Linköping University | Department of Physics, Chemistry and Biology
Master thesis, 60 hp | Educational Program: Physics, Chemistry and Biology
Spring term 2016 | LITH-IFM-x-EX—16/3234--SE

Behavioral effects of postnatal stress in domestic chickens (*Gallus, gallus*)

Marissa Soto

Examinator, Jordi Altimiras

LINKÖPINGS UNIVERSITET

Division, Department

Department of Physics, Chemistry and Biology
Linköping University

Contents

Language

- ☐ Svenska/Swedish
- ☒ Engelska/English
- ☐ _______________

Report category

- ☐ Licentiatavhandling
- ☒ Examensarbete
- ☐ C-uppsats
- ☐ D-uppsats
- ☐ Övrig rapport
- ☐ ____________

URL för elektronisk version

ISBN

ISRN: LITH-IFM-x-EX--16/3234--SE

ISSN

Title of series, numbering

Title

Behavioral effects of postnatal stress in domestic chickens (*Gallus, gallus)*

Author

Marissa Soto

Abstract

Disruptions during early development can induce both short and long-term effects. Studies have shown that postnatal stress can affect an organism's response to stress and induce anxiety and fear-related behaviors later on in life. Domestic White Leghorn chickens (*Gallus gallus*) that came from a commercial egg hatchery, where they were exposed to many potential stressors on their first day of life were compared to a control group, which were hatched at Linköping University and did not undergo commercial hatchery procedures. When the chicks reached sexual maturity, various behaviors were measured and analyzed by a series of behavioral tests that included an emergence test, a social regrouping test and a novel object test to assess long-term behavioral effects. In the emergence test, no significant differences were found between hatchery stress (HS) and control birds although, females emerged significantly more than males ($p = 0.02$). In the social regrouping test, no treatment effects were found, but several behaviors were significantly affected by the social regrouping of the birds. Females were also generally more active than males. In the novel object test, no treatment effects were found, although males fed and foraged more than females ($p = 0$). These results indicate that exposure to stress on the first day of life has no long-term behavioral effects in domestic chickens.

Keyword

Early stress, behavior, social behavior, chickens, hpa-axis, fearfulness

Contents

1 Abstract

Disruptions during early development can induce both short and long-term effects. Studies have shown that postnatal stress can affect an organism's response to stress and induce anxiety and fear-related behaviors later on in life. Domestic White Leghorn chickens (*Gallus gallus*) that came from a commercial egg hatchery, where they were exposed to many potential stressors on their first day of life were compared to a control group, which were hatched at Linköping University and did not undergo commercial hatchery procedures. When the chicks reached sexual maturity, various behaviors were measured and analyzed by a series of behavioral tests that included an emergence test, a social regrouping test and a novel object test to assess long-term behavioral effects. In the emergence test, no significant differences were found between hatchery stress (HS) and control birds although, females emerged significantly more than males ($p = 0.02$). In the social regrouping test, no treatment effects were found, but several behaviors were significantly affected by the social regrouping of the birds. Females were also generally more active than males. In the novel object test, no treatment effects were found, although males fed and foraged more than females ($p = 0$). These results indicate that exposure to stress on the first day of life has no long-term behavioral effects in domestic chickens.

2 Introduction

Behaviorally, chickens (*Gallus gallus*) can be described as being highly sociable, establishing complex hierarchies and living in small stable family groups (Siegel, 1976). Wild Red Junglefowl (RJF), ancestors of domestic chickens, live in social groups with strict pecking orders (Guhl, 1968). Their group dynamics are formed and maintained by dominant hierarchies through many agonistic behaviors and interactions such as fighting, pecking and threatening in order to keep their stable social structures (Ghul, 1962; 1968). Domestic fowls, such as the White Leghorn (WL) are considered a subspecies from its ancestor the RJF (Fumihito et al. 1994; Appleby;Mench 2004). Domestic chickens share several social behaviors and patterns with their wild ancestors (Wood-Gush, 1989) however, some major differences are evident when comparing morphology and behaviors. For example, Schütz and Jensen (2001) found Red Junglefowls to be more active, more social and perform more intensive feeding and foraging strategies when compared to selected breeds such as the WL. Väisänen (2005) found White Leghorns to be more aggressive after regrouping into new social dynamics compared to Red Junglefowls, indicating that White Leghorns have a decreased ability to cope with group disruptions or changes. These behavioral differences may directly be from the effects of domestication.

Domestication can be defined as the process by which animals adapt to a life with humans by means of selection (Price, 2002). Domestication has altered certain behaviors such as fearfulness and social behaviors (Price, 1984). Some of the changes resulting from selection pressures have made animals better adapted to their environments, while on the other hand negative effects have also been found as a result. In the farming industry, animals are selected for specific production traits. For example, egg-laying hens are bred for high egg production. Increased production may result in higher yields, but also affect health, behavior and physiology in adverse ways (Rauw et al., 1998). One possible evolutionarily explanation to the changes seen in domestication is the resource allocation theory presented by Beilharz and Nitter (1998). This theory suggests that when selection pressure for other costly traits increase, energy demanding traits may decrease. In other words, when animals are selected for high production traits, fewer resources may be left for biological processes. Some farm animals may lose the ability to cope with stressors or the ability to adapt to new environments.

In the poultry industry, chickens encounter many potential stressors such as overcrowding, rough-handling, fear of human caretakers and injury during transport (Janczak; Riber, 2015). Particularly, in egg production, domestic chicken breeds endure a variety of potential stressors, such as being removed by their eggshells by machines, sex sorting, needle vaccinations, transport, overcrowding and human handling (Appleby; Mench, 2004). The stressful procedures start all upon their first day of life.

Postnatal stress can be defined as any stimuli or sudden disruption an organism experiences after birth and during development that acts to increase the exposure of glucocorticoids (Schoech et al., 2011). A stimulus can be either environmental or behavioral and can cause physiological and/or behavioral responses (Creel, 2001). Sudden disruptions from an external stimulus invoke a series of internal neuroendocrine events mediated by stress systems such as the HPA axis.

Previous research has shown that disturbances in the early environment can be potentially harmful for various traits inducing both short and long-term effects. Postnatal stress exposure has been shown to affect growth rates, reproduction, cognition and important behavioral traits that can affect an animal’s fitness. Lindqvist et al. (2008) found reduced abilities to solve a spatial learning task in chickens raised in a stressful environment due to unpredictable lighting, compared to controls. Birds exposed to early stress show more fearful behavior such as significant reduced latencies to approach novel objects and significant reductions in their competitive dominance abilities (Spencer; Verhulst, 2006). In another study, researchers found male chickens exposed to early stress displayed more anxious behaviors than birds that were not stressed early in development.

Studies have shown that the early environment can also program the hypothalamo-pituitary-adrenal (HPA) axis shaping the way an animal responds to or reacts to stressors as an adult (Matthews, 2005). A normally functioning HPA axis is essential in maintaining bodily equilibrium and regulating the stress-response. It also plays an important role in controlling feeding behaviors and energy metabolism (Penke et al., 2001). However, studies have shown that repeated activation of the HPA axis, can cause damaging physiological effects that then may have many negative impacts on brain function and behavior (McEwen, 2000; Schoech et al., 2011; Spencer; Verhulst, 2006).

