SHORT TERM SCIENTIFIC MISSION (STSM) – SCIENTIFIC REPORT

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

Action number: CA15134
STSM title: Investigating how feather peckers choose their victims: can they detect asymmetry?
STSM start and end date: 15/02/2018 to 16/03/2018
Grantee name: Fernanda Machado Tahamtani

PURPOSE OF THE STSM/

Feather pecking has been studied from several aspects e.g. aggression, litter supply, light intensity, environmental enrichment, etc. (Bilcik and Keeling, 1999; Kjaer and Vestergaard, 1999; Sedlackova et al., 2004; McAdie et al., 2005; Tahamtani et al., 2016). However, no research has been conducted on whether chickens use fluctuating asymmetry to select their victims of feather pecking. Indeed, research investigating the visual cues that trigger feather pecking is very limited.

Deviation from perfect symmetry in bilateral traits can be a result of developmental instability, which is influenced by both genetics and environmental stress (Tuyttens, 2003). Fluctuating asymmetry can be defined as the randomly directed deviations (difference between left and right side) from perfect symmetry in bilateral traits what would have been expected if a perfect control of the morphological development had occurred (Tuyttens, 2003). One study suggests that feather pecking can be triggered by visual cues, and that pecks are significantly more often directed towards feathers that are trimmed by the researchers, compared to intact feathers, that feather pecking and cannibalism spread through the flock after this manipulation (McAdie and Keeling, 2000). However, no work has tested whether chickens can actually detect asymmetry, and to what degree. Studies with other bird models suggest that, when discriminating asymmetry of simple patterns like the length of two bars, European starlings (*Sturnus vulgaris*) could detect asymmetry as low as 1.8%, and pigeons (*Columba livia*) as low as 2% (Schwabl and Delius, 1984; Swaddle, 1999). The main questions are "Can chickens visually detect asymmetry?" and, if so, "how low levels of asymmetry can they detect?"

The main aim of the STSM was to test the possibility of using the equipment and technical expertise of the host institution, namely the software "The Biopsychology Toolbox" and custom made Skinner boxes (Rose et al., 2008; Horvath et al., 2016), to answer these questions.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

Animals and Housing

Twenty-four Dominant black (Dominant žíhaný) laying pullets were obtained at 5 weeks of age, before the arrival of the grantee, and housed in the experimental facilities of the Slovak Academy of Sciences. The pullets were housed in three custom pens (1m x 1.5m x 2m WxDxH). Two pens housed 10 pullets each, the third housed four pullets. Each pen provided *ad libitum* feed and water, litter in the form of wood shavings, two perches (at 40cm and 75 cm height) and two nest boxes. The pullets were fed commercial pelleted feed for the growing phase. The temperature of the housing room was kept between 18° and 22° Celsius. The relative humidity was maintained between 40RH and 80RH.

Skinner Boxes

Two custom-built Skinner boxes were used in this STSM (Fig. 1). Each box consisted of an outer sound attenuation chamber in dense fibreboard (Campden Instruments, UK, internal dimensions 52cm x 47cm x 41cm WxDxH) with an interior protractible drawer. On the drawer was placed the Skinner box aluminium frame (45cm x 50cm x 40cm WxHxD). The front side of the frame has a cut-out that fits a computer touchscreen (Elo 1529L Touchmonitor, Elo TouchSystem, USA). Immediately bellow the touch screen, on the outside of the frame, sits a custom built automatic dispenser of mealworms. The mealworm dispenser consists of three circular panes of plexiglass (12cm in diameter) forming 40 small compartments inside each fits one mealworm. The bottom pane of plexiglass has one hole, the same shape as each of the compartments, and is positioned directly on top of a feed trough situated in the skinner box frame. When a hen pecks at the touch screen, the dispenser turns, bringing one mealworm to this hole, falling into the feed trough. The Skinner box is operated with “The Biopsychology Toolbox” software via MATLAB (MathWorks). For each trial, the pullets were individually carried from the adjacent housing room to the test room and placed inside of the Skinner box. The box drawer was retracted and the outer door closed. The pullets remained inside the box until the end of each trial, at which point they were returned to their home pens.

