

## SHORT TERM SCIENTIFIC MISSION (STSM) – SCIENTIFIC REPORT

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

**Action number: CA15134**

**STSM title: Damaging behaviour in poultry – “Using TrackLab to measure the activity in two selection lines of laying hens (high and low feather pecking) and the role of the microbiome.”**

**STSM start and end date: 09/07/2018 to 18/07/2018**

**Grantee name: Jenny Stracke**

### PURPOSE OF THE STSM

Feather pecking (FP) can be considered to be one major behavioural problem in the egg production industry. This is reflected by a large number of studies published on this topic (for a review see Jung and Knierim, 2018 and Rodenburg et al., 2013). However, up to now, underlying mechanisms are not fully understood. One most popular hypothesis would be that FP might represent misdirected foraging behaviour (Cronin et al., 2018) with deficiencies in the husbandry systems to be major causes for this behavioural disorder. However, a second hypothesis discusses the role of stress triggering FP (Rodenburg et al., 2013). Various studies hint to stress being highly related to FP, among others confirmed by studies using an animal model with two strains of laying hens selected for and against feather pecking (HP=high feather pecking line; LP=low feather pecking line) (Kjaer et al., 2001). There is strong evidence that both lines differ in their behavioural characteristics especially in relation to stress and fearfulness (see Rodenburg et al., 2008 and van der Eijk et al., 2018 for an overview).

When talking about stress, there is growing evidence highlighting the importance of the gut-brain axis, with the microbiota (e.g. the composition of microbes like bacteria, archaea, yeasts, helminth parasites, viruses, and protozoa) being a key player in various processes (Rea et al., 2016). Especially in the regulation of stress-related (behavioural) responses, the microbiome was found to play a superior role (Foster et al., 2017) and in consequence might be an interesting factor when studying underlying mechanisms of FP. Indeed there are hints of differences in the microbiome of laying hens providing high and low feather pecking (Meyer et al., 2013), however, how the microbiome can alter behaviour is not clarified yet.

The STSM was implemented within the PHD project of Jerine van der Eijk, here analysing the role of the microbiome of laying hens in terms of FP and other behavioural characteristics like fear behaviour, tested in different behavioural tests. Purpose of my research during the STSM was to analyse the role of the microbiome according to the activity/locomotion behaviour of the birds, using above mentioned selection lines. As it is known that both lines differ in their locomotion and activity (Kjaer, 2009; Rodenburg et al., 2017), we hypothesized differences in the microbiome of both lines might be one key factor in regulating these contrasting behaviours.

For this purpose we used an ultra-wideband tracking system (TrackLab, Noldus, Wageningen, The Netherlands), recently established in the Behavioural Ecology Group of Bas Rodenburg. Here active tags

are attached to the animals, with calculating the location of each bird on an individual level, based on beacons receiving data on “Time of Arrival” and “Angle of Arrival”. The included software of the TrackLab system processes data (x, y, z coordinates) automatically, which results in information of different activity parameters like the distances and speed of movement of each animal.

## DESCRIPTION OF WORK CARRIED OUT DURING THE STSM

### *Animals*

We worked with 180 laying hens from two feather pecking selection lines (high (HP, n=90) and low (LP, n=90)). Microbial treatment was conducted previously to the STSM. Here, hatching of the chicks was monitored from embryonic day 19 on, with checking hatching every six hours. Directly after hatching each chick received a neck tag containing a number for individual identification, additionally each chick was weighed. Treatments were administered orally, consisting of three treatments: the control treatment (sterile saline solution; CON), the adult microbiota pool from the high feather pecking line (HL) and the adult microbiota pool from the low feather pecking line (LL). Treatments were balanced over chicks as equally as possible. Microbiota was administered from embryonic day 21 on (day 0 of age), with each chick receiving the treatment daily during the first two weeks post hatch (until day 14 of age).

### *Activity Testing*

Activity testing was conducted with birds being 17 weeks of age. Birds were divided in two batches while tests for batch 1 were run in June 2018 and for batch 2 in July 2018 respectively. Tests were run at the Carus research facility of the Animal Sciences Group of Wageningen University, The Netherlands. The test-room measured 7x6 meters and was equipped with four Ubisense beacons. Birds wore backpacks (providing an additional individual marking by numbers) which were attached to the back of the hen using elastic loops around the wing base, containing active sending tags of Ubisense with a 12V battery (3.5 \* 3.5 cm, ± 29 grams).

Birds were tested in their normal group, with the order of pens tested, being randomized. The backpacks were attached to the birds of each separate pen 15 minutes prior to testing, giving them time to get habituated. For testing, all hens of one pen were placed in the middle of the test-room, here again given them five minutes to get used to the new environment. Afterwards the individual hens were tracked using the TrackLab system for 15 minutes. Parallel to the automatic recording by TrackLab video recordings were implemented.