In an experiment performed on rat pups, Seymour Levine (1957, 1967) found that when neonatal rat pups were handled and separated from their mother for shorter periods of time (3-15 minutes per day), they showed a significant reduction in anxiety-like behaviors later as adults. They were more explorative and had lower levels of corticosterone when compared to rat pups that were not handled. Although in contrast, studies have found when rat pups were separated from their mother for longer periods (3-6 hours per day) during early development, they showed permanent increases in anxiety-like behaviors as adults, thus having greater stress responses (Van Oers et al., 1998; Plotsky;Meaney, 1993). Since Levine, many more studies have shown similar results of early life stress impacts on the HPA-axis in adulthood in a variety of species, including birds. These studies also address the importance of duration, the type of stress and developmental timing on the impact of postnatal stress.

The aim of this project was to investigate long-term behavioral effects in domestic chickens as a result of exposure to hatchery stress on their first day of life. More specifically, as the chickens reached sexual maturity a series of behavioral tests were carried out to assess their behavioral responses to stressful stimuli and novel situations.

I hypothesize that early stress exposure will have long-term effects on domesticated chickens' behavior. I predict there will be significant behavioral differences between chickens that were exposed to stress on their first day of life and those that did not go through the hatchery procedures. Particularly, I expect to see significant effects between the two groups in their coping abilities and responses in the behavioral tests implemented. This includes differences in their activity patterns, feeding and foraging behaviors and levels of aggression and fearfulness.

3 Material & methods

3.1 Birds and Housing

The birds used in this experiment were domestic White Leghorns (*Gallus gallus domesticus)* from the commercial strain *Dekalb White.* This strain has been selected to produce the maximum number of eggs over various environmental and production conditions. All birds came from the same maternal flock kept at the hatchery.

35 male and 35 female birds were hatched at the Swedfarm hatchery in Linghem, Sweden and underwent standard commercial hatchery routines including multiple human handlings, sex-sorting, crowding, vaccinations, and being transported from the hatchery. These birds were assigned to the "hatchery stress" treatment group (HS) that were selected by the hatchery. When the HS group arrived at the university on Day 1, all birds were weighed, wing tagged and placed in 6 identical neighboring pens measuring 0.70 x 0.73 m equipped with feed, water, wood shavings, and heat blocks.

The control treatment group (C) came from the same hatchery as the HS. However, on incubation day 18, 75 eggs were collected that were assigned from the hatchery company and were incubated and hatched at Linköping University, 63 eggs (32 females, 31 males) hatched. This group did not undergo any of the commercial post-hatch procedures on day 1 as the HS did. Wing-tagging for identification and vaccination against Mareks' disease was however inevitable. They were placed in identical pens in the same room as the HS group.

At the age of 5 weeks, all chickens were moved to the "Wood-Gush" research facility, 10 km outside of Linköping. The chickens were separated by sex (mixed among treatment groups) and housed in two identical pens measuring 3.0×2.5×3.0 m (l × w × h) with full visual and auditory contact between the pens. The pens contained food and water ad libitum, perches, nest boxes and wood shavings on the floor. The chickens were kept on a 12:12 h light:dark cycle and the room temperature was 20°C.

The project was approved by the Linköping Council for Ethical Licensing of Animal Experiments; ethical permit number 50-13.

3.2 Behavioral tests

3.2.1 Emergence test

The test was a modified version of an emergence test frequently used in rodents to assess fearfulness (Pare et al., 2001). A more fearful animal will take longer to emerge and explore the novel arena outside the holding pen. The emergence test took place during four consecutive days when the birds reached sexual maturity and were about 25 weeks of age. The test setup consisted of a cardboard box measuring 57 x 35 x 41 cm (l × w × h) with a guillotine type door measuring 32 x 21 cm opening into an enclosed arena measuring 185 x 180 x 90 cm (Figures 1, 2A, 2B). The top of the arena was covered with metal fencing so no birds could escape. Four small lamps were attached to the roof of the arena to diminish any shadows. A video recorder was placed behind the cardboard box to observe the testing while remaining out of the bird's sight to limit any disturbances from the observer. All birds went through testing once. One bird at a time was placed in the box and allowed 2 minutes of acclimatization. The door was then raised and the time until the head emerged (HE) from the box was recorded, defined as showing the full comb visible outside the door. Full body emergence (FE) from the box was also recorded, defined as when a bird's full body including tail was observed. Each bird was given a total of five minutes to emerge or else it received the maximum score of 300 seconds.

