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The effect of hatchery routines on commercial leghorn chickens

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Sammanfattning
Abstract

Animal welfare and stress are entwind; in the presence of high stress animals will experience decreased welfare. Corticosterone (CORT), the primary stress hormone in chickens, can have detrimental effects on production factors, growth and immune response. Commercial hatcheries expose chickens to; loud noises, rough handling and long transportations. The present study explores acute and chronic effects of hatchery routines on commercial laying chickens and compares differences between sexes. The hatchery group experienced normal commercial hatchery routines, whereas the control group were taken from the hatchery prior to hatching. Control chickens were removed from the incubator and placed straight into a home pen once hatched. 83 control and 85 hatchery chickens were tested at <1 week and 6 weeks of age in novel arena tests, tonic immobility (TI) tests and restraint tests. Egg data, feather scoring and gonadal hormone analysis was also conducted. The present study shows that acute effects of hatchery routines include; stronger CORT reaction, decreased exploration and comfort behaviours. The chronic effects include; stronger CORT reaction, increased feather damage, increased comfort behaviours in females, more eggs per day and higher estradiol levels. Comparing hatchery males to females: males performed fewer comfort behaviours at 2 days and 6 weeks, more vocalisations in TI at 6 weeks, and had increased feather damage. In conclusion the present study shows that current hatchery routines have negative impacts on chickens' early lives. In later life there are positive and negative impacts, with males reacting more severely to hatchery routines than females.

Nyckelord
Keyword
Chicken, stress, commercial, corticosterone, behaviour, production, welfare

Contents

1 Abstract

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Key words: chicken, stress, commercial, corticosterone, behaviour, production, welfare

2 Introduction

In 2012, there were five billion laying hens in commercial farms worldwide, which collectively produced over one trillion eggs (Nicol, 2015). Eggs are widely consumed for their nutritional content leading to egg production being highly economised (Gocsik et al, 2015). However, increasing public concerns for intensive production systems and animal welfare is driving new research into the stress animals face as a result of human consumerism (Zulkifli and Siti Nor Azah, 2004; Fraisse and Cockrem, 2006). The level of animal welfare experienced in commercial

farming is entirely dependent on human management (Fontana et al., 2016) and often animal welfare is sacrificed in favour of increasing productivity, therefore achieving a solution which balances these factors is an important research topic (Compendio et al., 2016). Animal welfare and stress are entwined, in the fact that welfare cannot occur in the presence of high levels of stress and a high level of stress does not occur under good welfare conditions (Scanes, 2016)**.** Therefore creating an understanding of the stress animals endure in commercial production settings is fundamental for the improvement of welfare of production animals (Ericsson et al., 2014).

2.1 Egg production

Red Junglefowl, the closest living relative to the domestic chicken, are not known for their egg laying abilities. However, over the last 8,000 years humans have selectively bred the ancestors of modern chickens for traits such as; fighting ability, body size and egg laying abilities, creating a plethora of different chicken breeds. Divergence between meat (broiler) and egg (layer) chickens began between the late 19th to early 20th century evolving into the current poultry practices of the 21st century. Almost all commercial laying chicken breeding is conducted by a handful of companies within Europe; Hendrix (Netherlands), Erich Wesjohann (Germany) and Group Grimaud (France). These three companies are responsible for the care and breeding of the European grandparent- and parental-flocks. These breeding populations are extremely valuable and contain a mixed sex population; where females and males are often different breeds in order to produce hybridised offspring. Except in the case of commercial leghorn chickens, where the parental flock are selected lines of leghorns. Fertile eggs from these grandparent- and parental-flocks are then shipped throughout Europe to hatcheries ready to incubate their own parental flocks or next generation of commercial laying hens (Nicol, 2015).

Commercial hatcheries function on an extremely large scale and are therefore an incredulously sterile environment, reducing the effect that any bacteria may have on the development of embryos. When eggs arrive at a hatchery they go through an egg washing machine before being placed in large cabinet incubators. These fan heated cabinet incubators often contain several thousand eggs at one time and are controlled automatically for temperature and humidity. The eggs remain in the incubators until day 19 of incubation. At this stage of the eggs are then removed from the incubators, transported by machinery into hatching trays and then placed in hatching machines. Trays of newly hatched chickens are removed from the hatchers and are taken to the next stage of the commercial hatchery. The

first stage of the hatchery process differs dependent on the breed of chicken, commercial brown chickens are sex-linked therefore can be sexed immediately through feather colouration. Female commercial browns are brown/orange and males are white/yellow. The males are discarded immediately after leaving the hatchers. However, the commercial white chickens have no colour differences upon hatching so both males and female are transported via conveyer belts to the sorting room. Once the white commercial chickens arrive in the sorting, they are manually sorted by looking at the developing wing feathers or in some cases cloacae sexed. The males are discarded from the process and transported to a different room where they are culled in a gas machine. The female chickens are then transported on the conveyer belt system to a vaccination station where they are vaccinated by automatic dispensing machines. In some countries the chickens are also beak trimmed in order to prevent feather pecking, however this is not practiced in Sweden. Once vaccinated the chickens are transported on another conveyor belt system, this time with multiple drops and differing speeds in order to prevent the chickens clustering so they can be counted by a machine and dropped into transportation crates containing 104 chickens. The chickens then remain in these transportation crates until the whole batch of up to 10,000 chickens have been through the sorting process. The newly hatched chickens are then loaded onto specially designed transport vehicles which maintain temperature and humidity levels for the chickens for a journey of up to a maximum of eleven hours.

Laying hens are generally raised in a different farm to that which they will live as an adult. The farms are called rearing farms or pullet breeders and the newly hatched laying hens generally stay on this farm until they are 15-18 weeks old. Laying poultry hens are often reared in similar enclosures to those of broilers; single layered pens with multiple food and water systems throughout. From arrival up to five weeks of age the hens are heated to simulate natural brooding temperatures, the temperature slowly decreases throughout this time until heating is no longer required. During the time spent at the rearing facility the laying hens receive further vaccinations and in some systems undergo beak trimming to reduce the occurrence of feather pecking and injurious pecking (Janczak and Riber, 2015).

Before laying hens start producing eggs, they are transported to laying hen facilities; sometimes this involves transport to an entirely different farm (Janzak and Riber, 2015). Since the banning of battery cages in the EU by legislation in 2012 most farms converted to tiered aviary systems, the next most economic system (Abrahamsson and Tauson, 1995). A comparatively small percentage of farms choose free-ranging and

organic poultry farming due to lack of economic incentives, only 3.8% of laying hens in Europe are housed in organic systems (Bestman et al., 2017). Egg size is generally larger multi-tiered aviaries and the qualities of eggs are similar to those produced in free-range and organic systems (Gocsik et al., 2015). Multi-tiered housing systems usually comprise of a ground level covered in litter for scratching and dustbathing, and multiple tiers of wired platforms. Each level also contains food and water stations and facilities which enable perching. Separate nesting areas are provided with roll away nest boxes covered in artificial grass to ensure eggs remain clean and undamaged (Abrahamsson and Tauson, 1995). Free range systems differ from traditional multi-tiered systems in that the hens are allowed access to outdoor areas during the daytime. Organic systems also require chickens to have access to outdoor areas and provided with organic feed and medications (Bullandey Scott et al., 2017). Laying hens remain within laying production systems until approximately 18 months of age when production has decreased to approximately 50%, at this point hens are culled and a new batch of point of lay pullets arrive to replace them (Nicol, 2015).

2.2 Stress

Stress is used as a primary measure of welfare in laying chickens however measuring welfare is a complex process due to variable commercial housing systems (Ralph et al., 2015; Graml et al., 2008). Stress is defined as ‘a state in which an animal is responding to a stressor’ (Fraisse and Cockrem, 2006). Stressors are literally defined as ‘anything that changes homeostasis’, or when an animal experiences a demand which exceeds the normal amount of resources available to cope with such demands (Morgon and Tronberg, 2007), whether the demand be physical or emotional (Fraisse and Cockrem, 2006). On a physiological level stress is defined as an activation of the hypothalamic-pituitary-adrenal axis (HPA-axis) with an increase in the secretion of glucocorticoids such as cortisol and corticosterone (Cockrem, 2007; Jensen et al., 2014).

The primary glucocorticoid produced, as part of the HPA-axis, in chickens is corticosterone (CORT) which is released from the adrenal cortex (Cockrem, 2007; Ferrante et al., 2016; Wang et al., 2013). In order to release CORT from the adrenal cortex there is an increase in the secretion of adrenocorticotropic hormone (ACTH) and corticotropin releasing hormone (CRH) (Scanes, 2016). CORT levels are mediated through a negative feedback loop to the hypothalamus which prevent stress response overreactions and aid in protecting the animals from injury due to

extreme fear responses (Wang et al., 2013; Wang et al., 2014). While the release of glucocorticoids is necessary for processes such as the fight or flight complex by increasing the blood glucose levels (Cockrem, 2007), prolonged exposure to high levels of glucocorticoids can have negative long term effects on the behaviour and physiology of animals (Ericsson et al., 2016; Jensen et al., 2014; Elfwing et al., 2015; Goerlich et al., 2012). Plasma corticosterone analysis is currently the most common method used to assess the welfare of commercial chickens; however other measures of stress may be required to fully understand the extent that commercial stressors affect commercial laying hens (Ralph et al., 2015).

Commercial chickens are exposed to a multitude of stressors throughout their life such as; temperature stress, social stress, novel environments and transportation stress (Ericsson et al., 2016). Heat and cold stress can adversely affect the welfare and production of commercial laying hens (Scanes, 2016; Goerlich et al., 2012; Wang et al., 2014; Matur et al., 2016). Cold stress has greater effects on commercial chickens than commercial mammals due to birds having a naturally higher body temperature, making them more sensitive to cold stress (Xie et al., 2017). Cold stress can cause a decrease in egg production; alter the efficacy of the nervous system, which ultimately reduces feed intake and growth rates. These factors contribute to an economic loss in commercial farms (Xie et al., 2017). Heat stress has also been shown to have detrimental effects on commercial chickens (Mignon-Grasteau et al., 2015). Heat stress can decrease egg production, egg quality and compromise immune-efficiency leading to higher mortality levels amongst flocks of commercial laying hens (Mignon-Grasteau et al., 2015). A study by Saint-Pierre et al., (2003), found that egg production can be reduced up to 7.2% in heat stressed laying hens, mortality rates were increased by 0.98% which caused an economic loss of $98.1 billion dollars annually in the USA.

Commercial chickens usually experience social separation and regrouping several times between hatching to reaching adulthood (de Haas et al., 2012; Goerlich et al., 2012; Warnick et al., 2005; Jones, 1996). Regrouping chickens may lead to social instability, resulting in the chickens re-establishing the pecking order of the group due to new flock mates. More dominance-aggression is observed after regrouping, leading to an increase in overall flock stress levels (de Haas et al., 2012). Separating an individual from its flock mates can also be a potent stressor (Jones, 1996). Stress response to separation from conspecifics can be measured in chickens as young as 7 days old, chickens separated from their group performed a high frequency of distress vocalisations (Fontana et al., 2016), and exhibited analgesia and CORT responses (Warnick et al., 2005).

One of the most common stressors experienced by commercial laying hens is exposure to novel environments and objects (Jones, 1996; de Marco et al., 2013). Chickens are frequently moved into novel environments, for example from a commercial hatchery to a rearing farm and then to an adult egg production farm, each move exposing the chickens to novel environments requiring habituation periods (Jones, 1996). Chickens are also exposed to novel objects, including feed and farm machinery, novel odours and novel noises, each of which can elicit stress responses (Jones, 1996; de Marco et al., 2013). Jones, (1996), reported that chickens often cannot habituate to some husbandry practices, such as dusting the fronts of cages, meaning they elicit a stress response each time which may have long term implications of productivity of commercial laying hens.

Another stressor which all commercial laying hens experience is transportation between facilities which can often be long distances (Matur et al., 2016; Goerlich et al., 2012; Jones, 1996; Compendio et al., 2016). Laying hens experience many transport events throughout their first year of life, from a hatchery to a rearing facility, to a laying hen facility, then finally to slaughter when their peak production period ends (Matur et al., 2016). Transportation itself creates a host of additional stressors to the chickens including; temperature variability, vibrations and loud noises (Matur et al., 2016). Compendio et al., (2016) suggests that vibrations produced by vehicles during transportation are negative experiences for poultry leading to stress which reduces productivity, and may increase mortality rates of commercial laying hens, both leading to decreased profit from the commercial flock.

2.3 Early stress

Day old chickens, destined to become laying hens in commercial egg production systems are subjected to a host of different stressors from the moment they hatch; these include rough human handling, sex sorting, being in crates for long periods of time, and being transported long distances (Ericsson and Jensen, 2016; Ericsson et al., 2016). This early stress can have serious implications on chicken behaviour, physiology and immune-function (Gross and Siegel, 1980; Jensen, 2014), creating problems which later affect the production and welfare of laying hens (Archer and Mench, 2014). The early developmental period after hatching or birth, in which vertebrates are particularly susceptible to stress due to accelerated speed and increased complexity of brain development during this period (Ericsson et al., 2016). In chickens it has been found that stressful events

experience early in life can influence the behavioural and physical responses until at least adulthood, whereas stress experienced later in life may only last a few days (Gross and Siegel, 1980). Stress may also alter HPA-reactivity and hypothalamic gene expression, which can also pass transgenerationally (Ericsson et al., 2016; Gross and Seigel, 1980). Chickens which have experienced early stress have increased lateralisation of the hippocampus; this has been correlated with increased expression of severe feather pecking and cannibalism in rearing and adult flocks of laying hens (Nordquist et al, 2012).

Stressors faced by newly hatched laying hens include maternal deprivation and hatching in artificial conditions. Maternal deprivation in precocial birds, such as chickens, is not as widely researched as the affect of maternal deprivation on altricial birds (Elfwing et al., 2015), however research has shown that chickens brooded by a hen showed reduced fear responses when compared with artificially brooded chickens (Edgar et al., 2015). Chickens brooded artificially have also been seen to respond to recorded proximity-indicating maternal vocalisations, producing calming effects on the chickens (Nicol, 2015). Rearing in artificial conditions may decrease a chicken's ability to deal with stressful situations and therefore be more susceptible to stressors and stress related conditions in later life (Ericsson and Jensen, 2016). In addition to the artificial environments and maternal deprivation, other stressors such as lack of perches and unsuitable flooring can also have long term effects on laying hens (Ericson et al., 2016). Handling of chicks is also a stressor; therefore the rough handling that day old chickens may experience in commercial hatcheries is likely to induce a stress reaction (Zulkifli and Siti Nor Azah, 2004).

