SHORT TERM SCIENTIFIC MISSION (STSM) – SCIENTIFIC REPORT

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

Action number: CA15134 Synergy for preventing damaging behaviour in group housed pigs and laying hens (GroupHouseNet)
STSM title: Influence of naturally-inspired lighting schedule on domestic chick behaviour and welfare
STSM start and end date: 19/11/2017 to 17/12/2017
Grantee name: Zuzana Skalná

PURPOSE OF THE STSM/ AIMS AND OBJECTIVES OF STSM

(My short term scientific mission at the School of Clinical Veterinary Science, University of Bristol, funded by the COST Action CA15134 Synergy for preventing damaging behaviour in group housed pigs and laying hens (GroupHouseNet) was carried out from November 19th till December 17th 2017. During my stay I have under the supervision of Dr. Joanne Edgar participated in a short experiment, focused on effects of intermittent lighting schedule on laying hens behaviour and welfare.

Experiment 1 Effects of intermittent lighting schedule on chick's behaviour and welfare

Maternal care has a strong beneficial effect on behavioural development in domestic chickens (Edgar et al., 2016). However, allowing chicks to be brooded naturally is not commercially viable. Naturally brooded chicks learn from their mother a great deal, like what to peck, when to rest. Mother hen gives the possibility for inactive chicks to collect under her, gain her warmth in relative darkness, and separates them from active chicks (Nielsen et al., 2008). In commercial setting, there are billions of chicks worldwide receiving only artificial radiant heat from static dark brooders, and experiencing continuous light for up to 23 hours per day (De Oliveira and Lara, 2016). Intermittent lighting schedules have the potential to mimic natural brooding and synchronise chick's behaviour. Lack of behavioural synchrony in non-brooded chicks leads to active chicks disturbing and directing feather pecks towards resting conspecifics, representing potential risk factor for the development of feather pecking (Shimmura et al., 2015). Jensen et al. (2006) used dark brooders (which mimic the warmth and dark of a mother hen) in the rearing of laying hens, succeeding in reducing the incidence of feather pecking in later life.

The aim of the first experiment was to determine the influence of intermittent lighting schedule on:

- synchronisation of chick's behaviour,
- feed conversion ratio and weight gain of chicks,
- welfare of chicks (human approach test, novel object test, tonic immobility test).

Experiment 2 Socially-mediated arousal after presentation of positive emotional stimulus

Farmed animals are exposed to situations which may reduce their welfare and the social environment has the potential to either alleviate or magnify this effect. Indeed, for domestic chickens, both stress and unwanted behaviour (e.g. feather pecking) may spread quickly across large groups of individuals, through socially-mediated arousal and social facilitation. Conversely, conspecifics can provide an important source of social learning (e.g. locating food) and can alleviate stress (e.g. social buffering). Despite being inter-related, no studies have assessed associations between these social phenomena. We hypothesise that individuals with greater capacity for socially-mediated arousal will show greater ability to learn from conspecifics. This would form a basis for further studies to understand the spread of damaging behaviours in commercial flocks.

Domestic hens show socially-mediated arousal when watching their chicks receiving an aversive air puff (Edgar et al., 2011), phenomena that are not apparent when adult hens watch other familiar adults receive an air puff (Edgar et al., 2012). However, until now only empathy for negative emotions was tested in domestic chickens. We decided to test whether the reward anticipation, induced by the trace classical conditioning (Moe et al., 2009), that is hypothesized to reflect an appetitive type of positive affective state, can cause socially-mediated arousal.

The aims of the second experiment were:

- to test whether young laying hens show any evidence of socially-mediated arousal after the presentation of positive emotional stimulus (anticipation of food reward),
- to test whether socially mediated arousal differs in chicks reared under intermittent lighting schedule in comparison with those reared under continuous lighting schedule.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSM

Experiment 1

Eighty one day old Hy-Line Brown chicks from a commercial layer breeding hatchery were randomly assigned into groups of 5. Each group was kept in a circular pen with diameter approximately 130 cm, with floor covered by wood shavings, and containing feeder, drinker and a non-light emitting heat source maintaining 30°C in pens. The room temperature was maintained around 24°C. Lights with 200 Lux LEDs were used. There were 4 pens per room with 4 rooms used.