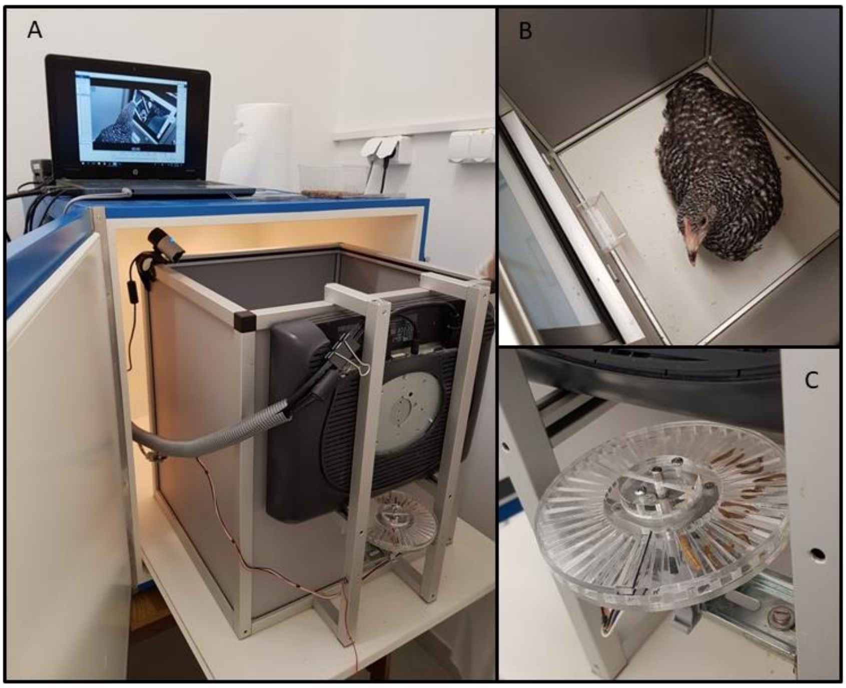

Figure 1: The Skinner box consisted on an outer sound attenuation chamber with a protractible drawer on which sits an aluminium frame equipped with a touch screen and a custom built automatic dispenser of mealworms (panel A). Panel B shows the inside of the frame, containing the touchscreen, a feed trough directly below it, and one of the test pullets. Panel C shows a close up of the mealworm dispenser, with some mealworms loaded inside of it. Photo credit: Fernanda Tahamtani.

Training phases

I. Habituation and AutoShaping

At 9 weeks of age, the pullets were initiated in the habituation and AutoShaping phase of the training. The pullets were placed inside the Skinner box and an animation of a mealworm against black background was displayed on the touchscreen for 5 secs, at 10-second intervals. After the 5 seconds, the mealworm image would disappear and a real mealworm would be delivered in the feed trough. If the pullets pecked at the mealworm on the screen, the real mealworm would be delivered right away. This procedure was repeated in two trials, each trial containing 30 presentations of the mealworm animation. The aim of this step is to habituate the hens to the box and to focus their attention to the screen and teach them to peck at it. As the normal reaction of chickens to the sight of mealworms is to peck at them, this part of the training is called AutoShaping.

II. Fixed interval

In this phase, mealworms are only delivered to the pullets if they peck at the image on the touchscreen. For the first two trials of this phase, the animation of a mealworm was still used. After for the following 10 trials, the image shown on the touch screen was that of a white bar (5mm x 13mm WxH) against a black background. The image was presented for a maximum of 5 seconds, at 10 seconds intervals. Each trial consisted of 30 presentations of the stimulus image. The inclusion criteria for the next phase was that pullets must peck at a minimum of 80% of the presentations.

III. Discrimination training

Eighteen pullets met the criteria set for the fixed interval phase and where therefore, advanced to the discrimination phase of the study. In this phase, the pullets were presented with two white bars on the touchscreen at the same time (Fig. 2). One bar was 10mm tall; the other was 16mm tall. Both bars were 5mm wide. Half of the pullets were randomly assigned the short bar as the positive stimulus, the other half the tall bar. The bars where aligned on the bottom. A reward in the form of a mealworm would be delivered if the pullets pecked at the positive stimulus bar. A punishment in the form of white noise (5 sec, 75dB), was played if the hens pecked at the negative stimulus bar. The position of each stimulus (right vs left side) was randomised. It was expected that the pullets would learn to discriminate the short from the tall bar and only peck at positive stimulus. For the pullets to advance to the testing phase of the study, they were expected to peck at the positive stimulus at a minimum of 80% of the presentations of each trial, and to do this for 3-4 consecutive trials.