2.4 Aims

The aim of this Master's project was to assess the acute effects of hatchery routines on day old commercial laying chickens through blood corticosterone analysis and behavioural tests up to one week of age. A secondary aim of this project was to assess whether the impacts of hatchery routines continue to negatively impact chickens throughout developmental stages such as puberty and sexual maturation. Another aim of this project was to compare the impact of hatchery routines on male and female white leghorn commercial chickens.

3 Methods

3.1 Ethical Note

This study was approved by Linköping Council for Ethical Licensing of Animal Experiments, ethical permit no. 50-13.

3.2 Overview of Procedure

Table 1. Overview of experimental procedure

First stage	Second stage	Third stage
-3 Days: Collect eggs from hatchery	**Week 5**: Move to adult facility	**Week 15**: Sex hormone sampling 1
Day 1: Hatchery samples and collect animals	**Week 6**: Novel Arena 2	**Week 19**: Sex hormone sampling 2
Day 2: Novel Arena 1	Tonic Immobility 2	**Week 18-20**: Eggs collected
Day 5: Tonic Immobility 1	Restraint Test 2	**Week 20**: Feather scoring
Day 7: Restraint test 1		
Day 8: Weekly weighing begins		

3.3 Animals

130 eggs and 130 day old chickens were obtained from Gimranäs AB commercial hatchery. Eggs collected on day 19 of incubation and were transported in a portable incubator with a thermostat to maintain a temperature of 37°C throughout the three hour journey to Linköping University (LiU) hatchery. The eggs were then placed in a hatching incubator set to 37.5°C. Day old chickens were collected from the same commercial hatchery three days later immediately after completing the commercial hatchery process and then transported for three hours to LiU hatchery. All chickens were *Lohmann Selected Leghorn* strain from Lohmann Tierzucht, Germany grandparental stock. All chickens were wing marked at eight days old after the first round of behavioural tests had concluded. At this stage control chickens were vaccinated, the hatchery chickens underwent pseudo-vaccination to maintain similarity of treatment between groups. A total of 83 control chickens and 85 hatchery chickens

remained after week 1 testing; birds not used in tests were culled. On week six of the experiment all chickens were transported from the chicken hatchery facility to the adult chicken facility, remaining in the same flock as the previous facility.

3.4 Housing

All chickens were housed in identical pens.Approximately 40 birds were housed per pen of mixed sex, pen 1 and 2 contained control chickens and pen 3 and 4 hatchery chickens. The pens originally measured 90cmx90cm in size (Figure 1), increasing incrementally as the chickens grew bigger and needed more space. The final pens were constructed using nine 1m panels for each pen. Pens contained feed and water ad libitum, the floor was covered in corrugated cardboard with wood shavings on top and all chickens had access to perches from 1 week of age.

Figure 1. Home pen set up for arrival of day old chickens

3.5 Blood Sampling and Restraint tests

Within the commercial hatchery 10 chickens were culled for blood sampling directly out of the incubators, 10 were culled after the hatchery process was complete and a further 10 individuals were culled upon arrival to the animal housing facility in Linköping. 30 control birds were also killed for blood samples, to compare baseline CORT levels with the hatchery samples. 10 were culled after incubation, 10 after 15 minutes in home pen and the remaining 10 were culled when the hatchery birds arrived at the animal housing facility. Blood was collected from each chick by decapitation.

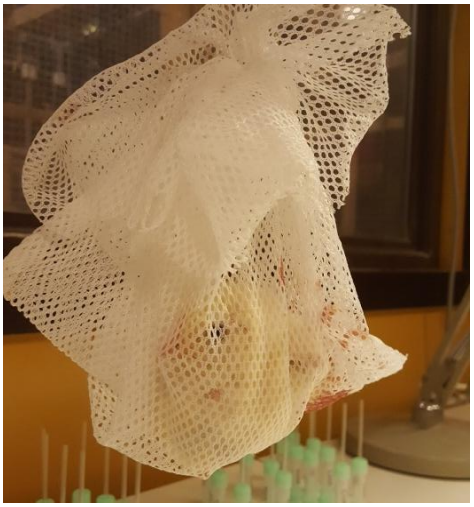


Figure 2. A 6 day old chicken being restrained in a washing bag

Restraint tests were conducted at day 7 and week 6, 26 control and 26 hatchery chickens were used at day 7 and 23 control and 25 hatchery birds were tested at week 6. All chickens were selected randomly from their home pen and then immediately tested. Once the chicken was removed from the home pen a blood sample was taken from the brachial vein within 3 minutes of capture, in order to establish a baseline level of CORT. The chickens were then suspended in a net washing bag for a period of 3 minutes (Figure 2). After suspension a second blood sample was taken to record CORT reaction levels.

Blood samples were also take from 10 males and 10 females from each pen for sex hormone analysis at age 15 and 19 weeks.

All blood samples were collected using a microvette heparin coated tube which holds 200 µl of blood. The blood samples were stored on ice or in a refrigerator until ready for centrifugation in the lab. The plasma was separated from the blood samples and frozen in storage at -40C until the time of analysis using a corresponding ELISA test.

3.6 Novel Arena

Novel arena testing was conducted on day 2 and week 6, 28 control and 28 hatchery birds were tested at day 2 and 24 control and 24 hatchery birds were tested at week 6. All birds were selected at random from their home pen at the time of testing. The arenas were assembled 2x2 (see Figure 3) and all 4 arenas were used simultaneously. Four chickens were placed in

each arena, within a start box with a sliding door closed. As well as a start box, each arena also contained food, water, hay and a novel object (blue pot at day 2 and glove at week 6). At the beginning of the test all chickens were placed in the dark start box and were marked with either a head dot, back dot, tail dot or no dot. Cameras were suspended above the arenas to record the test. Once the cameras were set to record, the sliding doors to the start boxes were opened. The test began when the boxes were opened. The experimenters then left the test room and closed to door to avoid their influence on the tests. After a period of 30 minutes the test finished and the experimenters re-entered the test room, stopped the recordings and reset the arenas for the next group of chickens. All tests were conducted on the same day and each arena contained chickens from the same pen. All recordings were reviewed by one experimenter at a later date using Observer 13 software. For behaviours recorded see Table 2.

Figure 3. Novel arena set ups at 2 days old (left) and 6 weeks old (right)

Table 2. Ethogram showing behaviours recorded during Novel Arena video analysis.

Behaviour	Description
Time spent in start box	Moment of emergence from start box recorded as the time the entire chicken has left the box
Transverses	The number of times the chicken crosses to a different quarter of the arena during locomotion
Distress Vocalisation	A high frequency vocalisation indicating distress, usually observed whilst in an alert state with neck extended and scanning the surrounding area
Escape	Jumping motion at walls, usually accompanied by distress vocalisation
Out of Arena	Chicken successfully escapes arena
Conspecific Peck	A pecking motion, striking a conspecific with beak, non-aggressive, without feather pecking
Activity Patterns	
Stand Relaxed	Standing with no alert head movements, eyes may be partially closed with reduced attention to surrounding, may include performance of foraging or comfort behaviours
Stand Alert	Standing with head extended fully eyes open, attending to the surrounding area, may include performance of distress vocalisation.
Walk	Two or more steps at a slow pace
Run	Two or more steps at a fast pace
Sit	Sitting, legs bent with body touching the ground
Sleep	Stand or sit with eyes closed, neck retracted with no head movements
Exploration	
Ground Peck	A pecking motion with the beak, directed towards the ground or a non-specific object
Food Peck	A pecking motion with the beak, directed specifically towards a food item
Object Peck	A pecking motion with the beak, directed specifically towards a non-food related object
Explore Ground	Head extended downwards, inspecting the ground
Explore Food	Head extended downwards, inspecting food items
Explore Object	Head extended towards object, inspecting object
Bill Rake	Wiping beak in feed, on the ground or against objects
Manipulate Object	Using beak to lift, move or otherwise manipulate object
Food Run	Runs with a food item in beak, usually chased by another individual
Drink	Dipping beak into water source and drinking water
Comfort	
Preen	Using beak to clean and rearrange feathers
Scratch Body	Using feet to scratch body
Stretch Leg	Stretch leg outwards away from body
Stretch Wing	Stretch wing backwards, usually performed with leg stretch
Yawn	Opening the beak, gaping without vocalisation
Feather Ruffle	Erect feathers and shakes body
Dustbathe	Vertical wing shake in seated position, proceeded by rubbing substrate into feathers, bill rakes, followed by feather ruffle to remove substrate

3.7 Tonic Immobility

Tonic immobility tests were conducted at day 5 and week 6, 26 control and 27 hatchery chickens were used at day 5 and 25 control and 24 hatchery chickens were tested at week 6. All chickens were selected randomly from their home pen and carried into the test room. One experimenter was responsible for inducing all tonic immobility and the second experimenter was responsible for timing the experiment. Once in the test room the chicken was placed on its back on a cradle (Figure 4), light pressure was applied to the body with 2 hands for 10 seconds to induce tonic immobility. If the chicken righted itself within 5 seconds it was deemed tonic immobility was not established, and the process was repeated for up to another two times. The time of first vocalisation, first head movement and rightening was recorded as well as the frequency of vocalisations whilst in tonic immobility. Tests were recorded in case there was any uncertainty in timing so it could be reviewed, if needed, at a later date.

Figure 4. A 6 week old male chicken during tonic immobility testing

3.8 Weight

Chickens were first weighed at 1 week old using a scale measuring to the 0.1 g accuracy. The chickens were then weighed weekly until they were 12 weeks old, and then were weighed again at 14 and 16 weeks old. When comparing weight between hatchery and control chickens, males and females were compared separately to allow for weight differences due to sexual dimorphism.

3.9 Hormone Analysis

3.9.1 Corticosterone

CORT analysis was conducted using ELISA kits from ENZO Life Sciences using 96 well plates. Blood plasma samples were thawed at room temperature for 30 minutes prior to beginning the procedure and the ELISA kit was warmed at room temperature for the same period of time. Prior to starting the experiment assay buffer, wash buffer and 1:100 steroid displacement reagent (SDR) was prepared. Blood plasma samples were diluted to requirement and standards of known CORT concentration (pg/mL) (20000, 4000, 800, 160, 32) were prepared immediately before the experiment and used within 1 hour of mixing. 150 µl assay buffer was added to non-specific binding (NSB) wells. 100 µl assay buffer added to Blank wells. Standards and Samples were added to assigned wells. Conjugate was added to all wells except Total and Blank wells. Antibody was then added to all wells except Total, Blank and NSB wells. The well plate was then covered with a plastic sheet and placed on plate shaker for 2 hours incubation at 500 rpm at room temperature. The plate was then aspirated and washed with wash buffer adding 200 µl wash buffer to each well 3 times, inverting between each time and patting dry. 5 µl of Conjugate was then added to Total wells. 200 µl pNpp Substrate added to all wells, covered with plastic and aluminium foil to block the light. Well plate incubated for 1 hour without shaking at room temperature. Stop solution was then added all wells to stop reaction and absorbance at 405 nm immediately read using a plate reader. Samples were then compared to a standard curve; CORT concentrations were extrapolated and then multiplied by their dilution factor.

3.9.2 Testosterone and Estradiol

Testosterone and Estradiol were analysed using corresponding ELISA kits from MyBioSource using the same protocol for both hormones. Samples were thawed and all reagents were warmed to room temperature before use for a period of 30 minutes. A wash buffer was prepared by diluting 15 ml of concentrated wash buffer with 285 ml deionised water. Firstly the Blank wells were assigned to the plate, 50 µl of Standard or Sample were then added to their assigned wells. 50 µl HPR-Conjugate was added to all wells except Blank and then 50 µl Antibody was added to all wells. The plate was then covered with adhesive film and incubated for 1 hour at 37 °C. All wells were then aspirated three times with 200 µl wash buffer administered using a multichannel pipette. Liquid was completely removed each time by inverting plate gently and patting dry. 50 µl Substrate A and 50 µl Substrate B was added to all wells, mixed well; then incubated for 15 minutes at 37 °C. The plate was covered in aluminium foil for this incubation period in order to block light. Finally 50 µl of Stop Solution was added to each well, the highest concentrated standards should develop an obvious blue colour. Optical density was then determined by using a microplate reader set to 450 nm and hormone levels were compared to the standard curve.

3.10 Egg Production

Eggs were collected daily from the onset of lay and were labelled according to the day they were laid and which pen they came from. Eggs were stored in a refrigerator at 15 °C before weighing. All eggs were then weighed using a scale with accuracy to the nearest 0.01 g.

3.11 Feather scoring

All chickens were feather scored for damage to feathers, combs and wattles at 19 weeks of age. They were scored on a scale of 0 – no damage to 3 – severe damage, missing feathers or bleeding wounds on five body parts (head, back, tail, underbelly and wings). Combs and wattles were scored on bruising, number of bruises and open wounds. Feathers were scored on the amount of damage and whether any feathers were missing.

3.12 Statistical Analysis

Results were analysed using R 3.3.2, R Studio, IBM SPSS statistics 24 and Microsoft Office Excel 2007. All data was tested for normality using a Shapiro-Wilks test or visually analysing the data using frequency density plots. Significant levels shall be depicted on graphs using stars (* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$), and "T" shall signify a tendency towards a significant difference. CORT, Estradiol and Testosterone data was extracted from nanoplate reader using a 4-parameter regression curve to extrapolate values from percentage of bound antibodies. Hatchery CORT data was analysed using a Wilcoxon rank sum test with continuity correction comparing both treatment groups and sex. Restraint CORT data, Estradiol and Testosterone data were analysed using a repeated measures ANOVA using auto-correlation function, testing for fixed effects and pseudo R-squared effect. Both treatment and sample (baseline CORT or reaction to physical restraint) were used as factors. The interaction between treatment and sample was further analysed using a leastsquares post-hoc Tukey-test. Tonic immobility data was censored due to a number of individuals remaining in tonic immobility for the maximum time. Cox proportional hazard analysis was used to compare rightening time, first head movement and first vocalisation between control and hatchery chickens. Vocalisation frequency was normalised for the amount of time each individual spent in tonic immobility and then analysed using a Wilcoxon rank sum test with continuity correction comparing treatments and sexes. Novel arena data extracted from Observer 13 into Microsoft excel. The data was then normalised for time each individual spent outside of the start box, then a Wilcoxon rank sum test with continuity correction was used to compare treatments and sexes Egg data was separated into egg weight and number of eggs laid. Number of eggs laid was analysed by normalising the number of eggs laid with the number of hens per pen and then a two-way ANOVA was conducted using both pen number and day in which the egg was laid as factors. A post-hoc Tukey-test was used to analyse the interactions between the numbers of eggs laid per pen per day. Egg weight data was analysed using a Kruskal-Wallis test using pen and day as a factor. A post-hoc Dunn-test with a Benjamini-Yekuteili correction was used to assess the interaction between weight and pen, and weight and day. Feather score data was analysed using a Kruskal-Wallis test using pen and sex as a factor. A Dunn-test was conducting with Benjamini-Yekuteili correction to assess interactions between feather damage and pen.