Two rooms with totally 40 chicks were under continuous lighting schedule (C) with chicks experiencing lights for 14 hours per day (7:00 AM – 9:00 PM) and darkness 10 hours per day (14L : 10D). The other two rooms containing 40 chicks were treated by intermittent lighting schedule (IT), which was predetermined from video observations of 27 broody hens and their chicks. The lighting schedule was adjusted as chicks were growing old (as shown in Table 1).

Table 1: Intermittent lighting schedule.

days of age	IT schedule	extra dark period per day (from 0:00 AM)
1 – 5	8 min L : 8 min D	2 hours
6 – 9	10.5 min L : 4 min D	4.5 hours
10 – 16	11 min L : 4 min D	4.5 hours

16 – 21	20 min L : 4 min D	7.1 hours
From day 21	30 min L : 4 min D	8.5 hours

Feed was weighted 5 times per week (to calculate feed conversion and efficiency) and chicks were weighted 3 times per week (to ensure adequate weight gain).

The behaviour of chicks was recorded with 1 camera per pen for 24hours daily. Behaviour was analysed on day 1 (practice), day 2, day 7 and day 14 from 16:30 till 21:00. Chicks were observed in around every 30 minutes at set time points, which were taken from half way through the light phase of the IT group. We were looking after inactive (sitting, standing) and active behaviours (drinking, feeding, ground scratching/ pecking, preening, dustbathing, walking, others), location of chicks one from another (clustered/ alone) and synchronisation in behaviour of the group.

On day 16 chicks underwent welfare measurements which consisted of 3 testing procedures. The first was the human approach test with a person standing in the corner of a pen for 5 minutes and with distance and behaviour analysed in 1 min intervals. The second was the novel object test with colourful toy placed in the centre of the pen for 10 minutes and with distance and behaviour analysed in 1 min intervals. The third was the tonic immobility (TI) test with 3 out of 5 chicks from every pen. Each chick was placed gently on its side, with one hand restraining its body and another restraining its head and released after 15 sec. The induction of TI was considered as successful if the chick remained lying down for 15 sec after releasing. There were 3 attempts if unsuccessful. The maximum time for TI latency was set at 5 minutes (300 sec).

Experiment 2

In Experiment 2 we were looking for evidence of a rudimentary empathy (socially-mediated arousal) in chicks. Arousal was induced by the anticipation of food reward.

Chicks reared under both lighting schedules (IT and C) were divided into 4 groups. For each lighting schedule, there was a group of chicks in which reward anticipation was induced by the trace classical conditioning method as described by Moe et al. (2009) (trained demonstrators, TD, n = 4) and trained demonstrators observers (TDO, n = 4) and a group of chicks without training (untrained demonstrators, UD, n = 4) with untrained demonstrators observers (UDO, n = 4).

Testing apparatus was 100 x 50 cm wooden structure divided into two chambers (one for demonstrators and one for observers) by a perspex screen. Testing cameras were attached to the corner of each chamber. For delivering food rewards (meal worms) we used plastic tube.

Trained demonstrators were trained to associate the unconditioned stimuli (US, green light) with conditioned stimuli (CS, meal worm) while UD were only habituated to the US (green light). Demonstrators were trained/ habituated for 5 consecutive days (days 19-23 of life). Twenty min session for TD consisted of 5 min habituation, 14 min of trace conditioning to associate the US with CS, with 8 presentations of US with an increasing time interval between the US and CS, and 1 min behavioural recording at the end of the session (as shown in Table 2). Session for UD consisted also of 5 min habituation with no stimuli presentation, 14 minutes of habituation to 8 US presented in increasing time intervals, and 1 min of behavioural recording (as shown in Table 2).

