SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

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

Action number: CA15224
STSM title: Technology transfer of measurements for the detection of genetic factors influencing bone quality (including keel bone) (n°9)
STSM start and end date: 17/07/2017 to 28/07/2017
Grantee name: Beryl Eusemann

PURPOSE OF THE STSM:

The aim of this STSM was to learn methods of bone quality assessment. Ian Dunn's working group has a lot of expertise in this field because they have been working on bone quality in laying hens for a lot of time. I was especially interested in learning about bone histology and bone strength measurement. Moreover we wanted to compare our methods of bone radiographic density assessment. In our working group we have measured radiographic density in live birds while at the Roslin Institute radiographic density is measured in isolated bones. The aim was to have a look on the comparability of both methods.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSM

Radiographic density measurements

During my first week at the Roslin Institute we were focusing on bone radiographic density measurements. Pete Wilson and I took X-rays of tibiotarsi, humeri and keel bones of different laying hens. 10 to 14 bones were put together on a digital scanner and X-rayed using a cabinet X-ray system. Together with the bones there were labelling elements on the scanner. These included the letter of the plate and the number of the bone on the plate. Moreover, there was an aluminium step-wedge for calibration purposes. It consists of 17 steps which alter in height. The system was then switched on and set at 52 kV. It took about 5 minutes to scan the plate.
After having scanned the bones, the radiographic density of each bone was measured using the image processing programme ImageJ. For each image a density calibration was required. Therefore, the mean grey value of each step of the aluminium step-wedge was measured. Afterwards, the height of each step was linked with the correspondent mean grey value and a calibration function was generated. With this function it was then possible to determine the radiographic density of all bones in this image (i.e. all bones scanned on the same plate) with the unit "mm Al equivalent".

Bone breaking strength

Pete Wilson also showed me how to measure bone breaking strength. Different long bones (the humeri and tibiotarsi which we had already X-rayed) were laid down on a machine and a defined load was put on the bone until the bone broke. The power which was necessary to break the bone was determined and recorded by a computer programme called NEXYGEN Batch Document.

Histology

My second week at the Roslin Institute was dedicated to histology. On Monday Heather McCormack, Maisarah Maidin and I cut bone samples which were already embedded in paraffin. The bones had been collected from different laying hens of projects of Ian Dunn's working group. These hens differed in age which allowed us to cut bones from different stages, i.e. before and after the onset of lay and thus with different amount of medullary bone. On Thursday my supervisor from the FLI, Stefanie Petow, visited me at the Roslin Institute and brought embedded bone samples of our working group so that we could cut them there. These were femora and keel bones of different laying hens. After the cutting, the slides with the sections were dried in the oven at 55°C overnight and stained the other day. For staining we used two different protocols: H&E and Toluidine Blue staining. We took the mounted sections with us to the Bio-Imaging facility. Here, Bob Fleming helped us to have a look on them and to take photos with optical as well as fluorescence microscopes. Afterwards we used ImageJ for different analyses such as measurement of the amount of medullary and structural bone.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

Radiographic density measurements

We compared the method to measure radiographic density of the Roslin Institute with our method. We found one difference concerning the calibration: we used different calibration functions. Therefore we compared both functions in several images but we did not find any significant difference between the values obtained with one or the other function. Nevertheless, we decided that in our institute in Celle we are now going to use the same calibration function as used at the Roslin Institute so that our results are a 100% comparable.

Histology

We were very successful in cutting the embedded samples of our working group and the sections obtained were as good as the sections obtained by the samples of the Roslin Institute. That means that our decalcification and embedding method is good. However, in our working group we had never been as successful as in Edinburgh to cut the samples. It is thus important for us to adopt the cutting method which I've learned at the Roslin Institute.

FUTURE COLLABORATIONS (if applicable)

Radiographic density in live hens

Ian Dunn's working group is interested in measuring bone radiographic density in live laying hens as well. It is therefore interesting to continue our collaboration in this field as we have already experience with X-rays and radiographic density measurement in live hens. The idea is to figure out whether it is possible to get radiographic density of hens on a large scale, e.g. in laying hen farms. We have discussed about the possibility that Maisarah Maidin, who is going to start her PhD thesis on bone stability in laying hens at

the Roslin Institute this autumn, comes to our working group in Celle to get trained on X-raying live laying hens.

Histology

Heather McCormack and Bob Fleming kindly offered us to ask them about any problems we would face trying to establish their histological methods in our lab.