4 Results

4.1 Hatchery Day

A comparison was made between the CORT levels from the blood samples taken in the commercial hatchery with those taken from the control group. The CORT levels were significantly higher in hatchery chickens after both incubation ($W=16.5$, $p=0.01$) and processing ($W=0$, $p<0.001$) than for the control chickens. The CORT levels also appeared higher after the transportation stage for the samples from the hatchery chickens; however this was not statistically significant ($W=38$, $p=0.4$).

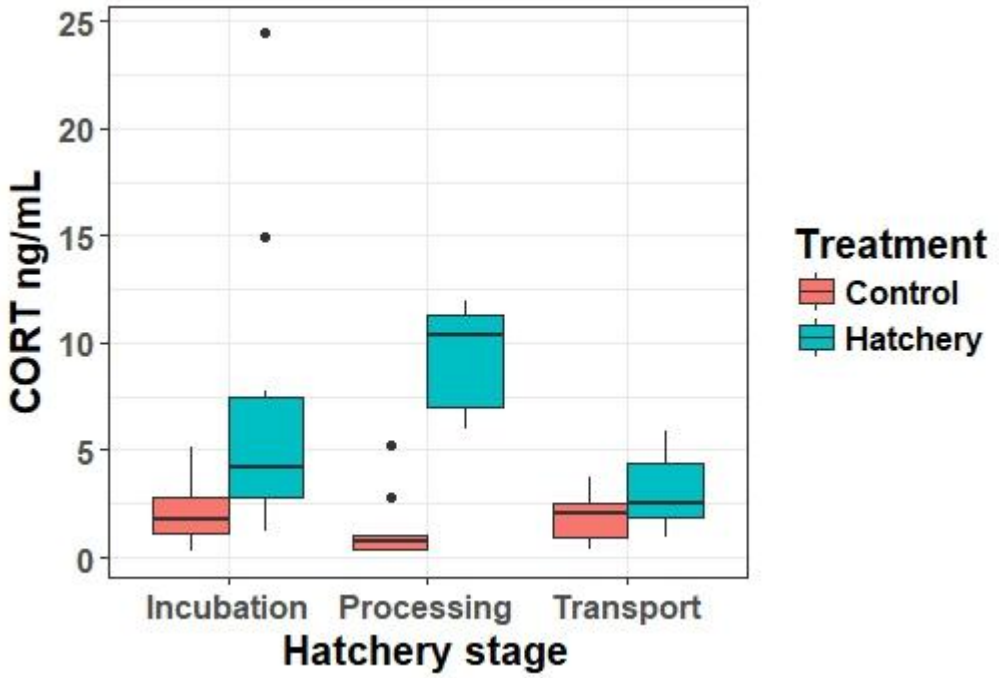


Figure 5. The effect of different stages of the commercial hatchery regime on Corticosterone (CORT) response of commercial hatchery chickens compared to a control group hatched at LiU hatchery. The control group were only moved from the incubator to a home pen. The box plot represents the median CORT values with the upper and lower quartiles and whiskers depicting variation outside of the quartile. Outlying data points are represented by black dots.

4.2 Weight

Visual inspection of the data showed that hatchery chickens, both male and female, weighed more than the control chickens for the duration of the study (Figure 6).

When comparing the male chickens, the hatchery chickens weighed significantly more on weeks: 1, 2, 3, 8, 9, 12, and 14 ($p<0.05$, see Appendix: Table 3 for full list of p values). There was also a tendency on week 10 for the hatchery males to weigh more than control chickens ($p<0.1$). There was no statistically significant difference in weight between hatchery and control chickens on weeks: 4, 5, 6, 7, 11, and 14 ($p>0.1$).

Comparison between female hatchery and control chickens showed that hatchery females weighed significantly more than control females on weeks: 1, 2, 3, 4, 5, 6, 8, 9, 12, and 14 ($p<0.05$). There were no statistically significant differences in weight between hatchery and control chickens on weeks: 7, 10, and 11 ($p>0.1$).

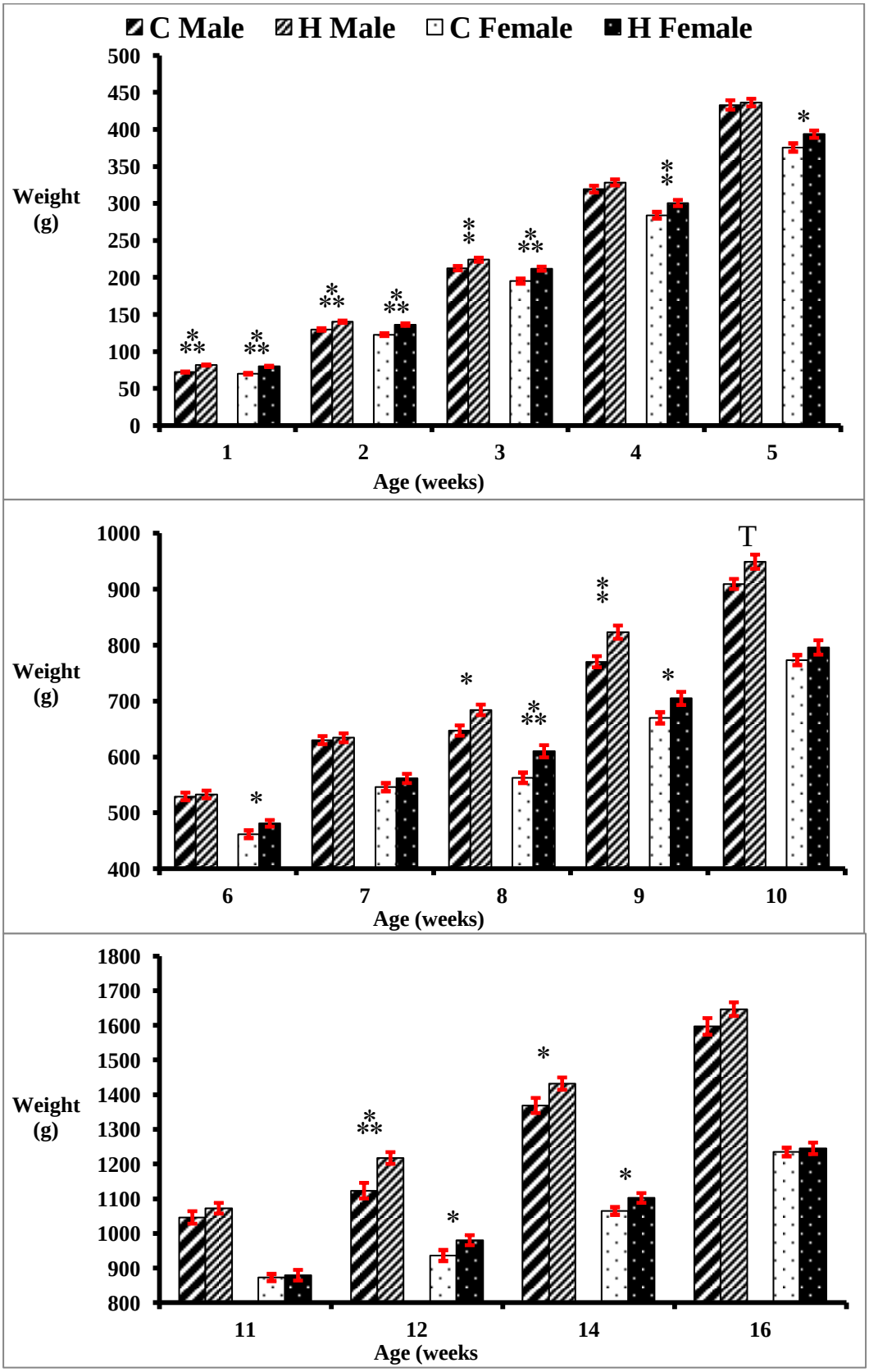


Figure 6. Mean weight (±SE) of male and female chickens weighed weekly comparing hatchery (H) chickens which underwent normal hatchery routines with a control group (C) exempt from hatchery routines.

4.3 Novel Arena

4.3.1 Novel arena testing conducted at 2 days old

When looking at the percentage time performing behaviours the hatchery chickens spent a significantly lower percentage time performing the following behaviours; total exploration (Figure 7: C), walking, standing relaxed (Figure 7: D), preening, dustbathing, exploring ground, exploring objects ($p<0.05$, for complete list of p values see: Appendix: Table 4). The hatchery group spent a significantly higher percentage time in start box ($p<0.05$) (Figure 7: A) and a tendency towards a higher percentage time escaping ($p<0.1$). Lower rate per minute; transverses (Figure 7: B), conspecific pecks (Figure 7: F), total explorative pecks, comfort behaviours (Figure 7: E), feather ruffle, ground pecks, object pecks ($p<0.05$).

When comparing hatchery males to control males it was found that hatchery males spent a lower percentage time performing; total exploration, standing relaxed, exploring ground, exploring object, preening ($p<0.05$). Hatchery males also spent a higher percentage time in start box ($p<0.05$). Hatchery males also had a lower rate per minute for the following behaviours when compared to the control males; total explorative pecks, comfort behaviours, ground pecks, object pecks ($p<0.05$). There was also a tendency towards hatchery males performing transverses and scratch body at lower rate per minute when compared to control males ($p<0.1$).

When comparing hatchery females to control females, hatchery females spent a significantly lower percentage time walking, standing relaxed, and exploring the ground ($p<0.05$). Hatchery females also had a tendency towards spending a higher percentage time in the start box and standing alert ($p<0.1$). Hatchery females also had showed tendency towards a lower percentage time spent sleeping ($p<0.1$). When compared to control females, hatchery females performed the following behaviours at a significantly lower rate per minute; feather ruffles, ground pecks, object pecks ($p<0.05$). Hatchery females also showed a tendency towards performing transverses and total explorative pecks at a lower rate per than control females ($p<0.1$).

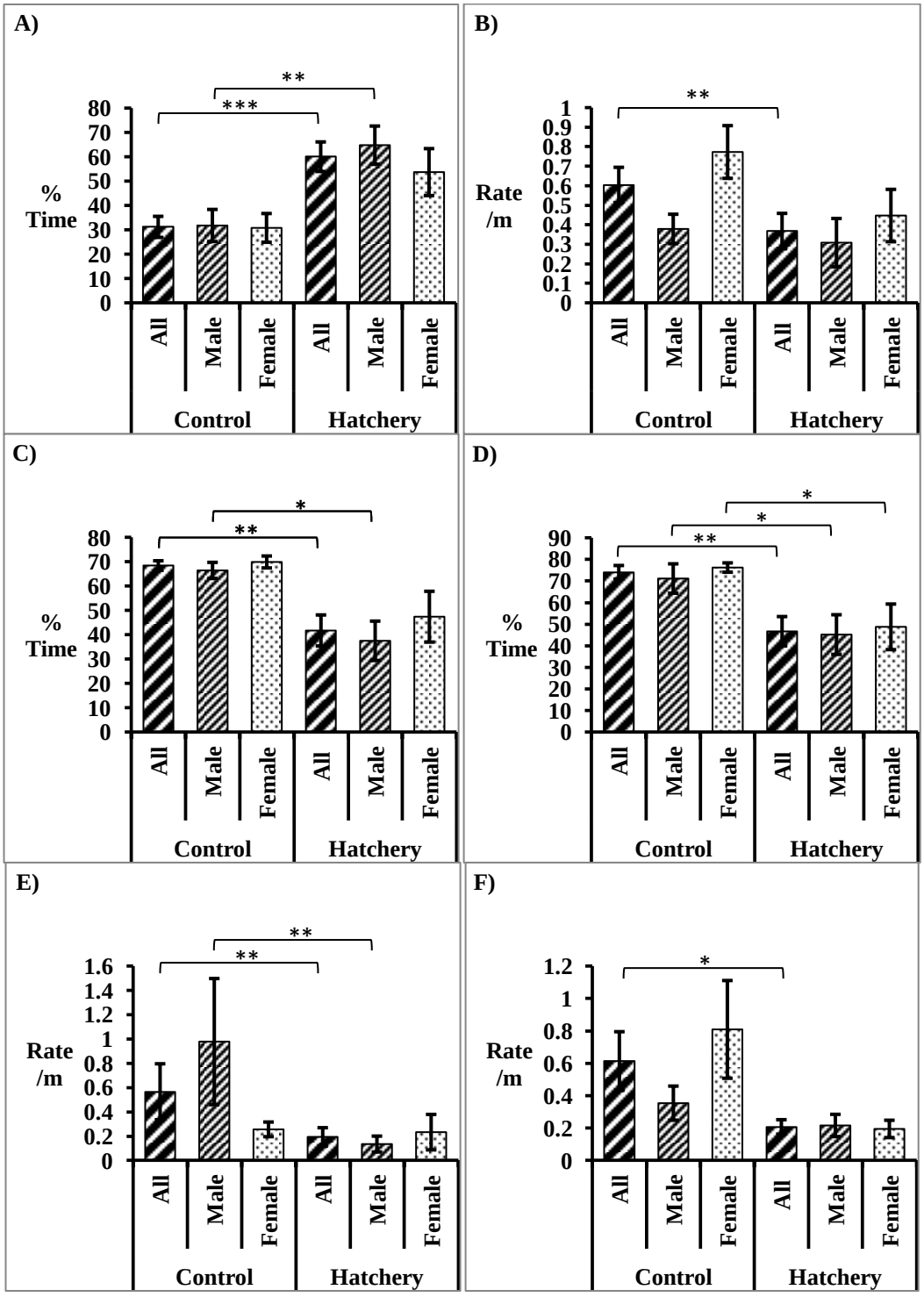


Figure 7. Behaviours observed from novel arena testing of 2 day old commercial leghorn chickens after experiencing hatchery routines compared to a control group which did not experience hatchery routines. A): Percentage time spent in start box for the duration of testing. B): The rate of transverses between different sections of the arena per minute spent outside the start box. C): Percent time spent performing exploration behaviours after leaving the start box. D): Percentage time spent standing in a relaxed state after exiting the start box. E): The rate of comfort behaviours performed per minute after leaving the start box. F) The rate of pecks towards a conspecific per minute after leaving the start box. The graphs depict either mean rate per minute or mean percentage time ± SE.