Table 2: Design of the 20 min session for trained demonstrators. Untrained demonstrators were exposed only to US with no CS presented.

5 min habituation	
14 min training	0 min after habituation – US-CS interval 3.5sec
	1 min after habituation – US-CS interval 3.5sec
	3 min after habituation – US-CS interval 5sec
	5 min after habituation – US-CS interval 6.5sec
	7 min after habituation – US-CS interval 8 sec
	9 min after habituation – US-CS interval 9.5 sec
	11.5 min after habituation – US-CS interval 11 sec
	14 min after habituation – US-CS interval 12.5 sec
1 min observation	

We have compared the effect of lighting schedule on the learning process of demonstrators, evaluated the presence of anticipatory behaviours in TD or no signs of fear response to the US in UD. Anticipatory behaviour was defined as standing still or walking with slow steps, moving towards the place where the meal worms were delivered, with legs, body and neck stretched upwards and eyes open, head moving up or tilting, and kept at an angle, sometimes but not always directed at the light source or feeding tube, and as pecking directed at the feeding tube.

All observers were habituated to the testing conditions as well. They were placed on 2 consecutive days (day 22-23 of life) for 10 min session into the testing apparatus. Each session consisted of 5 min habituation, 4 min with exposure to the 4 US with increasing time intervals between them and 1 min of behavioural recordings (as shown in Table 3).

Table 3: Design of the 10 min habituation session for observers.

5 min habituation	
4 min training	0min after habituation – US for 3.5 sec
	1min after habituation – US for 6.5 sec
	2.5min after habituation – US for 9.5 sec
	4min after habituation – US for 12.5 sec
1 min observation	

The behavioural response to US was analysed on both days to evaluate the habituation and extinction of fear responses. Fear response have been determined as vocalisations, freezing and locomotor activity after the US presentation.

After the training and adaptation to the testing apparatus, respectively, we tested demonstrators and observers for the indicators of socially-mediated arousal for 2 days (day 24-25 of life). Tests were 10 min long and consisted of 5 min habituation, 4 min testing with 2 US with duration 12.5 sec and 1 min of

behavioural observation (as shown in Table 4). Trained demonstrators were not rewarded with meal worm after US presentation and for preventing trained demonstrators from US-CS connection extinction, we have one more training session in first day after testing socially transferred arousal.

Table 4: Design of the 10 min test of socially-mediated arousal.

5min habituation	
4 min testing	1 min after habituation – US presented for 12.5 sec
	3 min after habituation – US presented for 12.5 sec
1 min observation	

We have recorded the behaviour indicating socially-mediated arousal in observing chicks (e.g. locomotion activity, vocalization, stress induced hyperthermia). Stress induced hyperthermia was measured using thermal camera FLIR SC300. Anticipatory behaviour in observers was defined as standing still or walking with slow steps, with head tilting while observing arousal in demonstrators and looking at them or the green light source or the feeding tube.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

Until now only data from the Experiment 1 have been analysed.

Behaviour and activity synchronisation was defined as percentage of observation during which have all five chicks either perform the same behaviour, or were active or inactive. Mean percentages of synchronisation in C and IT groups were tested for normality and compared using independent samples t-test. Behaviour was more synchronised in chicks with IT lighting condition in first two days (day 1 $p < 0.05$; day 2 $p < 0.001$), but this synchronisation disappears with time (as shown in Figure 1). It was very similar with activity with chicks from IT lighting schedule more active on day 1 ($p < 0.05$) and day 2 ($p < 0.001$) with no differences during day 7 and day 14 (as shown in Figure 2).