Figure 1. Bird in test box about to emerge into test arena

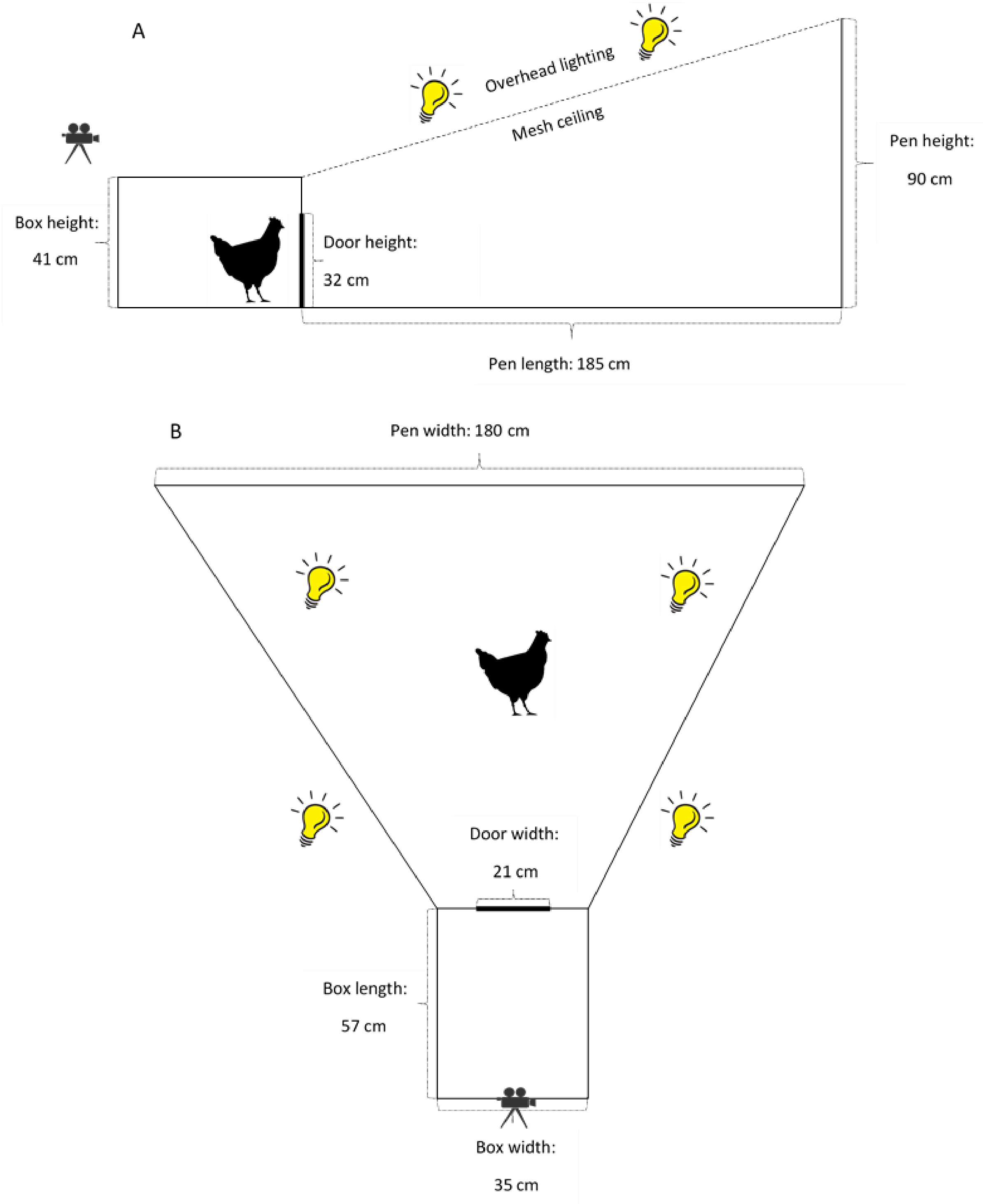


Figure 2. Schematic drawing of side view (A) and overhead view (B) of emergence test setup

3.2.2 Social regrouping test

A sub-set of birds (n = 60; 15 per sex and treatment) was randomly picked for the social re-grouping test at around the age of 40 weeks. This test lasted for a total of 26 days starting with the females, since males and females had to be tested separately. In the testing room, 10 pens (1 x 1 square meter) were built and furnished with wood shavings, a perch, mesh plastic roof to prevent birds from escaping and fed an ad libitum supply of commercial chicken food and fresh water. The chickens were kept on a 12:12 h light:dark cycle and the room temperature was 20°C.

Three birds from the same treatment group were housed together in each pen (Figure 3). Pens were set up next to each other and were alternated by treatment group (Figure 4). Birds were individually marked with colored leg bands for easy identification and given roughly 70 hours to adapt to their new environment before observational recordings began.

Observations were carried out using focal animal sampling with 1/0 sampling at 10-second intervals. Each bird was observed for a total of 3 minutes before rotating to the next bird. Each pen was observed for a total of 9 minutes in a different rotating and balanced pattern each testing day. Two 180 min observation periods were carried out, one in the morning and one in the afternoon, for 3 consecutive days, which were labeled as baseline recordings. Each bird ended up with a total of 162 minutes of observations where the frequency of various behaviors defined in the Ethogram (Table 1) were recorded.

After baseline recordings, all birds were regrouped into new pens with new cage-mates (staying within the same treatment groups and groups of 3 birds per pen) for 5 consecutive days each morning to expose animals to a period of stress. After the 5 days of rotation they were returned to their original home pens, with original cage-mates. Starting the following day, another 3 days of observational recordings took place in the same exact manner as used in baseline testing. This was labeled as the "post-treatment recordings." Figure 5 shows a timeline for the entire duration of the social regrouping test.

The same testing procedures as described in the social regrouping experiment were then carried out with the male birds in the same exact manner.

Figure 3. Birds housed together in pens during social regrouping test

Figure 4. Pens were built next to each other alternating treatments

Habituation	Pre-stress Recording	Regrouping of Birds	Post-stress Recording	Novel Object Test
Day 1-3	Day 4-6	Day 7-11	Day 12-14	Day 15

Figure 5. Schematic drawing of timeline in social regrouping test

3.2.3 Ethogram

The following behaviors were recorded during the social regrouping test (Table 1).

Table 1. Ethogram for social regrouping test

Functional Term	Explanation
Feeding/Foraging	
Feed	Eating from food container, food hopper, or other food source
Food peck	Distinct pecks at supplied feed
Bill rake	Wiping of the beak, in feed, ground or against objects
Ground Peck (GP)	Pecks at items (visible or not) on ground
Ground scratch (Gs)	Scratching at ground, often intermittent during bouts of Gp, often followed by one-two steps backwards after Gs
Drink	Drinking from water nipple, water bell or other water source
Exploratory	
Explore object	Head close to object of interest, eyes focusing on object
Object peck	Peck at object of interest, including fittings in environment
Explore Ground	Walking or standing with head close to ground (below back), eyes focusing on ground items
Manipulate object	Uses beak to lift, move or otherwise manipulate object
Food run	Runs with a food item in beak, usually followed by one or more other animals
Vigilant Behaviors	
Freeze	Stiff posture, stand, sit or lie motionless, vigilant, open eyes/attend to surroundings
Stand alert	Stand with eyes open and neck raised, attendant to the surrounding but not to floor, feed- or water-bell
Sit alert	Sitting (legs bent, body touches ground) with open eyes, attending to the surroundings
Locomotive Behaviors	
Walk	Two or more steps, but without focus on floor, feed- or water-bell
Run	Two or more steps in considerable faster tempo than Walk, body often stretched, head held in a more forward position than during Walk

Sit relaxed	Sitting (legs bent) with reduced attention, eyes may be partly closed, neck short, no alert head movements
Sleep	Stand or sit with eyes closed, neck short, no head movements
Perch	Sits in any position on a perch
Escape/flight	Attempt to escape out from the test arena by jumping or making fly attempts towards the roof
Comfort Behaviors	
Preen	Uses beak to trim and arrange feather
Scratch body	Uses feet to scratch, clean and preen feathers
Stretch wing	Stretches wing straight backwards
Stretch leg	Stretches leg
Yawn	Gape
Feather ruffle	Erects feathers, ruffles, and shakes body
Dust Bathe	Corresponds to vertical wing shake, and rubbing phase. Usually proceeded by scratching and bill raking, and followed by Fr and Pr.
Wing flap	Flaps wings while standing on ground or perch
Vocalizations	
Crow	Cockerel crowing
Social Behavior	
Non-aggressive peck	Pecking or manipulating gently at other
Aggressive Behavior	
Raised hackle threat	Body horizontal or in pecking position, head towards opponent, hackles raised
Attack	Bird runs, jumps or flies when approaching another bird in order to give one or more aggressive peck. The head is kept above the receiver's head.
Aggressive peck	Bird gives a fast peck, directed to an anterior part of another bird's body

3.2.4 Novel object test

After the social regrouping test, 20 males and 20 female birds from the social regrouping pens were tested individually in a separate test room. A pen identical in size and furnishings to the pen used in the social re-grouping experiment was built and used as the testing arena (Figure 6). Birds selected for testing were alternated by treatment.

