated by FTIR increases with age in both tissues. The collagen content, measured by the same technique, is stable up to 57 weeks in the cortical bone being higher at 90 weeks while increases from 17 to 28 weeks remaining stable afterwords in the medullary bone.
cHAp crystal size measured by XRD apparently changes in length and width being shorter and wider at 90 weeks in the cortical bone and bigger in size with the age in the medullary bone.
SEM and μCT images confirm the increasing mineralization degree with the age, especially for medullary tissue (Fig. 2).

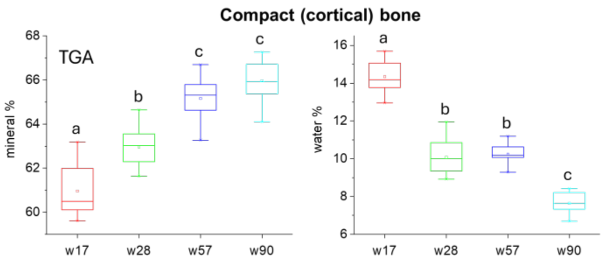

Fig 1*. TG analyses: mineral (left) and water (right) content (%) in hen tibiae bone samples as a function of age (17-90 weeks).*

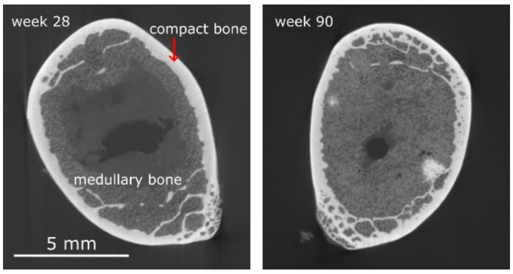

Fig 2*. μCT images of horizontal sections of hen tibiae bone samples of different ages.*

CONCLUSIONS

Through remodelling processes, the amount and composition of structural and more labile (medullary) bone tissues are regulated during the hen's life cycle. In younger hens (week 28-57), calcium for eggshell calcification is likely mobilized from both medullary and cortical tissues which are less mineralized. Higher mineralization and carbonate substitution with the age result in stiffer but more brittle bones (Boskey & Coleman, 2010). Further aspects, such as mineral/collagen arrangement, collagen structure and crystal shape, should be considered to have a full comprehension of the factors defining the mechanical properties of the ageing hen bone. For this reason, micro and nanoscale study (transmission electron microscopy, synchrotron techniques, atom probe tomography) of hen bones samples is the focus of an ongoing complementary investigation.

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