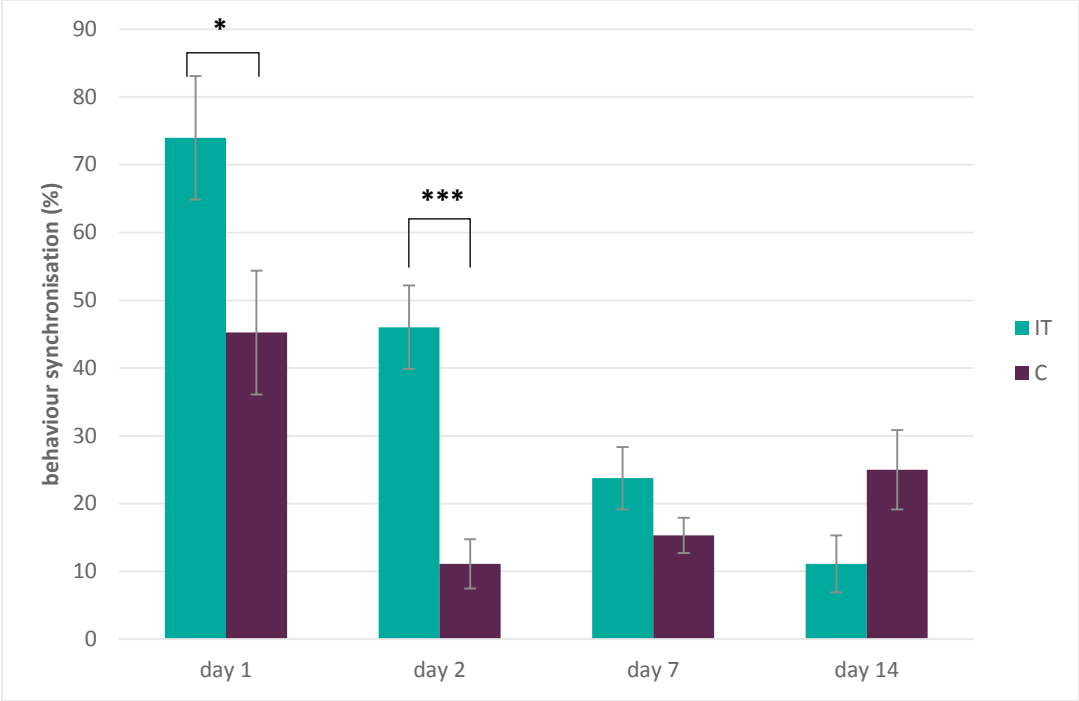

Figure 1: Behavioural synchronisation of chicks with intermittent lighting schedule (IT) and continuous lighting schedule (C). On day 1 and day 2 were IT chicks more behaviourally synchronised than C chicks (day 1 $p < 0.05$; day 2 $p < 0.001$).

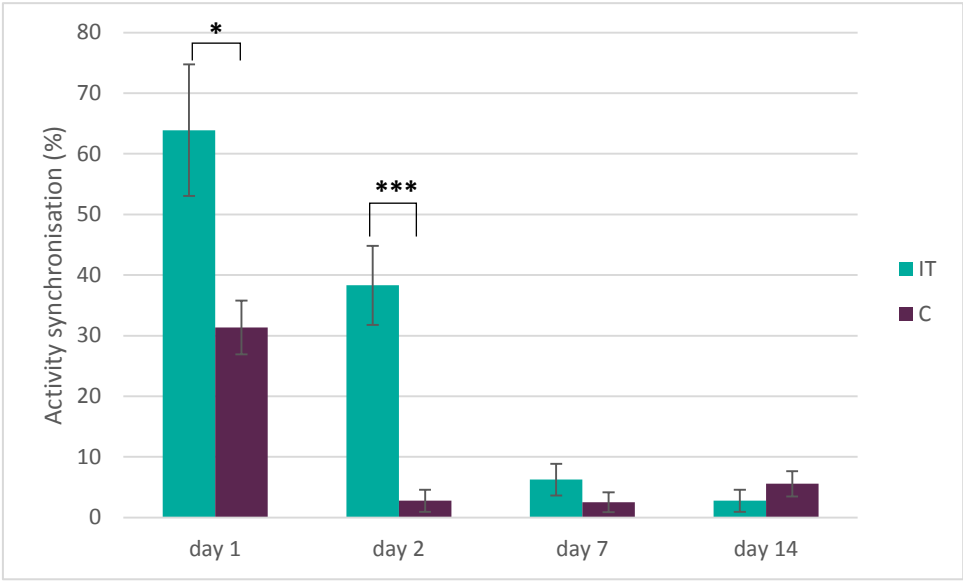

Figure 2: Activity synchronisation of chicks reared in intermittent lighting schedule (IT) and in continuous lighting schedule (C). On the first two days were IT chicks more synchronised than C chicks (day 1 $p < 0.05$; day 2 $p < 0.001$).

