

SHORT TERM SCIENTIFIC MISSION (STSM) - SCIENTIFIC REPORT

The STSM applicant submits this report for approval to the STSM coordinator

Action number: CA15224 STSM title: Identifying causes and solutions of Keel Bone Damage in laying hens STSM start and end date: 19/02/2018 to 24/03/2018 Grantee name: Lazarin Velikov Lazarov

PURPOSE OF THE STSM/

The purpose of this STSM was to extend my knowledge and practical skills in developing and validating techniques that can diagnose, assess, and distinguish anatomical characteristics of keel damage with a high level of accuracy and consistency across a variety of situations, which match specific needs of the research environment. Michael Toscano's group has a lot of expertise in this field. They have been working on bone damage in laying hens in aviary systems for a long time.

At the same time, this STSM offers possibilities that are unique for me because there is no equivalent in our university and our experience in this area is quite limited.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

Animal, housing, and diets. The ability of a CaT and HYD to reduce susceptibility to fracture has been tested using an ex vivo protocol developed by Michael Toscano's group (Toscano et al., 2013). In this system, collisions of quantifiable energetic loads are performed directly onto the keels of deceased birds allowing the likelihood of fracture to be assessed for respective treatments. Our expectation was that birds receiving a diet characterized by CaT, HYD, or a combination of the two will manifest a reduced risk of fracture which we can model statistically.

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For the experiment, 240 birds were utilized in total and were exposed to one of four treatment combinations in a 2X2 factorial design (Factor 1: CaT- CON/Treated; Factor 2: HYD-CON/HYD).

At population (18 wks), all birds were grouped housed in a commercial aviary and received a standard commercial diet until 33 weeks of age when hens were moved to a test barn and randomly assigned to one of eight pens (30 hens/pen). After 1 week of habituation (34 wks), all birds began receiving one of the four treatment combinations (n=2 replicates /treatment combination).

Bird-level production data. To provide bird-level egg production data, at 37 weeks of age, twenty focal birds/pen were selected and administered a small capsule containing a fat-soluble dye that allowed identification of eggs from birds given that dye (Appleby and McRae, 1983). By administration of various combinations of three different dyes over three different days (including 1 day without colour), individual eggs were traced back to the laying hen.

At +2, +3, +4, and +5 days following dye administration, all eggs were collected, and underwent quantification of biomechanical strength, eggs shell thickness and mass. All data was stored in a database allowing individual hen productivity measures to be combined with other study response measures, e.g., keel fracture susceptibility.

All experimental work had been performed at the ZTHZ Laying Hen Research Facility in Bern, Switzerland using a commercially relevant genetic line (LSL).

DESCRIPTION OF THE MAIN RESULTS OBTAINED

During the current STSM, many results have been gathered and will be discussed and processed statistically.

<u>Pen-level production data</u>: Daily egg production were quantified for each pen as it was feed the weekly feed through the weigh back of the feeder. Records of egg production and feed consumption for the period from 27 wks of age until death were kept also.

<u>Bird-level production data:</u> At +2, +3, +4, and +5 days following dye administration, all eggs were collected, and underwent quantification of biomechanical strength, eggs shell thickness and mass. At that time, blood samples of 20 hens per diet



were also taken for an analysis of bone formation and resorption. The plasma from these samples was separated in a timely manner, and the studies still to be done.

Impact Testing: Birds were underwent impact testing at 39 weeks of age (4 diet combinations X 60 birds/diet combination = 240 birds). Immediately before impact testing, birds were euthanized with delivery of a barbiturate and subsequent cervical dislocation, a method of killing eliminates convulsions during death that can often lead to broken bones, an outcome that could complicates our analysis, which seeks to induce fractures with a controlled collision.

After the collisions, keel and long bones (humerus, tibias) were removed, checked for damage, and prepared for biomechanical, histological and computer tomography testing to identify changes in the relevant bone measures.

All data was stored in a database allowing individual hen productivity measures to be combine with other study response measures, e.g., keel fracture susceptibility.

FUTURE COLLABORATIONS

The STSM was the perfect starting point of a fruitful and close collaboration between our institutions. In conclusion, I would like to say that I had the exclusive opportunity to introduce modern methods of research and to work in a laboratory fully equipped with high-tech equipment and with a team of great scientists like Michael Toscano, from which I received useful guidance and unconditional support in this action. This allows me to define the experience as invaluable for my future career.

I would like to give my special thanks to Michael Toscano for his efforts and support in realizing of STSM action.