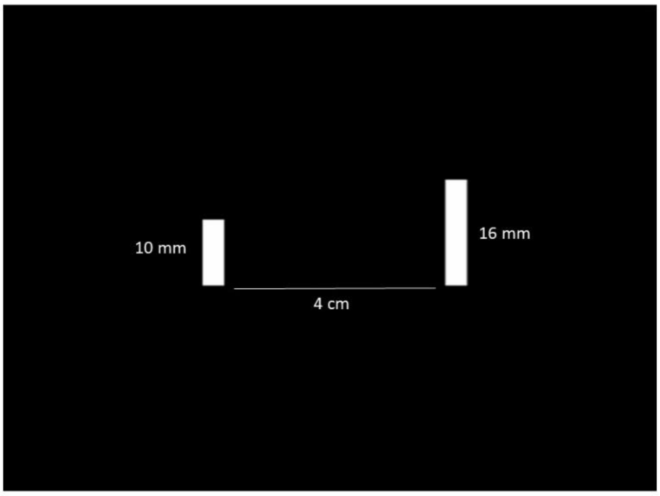

Figure 2: Diagram of initial discrimination training stimuli. Both bars were 5mm wide. Bar design based on Schwabl and Delius (1984).

IV. Testing

After meeting the criteria of the discrimination-training phase, the pullets were advanced to the testing phase. In this phase, the difference between the two bars was to decrease slowly, in each trial, until the pullets could no longer distinguish between the two.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

The discrimination training trials started on the 20th of February. Each trial lasted a maximum of 7.5 minutes. Two trials were carried out per day (morning and afternoon). Unfortunately, no pullet met the inclusion criteria to be advanced to the testing phase. Figure 3 shows the progression of the average performance of the pullets in each trial. In the first few trials, the response of the pullets was equally split between pecking at the positive stimulus (S+), pecking at the negative stimulus (S-), or not pecking at either (O). By the fifth trial, the rate of omissions decreased, and the pullets started almost always selecting one of the two stimuli to peck. However, despite 18 trials, their performance never achieved the levels of the inclusion criteria. Indeed, the average performance fluctuated around 50%. Of course, some pullets performed better than others did. One pullet in particular achieved a score of 80% on trial 14. However, her performance on the following trials decreased and did not reach the criteria level again. Furthermore, the average rate of omissions started to rise again around trial number 16. These results indicated that either the pullets could not distinguish between the two bars, or they did not yet understand the rules of the trials. Therefore, a new set of stimuli was used to test whether the pullets can understand the task and perform it well. The new stimuli chosen were 2 bars, both 5mm wide and 16 mm tall, but one white and the other 80% grey. These stimuli were chosen due to previous experience of the Slovak group on using discrimination of these colours to train and the test hens in a judgment bias test (Pichova and Kostal, 2016). When presented with the white and grey bars, the hens performed exceedingly well. After only two trials, the average S+ performance was 74.8%, with the individual score of nine pullets ≥ 80%. These results suggest that the pullets can understand and perform the task in the parameters stipulated, but have difficulty with the task when the bars differ in size.