4.3.2 Novel arena testing conducted at 6 weeks of age

The results of the novel arena testing conducted at 6 weeks of age showed that hatchery chickens spent a significantly lower percentage time running (Figure 8: C) and drinking (Figure 8: B) when compared to control chickens ($p<0.05$, for full list of p values see Appendix: Table 5). Hatchery chickens also showed a significantly lower rate of feather ruffles and conspecific pecks (Figure 8: D) per minute than control chickens ($p<0.05$), however hatchery chickens showed a tendency to perform a higher rate of wing stretches per minute than control chickens ($p<0.1$).

When comparing the results for hatchery males versus control males, hatchery males performed a significant lower percentage time running that control males ($p<0.05$). Hatchery males also showed a tendency to perform a lower rate of feather ruffles per minute than control males ($p<0.1$).

Hatchery females performed drinking behaviours for a significantly lower percentage time than control females ($p<0.05$). Hatchery females, however, showed a tendency to spend a higher percentage time sitting relaxed than control females ($p<0.1$). When looking at feather ruffle behaviours, hatchery females showed a tendency to perform this behaviour at a lower rate than control females ($p<0.1$). Hatchery females also performed leg stretching, wing stretching and all comfort behaviours (Figure 8: A) at a significantly higher rate per minute than control females.

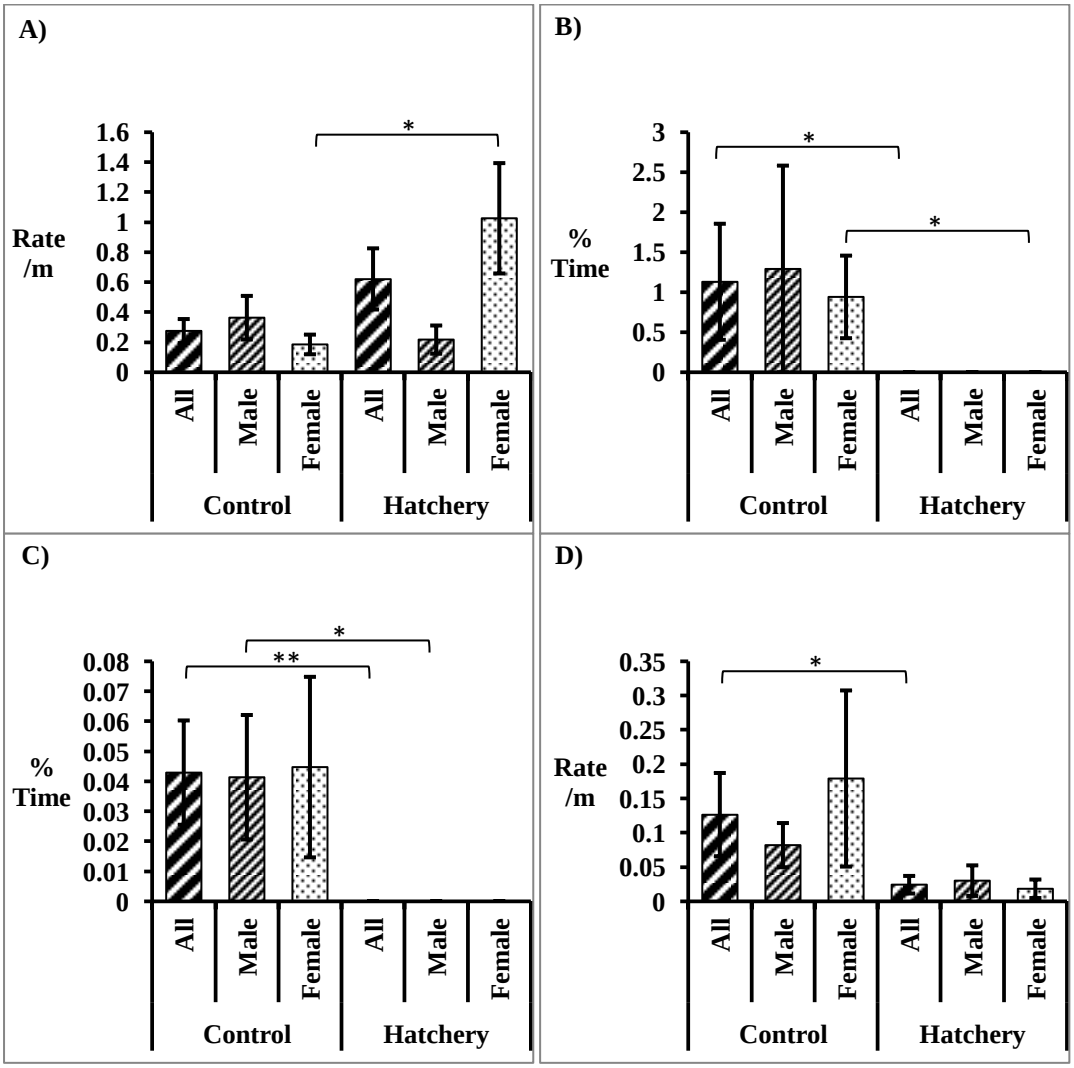


Figure 8. Behaviours observed during novel arena testing commercial leghorn chickens at 6 weeks of age after experiencing hatchery routines compared with those of a control group which did not undergo hatchery routines A) The rate in minutes of comfort behaviours performed after exiting the start box. B) Percentage time spent drinking after leaving start box. C) The percentage time spent running whilst outside start box. D) The rate of conspecific pecks performed after leaving start box. The graphs depict either mean rate per minute or mean percentage time ± SE.

4.4 Tonic immobility

4.4.1 Tonic immobility conducted at 5 days old

Tonic immobility testing conducted on day 5 of the experiment found that there was a tendency towards hatchery males having a longer latency until first vocalisation during tonic immobility that control males ($p<0.1$, for detailed p values see Appendix: Table 6) (Figure 9: left). There were no significant differences when comparing the hatchery group with control group, or hatchery females with control females, and when comparing males and females regardless of treatment group ($p>0.1$).

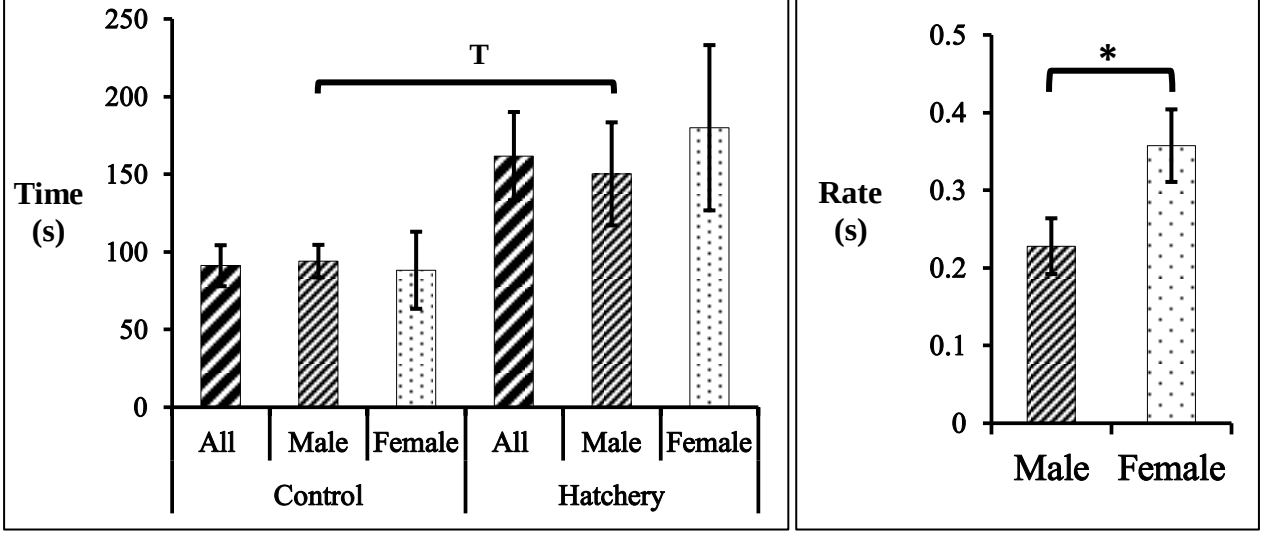


Figure 9. Tonic immobility testing conducted on 5 day old commercial leghorn chickens comparing chickens which underwent normal hatchery routines and a control group which did not undergo hatchery routines. Left: Average time of first vocalisation after tonic immobility has been induced. Right: Rate of vocalisations performed during tonic immobility, comparing males and females without taking treatment into account.

Analysis of vocalisation rate during tonic immobility showed a significant difference in rate of vocalisations between male and female chickens without taking treatment into account, with females vocalising at a higher rate than males ($p<0.05$) (Figure 9: right). When comparing the effect of sex within treatment groups a significant difference was found between control females and males; control females vocalised more frequently than control males ($p<0.05$). However no significant differences were found

when comparing treatments, between control males and stressed males control females and hatchery females ($p>0.1$).

No significant differences were found when comparing latency until first head movement or rightening time between control and hatchery chickens, control and hatchery males, control and hatchery females, or males and females regardless of treatment ($p>0.1$)

4.4.2 Tonic immobility conducted at 6 weeks of age

When analysing results for tonic immobility testing at 6 weeks of age hatchery males performed vocalisations at a significantly higher rate during tonic immobility than control males and hatchery females ($p<0.05$, a full list of p values in Appendix: Table 7) (Figure 10: left). A tendency was also found towards control males having a longer latency to righten than hatchery males ($p<0.1$) (Figure 10: right).

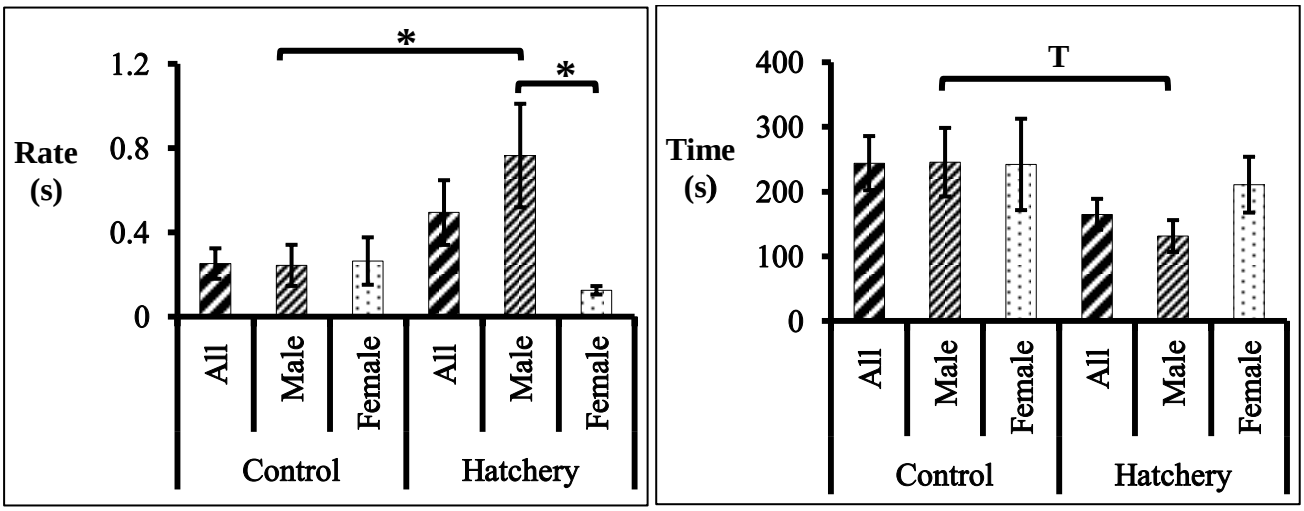


Figure 10. Tonic immobility testing conducted on 6 week old commercial leghorn chickens, comparing stressed chickens with a control group at 6 weeks of age. Left: Average vocalisation frequency per second during tonic immobility. Right: Average latency to righten after induction of tonic immobility.

There were no significant difference found for latency until first head movements and first vocalisation when comparing treatment groups, sexes within treatment groups, and sexes between treatment groups ($p>0.1$). No significant differences were found between control and hatchery group, control and hatchery females, males and females regardless of treatment group, and males and females within treatment groups for latency until first

vocalisation ($p>0.1$). There were also no significant differences between control and hatchery group, control and hatchery females, males and females regardless of treatment group, and males and female control chickens for vocalisation frequency during tonic immobility ($p>0.1$).

4.5 Restraint test

4.5.1 Restraint test conducted at 7 days old

The results of the restraint test conducted at day 7 shows that, logically, there is a significant difference in the least-square mean CORT levels between baseline and reaction to 3 minutes restraint ($\chi^2=37.6506$, $p<0.001$). There was also a significant difference between treatments, ($\chi^2=6.1321$, $p=0.01$), hatchery chickens had significantly higher CORT levels than the control chickens (Figure 11). When looking at the interaction between treatment and sample, there was a significant result ($\chi^2=6.7169$, $p=0.01$), meaning that both treatment and sample explains variation within the data. Conducting a post hoc analysis showed that there was no significant difference for baseline CORT levels between control and hatchery chickens ($t=0.575$, $p=0.9$). However there was a significant difference between control and hatchery reactions samples, which relates to CORT response to a 3 minute restraint ($t=3.184$, $p=0.01$).