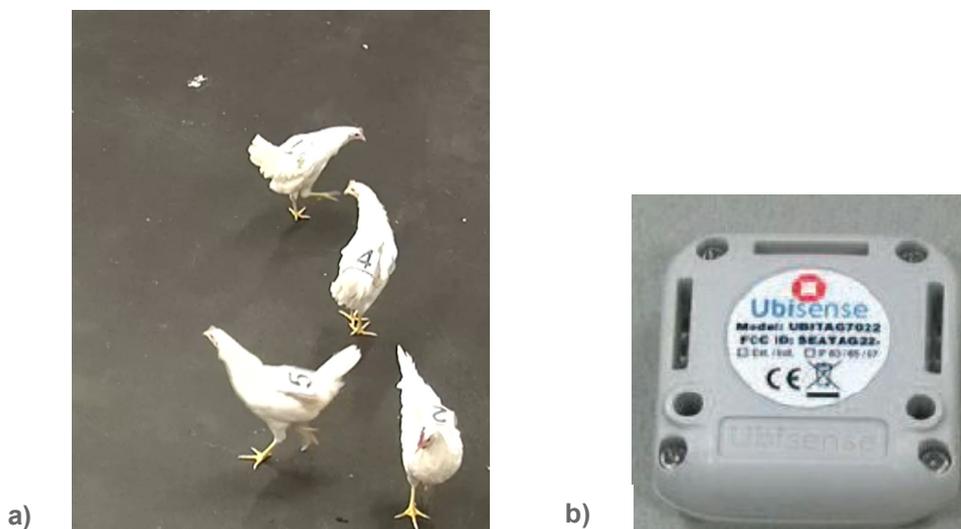


Figure1: a) Test-arena with group of hens equipped with backpacks b) Ubisense active sending tag

### *Coping with the backpack*

As video recordings revealed some of the birds were still struggling with the backpacks during the activity recordings, videos of the individual sessions were analyzed manually. Each bird was classified in one of four categories (1=normal walking; 2=impaired walking (birds try to move, movement appears disoriented, birds tumble over etc.) during the session recovery during the 15 minutes was recorded as well; 3=impaired walking which does not recover during the 15 minutes session; 4=no walking trials during the 15 minutes session (with hens lying the entire time of the activity recording period)

### *Analysis of the data*

Statistical analysis was done using SAS (SAS Version 9.4, SAS Institute Inc., Cary, NC, USA).

Analysis of the data for activity recording was performed for the parameters of distance (cumulative distance of two consecutive samples of the track), the average speed (division of two-sample distance by the time difference) and the moving distance (sum of distance between two consecutive samples while an object is moving, here correcting for “noise”). Analysis was provided on an individual (data/hen) level.

A generalized linear mixed model, using the GLIMMIX procedure was applied. Treatment (CON, HL, LL), selection line (HP, LP), their interaction and batch were considered as fixed factors, pen was considered as random effect. Multiple pairwise comparisons were done using the Tukey-Kramer test.

Data of the coping behaviour were analysed using the Genmod procedure. Treatment, line, their interaction as well as batch were considered as fixed factors, the repeated statement was specified for the pen. The DIST= option was set to multinomial, in compliance with using the link function cumlogit.

## **DESCRIPTION OF THE MAIN RESULTS OBTAINED**

Data were tested for normal distribution first. Results revealed no normal distribution. Therefore the DIST= option “poisson” and the link function “log” was specified in the GLIMMIX procedure.

Results for the parameter “distance” revealed a significant effect of the line ( $F=5.1$ ;  $p<0.05$ ) with the HP-birds showing a greater distance moved compared to LP-birds. Results also revealed a significant effect of the batch ( $F=9$ ;  $p<0.01$ ) with batch1 revealing a higher distance moved. The same could be found for the parameter of “moving distance” (line:  $F=4.2$ ;  $p<0.05$ ; batch:  $F=7.2$ ;  $p<0.01$ ). For the “average speed” no significant effect could be found (all  $F>0.02$ ; all  $p>0.05$ ) (Fig.2). No effect of the treatment or the interaction between treatment and line could be found (all  $p>0.05$ ).

Analysing the data for birds showing “normal walking” only, analysis for the “distance” revealed a significant effect for the treatment ( $F=3.2$ ;  $p<0.05$ ) (Fig.3), and an effect of the batch ( $F=7.3$ ;  $p<0.01$ ). Here no effect of the line nor the interaction between line and treatment could be found (all  $p>0.05$ ). For the parameter “moving distance” and “average speed” no significant effects could be found.