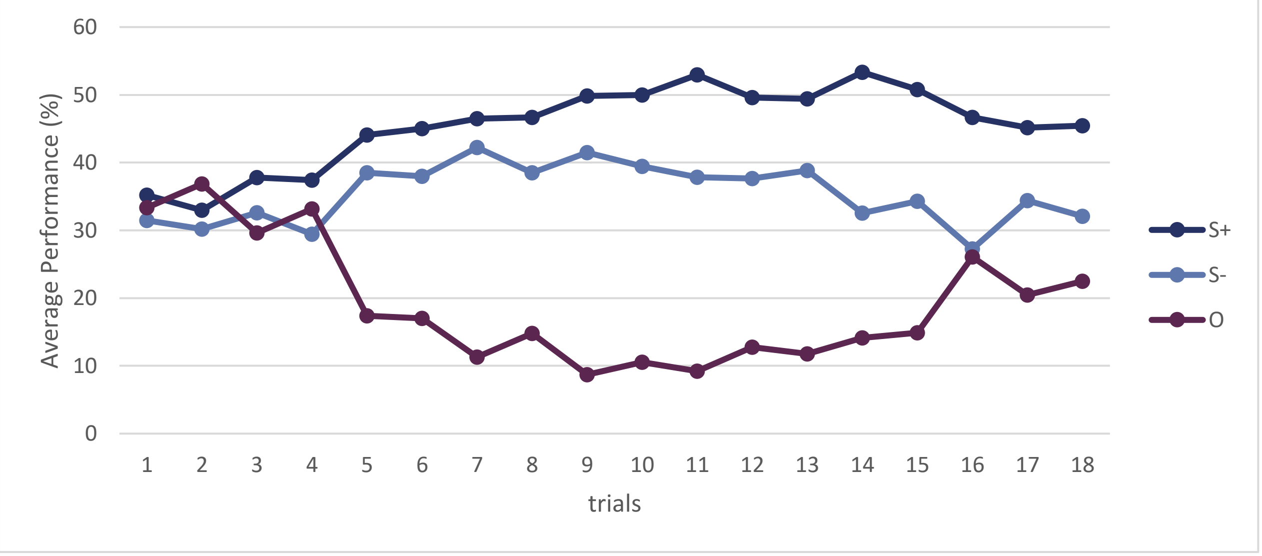

Figure 3: Average performance in the discrimination training trials (N=18). The stimuli were white bars on black background, 5mm wide, one at 10mm height, the other at 16mm height. The S+ curve represents the pecks at the positive stimulus, the S- at the negative stimulus. The O curve represents the omissions, or presentations during which the pullets did not peck at either stimulus.

Following the colour discrimination trials, a new set of stimuli, based on Swaddle (1999); Swaddle et al. (2004) was tried (Fig. 4). These new stimuli consisted of a pair of bars. In one stimulus, the bars were of the same length (16mm). In the other, one bar was shorter (10mm vs 16mm). At this point in the mission, two pullets had to be excluded from further training, one due to an injury to the beak, the other due to the high rate of omissions during the trials. The progression of the average performance using the double bar stimuli is shown in Figure 5. With the double bars, the performance of the pullets decreased considerably. In four trials, the average percentage of correct pecks decreased to 29.4%, while the average rate of omissions increased to 44.2%.

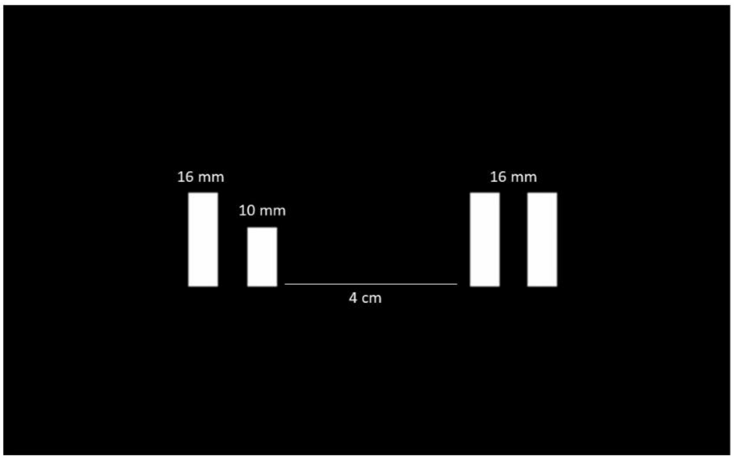

Figure 4: Diagram of double bar discrimination. All bars were 5mm wide. Design adopted from Swaddle (1999); Swaddle et al. (2004).

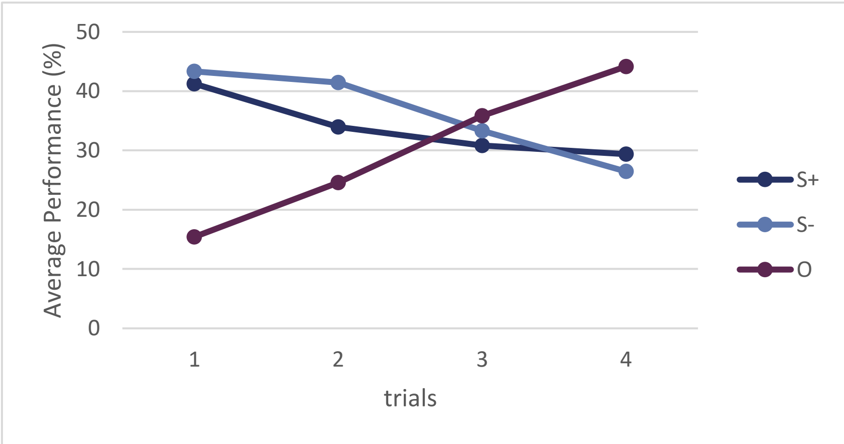

Figure 5: Average performance in the discrimination trials using the double bar stimuli (N=16).