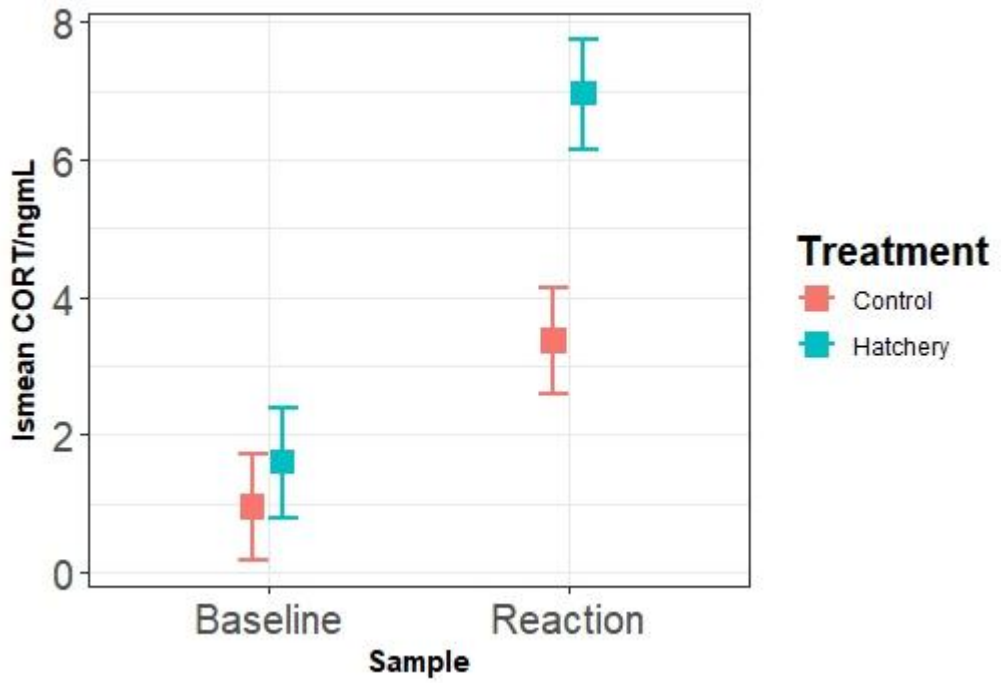


Figure 11. Leastsquare mean CORT levels of baseline and reaction blood samples taken before and after a three minute restraint test conducted on 7 day old commercial leghorn chickens. The results are comparing a hatchery group which underwent normal hatchery routines with a control group which did not go through hatchery routines.

4.5.2 Restraint test conducted at 6 weeks old

The results of the restraint test conducted at 6 weeks of age shows that hatchery chickens have a significantly higher least-square mean circulatory CORT than the control chickens (χ^2=3.7165, p=0.05) (Figure 12). When conducting a post-hoc analysis it showed that there was a significant difference between control and hatchery CORT values for sample 2, hatchery individuals had a significantly higher CORT level after 3 minutes of restraint than the control chickens (t=2.796, p=0.04). There was no significant difference between control and hatchery chickens for baseline CORT samples (t=0.093, p=0.9).

Predictably there was a higher level of CORT in the second sample, response to 3 minute restraint, than the baseline sample (χ^2=23.4029, p<0.001). There was also a significant interaction between treatment and sample (χ^2=4.1630, p=0.04), meaning that both treatment and sample account for variation within the data.

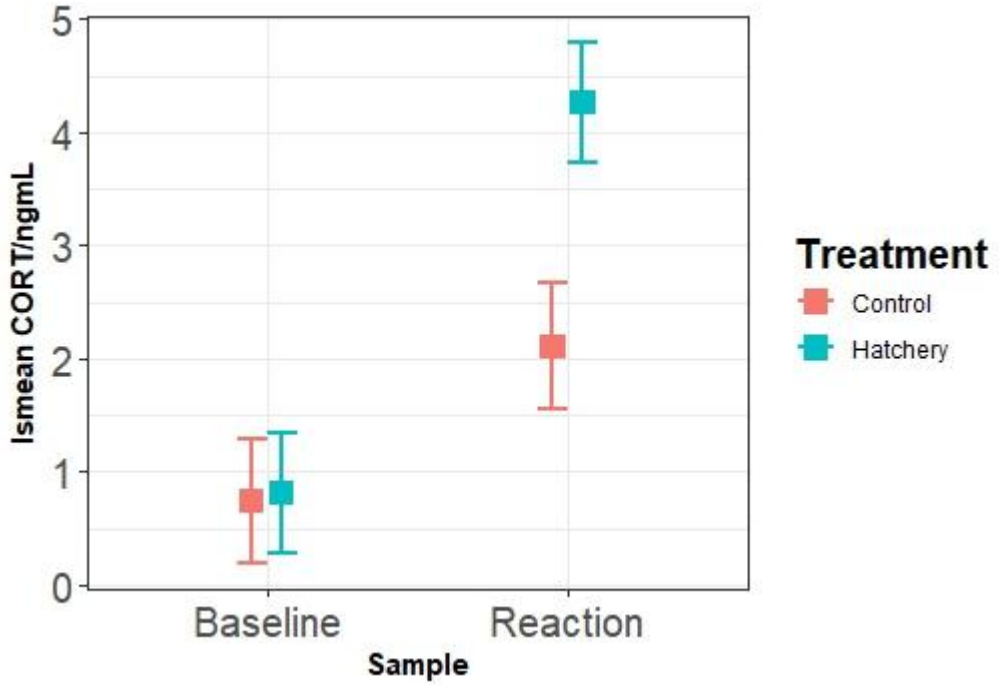


Figure 12. Leastsquare mean CORT levels of baseline and reaction blood samples taken before and after a three minute restraint test conducted on 6 week old commercial leghorn chickens. The results are comparing a hatchery group which underwent normal hatchery routines with a control group which did not go through hatchery routines.

4.6 Gonadal Hormone analysis

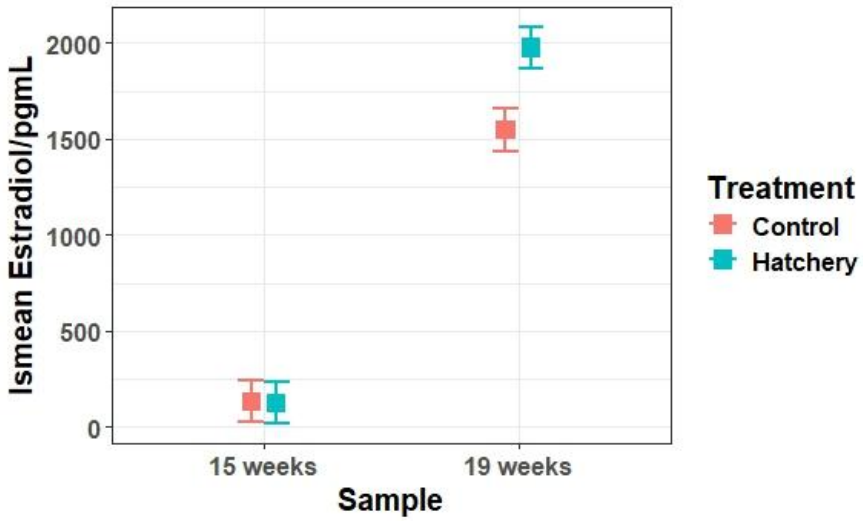
4.6.1 Estradiol

When comparing estradiol levels between control and hatchery females (Figure 13: A), there was a tendency that hatchery females have higher levels of estradiol than control females (χ^2=3.6674, p=0.06). There was also a significant interaction between treatment group and sample (χ^2=4.0231, p=0.04). After conducting a post hoc Tukey test, there was no significant difference between control and hatchery female for the sample taken at 15 weeks of age (t=-0.052, p=0.9). However there was a significant difference between control and hatchery for sample 2 taken at 19 weeks of age (t=-2.772, p=0.04).

The effect of home pen was also analysed (Figure 13: B), there was no significant differences between pens (χ^2=5.6142, p=0.1) and no interaction between pen and estradiol sample (χ^2=6.2133, p=0.1). The post

hoc analysis of the effect of pen on estradiol showed that there were no significant differences when comparing the results for the 15 weeks samples between pens ($p>0.05$). However when comparing pen differences for the 19 weeks sample there was a significant difference between pen 1 and 4 ($t=-3.258$, $p=0.05$). There were no other significant differences between the other pens when comparing the results for sample 2 ($p>0.05$).

(A)



(B)

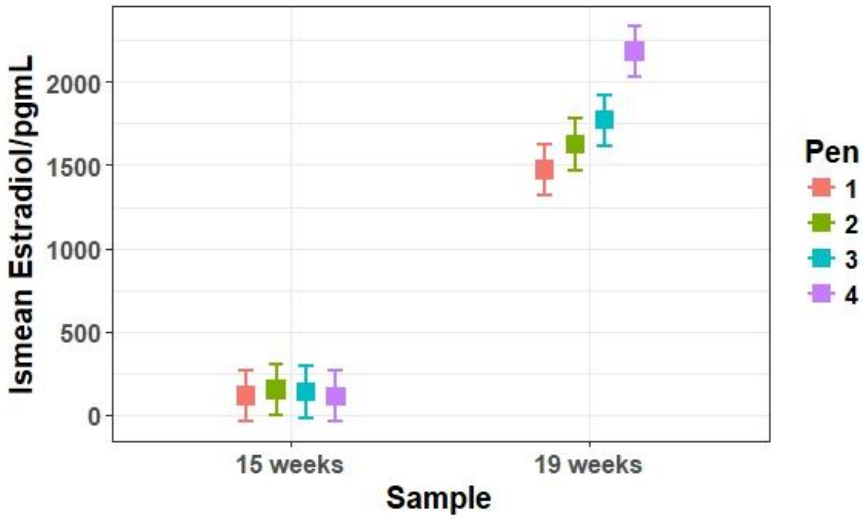


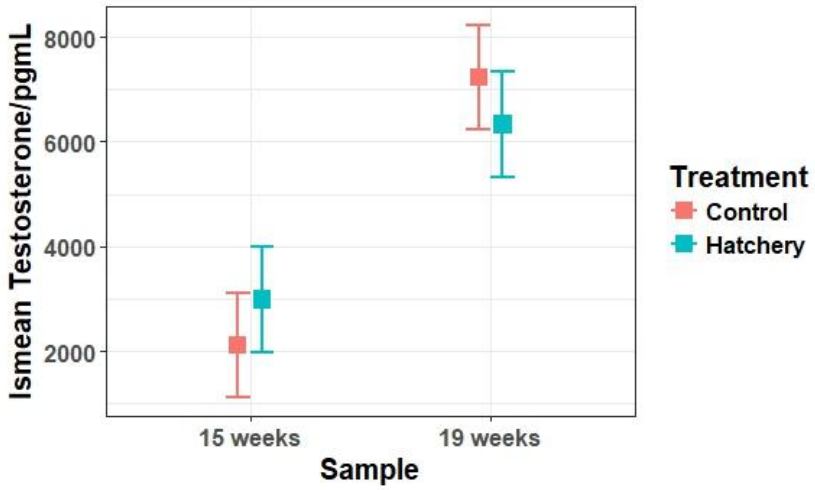
Figure 13. Estradiol levels taken at 15 weeks and 19 weeks of age from hatchery group chickens which underwent normal hatchery routines with control chickens which did not experience hatchery routines. A) Comparing control and hatchery females. B) Comparing differences in estradiol between pens (pen 1 and 2: control, pen 3 and 4: hatchery).

4.6.2 Testosterone

When comparing testosterone levels between control and hatchery males (Figure 14: A), there were no significant differences between the testosterone levels of control and hatchery males (χ^2=0.0001, p=0.9). After conducting a post hoc Tukey test, there was no significant difference between control and hatchery males for the sample taken at 15 weeks (t=-0.620, p=0.9) or at 19 weeks (t=0.635, p=0.9).

The effect of home pen was also analysed (Figure 14: B), there was a significant difference in testosterone between pens (χ^2=5.6142, p=0.02) and the interaction between pens and testosterone samples (χ^2=10.4755, p=0.01). The post hoc analysis of the effect of pen on testosterone showed that there were no significant differences when comparing the results for the sample taken at 15 weeks between pens (p>0.05). However when comparing pen differences for the sample taken at 19 weeks there was a significant difference between pen 2 and 3 (t=3.491, p=0.03), and pen 2 and 4 (t=-3.547, p=0.02). There were no other significant differences between the other pens (p>0.05).

(A)



(B)

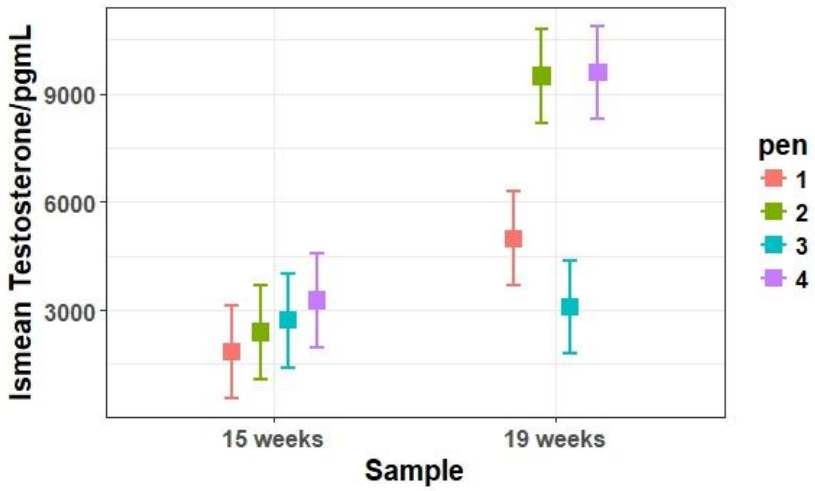


Figure 14. Testosterone levels from blood samples taken at 15 weeks and 19 weeks of age from hatchery chickens which underwent normal hatchery routines and a control group which did not. A: Comparing control and hatchery males. Right: Comparing differences in testosterone levels between pens (pen 1 and 2: control, pen 3 and 4: hatchery).

