

SHORT TERM SCIENTIFIC MISSION (STSM) SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

Action number: CA15224-42511

STSM title: The influence of sun light during rearing phase on keel bone damage

STSM start and end date: 12/11/2018 to 26/11/2018

Grantee name: Manja Zupan

PURPOSE OF THE STSM:

(max.200 words)

In the course of this project, the candidate will be introduced with the on-going national project investigating the influence of sun light, more precisely the UV-B radiation on KBD during the rearing phase which has been carried out at the Friedrich Loeffler Institut of Animal Welfare and Animal Husbandry. There the candidate will have the opportunity to learn about the use of several techniques like radiographical methods, ELISA and treatments for histological investigations to get more information why KBD occur. More specific, the candidate will be firstly introduced with technique to investigate the microstructure of the keelbones to see whether there is any metabolic disorder like osteoporosis and/or osteomalacia underlying keel bone deviations and fractures and at which time in the hens' life it occurs. Secondly, radiological images of the keel bones in living hens will be collected and the radio density of the keel bones will be analyzed with the aid of an aluminium step-wedge. Thirdly, the candidate will learn how to analyze plasma samples of involved hormones like 17- β -Estradiol and 1,25 dihydroxycholecalciferol, with the ELISA technique. The candidate will also participate during the sectioning process of 16 weeks old hens, where histological investigations will be done to measure the amount of osteoclasts and osteoblasts in the bone together with the measurements of area of the different bone types in each slice. The last main aim of the project is to write of the first draft of scientific paper focusing on the physiological (i.e., corticosterone in feathers, H/L ration) and behavior measurements (i.e. fear and sociality trait oriented behaviours) related to keel bone damage collected in Slovenia in 2018.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

(max.500 words)

I started with a 14 day STSM at the Friedrich Loeffler Institut of Animal Welfare and Animal Husbandry in Celle, Germany on Monday 12th November and finalized it Monday 26th November. My main intention here was to get be introduced with the activities in the Institute regarding KBD, to better understand good research management in avian basic science and to look for opportunities to collaborate.

During my STSM, Dr. Stefanie Petow who is an avian physiologist provided me a good working atmosphere in her research group and introduced me with her current experiment (Figure 1) investigating keel bone damage (KBD). By answering to the basic research questions, her main focus has been the histological investigation of keel bone to get more information why KBD happen when manipulating different hen environment. One of the activities in the Institute included the visit of EuroTier in Hannover, which is the biggest fair in animal production in the world. There, one of the new finding that I came across was the Swiss company called Herbonis who cells a commercial feed product called Pandonis and that according to them is a unique natural source of the bioactive form of Vitamin D. Together with Dr. Petow

we think this product should be further tested to know the application for the bird welfare, since no study on keel bone quality has ever been conducted.

In the current experiment, the research questions address the following topics, whether the number of Vitamin D receptors in the gut, size of medullary bone and cortex (Figure 2) differ between groups of hens kept under natural UV light compared to control groups kept indoors. As a start, I was able to be introduced with the use of radiography (Figure 3) where at the time of my STSM 40 hens were investigated at the age of 17 weeks. With this method the quality of keel bone was objectively proven and divided into fractures, deviations and bone density. On the following day, these hens were photographed, weighted, euthanized brain and eyes removed and weighted (Figure 4a-d) and preserved in nitrogen cooled tissue Tek (left eye). Right eye was put in formalin to test for a better method when investigating avian vision and eye development between these two groups. In the next step the hens were dissected with keel bone and tibia (right) were removed first (Figure 5 a, b) and placed in the formalin. Left tibia was placed in the plastic bag and used for further chemical analysis at Alejandro Navarro of the University of Granada. The removal of gut followed and the sample of a gut content was homogenized (Figure 5 a, b) and stored in a DNA stabilizing solution for further microbioma sequencing.

The following step included the cutting of tibia bone into half, taking a small middle sample of circa 0.5 cm for histological investigation looking at the quality of cortex and medulla bone (Figure 2). Slices were prepared using two different equipments (Figure 6, below left and right) and stained. An example of a previous experiment was shown to me in order to understand the interpretation of slices.



Figure 1: Indoor (left) and outdoors (right) conditions for laying hens.

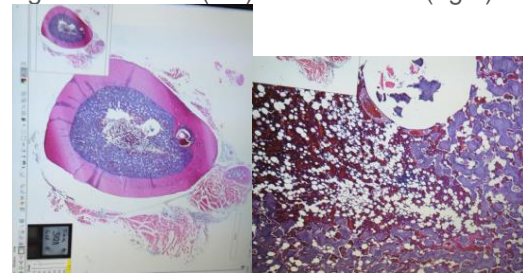


Figure 2: Scan of a slice of a tibia bone. Left picture presents cortex layers of the bone coloured pink and medulla bone in purple. In dark purple one can differentiate the bone marrow from the medullary bone, more brightly. Bigger the size of the cortex more stable is the bone.



Figure 3: Radiographic device (left) and its software (right) used for the investigation of keel bone damage in laying hens.

