

## PS4 - Customized feeding strategies

### PS4-337 The use of NanoSIMS and Synchrotron techniques to explain eggshell resistance in aged layers

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Old layer hens have eggs with a thinner shell, increasing the problems related to broken eggs and shell defects. The objectives of this study were to evaluate the effect of trace metal (TM) supplementation on eggshell quality and understand how TM are incorporated into eggshell and egg membrane of aged layers. 936 Leghorn layers (60 weeks of age) were distributed in three treatments with 12 replicates of 26 birds. Treatments were: negative control (NC) diet without TM supplementation (54 ppm Zn, 60 ppm Mn); Zn diet, NC + 90 ppm of Zn from HiZox; Mn diet, NC + 110 ppm of Mn from ManGrin (Animine, Annecy, France). After 20 weeks trial, eggs were collected and analyzed for fracture force, shell thickness and correlative imaging (X-ray tomography, Laser ablation LA-ICPMS, and NanoSIMS). Pieces of membrane were separated from the eggshell and analyzed by ATR-FTIR. Shell thickness was improved ( $P<0.05$ ) by both Zn and Mn supplementation (0.35 mm) in comparison to NC (0.34 mm). However, fracture force was more increased ( $P<0.05$ ) when hens received Mn diet (37 N) than Zn or NC diets (35 N in average). Correlative imaging results (X-ray tomography and LA-ICPMS) showed that Mn and Zn are located in the outer membrane of the eggshell and in the cuticle, whereas Ca is mainly found in the palisade and mammillary layers. NanoSIMS images revealed that Ca in eggshell seems to be more present in Mn supplemented diets, which may be related to the impact of Mn on calcium-binding proteins, and would explain the better fracture force in these eggs. Synchrotron radiation FTIR technique showed differences in the polysaccharide region of the spectra, increasing concentration in the membrane and nucleation site of the samples supplemented with Mn. This would also explain the better eggshell resistance in this treatment. In conclusion, TM supplementation improves eggshell quality but the effect of Mn seems to be more important than Zn. Mn can improve eggshell quality because it is incorporated in the membrane of the eggshell, improving its polysaccharide composition. A potential role of Mn on Ca-binding proteins requires further research.