4.7 Egg production

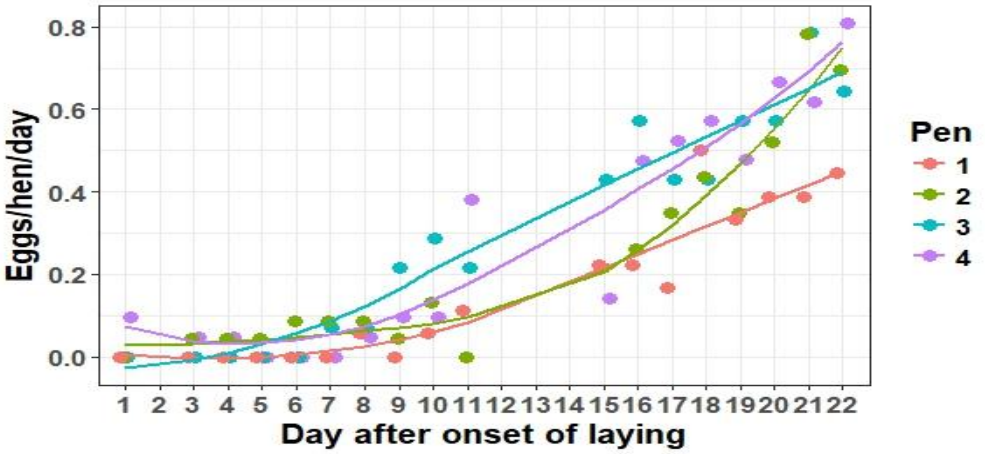
Comparison of the number of eggs laid per hen in different pens showed that there was a significant difference between pens ($F=19.249$, $p<0.001$). There was also a significant interaction between pen and day ($F= 4.97$, $p=0.007$). Visual inspection of the data shows that the number of eggs laid pen hens in pen 1 was lower for the duration of the egg collection (Figure 15: A). Pen 2, 3 and 4 appear to end up with a similar number of eggs per

hen laid each day; however the trends of laying uptake are visually different for each pen. A post hoc analysis of differences between pens showed that there was a significant difference between number of eggs laid in pens; 1 and 2 ($p=0.02$), 1 and 3 ($p<0.001$), 1 and 4 ($p=0.003$), 2 and 3 ($p=0.04$), and a tendency was found between pen 2 and 4 ($p=0.09$). There was no significant difference in the number eggs laid per hen between pen 3 and 4 ($p=0.9$).

The weight of eggs per day was also analysed. Visual inspection of the data shows that for each pen the weight of eggs increases over the sampling period (Figure 15: B). There is a significant difference between egg weight and the day in which the egg was laid ($\chi^2 = 89.233$, $p<0.001$). A post hoc analysis found a significant difference in egg weights ($p<0.05$) between days; 19 and 11, 20 and 10, 20 and11, 20 and 15, 21 and 10, 21 and 11, 21 and 15, 22 and 10, 22 and 11, 22 and 15, 22 and 16, 22 and 17, 8 and 19, 8 and 20, 8 and 21, 8 and 22, 9 and 21, 9 and 22.

When comparing the egg weight per pen there was a significant difference in egg weight between pens ($\chi^2=9.2882$, $p=0.03$) (Figure 15: C). A post hoc analysis found that there was a tendency when comparing egg weight between pen 2 and 4 ($p=0.07$). There was no significant difference between the other pens ($p>0.05$).

(A)



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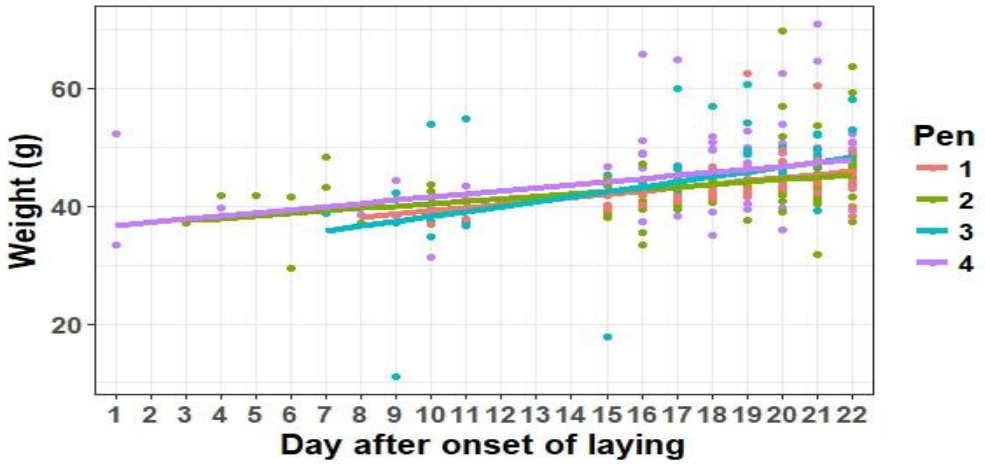


(C)

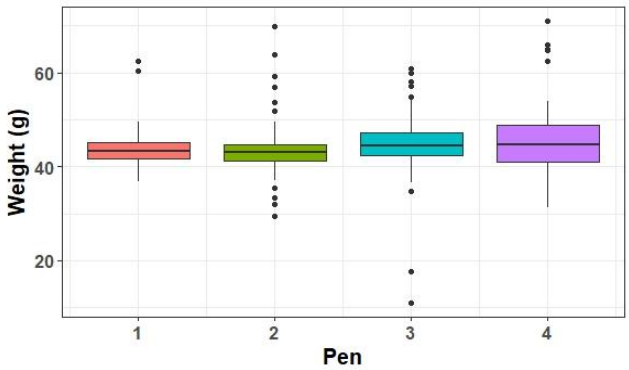


Figure 15. Analysis of egg data from point of lay commercial leghorn chickens between hatchery group (pen 3 and 4) which underwent normal hatchery routines and a control group (pen 1 and 2) which did not. A) Number of eggs laid per day for each pen after onset of laying with a trend line. B) Comparison of egg weight dependant on the day in which the egg was laid and from which pen showing a linear model. C) The effect of pen on egg weight, the box plot shows median egg weights, upper lower quartiles, whiskers and outliers.

4.8 Feather condition

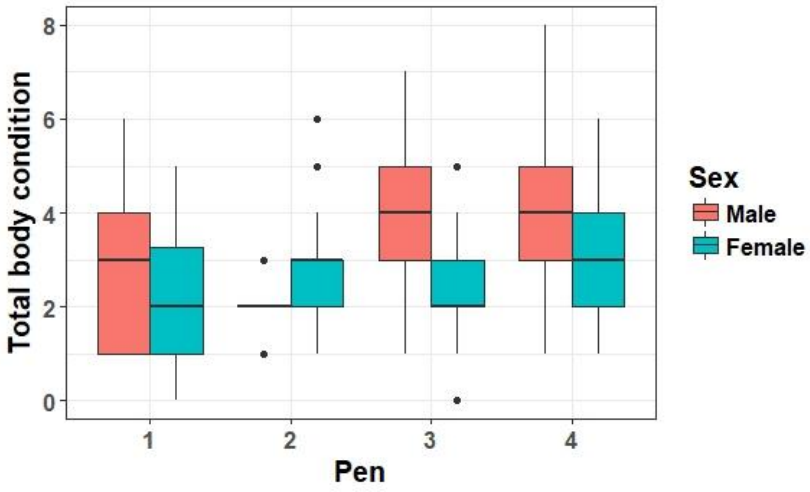
Assessment of body condition at the end of experimentation showed that there was a significant difference in combined feather, comb and wattle scores different pens ($p<0.5$, for full list of p values see Appendix: Table 8) (Figure 16: A). A post-hoc Dunn-test showed that hatchery pens had significantly higher feather damage than control pens ($p<0.05$). The results were not significant when comparing pens from the same treatment group ($p>0.1$).When comparing the total feather damage between pens for just males the difference was also significant. A post-hoc Dunn-test was also conducted on the male data, with significant or tendency towards differences between pens containing chickens from different treatment groups ($p<0.1$). There were no significant differences between pens of the same treatment groups ($p>0.1$). There were no significant differences between pens when comparing just females ($p>0.1$). When comparing sexes within treatment groups hatchery males had significantly higher feather condition scores than hatchery females ($p<0.05$), however, there was no significant difference between control male and females ($p>0.1$).

When analysing the feather scores separately, hatchery pens also had significantly higher feather damage than control pens ($p<0.05$) (Figure 16: B). A post-hoc Dunn-test showed significant differences between pen 2 and 3, and pen 2 and 4 ($p<0.05$). A tendency was found between pen 1 and 3 ($p<0.1$). There was no significant differences between; pen 1 and 2, pen 1 and 4, and pen 3 and 4 ($p>0.1$). Hatchery males also had significantly higher feather damage than control males ($p<0.05$). Analysis of pen differences found a significant difference between pen 2 and 3 ($p<0.05$), and tendencies were found between pen 1 and 3, and pen 2 and 4 ($p<0.1$). There were no significant differences between the other pens ($p>0.1$). There were also no significant difference when comparing females of different treatment groups, control males and females, and hatchery males and females ($p>0.1$).

Comb and wattle scores were also analysed separately (Figure 15: C). Hatchery chickens showed significantly higher comb and wattle damage than control chickens ($p<0.05$). After a post hoc analysis of pen differences a significant interaction was found between pen 1 and 4 ($p<0.05$), and a tendency was found between pen 2 and 4 ($p<0.1$). There were no other significant interactions between pen ($p>0.1$). Hatchery males also had significantly higher feather damage than control males and a significant interaction was found between pen 1 and 4, and pen 2 and 4 ($p<0.5$). There were no other significant interactions when comparing

males of different pens ($p>0.1$). There was also a tendency towards hatchery females having higher comb and wattle damage than control females ($p<0.1$), however upon further analysis there were no significant interactions between pens ($p>0.1$). When comparing sexes within treatment groups it was found that hatchery males had significantly higher comb and wattle damage than hatchery females ($p<0.05$), however, there was no significant differences when comparing control males and females ($p>0.1$).

(A)



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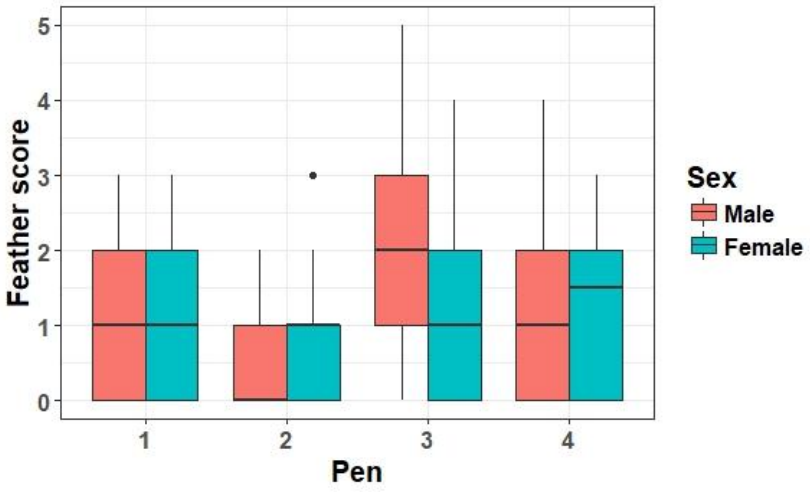

(C)

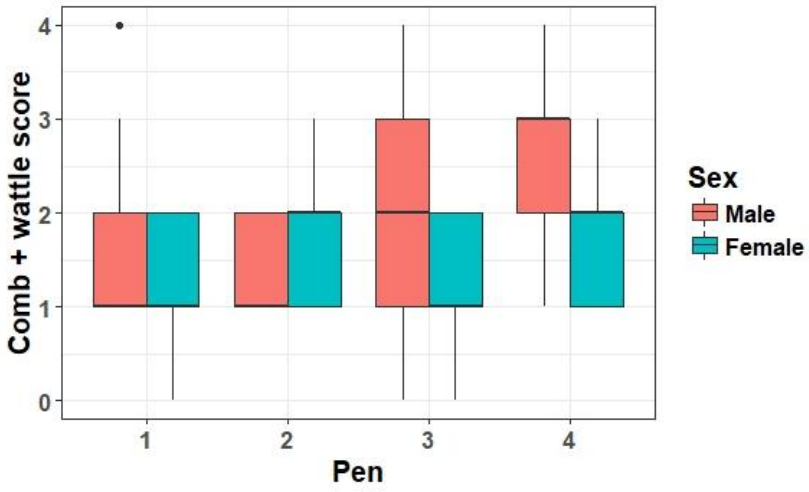

Figure 16. Body condition scoring conducted at 20 weeks of age on 2 pens of control (pen 1 and 2) and 2 pens of hatchery chickens (pen 3 and 4). The hatchery chickens underwent normal hatchery routines, whereas the control group did not. A) Combined feather, comb and wattle scores. B) Feather condition scores. C) Comb and wattle scores.

5 Discussion

The present study found that hatchery routines caused high levels of CORT after incubation and completion of hatchery routines when compared with the control group. The acute effects of hatchery routines include; stronger CORT reaction, decreased exploration and comfort behaviours. The chronic effects include; stronger CORT reaction, increased feather damage, increased comfort behaviours in females, more eggs per day and higher estradiol levels. Comparing hatchery males to females: males performed fewer comfort behaviours at 2 days and 6 weeks, more vocalisations in TI at 6 weeks, and had increased feather damage.

5.1 Effect of hatchery routines

In the present study it was found that hatchery chickens had higher levels of circulatory CORT than the control chickens for both the incubation samples and the samples take after the hatchery process was completed. One possible explanation for the hatchery chickens having higher CORT levels than the controls after being removed from the incubator; could be that in the commercial hatchery the incubators are much louder than the incubators used to hatch the control chickens. Commercial incubators contain large fans to circulate the air evenly throughout the incubator, which as a result cause high levels of noise for the developing and hatching chickens. Research into the effect of noise on embryonic development in chickens appears sparse; Stadelman (1958) as cited by Campo et al., (2008), suggests that there are no effects of sounds below 96dB on the developing chickens. In contrast to this, when comparing variables between the incubation methods for each treatment group, the biggest difference between egg treatment appeared to be the level of noise in a commercial hatchery compared to a research hatchery. Further research into this could include measuring noise levels between the treatment groups, to give a more accurate comparison, especially considering that noise stress in adult birds has been shown to increase the heterophil to lymphocyte ratio when a treatment group exposed to high levels of noise (90dB) compared to control group which experienced only baseline levels of noise (65dB) (Campo et al., 2005).

The differences in CORT levels between the hatchery group after hatchery processing compared to the control group, which remained in their home pen since removal from the incubator, may be easier to explain than the incubation samples. During the hatchery process in the present study the stressed treatment group were subjected to manual manipulation

multiple times, including; removal from hatching trays, wing sexing and vaccinations. This manual handling could be a source of stress for the hatchery treatment group. Handling of chickens during the hatchery process has been shown, in previous research, to increase levels of circulatory CORT in chickens (Ericsson and Jensen, 2016). Some companies have tried to eliminate this stress by almost completely mechanising the process with mechanical sorting of hatched chickens from egg shells, mechanical vaccination using either sprays or vaccinating *in ovo*, conveyer belt systems for transportation around the hatchery, and finally transported on ascending speed conveyer belts into an automatic chick counter. Full automation of the hatchery process means that the only time chickens would need to be handled manually would be when sex sorting (Appleby et al., 2004).

