SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

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

Action number: CA15134
STSM title: Potential sources of chronic stress: does foot pad dermatitis induce chronic negative affective states?
STSM start and end date: 29/09/2019 to 04/10/2019
Grantee name: Matthew Craven

PURPOSE OF THE STSM:

(max.200 words)

I have recently registered as a new PhD Student at Newcastle University, where I will be investigating the housing and health conditions that contribute to chronic stress in laying hens. It is known that chronic stress is a cause of damaging behaviours in group-housed hens (Rodenburg et al, 2013), and in order to reduce the prevalence of these damaging behaviours, it is essential that we understand the causes of chronic stress.

Foot pad dermatitis (FPD) is a source of pain and discomfort which is commonly experienced by commercially housed hens (Hothersall et al, 2016). The main aim of this STSM was to investigate the severity of FPD as a chronic stressor in laying hens. In order to accomplish this, the aim was to collect tissue samples to allow quantification of adult hippocampal neurogenesis, a biological marker of chronic stress.

In order to collect these samples, I travelled to ILVO in Belgium, where Professor Frank Tuyttens's research group have a well-studied flock of hens whose FPD statuses vary from absent to severe. While at ILVO I also aimed to gain experience of research early in my PhD and build a professional network with a European partner.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

(max.500 words)

Hens from an existing study at ILVO were scored for FPD severity on a scale of 0-2 on three occasions (May, July, and September 2019), where 0 = no FPD, 1 = necrosis only, and 2 = swelling that can be seen from above the foot. Only birds which had a score of 0 on all three sampling occasions were selected as control birds (n=9). The birds with the most severe FPD were those which had a score of 2 on all three occasions, or a score of 1 in May and 2 in July and September (n=12). At the end of the study at ILVO, when the birds were 70 weeks of age, the selected individuals were culled over two days (9 on Monday, 12 on Tuesday) using an overdose of sodium pentobarbital by intravenous injection. Birds were culled one at a time, and immediately after death the brain was collected and dissected.

The total mass of each brain was recorded, then one hemisphere of each brain was fixed in 4%

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paraformaldehyde for 48 hours, then transferred to 30% sucrose solution in 0.1M PBS and stored at 4°C. The fixed hemispheres were shipped to Newcastle University, where they are being processed for immunohistochemistry. Over the next 6 months, adult hippocampal neurogenesis will be quantified in order to assess whether FPD is a chronic stressor in commercially housed hens.

The hippocampus was removed from the other hemisphere. The hippocampus was divided equally into rostral and caudal sections, which were stored in vials of RNAlater for analysis using molecular biology. The remainder of the brain was dissected into 3 regions (cerebral hemisphere, optic tectum, and cerebellum) and the mass of each of these regions was recorded.

The keel bones of the birds were assessed for damage, and photographs of the feet were taken so that further analysis of FPD severity can be carried out at a later date if necessary. Two researchers from the ILVO (Elske De Haas and Dimitri Van Grembergen) dissected the gut of each bird, and the length of the duodenum, jejunum and ileum were recorded. One caecum from each bird was collected and placed on dry ice, then stored at -80°C at the end of the day. These caecae were also transported to Newcastle for storage. In a future study, these samples may be used to analyse the relationship between stress caused by FPD and the microbiome of the gut.

In addition to collecting tissue samples to analyse so early in my PhD, the opportunities for knowledge sharing and networking during my STSM were invaluable. I shared my knowledge of dissecting the brain and using AHN as a marker of chronic stress with researchers from Professor Tuyttens's research group, and I benefitted from practical training in FPD severity scoring and behavioural welfare assessment of laying hens. I also learned about the experiment from which the birds had been taken. Knowledge of the conditions under which my study subjects were reared will help me when analyzing the data collected during the STSM.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

Measurements of the mass of brain regions that I recorded during the STSM were shared with ILVO to add to their measurements that were taken during the postmortems. Between the control birds and those with FPD, there was no significant difference in body mass (P=0.072), total brain mass (P=0.426), or the mass of the cerebellum (P=0.479), the optic tectum (P=0.744), or the cerebral hemisphere (P=0.471).

Brain and caecum samples were transported back to Newcastle University, where analysis of these samples is ongoing.

FUTURE COLLABORATIONS (if applicable)

It is hoped that the results obtained from this STSM, and the networks built, will lead to future collaborations between Newcastle University and ILVO. There will be ongoing discussion about the use of AHN as a marker of welfare states in laying hens, and it is hoped that in due course our collaboration will result in a published scientific paper.