One bird was placed in the test pen and the lights were turned off for 2 min for the bird to acclimate. After 2 min the lights were turned on and an aluminum soda can was placed in the pen. Each bird was given a total of 5 min and various behaviors were recorded using 1/0 sampling with 10 sec intervals (listed in Ethogram Table 2) as well as the latency to peck the can.

Figure 6. A bird in testing pen during novel object test

3.2.5 Ethogram

The following behaviors were recorded during the novel object test (Table 2).

Table 2. Ethogram for novel object test

Functional Term	Explanation
Feeding/Foraging	
Feed	Eating from food container, food hopper, or other food source
Food peck	Distinct pecks at supplied feed
Bill rake	Wiping of the beak, in feed, ground or against objects
Ground Peck (GP)	Pecks at items (visible or not) on ground
Ground scratch (Gs)	Scratching at ground, often intermittent during bouts of Gp, often followed by one-two steps backwards after Gs
Drink	Drinking from water nipple, water bell or other water source
Exploratory	
Explore feed	Head close to ground (below back), eyes focusing on feed or other edible objects
Explore Ground	Walking or standing with head close to ground (below back), eyes focusing on ground items

Explore object	Head close to object of interest, eyes focusing on object
Object peck	Peck at object of interest, including fittings in environment
Vigilant Behaviors	
Freeze	Stiff posture, stand, sit or lie motionless, vigilant, open eyes/attend to surroundings
Stand alert	Stand with eyes open and neck raised, attendant to the surrounding but not to floor, feed- or water-bell
Locomotive Behaviors	
Walk	Two or more steps, but without focus on floor, feed- or water-bell
Relaxed Behaviors	
Stand relaxed	Standing (legs erect) with reduced attention, eyes may be partly closed, neck short, no alert head movements
Comfort behaviors	
Preen (Pr)	Uses beak to trim and arrange feather
Stretch wing	Stretches wing straight backwards
Yawn	Gape
Feather ruffle (Fr)	Erects feathers, ruffles, and shakes body
Vocalization	
Crow	Cockerel crowing

3.3 Data analyses

All data was analyzed using the statistical software package SPSS 23.0 (IBM). A Kaplan-Meier survival analysis test was used to compare the latency times for birds to emerge from the box between the two treatment groups. All behavioral variables were plotted and checked for normality prior to further analysis for all behavioral tests. To compare means between the treatments and sexes, a chi-square test was used. To evaluate any changes in behavior during the social regrouping test, a repeated measures ANOVA was performed to compare baseline recordings from post stress recordings for all behaviors observed. To look closer at baseline behaviors between the two treatment groups and then between sexes, a one-way ANOVA test was used. For additional analysis, all behaviors listed in the Ethogram were first analyzed separately, but then later grouped into categories as indicated in the Ethogram (Tables 1 and 2) where another ANOVA with repeated measures test and one-way ANOVA was performed. For the novel object test, all behaviors were categorically combined and analyzed using a one-way ANOVA. For the social regrouping and novel object test, results are reported from combined behaviors.

All data was presented as means and the standard error of the mean (SEM). The differences were considered significant if P-values were below 0.05.

On the third day of baseline recordings during the social regrouping test, a hen (control) had to be removed from one of the pens due to an injury. Her data was excluded and the average score was taken for the two remaining birds. A third bird was added as a replacement to keep the social dynamics during the study, but no recordings were used for the replacement bird.

4 Results

4.1 Emergence test

Hatchery stress and control birds were both fearful with only 17 birds fully emerging (FE) from the box out of the 90 birds tested in total. 12 birds emerged from the HS group and 5 from the C group. No significant differences were found in latency times for birds to emerge from the test box between HS and control treatment groups or between male and female birds (Kaplan-Meier survival analysis, X^2 (df = 1) = 2.43, P = 0.19; Figures 7A, 7B, 7C).

Females were significantly less fearful than males (Kaplan-Meier survival analysis, X^2 (df = 1) = 5.66, P = 0.017; Figure 7D) with 13 females and 4 males fully emerging from the test box.

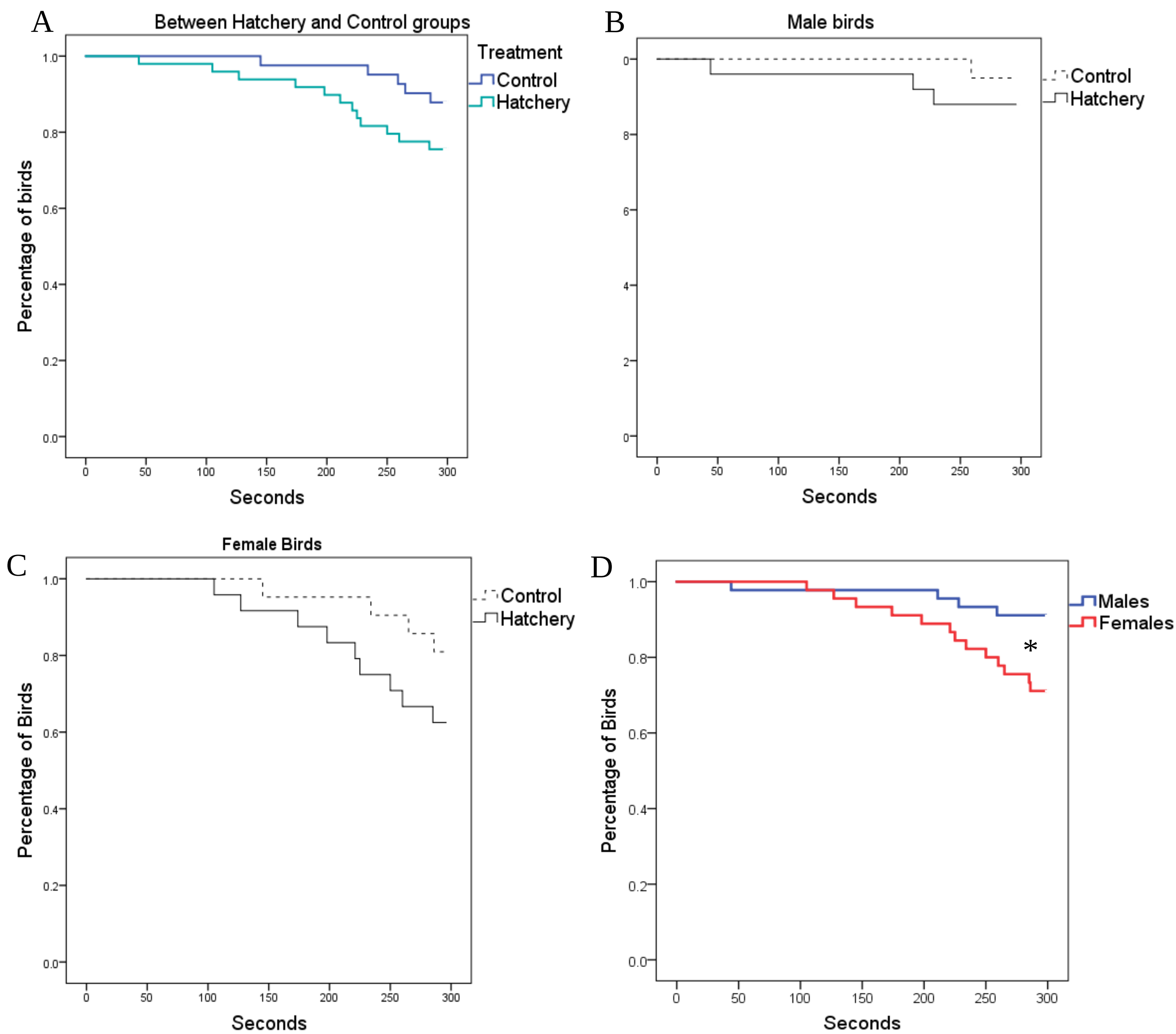
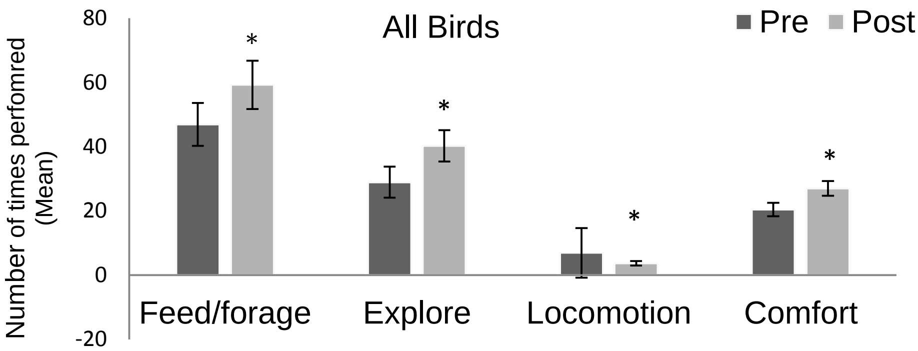