Although visually there was a difference in CORT levels between control and stressed chickens after transportation, this result was not statistically significant. One explanation for this could be that during the 3 hour transportation time between the commercial hatchery and the Linköping University (LiU) hatchery, the hatchery chickens may have become habituated to their transportation boxes. Another possible explaination to the non-significant levels of CORT after transportation is that by the time of arrival at LiU hatchery the stress experienced had caused a decrease in the level of circulatory CORT due to the negative feedback loop associated with the HPA-axis (Wang et al., 2014). In order to prevent overreactions to CORT which can cause injury, homeostasis must be re-established (Wang et al., 2013); as a result any further stressors may cause blunted activation of the HPA-axis (Morgan and Tronberg, 2007).

5.2 Acute effects of hatchery routines

Previous research has found that stress experience in early life can cause prolonged glucocorticoid release can have a plethora of acute negative consequences on development, including decreased weight, changes in metabolism, and increased fearfulness (Scanes, 2016). Contrary to the previous statement, in the present study it was found that the hatchery chickens weighed more than the control birds, not only initially, but for the duration of the study. Research into the effect of stress, more specifically the effect of CORT, on weight is very contradictory. Wang et al. (2013) found that administering CORT to broiler chickens water decreased the weight for the remainder of the experiment when compared to control groups which were not administered CORT. Another study found that,

although there was no difference in the weight at hatch, between chicks which were injected with CORT in the egg and control, CORT injected chicks weighed less at both 1 and 4 weeks than control (Janczak et al., 2006). Both these studies contradict the findings in the present study.

One explanation for increased weight in the hatchery group could relate to a possible increase of feed efficiency due to early life stress. Gross and Siegel (1980) found that early life stress in chickens increased feed efficiency and increased weight for a line selected for low antibody reaction compared to a group selected for high antibody reaction. Increased feed efficiency in early stressed chickens would most likely account for increased weights. Further research into the effects of hatchery related stress could involve feed efficiency calculations by measuring the volume of food consumed by each treatment group.

Another possible explanation for hatchery individuals weighing more throughout the present study compared to the control chickens could relate to conditioning hormesis. Conditioning hormesis refers to exposure to mild stressors, in the present study this would be hatchery related stress, enabling an individual to be able to cope with subsequent exposures to stress more effectively than a control group (Constantini, 2014; Monaghan and Haussmann, 2015). This would suggest that by exposing chickens to hatchery related stress, they are more tolerant to stressors, such as behavioural testing, weighing and handling in later life than a control group. Therefore if the control group are less able to cope with stressors, it explains why they might weigh less than the hatchery group. The weight data in the present study further supports this notion due to an increase in the differences in weight between the hatchery group and the control group of chickens after all birds were moved from the hatchery facility to the adult chicken facility (at 5 weeks old), and after the second round of behavioural testing (at 6 weeks old).

Hatchery routines appears to have acute effects on the behaviour of chickens up to 1 week of age. In the present study when comparing hatchery chickens with control chickens by challenging them in a novel arena test, hatchery chickens appeared to show more fearfulness or stress when exposed to a novel environment. Hatchery individuals took significantly longer to emerge from the start box than the control chickens, in fact some hatchery individuals did not leave the start box for the entire duration of the experiment (n=8). This would suggest that hatchery individuals were more fearful of entering a novel environment. Hatchery individuals also distress vocalised and attempted to escape the arena at a higher rate than control chickens. Control chickens were more relaxed throughout the novel arena test compared to hatchery chickens, they

performed more exploration behaviours, spent more time in a relaxed state, performed more comfort behaviours and transversed between different sections of the arena more frequently than hatchery chickens. These results would suggest that hatchery routines have an acute negative impact on the behaviour of chickens. A study conducted by Nicol et al. (2015) suggests that exploration and comfort behaviours in chickens are important indicators of not only negative welfare, but also positive welfare. Therefore, hatchery individuals performing less exploration and comfort behaviours suggests that they may have had their welfare compromised by hatchery routines compared to control chickens.

A similar occurrence was observed by Elfwing et al. (2015) in a study looking at the effects of early stress on open field behaviour. This study found that early stressed birds, particularly males, moved and explored in the open field less than non-stressed birds. This result was in contradiction to previous research within the same group by Goerlich et al. (2012) which found no effect of stress on open field behaviour. To further support the findings in the present study, it has been found that chickens treated with corticosterone performed a higher frequency of distress vocalisations during an open field test compared to a control group (Freire et al., 2006), this corroborates with the occurrence of a higher frequency of distress vocalisation performed by hatchery chickens in the present study.

The results of the first TI test conducted in the present study at 5 days old may also contribute to the hypothesis that hatchery individuals are have a stronger acute reaction to stress than the control chicken. Although there was no difference between the latency to righten and first head movement between control and hatchery treatments there was a tendency when comparing latency to first vocalise between males of both treatments. Upon visual inspection of the data it also shows that hatchery individuals had a longer latency to first vocalise than control individuals. This might suggest that control individuals began to wake from tonic immobility earlier than hatchery individuals, meaning they were less stressed than the hatchery individuals. In contrast to the present study, research conducted by Ericsson et al. (2016), found that an early stressed group had a shorter latency to righten and first move head compared with a control group. One possible explanation for the differences between the results from this study and the present is the stressor. In Ericsson et al. the stressor was food frustration on 2 separate days and social isolation for 2 separate days, in the present study the stressors received throughout the commercial hatchery process may have cause higher stress responses than the afore mentioned study. Elfwing et al. (2015) found that early stressed males during TI had a shorter latency to move their head than control males, suggesting that early

stressed individuals do not respond similarly to control individuals in test measuring the effects of acute stress. The findings by Elfwing et al. also contract the findings in the present study that hatchery individuals respond more strongly to tonic immobility testing than control individuals.

The present study also supports the hypothesis that hatchery chickens are more stressed than control chickens during early life, through the results from the first restraint test at 7 days old. Hatchery individuals showed a higher level of circulatory CORT levels after restraint than the control individual. This shows that they have higher HPA-reactivity to a 3 minute restraint period than control chickens. This could be explained by either, hatchery chickens produce greater amounts of CORT in response to a stressor or they may have altered HPA-activity as a result of CORT exposure. Having fewer binding sites could be the result of early physiological changes due to the stress (elevated glucocorticoid levels) experienced within the commercial hatchery. A study conducted by Wang et al. (2013) explored the effect of CORT administration on gene expression relating to HPA-activity. In this study it was found that low TI broilers had increased numbers of glucocoticoid receptors in the hypothalamus, the main area contributing to the negative feedback loop in the HPA-axis. This insinuates that short TI broilers had a more efficient feedback when exposed to high levels of corticosterone. It is possible that exposure to stress from commercial hatchery routines in the present study altered the expression of HPA related genes, leading to the stressed chickens to have different HPA reactivity to the control chickens.

5.3 Chronic effect of hatchery routines

The chronic effects of hatchery routines appears to differ from the acute effects of hatchery routines somewhat. When looking at the results of the second novel arena tests conducted 6 weeks of age, there appears to be some stark differences between the results found from the first novel arena test conducted. Although visual inspection of the data showed that stressed chickens spent more time in the start box, this result was no longer significant. There were also no longer any observed differences between control and stress groups in regards to the amount of time spent exploring the arena after leaving the start box. In contrast with the previous novel arena test conducted at 2 days old, the second test conducted at 6 weeks old found that hatchery females performed comfort behaviours at a significantly higher rate than control females. However this result was not significant between males. This would suggest, somewhat, that hatchery females were less stressed by being placed in a novel environment than

control chickens. This could be supported by the conditioning hormesis theory, exposure to early stress primed individuals for later stresses in life such as exposure to novel environments (Constantini, 2014). Drinking and running behaviours were only observed in control chickens. Drinking during a novel arena test may suggest that the control chickens were less stressed than the hatchery individuals, or simply that they were fulfilling the motivation of thirst whilst the stressed individuals were satiated before initiation of the experiment. Due to thirst being a basic biological function, with chickens only drinking when they are thirsty (Kyriazakis and Savoy, 1997, as referenced in Appleby et al., 2004), it may not be a reliable measure to compare behaviours between a stressed and control group. Running may be attributed towards exploration behaviours, or stress behaviours. An individual may run towards an item which interests them, or away from an aversive stimulus. In the case of this experiment, there were no recorded distress vocalisations or escape attempts from the arena suggesting that running may be an explorative rather than aversive behaviour. However when looking at explorative behaviour there was no significant difference between the amount of exploration between the hatchery chickens and the control chickens. It is possible that by 6 weeks of age, the immediate effects of the hatchery related stress are diminished and they react similarly to novel environments as the control group does.

The restraint test conducted at 6 weeks old yielded similar results to that at 7 days old with hatchery individuals having a higher level of circulatory CORT after a period of restraint than the control chickens. There was little difference between baseline CORT levels between treatments, suggesting there are no effects of hatchery routines on the level of CORT when not receiving a stress challenge. As previously discussed the difference in CORT reactions between treatments could be as a result of either the hatchery group releasing more CORT in response to a stressor or having a physiologically altered HPA-axis in which they do not uptake CORT to receptors at the same rate as the control chickens.

The results from the second TI test conducted at 6 weeks of age somewhat supports the findings from the second novel arena tests. Visual analysis of the data showed that control chickens appeared to have a longer latency to righten from tonic immobility test compared to the hatchery individuals, with a tendency between males. This suggests that control chickens may have a stronger stress response to acute stress than the hatchery chickens. This further supports the hypothesis that early stress within the commercial hatchery caused a conditioning hormetic effect and served to prime the chickens to deal more efficiently with additional sources of stress than non-stressed individuals. However when inspecting

the data for vocalisation frequency hatchery males performed distress vocalisation at a much higher rate than control males, this contrasts with the results for rightening latency. This shows that there may be an effect of sex on stress response.

Another aspect the present study which supports the hypothesis that hatchery chickens are more stressed than control chickens is looking at feather condition. Feather condition is mainly linked with prevalence of feather pecking, a stereotypical behaviour heavily linked with stress. Therefore in the present study finding that hatchery chickens had poorer feather condition, increased prevalence of feather pecking, shows that they were likely more stressed than control chickens. In support of this hypothesis Kjaer and Guémené (2009) found that chickens selected for high feather pecking had increased levels of CORT reactivity compared to low feather peckers, this suggests that there is a link between these two factors. El-Lethy et al. (2001) also link increased CORT with a higher rate of feather pecking when administering CORT in feed.

When looking at the negative impacts of stress on commercial laying hens, research states that the presence of stress and negative welfare decreases production parameters such as egg production (Monaghan and Haussmann, 2015). Dei (2014) found that heat stressed chickens laid fewer eggs than those which were offered cold water to cool them, suggesting that heat stress negatively impacts production. In the present study it was found that there were differences in the number of eggs laid daily per hen in each pen. More eggs were laid per hen in pen 3 and 4 than both control pens (1 and 2), this may suggest that the hatchery chickens had a faster uptake of laying eggs than the control chickens. By the end of egg collection chickens in pen 2 were laying a similar amount of eggs as both of the treatment pens. However it is a possibility that the differences in the amount of eggs laid daily per pen could have been a pen effect rather than the effect of treatment. Considering all pens were treated similarly with equal lighting would give enough credence to the hypothesis that the hatchery chickens had a faster uptake of laying eggs than the control chickens. When critically analysing the data for egg weight, the present study showed that eggs became heavier over time. This result was as expected due to egg weight generally increasing from onset of lay at 20 weeks old to approximately 40 weeks old at peak production (Appleby et al., 2004). There was also a significant difference in egg weight between pens, with a tendency result in weight with pen 4 producing heavier eggs than pen 2. It is also possible that there may be an effect of pen on eggs weight rather than just treatment, however there is some research showing that early stress increases egg weight. One study that supports this

hypothesis is Ericsson et al. (2016) found that out of three treatment groups; stressed at 2 weeks, stressed at 8 weeks and a control group, the group stressed at 2 weeks old laid heavier eggs than the other two treatments.

The results of the estradiol analysis showed that hatchery females had higher levels of estradiol at 19 weeks of age; this is considered point of lay for commercial chickens (Nicol, 2015). These results corroborate with the findings that hatchery related stress increases the number of eggs laid. This result contradicts reports that stress negatively impacts production factors (Monaghan and Haussmann, 2015). Research into stress and its effects on production factors has looked into temperature stress, both heat and cold stress negatively impacts egg production (Saint-Pierre et al., 2003; Mignon-Grasteau et al., 2015; Xie et al., 2017). A different story was shown when comparing testosterone levels, there was no statistical difference between treatment groups. This shows that hatchery related stress may have had no effects on male reproductive hormones; however there were significant differences between pens. Other factors such as number of males or females in the pen may have had an effect. The results of the present study contradict those of Elfwing et al. (2015) which found that early stress delayed sexual maturation in males; the reason for this difference may be due to not controlling for number of males and females in each pen, or having mixed sex pens.

5.4 Sex difference between hatchery chickens

One aim of the present study was to assess whether there was difference in the impact of hatchery routines when comparing males and females. There is zero selection on commercial laying chickens, as males are discarded at one day old if not being used as a parental bird and ex-laying hens do not get used as parental birds either (Nicol, 2015). Therefore it was unsure as to whether there would be differences between sexes. When analysing the results from the first novel arena test at two days old, hatchery females performed more comfort behaviours than hatchery males. This might suggest that hatchery males coped less well with high levels of stress during the commercial hatchery, than the hatchery females. Lower performance of comfort behaviours means that hatchery males' welfare was more compromised than females (Nicol et al., 2015). The same result was found in the novel arena tests conducted at 5 weeks old, hatchery females performed comfort behaviours at a higher rate than males. This finding shows that the same behavioural pattern caused by hatchery related stress may persist over time.