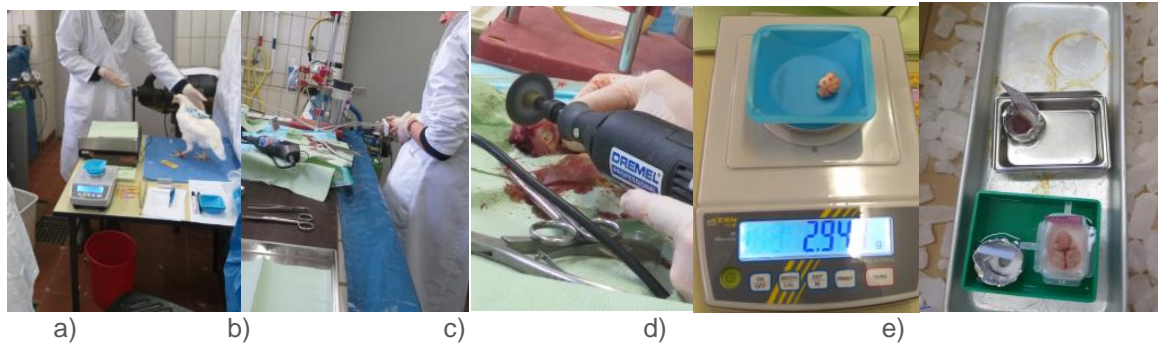


Figure 4: The procedure of removing the brain and eye out of a chicken and place it in a formalin and nitrogen cooled Tissue Tek.

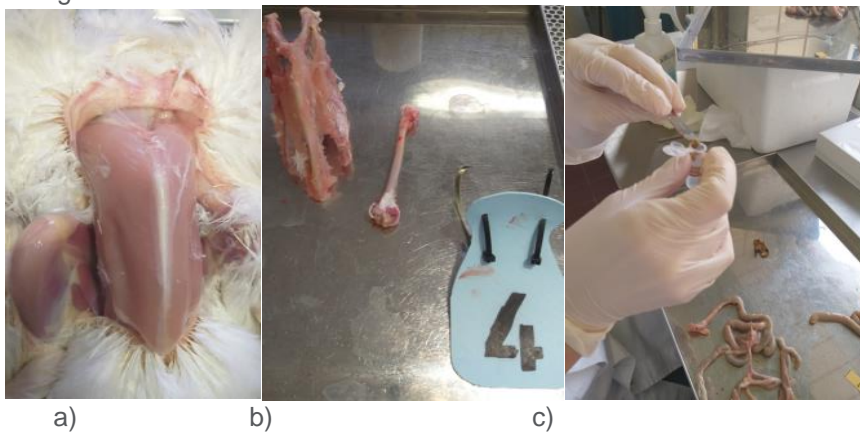


Figure 5: The body of a dead bird (a), removal of tibia and keel bone (b) and sampling of gut material in the appendix part of the intestine (c).



Figure 6: The investigation of histology of tibia bone and the equipment used (tibia moved from formalin, cut in half, small sample placed in histological boxes (upper photos, respectively), then the samples were decalcified, drained and embedded paraffin. After all they were cut with a microtome and stained. In tibiae the relation between cortices and medullary bone area is built and compared between the groups with the software for morphometric measurements (bottom right).

DESCRIPTION OF THE MAIN RESULTS OBTAINED

During my stay, our next goal was to look at the blood samples collected in Slovenia during Dr. Petow stay there and investigate immune responses of laying hens pending on their KBD. The number of lymphocytes, big lymphocytes, pseudo and eosinophyle granulocytes and trombocytes was counted and later the H/L ratio was calculated based on the number of eosinophyle granulocytes and lymphocytes. Our results revealed no correlation ($P=0.093$) with the KBD or fear responses in the same birds. Therefore our initial motivation to write a joined scientific paper has been changed.

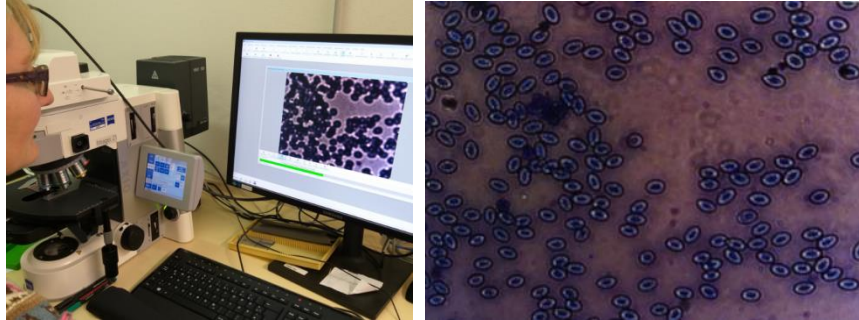


Figure 7: Counting the number of different blood cells of laying hens to investigate H/L ratio.

FUTURE COLLABORATIONS (if applicable)

We plan to find a second change in collaborating in 2019 when in Slovenia a second keel bone damage study will be starting and again took at the immune responses of around 200 birds. The focus will also be in relation to vit D use in poultry housing.