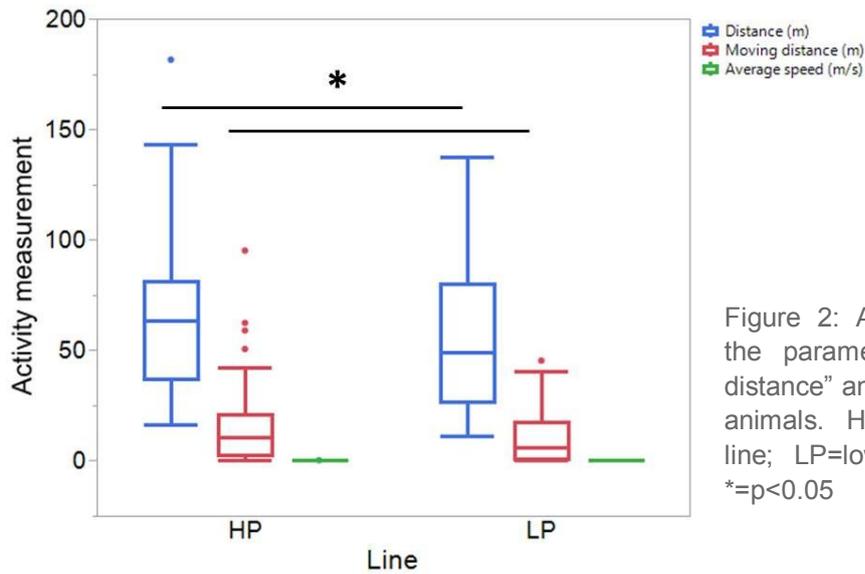


Figure 2: Activity measurement for the parameter “distance”, “moving distance” and “average speed” for all animals. HP=high feather pecking line; LP=low feather pecking line; \*=p<0.05

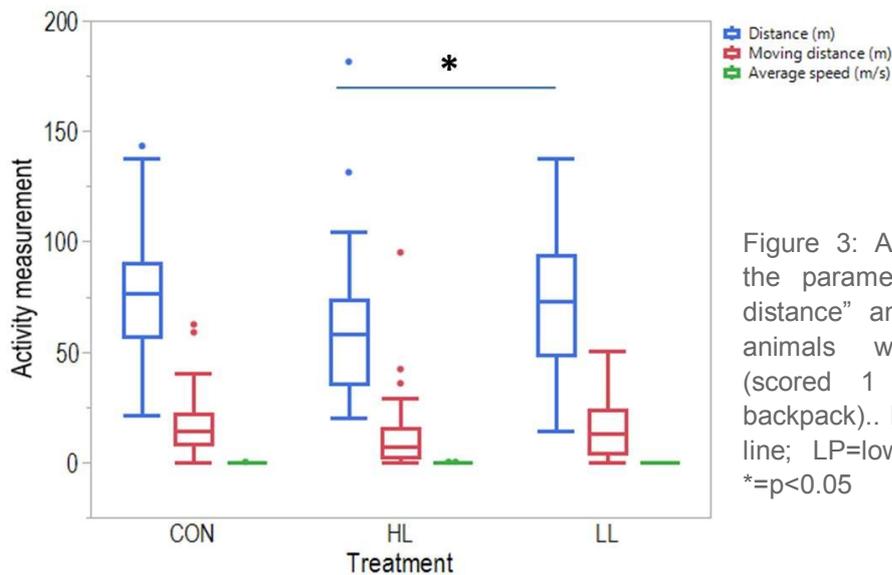


Figure 3: Activity measurement for the parameter “distance”, “moving distance” and “average speed” for animals with “normal walking” (scored 1 for coping with the backpack).. HP=high feather pecking line; LP=low feather pecking line; \*=p<0.05

Results for the coping behaviour revealed a significant effect of the line ( $p<0.05$ ), with LP-bird showing less “normal walking” while a higher number of animals were scored 3 and 4 according to the “coping”-categorization. The batch revealed a significant effect here as well ( $p<0.05$ ). No significant effect of the treatment ( $F=0.9$ ;  $p=0.63$ ) nor for the interaction between treatment and line ( $F=3.7$ ;  $p=0.16$ ) could be found.