*Figure 7. Kaplan-Meier survival analysis plot for variable "birds fully emerging" in emergence test A) for the control group (n = 41) and the hatchery stress group (n = 49) and B) for the male control (n =20) and hatchery stress (n =25) groups and C) for the female control (n =21) and hatchery stress (n =24) groups and D) for females (n = 45) and males (n = 45). * P < 0.05*

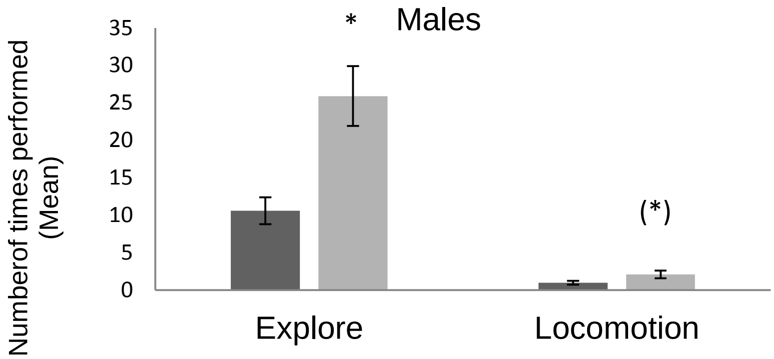
4.2 Social regrouping test

A repeated measures ANOVA test found no significant interaction effects between the HS and control birds or when the sexes were analyzed separately. Significant time effects were found in many behaviors as shown in Figure 8A, determining that the stressor (regrouping) had a significant impact on feeding, foraging and activity patterns in both treatments and when sexes were analyzed separately (Figures 8B, 8C).

A



B



C

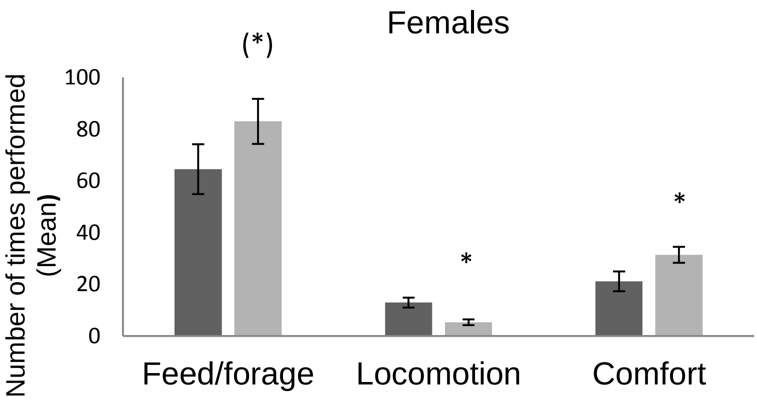


*Figure 8. Bar charts of pre and post stress scores A) for all birds (n = 20) B) for male treatments (n = 10) and C) female treatments (n = 10) when behaviors were combined in the social regrouping test. Significant differences between the groups are indicated (Repeated measures ANOVA, *: P = < 0.05, (*): P = <0.1)*

A one-way ANOVA test was used to analyze baseline values before the social regrouping was implemented. No significant behavioral differences were found between HS and control birds. When sexes were analyzed separately, no significant behavioral effects were found between treatments, but a tendency was found in male control birds being more "relaxed" than HS males ($F(1,10) = 4.1$; $P < 0.1$) and female control birds showed a tendency in acting more vigilantly than HS females ($F(1,10) = 4.5$; $P < 0.1$).

Significant sex differences were found in baseline values (pre-stressor) where females were more active by showing more "feeding and foraging" $F(1,10) = 10.2$; $P < 0.05$, "exploring" $F(1,10) = 64.7$; $P < 0.05$ and "locomotion" $F(1,10) = 40$;$P < 0.05$ behaviors than males, while males showed more "relaxed" $F(1,10) = 46.7$;$P < 0.05$ behaviors. Females were also more "aggressive" than males $F(1,10) = 9.7$; $P < 0.05$ (Figure 9).

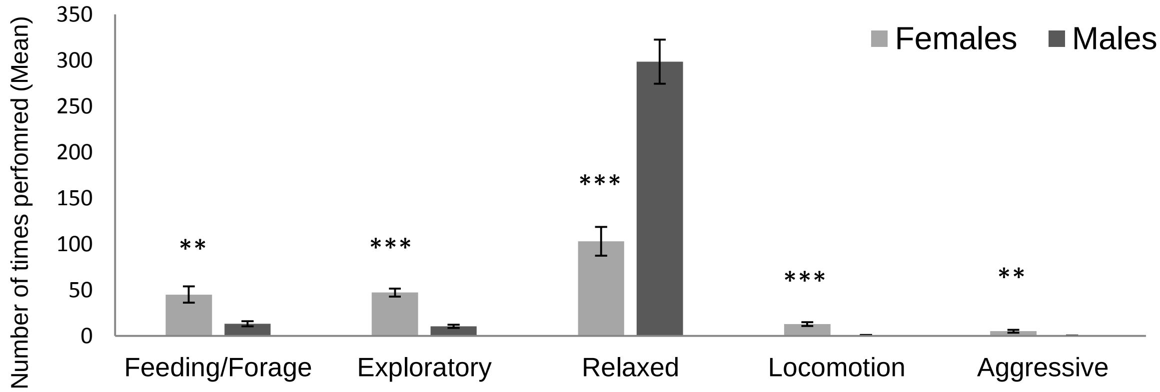


Figure 9. Bar chart on baseline (pre-stressor) behavioral responses when data was combined in the social regrouping test between males (n = 10) and females (n = 10) (One-way ANOVA,: P = 0.05- 0.01, **: P = 0.01- 0.001, ***: P < 0.001)*

4.3 Novel object test

No significant behavioral differences were found between HS and C birds or in the latency for birds to peck the novel object. Only 1 bird out of all test subjects pecked the novel object.