Following the double bars trials, several other ideas for small changes to the training stimuli were brainstormed by the group. Due to only a few days remaining in the STSM, it was decided that the 16 pullets still used in the training would be divided into four groups of four, in an effort to try as many alternatives as possible. Figure 6 describes the parameters of the stimuli in each group. Due to the small sample size of each group (n=4), the results are presented in descriptive statistics only. Figure 7 presents the progression of the average performance during training for the four groups.

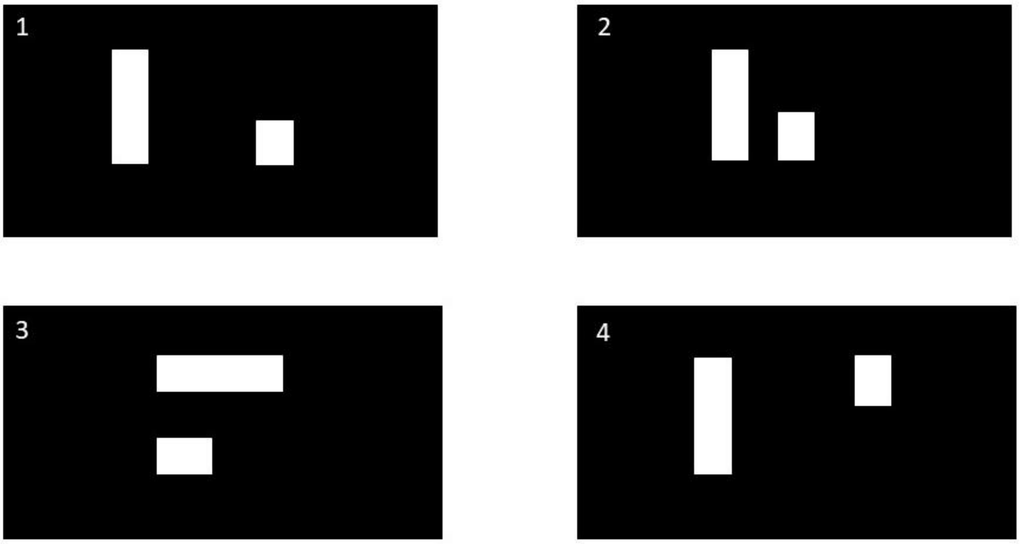

Figure 6: Representative diagrams of the slight modifications of the original stimuli. The length difference between the bars in each group was increased relative to the original set of stimuli. The bars are now 10mm and 20mm long. Group 1: increased length difference. Group 2: increased length difference, reduced distance between bars (2cm). Group 3: increased length difference, 4cm apart, horizontal orientation of the bars. Group 4: increased length difference, 4cm apart, top aligned.