We were looking for differences in inter-individual distance between groups using independent samples t-test. There was no significant difference in time spend clustered between groups from different lighting condition.

We have calculated mean feed conversion ratio (FCR) of chicks from different lighting schedule and we tested it for normality and groups were compared with independent samples t-test. Chicks from IT

lighting schedule have smaller feed consumption with higher weight gain during the whole time period (day 8 $p < 0.001$; day 15 $p < 0.001$) (as shown in Figure 3).

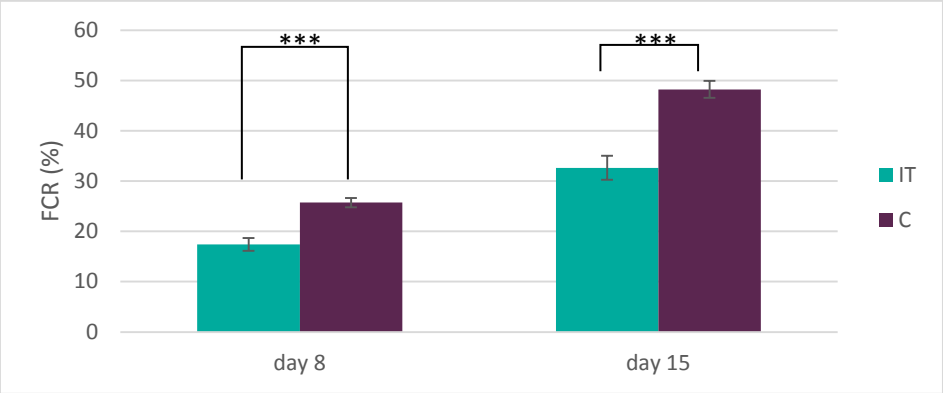

Figure 3: Mean feed conversion ratio (FCR) of chicks kept in intermittent lighting schedule (IT) and in continuous lighting schedule (C). Chicks from IT shown greater weight gain with smaller feed consumption on day 8 ($p < 0.001$) and day 15 ($p < 0.001$).

Welfare outcomes were also tested for normality and compared with independent samples t-test. There was no significant difference between IT and C chicks in the human approach, novel object test, or in tonic immobility test.

Remaining data have not been analysed yet. They will be by the STSM awardee in her home laboratory. The data have been already successfully transferred. The progress of analysis of the data will be coordinated with the STSM supervisor.

FUTURE COLLABORATION POSSIBILITIESWITH THE HOST INSTITUTION

Both laboratories expressed their interest in future collaboration on topics related to poultry behaviour and welfare, using possible future COST GroupHouseNet STSMs and other existing exchange schemes.

FUTURE PLANS, INCLUDING POTENTIAL FUTURE PUBLICATIONS

Data collected during the STSM can form the basis of the potential 1-2 research papers.

OUTPUTS PRODUCED (E.G. ACADEMIC PAPER, FUNDING APPLICATION, NEW DATASET ETC.)

Collected data could form the basis of 1-2 papers. The first one will be related to the effect of different lighting schedules (IT and C) on chick's behaviour. In the STSM the Hy-Line chick were used, but in the host laboratory similar experiment was done using the Lohmann classic chicks. Experimental design for both studies was the same, preliminary results indicate that the effects were very similar, and therefore maybe the data will be combined.

The second potential paper could be related to the reward anticipation in laying hen chicks younger then 25 days and socially-mediated arousal induced by positive emotional stimulus.

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