A one-way ANOVA determined a couple of behavioural sex differences. Males significantly fed and foraged more $F(1, 37) = 25.8$, $P = 0$ and females behaved more vigilantly $F(1, 37) = 11.1$, $P = .002$ (Figure 10).

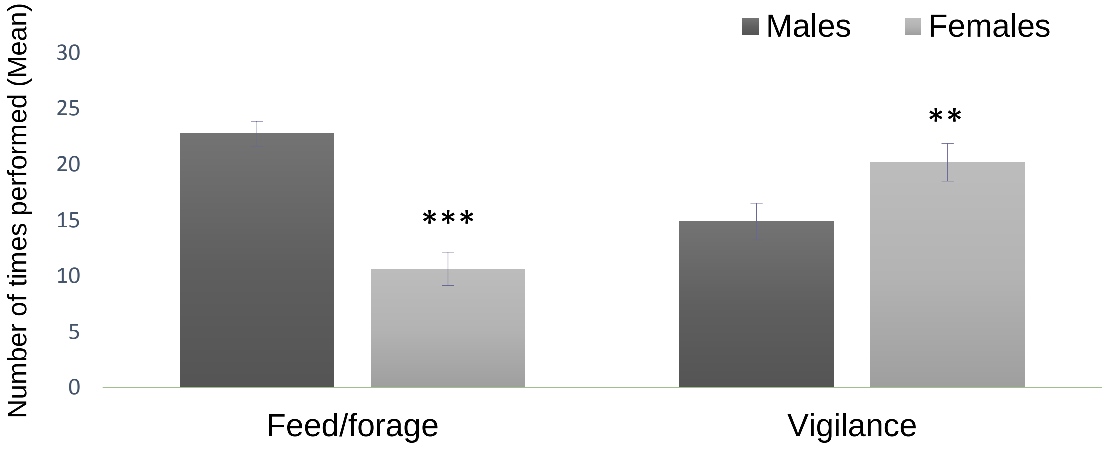


*Figure 10. Bar chart of behavioral responses in the novel object test between males (n = 20) and females (n = 20) when behaviors were combined. Significant differences between the groups are indicated (One-way ANOVA, *: P = 0.05 - 0.01, **: P = 0.01 - 0.001, ***: P < 0.001)*

5 Discussion

The purpose of this study was to examine whether postnatal stress on the first day of life affected long-term behaviors in domestic chickens. In this study we wanted to assess behavioral effects from stress administered immediately post hatch and directly from commercial egg-hatchery procedures. It was expected that birds exposed to early stress would display different behaviors in response to stressful stimuli and situations than birds that were not exposed to early stress.

In general, this study did not find exposure to postnatal stress on post hatch day one had any long-term behavioral effects in White Leghorn chickens. No significant treatment effects were found in all three behavioral tests. One possibility to why could be that the chicks' HPA-axis was not fully functional on day 1 post hatch. Some authors suggest that there is a "stress-non-responsive period" where stress fails to activate adrenal cortex secretion during the first few days or even week of postnatal life (Schapiro et al., 1962; Freeman, 1981). However, other studies have shown chickens and ducks to have well-developed adrenocortical responses at time of hatching (Ericsson & Jensen, unpublished; Carsia et al., 1987). Another possible explanation is that the stress the chicks were exposed to was not severe enough or not induced at the right time to cause any long-term effects. Results found in other studies of early stress also vary according to the timing and type of stress. Some examples of different types of stressors used are nutritional stress (Krause et al., 2009), handling, social isolation (Goerlich et al., 2012), and corticosterone injections (Lynn et al., 2014). In a study with rats, researchers found that long-term behavioral effects depended on the specific type of stress, the duration and when rats were exposed to early stress (Traslaviña et al., 2014). Also, it is impossible for the control group not to be exposed to some degree of stress on their first day of life. Conditions were controlled as much as possible, but chicks did have to go through some degree of handling and vaccinations. Maybe what the treatment and control group endured was not that different.

A series of behavioral tests (emergence, social regrouping and novel object test) were used to assess various behaviors and fear responses. Adverse environments during growth and development have been linked to changes in fear-related behavior. Therefore, it was important that this study used methods that assessed fearfulness (Spencer; Verhuslt, 2006). Fear has been associated with characteristics such as freezing, silence, and inactivity in open field tests (Jones, 1983), as well as reduced vocalizations and latency to emerge from a box in domestic chickens and quails (Jones, 1979). Fearfulness is also considered by some researchers as a trait associated with coping styles to stressors (Cockrem, 2007).

Particularly, the emergence test is less frequently used in chickens, but makes a valid alternative to other fear tests (Forkman et al., 2007; Jones; Mills, 1983). Each animal is tested individually and the latency to emerge is compared. It can be inferred that the longer an animal takes to emerge from a small compartment into a larger one, the more fearful the individual is. The test was set-up to mimic other studies that have used emergence tests with significant results (Jones, 1983; Ghareeb et al., 2008). No treatment effects were found in the latency to emerge from the box. In general, White Leghorns were fearful whether from the HS (treatment) or C group. The majority of birds did not emerge from the start box into the novel arena. This is not consistent with other studies, which have found significant numbers of animals emerge from a start pen (Jones et al., 1991; Paré et al., 2001). One possibility why not many birds emerged could be the age of the birds. Ghareeb et al. (2008) used the emergence test in a study with domestic chickens and found that their latency scores depended on their ages. The birds had shorter latency times at 20 weeks old. During this test, birds were about 25 weeks old.

Females were less fearful than males and emerged from the box significantly more. This correlates with other studies that have found female chickens to be more explorative and active than males (Jones; Black, 1980; Nätt et al., 2014). In a similar test called a “hole-in-the-wall timidity test”, Jones (1979) found males showed longer latencies to emerge from the hole than females. Banks et al. (1979) also found female domestic chicks to be less fearful than males when placed in a novel arena. In another study males were more fearful than females in a tonic immobility test (Janczak et al, 2007). Studies have also found males to be more responsive to stress than females (Madison et al., 2008).