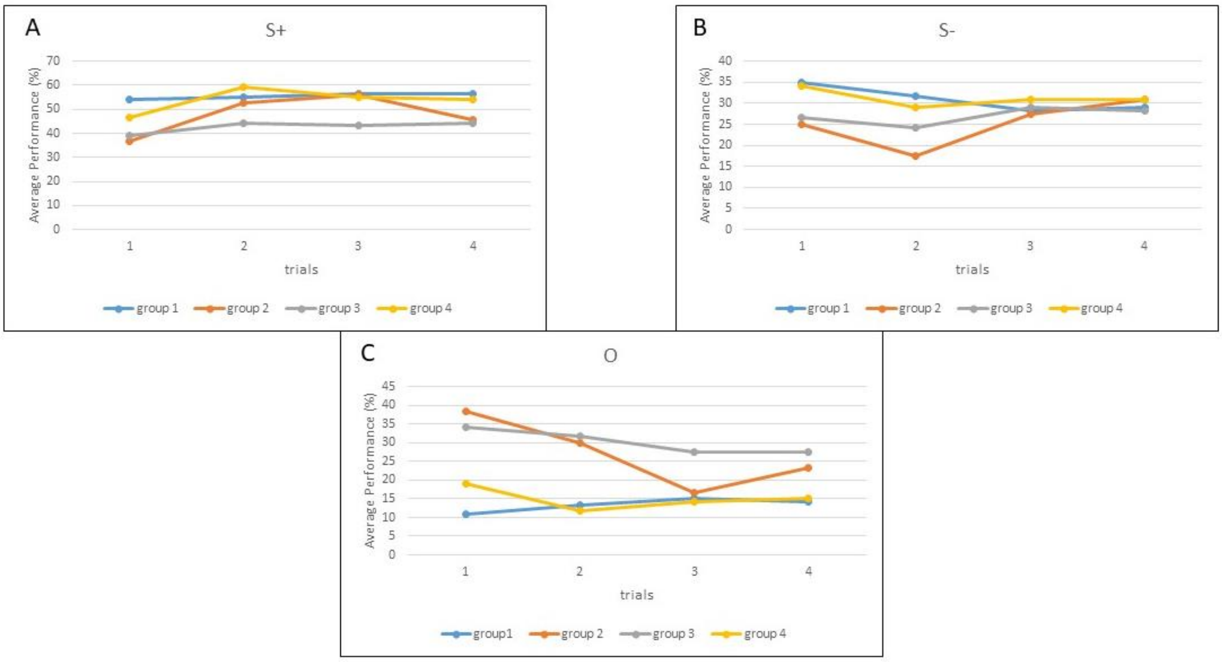

Figure 7: Average performance in the discrimination trials of the four training groups (n=4). Panel A presents the percentage of pecks to the positive stimulus. Panel B presents the percentage of pecks to the negative stimulus. Panel C presents this percentage of omissions (i.e. no peck at either stimuli).

The changes applied to the stimuli in groups 1-4 did not appear to greatly improve the performance of the pullets in the discrimination task. One pullet, 2RG from group 1, got scores of 86.6% in trial 2 and trial 3. However, on trial 4, her score decreased to 73.3%. Therefore, along the duration of the mission, no pullet achieved the minimum criteria for inclusion in the testing phase.

The main aim of this STSM was to test whether it is possible to use the equipment and software of the host institution to answer the questions “can chickens visually detect asymmetry?” and “how low levels of asymmetry can they detect?”. Several previous studies have successfully investigated symmetry discrimination in chickens, often using stimuli arguably more complex than the stimuli used in the present pilot study (Jansson et al., 2002; Forsman and Herrstrom, 2004; Clara et al., 2007; Mascalzoni et al., 2012). Therefore, despite the poor performance of the pullets in the present pilot study, it is likely that it is possible to train chickens with the use of the Skinner box and associated software on this discrimination task. The results from the pilot provide information on how to conduct the training and how long the training phase can be expected to last, as well as stimuli design. All these findings and practical experience gained will be valuable both for preparing a grant proposal to conduct the study in full scale and to perform the study successfully.

FUTURE COLLABORATIONS

The results from this pilot study conducted for this STSM suggest that it is possible to use the equipment and software developed by Dr Kostal’s group to train chickens in operant conditioning and test the limits to their visual detection of simple length asymmetry. The pilot has provided important information on stimulus design, the realistic time frame for training, and specific practical techniques in training. The future plans for this line of study consists of continued collaboration with the host institution in developing a grant proposal to conduct this study in full scale (i.e. larger sample size, longer study period, different layer hybrids, etc.). Furthermore, the grantee aspires to install a set of Skinner boxes in the home institution of Aarhus University, expanding the opportunity for future collaborations and for dissemination of the techniques.

In addition, the grantee plans to submit the present pilot study, with the collaboration of colleagues from the Slovak Academy of Sciences, as an oral or poster presentation to the XVIth European Poultry Conference, in 2019.

OUTPUTS PRODUCED

As mentioned above, this pilot study yielded much knowledge on the practical aspects of the study design, which will assist the development of a grant proposal for the full-scale study. In addition, during the STSM, the grantee greatly availed of opportunities for learning new skills and for networking. The grantee presented her work both to the research group at the Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences. Furthermore, the grantee gave an invited guest lecture on her STSM work to the Division of Livestock Sciences of the University of Natural resources and Life Sciences, Vienna, Austria.

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