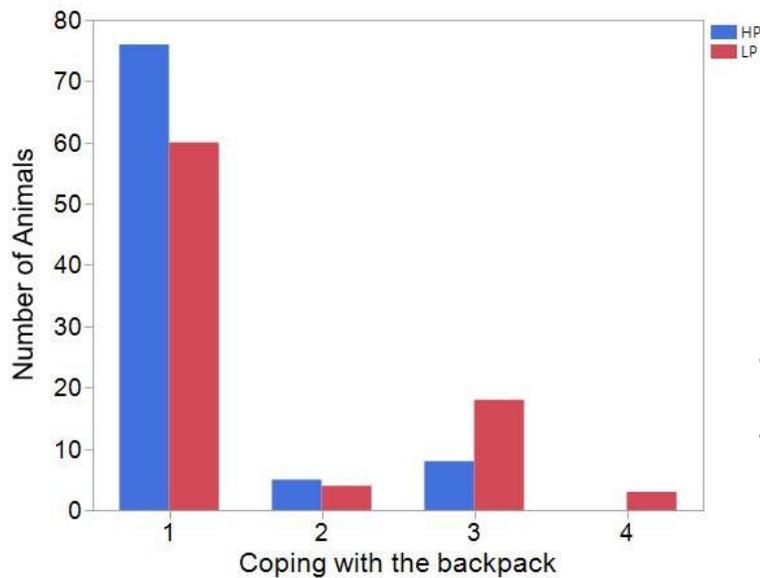


Figure 4: Coping with the backpack, number of animals (Score 1=normal walking; 2=impaired walking and recovering; 3= impaired walking; 4=no walking); HP=high feather pecking line; LP=low feather pecking line

### Conclusion

The presented results indicate a significant effect of the line (HP/LP) in both, activity - measured with the TrackLab system - as well as coping behaviour regarding the habituation to the backpack. However, when analysing the activity of the animals excluding animals which seemed to be struggling with the backpacks, the significant effect of the line could not be confirmed, here a significant effect of treatment for the distance moved could be found.

Differences in activity, where HP-birds were more active compared to LP-birds are known from literature. Assuming the coping with the backpack to be associated to fear, results here as well resemble results in literature. However, our data could not provide clear evidence for the microbiome being responsible to alter the activity in the two genetic selection lines. As the adaption to the backpacks was found to alter movement of the birds, results should be interpreted with caution - further studies where animals are habituated to the backpacks might give clearer results here.

### References

- Cronin, G.M., Hopcroft, R.L., Groves, P.J., Hall, E.J.S., Phalen, D.N., Hemsworth, P.H. (2018). Why did severe feather pecking and cannibalism outbreaks occur? An unintended case study while investigating the effects of forage and stress on pullets during rearing. *Poultry science* 97, 1484-1502.
- Foster, J.A., Rinaman, L., Cryan, J.F.. (2017). Stress & the gut-brain axis: Regulation by the microbiome. *Neurobiol Stress*, 19,124-136.
- Jung, L. and Knierim, U. (2018). Are practice recommendations for the prevention of feather pecking in laying hens in non-cage systems in line with the results of experimental and epidemiological studies? *Applied Animal Behaviour Science* 200, 1-12.
- Kjaer, J.B. (2009). Feather Pecking in Domestic Fowl is Genetically Related to Locomotor Activity Levels: Implications for a Hyperactivity Disorder Model of Feather Pecking. *Behav Genet*, 39, 564-70.
- Meyer, B., Zentek, J., Harlander-Matuschek, A. (2013). Differences in intestinal microbial metabolites in laying hens with high and low levels of repetitive feather-pecking behavior. *Physiol Behav*, 117,110-111.

Rea, K., Dinan, T.G., Cryan, J.F. (2016). The microbiome: A key regulator of stress and neuroinflammation. *Neurobiol Stress*, 4, 23-33.

Rodenburg, T.B., Van Krimpen, M.M., de Jong, I.C., de Haas (2013). The prevention and control of feather pecking in laying hens: identifying the underlying principles. *World's Poultry Science Journal*, 69, 361-374.

Rodenburg, T.B., Eijk, J.A.J. van der, Pichova, Katarina, Mil, B. van, Haas, E.N. de (2017). PhenoLab: automatic recording of location, activity and proximity in group-housed laying hens. *Proceedings of the ISAE Benelux conference 2017 International Society for Applied Ethology (ISAE)*, 21 - 21.

Rodenburg, T.B., Komen, H., Ellen, E.D., Uitdehaag, K.A., van Arendonk, J.A.M. (2008). Selection method and early-life history affect behavioural development, feather pecking and cannibalism in laying hens: A review. *Applied Animal Behaviour Science*, 110, 217-228.

van der Eijk, J.A.J., Lammers, A., Li, P., Kjaer, J.B., Rodenburg, T.B. (2018). Feather pecking genotype and phenotype affect behavioural responses of laying hens. *Applied Animal Behaviour Science* 205, 141-150.