The novel object test was another method in assessing behavioral responses in a novel or fearful situation. Many researchers find the novel object test to be a reliable way to assess fearfulness (Forkman et al. 2007). No treatment effects were found in the latency to peck the can or in various behaviors observed. Since only one bird pecked the can, it could mean that in general, all birds were fearful in this test or this test was not sensitive enough to detect the bird's responses and therefore unreliable. One reason why the test became unreliable could be that birds were tested immediately after the social regrouping test. Some birds may have been underfed due to being caged with other birds and having to share one feeder during the regrouping test. Hierarchies were most likely established on the first day when they were homed in test pens since birds were observed during the social regrouping test chasing and pecking other birds. In a study observing Red Junglefowls, dominant birds chased others away at prime feeding spots (Collias, 1967). The novel arena may have given birds a chance to feed alone for the first time in two weeks, especially the males since they fed more during novel object testing.

Females were found to be significantly more active than males in the social regrouping test, but this was not shown during the novel object test. These findings also contradict many other studies that have found females to be more active than males when placed in fearful situations (Schuetz et al., 2001). In this test, males fed and foraged more, which is also in contrast to previous studies. Jones (1978) suggests there is evidence that females feed more than males when both are placed in novel environments.

Further, a social regrouping test was performed to induce a period of stress by disrupting the birds' social dynamics. This test was used to further evaluate how the chickens would cope and behaviorally respond to acute stress. Agonistic encounters with unfamiliar birds are regarded as a powerful cause of social stress in poultry (Craig et al., 1969; Gross, 1972). Being a highly social species, chickens are sensitive to social disruptions, wherefore we expect social regrouping to be an effective stressor (Jones; Harvey, 1987; Väisänen; Jensen, 2004).

When chickens were exposed to a period of stress during the social regrouping test, no differences were found between treatment groups, but many behaviors were affected as a result in all birds showing social disruption to be an effective method for inducing stress upon chickens. This is consistent with other studies that have shown social regrouping to be stressful for chickens (Jones and Mills, 1999; Väisänen et al., 2005). Since the social regrouping test seemed to successfully stress the birds, it seems unlikely this test would fail to show behavioral differences or responses between treatment groups if there were any.

Many sex differences were also found during the social regrouping test. Females were found to be more active, explorative and aggressive than males. Many studies have found similar results in both domestic female chickens and their ancestors the Red Junglefowl with being more active and aggressive. In a behavioral study with Red Junglefowl, researchers found females foraged and explored open arenas more, while males displayed more comfort behaviors and perched more (Nätt et al., 2014). In a previous study, domestic male chickens displayed more comfort behaviors such as preening, feather ruffling and scratching body, which were associated with positive anticipation (Zimmerman et al., 2010). This may indicate that comfort behaviors are signs of non-stressed individuals.

5.1 Conclusions

This study found that birds exposed to early stress did not behave or respond differently than control birds when exposed to fearful situations or stress implemented by the emergence, social regrouping or novel object tests. This indicates that exposure to postnatal stress on Day 1 has no long-term behavioral effects. Social regrouping of chickens can affect a variety of behaviors. Many behavioral differences were found between male and female chickens with female birds generally being more active and explorative than males.

5.2 Societal and ethical considerations

This study is not only important for gaining more understanding of the impacts early stress may have on physiology and behavior later on in life, but also for animal welfare. Understanding how birds cope with stress and what long-term impacts it may have as a result is crucial for the improvement of their welfare. Animals that have difficulty coping with stress or to their environment are thought to have poor welfare (Broom, 1991). If we can better understand the long-term impacts of how animals are treated early on and how it affects their coping abilities, then maybe changes can be made to the rearing process in farm animals. It can also help diminish the development of abnormal behaviors commonly seen in the animal production industry. It is also well documented that the more fearful animals are, the higher their elevated levels of stress hormones are. If early stress exposure makes an animal more fearful throughout its life, then this may affect productivity in farm animals and also have harmful effects on organisms themselves in the long-term.

6 Acknowledgement

I would like to give a special thank you to my supervisor, Professor Per Jensen for all his support and insightful feedback on this project. I would also like to extend my gratefulness to Mia Ericsson, my co-supervisor who helped tremendously with very rough drafts, test set-ups, advice and support. I also would like to thank the AVIAN research group for welcoming us Master students into the group and supporting our on-going projects.

7 References

Appleby, M., & Mench, J. (2004). *Poultry behaviour and welfare* (pp. 184-186). Wallingford, Oxfordshire, UK: CABI Pub.

Banks, E. M., Wood-Gush, D. G., Hughes, B. O., & Mankovich, N. J. (1979). Social rank and priority of access to resources in domestic fowl. *Behavioural processes*, *4*(3), 197-209.

Beilharz, R. G. (1998). Environmental limit to genetic change. An alternative theorem of natural selection. *Journal of Animal Breeding and Genetics*,*115*(1-6), 433-437.

Broom, D. M. (1991). Animal welfare: concepts and measurements. J. Anim. Sci. 69:4167–4175.

Carsia, R. V., Morin, M. E., Rosen, H. D., & Weber, H. E. L. E. N. (1987, April). Ontogenic corticosteroidogenesis of the domestic fowl: response of isolated adrenocortical cells. In *Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, NY)* (Vol. 184, No. 4, pp. 436-445).

Cockrem, J. F. (2007). Stress, corticosterone responses and avian personalities. *Journal of Ornithology*, *148*(2), 169-178.
Collias, N. E., & Collias, E. C. (1967). A field study of the red jungle fowl in north-central India. *The Condor*, *69*(4), 360-386.

Craig, J. V., Biswas, D. K., & Guhl, A. M. (1969). Agonistic behaviour influenced by strangeness, crowding and heredity in female domestic fowl (Gallus gallus). *Animal Behaviour*, *17*, 498-506.

Creel, S. (2001). Social dominance and stress hormones. *Trends in Ecology & Evolution*, *16*(9), 491-497.

Jensen, P. (2015). Early stress causes sex-specific, life-long changes in behaviour, levels of gonadal hormones, and gene expression in chickens.*PloS one*, *10*(5), e0125808.

Forkman, B., Boissy, A., Meunier-Salaün, M. C., Canali, E., & Jones, R. B. (2007). A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. *Physiology & Behavior*, *92*(3), 340-374.

Freeman, B. M., Manning, A. G. G., & Flack, I. H. (1981). The effects of restricted feeding on adrenal cortical activity in the immature domestic fowl.*British poultry science*, *22*(3), 295-303.

Ghareeb, K., Niebuhr, K., Awad, W. A., Waiblinger, S., & Troxler, J. (2008). Stability of fear and sociality in two strains of laying hens. *British poultry science*, *49*(5), 502-508.

Goerlich, V. C., Nätt, D., Elfwing, M., Macdonald, B., & Jensen, P. (2012). Transgenerational effects of early experience on behavioral, hormonal and gene expression responses to acute stress in the precocial chicken.*Hormones and behavior*, *61*(5), 711-718.

Gross, W. B. (1972). Effect of social stress on occurrence of Marek's disease in chickens. *American journal of veterinary research*, *33*(11), 2275-2279.

Guhl, A. M.. (1968). Social Behavior of the Domestic Fowl. *Transactions of the Kansas Academy of Science (1903-)*, *71*(3), 379–384. http://doi.org/10.2307/3627156.

Janczak, A. M., Heikkilä, M., Valros, A., Torjesen, P., Andersen, I. L., & Bakken, M. (2007). Effects of embryonic corticosterone exposure and post-hatch handling on tonic immobility and willingness to compete in chicks.*Applied Animal Behaviour Science*, *107*(3), 275-286.

Janczak, A. M., & Riber, A. B. (2015). Review of rearing-related factors affecting the welfare of laying hens. *Poultry science*, *94*(7), 1454-1469.

Jones, R. B. (1978). Activities of chicks in their home cages and in an open field. *British Poultry Science*, *19*(6), 725-730.

Jones, R. B. (1979). The hole-in-the-wall test: its validity as a measure of the ‘timidity’aspect of fear in the domestic chick. *IRCS Med. Sci.*, *7*, 167.

Jones, R. B., & Black, A. J. (1980). Feeding behaviour of domestic chicks in a novel environment: effects of food deprivation and sex. *Behavioural processes, 5*(2), 173-183.

Jones, R. B., & Mills, A. D. (1983). Estimation of fear in two lines of the domestic chick: correlations between various methods. *Behavioural Processes, 8*(3), 243-253.

Jones, R. B. (1983). Fear responses in domestic chicks as a function of the social environment. *Behavioural processes*, *8*(4), 309-325.

Jones, R. B., & Harvey, S. (1987). Behavioural and adrenocortical responses of domestic chicks to systematic reductions in group size and to sequential disturbance of companions by the experimenter. *Behavioural processes*,*14*(3), 291-303.

Jones, R. B., Mills, A. D., & Faure, J. M. (1991). Genetic and experiential manipulation of fear-related behavior in Japanese quail chicks (Coturnix coturnix japonica). *Journal of Comparative Psychology*, *105*(1), 15.

Jones, R. B., & Mills, A. D. (1999). Divergent selection for social reinstatement behaviour in Japanese quail: effects on sociality and social discrimination. *Poultry and Avian Biology Reviews*, *10*(4), 213-223.

Krause, E. T., Honarmand, M., Wetzel, J., & Naguib, M. (2009). Early fasting is long lasting: differences in early nutritional conditions reappear under stressful conditions in adult female zebra finches. *PloS one*, *4*(3), e5015.

Levine, S. (1967). Maternal and environmental influences on the adrenocortical response to stress in weanling rats. *Science*, *156*(3772), 258-260.

Lindqvist, C., & Jensen, P. (2008). Effects of age, sex and social isolation on contrafreeloading in red junglefowl (Gallus gallus) and White Leghorn fowl.*Applied Animal Behaviour Science*, *114*(3), 419-428.

Lynn, S. E., Kern, M. D., & Phillips, M. M. (2013). Neonatal handling alters the development of the adrenocortical response to stress in a wild songbird (eastern bluebird, Sialia sialis). *General and comparative endocrinology*, *186*, 157-163.

Madison, F. N., Jurkevich, A., & Kuenzel, W. J. (2008). Sex differences in plasma corticosterone release in undisturbed chickens (Gallus gallus) in response to arginine vasotocin and corticotropin releasing hormone. *General and comparative endocrinology*, *155*(3), 566-573.

Matthews, S. G. (2002). Early programming of the hypothalamo–pituitary–adrenal axis. *Trends in Endocrinology & Metabolism*, *13*(9), 373-380.

McEwen, B. S., & Lasley, E. N. (2002). *The end of stress as we know it*. Joseph Henry Press.

Nätt, D., Agnvall, B., & Jensen, P. (2014). Large sex differences in chicken behavior and brain gene expression coincide with few differences in promoter DNA-methylation. *PloS one*, *9*(4), e96376.

Paré, W. P., Tejani-Butt, S., & Kluczynski, J. (2001). The emergence test: effects of psychotropic drugs on neophobic disposition in Wistar Kyoto (WKY) and Sprague Dawley rats. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *25*(8), 1615-1628.

Penke, Z., Felszeghy, K., Fernette, B., Sage, D., Nyakas, C., & Burlet, A. (2001). Postnatal maternal deprivation produces long-lasting modifications of the stress response, feeding and stress-related behaviour in the rat.*European Journal of Neuroscience*, *14*(4), 747-755.

Plotsky, P. M., & Meaney, M. J. (1993). Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. *Molecular brain research*, *18*(3), 195-200.

Price, E. O. (1984). Behavioral aspects of animal domestication. *Quarterly Review of Biology*, 1-32.

Price, E. O. (2002). *Animal domestication and behavior*. Cabi.

Rauw, W. M., Kanis, E., Noordhuizen-Stassen, E. N., & Grommers, F. J. (1998). Undesirable side effects of selection for high production efficiency in farm animals: a review. *Livestock Production Science*, *56*(1), 15-33.

Schapiro, S., Geller, E., & Eiduson, S. (1962). Neonatal adrenal cortical response to stress and vasopressin. *Experimental Biology and Medicine*,*109*(4), 937-941.

Schoech, S. J., Rensel, M. A., & Heiss, R. S. (2011). Short-and long-term effects of developmental corticosterone exposure on avian physiology, behavioral phenotype, cognition, and fitness: a review. *Curr Zool*, *57*(4), 514-530.

Schütz, K. E., Forkman, B., & Jensen, P. (2001). Domestication effects on foraging strategy, social behaviour and different fear responses: a comparison between the red junglefowl (Gallus gallus) and a modern layer strain. *Applied animal behaviour science*, *74*(1), 1-14.

Siegel, P.B., 1976. Social behavior of the fowl. Poult. Sci. 55 □1), 5±13.

Spencer, K. A., & Verhulst, S. (2006). Delayed behavioral effects of postnatal exposure to corticosterone in the zebra finch (Taeniopygia guttata).*Hormones and Behavior*, *51*(2), 273-280.

Spencer, K. A., Evans, N. P., & Monaghan, P. (2009). Postnatal stress in birds: a novel model of glucocorticoid programming of the hypothalamic-pituitary-adrenal axis. *Endocrinology*, *150*(4), 1931-1934.

Traslaviña, G. A. A., de Oliveira, F. L., & Franci, C. R. (2014). Early adolescent stress alters behavior and the HPA axis response in male and female adult rats: the relevance of the nature and duration of the stressor.*Physiology & behavior*, *133*, 178-189.

Väisänen, J., Håkansson, J., & Jensen, P. (2005). Social interactions in Red Junglefowl (Gallus gallus) and White Leghorn layers in stable groups and after re-grouping. *British poultry science*, *46*(2), 156-168.

Van Oers, H. J., de Kloet, E. R., & Levine, S. (1998). Early vs. late maternal deprivation differentially alters the endocrine and hypothalamic responses to stress. *Developmental Brain Research*, *111*(2), 245-252.

Wood-Gush, D.G.M. (1989) The Behaviour of Domestic Fowl (Hants, UK, Nimrod Pres

Zimmerman, P. H., Buijs, S. A. F., Bolhuis, J. E., & Keeling, L. J. (2011). Behaviour of domestic fowl in anticipation of positive and negative stimuli.*Animal behaviour*, *81*(3), 569-577.