Another result from the present study which supports the hypothesis that males are more susceptible to hatchery related stress than females was when analysing vocalisation frequency during the second tonic immobility test. Hatchery males performed vocalisations at a higher rate than females, this support that hatchery males were more stressed than females. However when looking at chronic effects of stress tonic immobility may not indicate differences due to chronic stress, because tonic immobility may measure more acute effects of stress. This being said, there is much research where tonic immobility tests correlate with other fear tests such as emergence tests and novel arena tests, additionally there are many studies supporting tonic immobility testing and its correlation with other stress parameters (Forkman et al., 2007).

Feather pecking has also been used as a stress parameter in commercial laying hen flocks and correlates with other production limiting factors (de Haas et al., 2013), the results of this study found that hatchery males had higher feather scores than hatchery females. A study by Jensen et al. (2005) contradicts this finding, suggesting a higher prevalence of feather pecking within females than males. One potential reason the results of this paper differs from the present study is that the chickens were not exposed to a strong early stressor such as the chickens in the present study. This supports the hypothesis that hatchery males had a stronger response to hatchery related stress compared to hatchery females.

5.5 Conclusion

In the present study it was found that hatchery chickens are exposed to high levels of stress within a commercial hatchery, which may have detrimental effects later in life. Looking at acute effects of stress, hatchery chickens appeared at a disadvantage to control chickens, showing higher stress reactions. They were less explorative and performed less comfort behaviours than control chickens, as well as having a higher CORT reactivity to a three minute restraint test. However, the chronic effects of stress paint a different picture. Hatchery related stress appeared to cause a hormetic effect with hatchery individuals performing more comfort behaviours than controls, having a shorter tonic immobility and producing more eggs which were heavier. Although hatchery chickens still showed higher CORT reactivity than control birds and also had higher feather damage scores, and these are commonly observed within commercial chicken flocks (Costa et al., 2012). When looking at the effect of hatchery related stress when comparing males and females, in the present study

males reacted more severely, both acutely and chronically, than the hatchery females. This suggests that females have a higher stress tolerance. In general, whilst hatchery related stress may have negative implications, the present study has shown that this early stress may prime individuals to be able to better deal with stressors faced in later life.

5.6 Societal and ethical considerations

Public concerns for animal welfare, especially production animals, have never been greater. Increasing numbers of people are becoming selective as to what they consume, selecting organic and free-range over other options. This is leading to a surge in research regarding farm animal welfare. This consumer conscientiousness has already led to changes in legislation, especially in laying hens. In the majority of Europe it is now against legislation to keep hens in battery cages, with farms now converting to aviary type enclosures. The research in the present study has significant importance for both ethical and societal aspects.

Ethically, research into production animal behaviour must seek to improve animal welfare. Any prevalence of stress related behaviours, such as feather pecking and cannibalism, is indicative of an aspect of the production process which must change. Therefore it is important to break the production process into different stages in order to pinpoint which part is more stressful and where there is room to improve the lives of the animals. By taking one aspect, such as the commercial hatchery process, and looking at the effects that it alone has on behaviour and growth of laying hens, it is possible to then eliminate it as a source of stress or use it as the first stage for improving the welfare of hens.

Improvement of laying hen welfare then links directly with the societal impact. Improving animal welfare is likely to improve public opinion concerning consumption of animal products, leading to a mutually beneficial improvement for consumer and producer as well as a decrease of negative welfare in laying hen production. By showing the producers that an aspect of laying hen production is stressful and can have long term negative implications on welfare it enables the producers to make changes in their management in order to combat stress in the future.

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Appendix

Table 3: Results of weekly weighing comparing control and hatchery males and females, hatchery group chick

Week	Sex	t value	p value	sig. level
1	Male	-7.7751	<0.001	***
	Female	-7.6365	<0.001	***
2	Male	-4.2887	<0.001	***
	Female	-5.6049	<0.001	***
3	Male	-2.7438	0.007	**
	Female	-3.9129	<0.001	***
4	Male	-1.4564	0.1	NS
	Female	-2.7019	0.008	**
5	Male	-0.39311	0.7	NS
	Female	-2.4215	0.02	*
6	Male	-0.3456	0.7	NS
	Female	-2.1281	0.04	*
7	Male	-0.3318	0.7	NS
	Female	-1.4246	0.2	NS
8	Male	-2.2442	0.03	*
	Female	-3.3201	0.001	***
9	Male	-2.7397	0.007	**
	Female	-2.2538	0.03	*
10	Male	-1.8707	0.07	T
	Female	-1.4387	0.2	NS
11	Male	-1.1178	0.3	NS
	Female	-0.3654	0.7	NS
12	Male	-3.338	0.001	***
	Female	-2.0718	0.04	*
14	Male	-2.2474	0.03	*
	Female	-2.1104	0.04	*
16	Male	-1.6036	0.1	NS
	Female	-0.50959	0.6	NS

*Table 4: Behaviours recorded during novel arena tests conducted at 2 days of age comparing hatchery group with control group, hatchery males with control males, and hatchery females with control females. Significant levels (sig.) are defined as: ***=p<0.001 **=p<0.01 *=p<0.05 T=p<0.1 NS=p>0.1 NA=behaviour not observed.*

Behaviour	**Combined**			**Males**			**Females**		
	W	**p**	**sig.**	**W**	**p**	**sig.**	**W**	**p**	**sig.**
In box	179	<0.001	***	37	0.006	**	55	0.06	T
Transverses	551	0.009	**	136	0.07	T	136	0.08	T
Distress vocalisation	318.5	0.1	NS	72	0.2	NS	85.5	0.5	NS
Conspecific pecks	255.5	0.02	*	64	0.1	NS	61.5	0.1	NS
Explorative pecks	239	0.01	**	54	0.05	*	59	0.09	T
Comfort	589	0.001	***	162	0.002	**	126	0.2	NS
Total exploration	208	0.003	**	46	0.02	*	55	0.06	T
Walk	203	0.002	**	62.5	0.1	NS	41	0.01	**
Run	431.5	0.5	NS	119	0.2	NS	98.5	0.9	NS
Stand relaxed	198.5	0.002	**	48.5	0.03	*	52	0.04	*
Stand alert	473	0.2	NS	101.5	0.8	NS	134	0.08	T
Escape	334.5	0.08	T	78	0.1	NS	85	0.4	NS
Preen	487	0.06	T	131	0.05	*	108	0.5	NS
Food run	406	0.3	NS	96	NA	NA	102	0.4	NS
Manipulate object	405	0.7	NS	98.5	0.8	NS	99	0.8	NS
Ground scratch	465.5	0.2	NS	113.5	0.4	NS	119	0.2	NS
Sit	407	0.6	NS	97.5	0.9	NS	101	0.7	NS
Scratch body	480	0.1	NS	132	0.07	T	103	0.8	NS
Drink	406	0.6	NS	96	NA	NA	100	0.8	NS
Bill rake	397	0.9	NS	97.5	0.9	NS	90	0.7	NS
Out of arena	378	0.3	NS	96	NA	NA	88	0.3	NS
Stretch leg	375	0.7	NS	85.5	0.4	NS	97	0.9	NS
Yawn	418.5	0.3	NS	105	0.5	NS	102	0.4	NS
Feather ruffle	479	0.03	*	109	0.4	NS	136	0.04	*
Dustbathe	448	0.04	*	112	0.1	NS	108	0.2	NS
Ground peck	697.5	<0.001	***	312	<0.001	***	159.5	0.003	**
Food peck	410	0.8	NS	113	0.4	NS	89	0.8	NS
Object peck	598	<0.001	***	146	0.02	*	138.5	0.05	*
Explore ground	674	<0.001	***	187	<0.001	***	156	0.006	**
Explore food	409	0.8	NS	113	0.4	NS	94	0.9	NS
Explore object	581	0.002	**	152	0.009	**	152	0.2	NS
Sleep	414.5	0.6	NS	84.5	0.4	NS	120	0.07	T

*Table 5: Behaviours recorded during novel arena tests conducted at 6 weeks of age comparing hatchery group with control group, hatchery males with control males, and hatchery females with control females. Significant levels (sig.) are defined as: ***=p<0.001 **=p<0.01 *=p<0.05 T=p<0.1 NS=p>0.1 NA=behaviour not observed.*

Behaviour	**Combined**			**Males**			**Females**		
	W	**p**	**sig.**	**W**	**p**	**sig.**	**W**	**p**	**sig.**
In box	231	0.2	NS	60.5	0.3	NS	52.5	0.4	NS
Transverses	328	0.4	NS	97	0.3	NS	67.5	0.9	NS
Distress vocalisations	379	0.3	NS	78	NA	NA	60.5	0.4	NS
Conspecific pecks	372	0.03	*	99	0.2	NS	63.5	0.9	NS
Explorative pecks	305	0.7	NS	70	0.6	NS	80.5	0.4	NS
Exploration	307	0.7	NS	74	0.8	NS	80.5	0.4	NS
Walk	268	0.7	NS	62	0.3	NS	66.5	1	NS
Run	216	0.01	**	54	0.05	*	54	0.1	NS
Stand relaxed	305	0.7	NS	71	0.7	NS	79.8	0.4	NS
Stand alert	295	0.9	NS	69	0.6	NS	73.5	0.7	NS
Preen	336	0.2	NS	97.5	0.2	NS	71.5	0.7	NS
Food run	312	0.2	NS	90	0.2	NS	66	NA	NA
Manipulate object	222.5	0.2	NS	300	0.8	NS	93	0.4	NS
Ground scratch	291	0.9	NS	79	0.9	NS	66	1	NS
Sit alert	275	0.5	NS	84	0.4	NS	55	0.2	NS
Sit relaxed	262.5	0.3	NS	84	0.4	NS	49.5	0.09	T
Scratch body	317.5	0.5	NS	90	0.5	NS	70	0.8	NS
Drink	348	0.02	*	84	0.4	NS	90	0.03	*
Bill rake	246.5	0.4	NS	72	0.7	NS	53.5	0.4	NS
Stretch leg	237	0.3	NS	85	0.7	NS	35	0.05	*
Stretch wing	204.5	0.06	T	74	0.8	NS	29	0.02	*
Yawn	299	0.6	NS	90	0.2	NS	60.5	0.4	NS
Feather ruffle	360	0.01	**	96	0.09	T	84	0.07	T
Ground peck	310	0.6	NS	159.5	0.9	NS	63.5	0.9	NS
Food peck	292	0.9	NS	82	0.8	NS	63.5	0.9	NS
Object peck	288	1	NS	95	0.3	NS	55.5	0.5	NS
Explore ground	313	0.6	NS	95	0.3	NS	64.5	0.9	NS
Explore food	285	0.9	NS	79	0.9	NS	62.5	0.8	NS
Explore object	300	0.8	NS	92	0.4	NS	61.5	0.8	NS
Sleep	312	0.2	NS	90	0.2	NS	66	NA	NA
Freeze	276	0.3	NS	78	NA	NA	60.5	0.4	NS
Comfort	241	0.3	NS	88	0.6	NS	32.5	0.04	*

Table 6: Results of tonic immobility testing conducted at 5 days old. C=control group, H=hatchery group, M=male, F=female, NS=not significant, T=tendency

	1st Vocalisation			**1st Head Movement**			**Rightening**			**Vocalisation Frequency**		
	z	p	sig.	z	p	sig.	z	p	sig.	W	p	sig.
C/H	-1.49	0.1	NS	-0.76	0.5	NS	0.24	0.8	NS	278	0.3	NS
CM/HM	-1.89	0.06	T	-0.32	0.8	NS	0.24	0.8	NS	95	0.7	NS
CF/HF	-0.73	0.5	NS	-0.62	0.5	NS	-0.15	0.9	NS	79.5	0.4	NS
M/F	-0.98	0.3	NS	-0.76	0.5	NS	-1.37	0.2	NS	215	0.03	*
CM/CF	-0.73	0.5	NS	-0.32	0.8	NS	-0.88	0.4	NS	45	0.04	*
HM/HF	-0.81	0.4	NS	-0.78	0.4	NS	-0.93	0.4	NS	59.5	0.3	NS

Table 7: Results of tonic immobility testing conducted at 6 weeks old. C=control group, H=hatchery group, M=male, F=female, NS=not significant, T=tendency

	1st Vocalisation			**1st Head Movement**			**Rightening**			**Vocalisation Frequency**		
	z	**p**	**sig.**	**z**	**p**	**sig.**	**z**	**p**	**sig.**	**W**	**p**	**sig.**
C/H	0.65	0.5	NS	0.58	0.6	NS	1.2	0.2	NS	422	0.1	NS
CM/HM	1.21	0.2	NS	0.98	0.3	NS	1.72	0.09	T	165	0.03	*
CF/HF	0.14	0.9	NS	-0.38	0.7	NS	-0.05	0.9	NS	64	0.8	NS
M/F	-0.27	0.8	NS	-0.05	0.9	NS	-0.58	0.6	NS	398	0.2	NS
CM/CF	-0.15	0.9	NS	0.47	0.6	NS	0.51	0.6	NS	72	0.6	NS
HM/HF	-0.46	0.7	NS	-0.66	0.5	NS	-1.2	0.2	NS	127	0.02	*

Table XX: Results of feather damage scoring conducted at 20 weeks of age comparing two pens of hatchery chickens (pen 3 and 4) with two pens of control chickens (pen 1 and 2)

Total feather scores

	Combined			Male			Female		
	χ^2	p	sig.	χ^2	p	sig.	χ^2	p	sig.
Treatment	18.287	<0.001	***						
Pens	19.146	<0.001	***	20.388	<0.001	***	3.5135	0.3	NS
1-2		0.5	NS		0.1	NS		>0.1	NS
1-3		0.02	*		0.03	*		>0.1	NS
1-4		0.02	*		0.06	T		>0.1	NS
2-3		<0.001	***		<0.001	***		>0.1	NS
2-4		0.001	***		0.001	***		>0.1	NS
3-4		0.9	NS		0.9	NS		>0.1	NS
Male vs Female									
Control	0.06873	0.8	NS						
Hatchery	9.5858	0.002	**						

Feather damage only

	Combined			Male			Female		
	χ^2	p	sig.	χ^2	p	sig.	χ^2	p	sig.
Treatment	11.89	<0.001	***						
Pens	17.087	<0.001	***	15.72	0.001	***	3.3533	0.3	NS
1-2		0.1	NS		0.1	NS		>0.1	NS
1-3		0.07	T		0.06	T		>0.1	NS
1-4		0.5	NS		0.7	NS		>0.1	NS
2-3		<0.001	***		<0.001	***		>0.1	NS
2-4		0.06	T		0.07	T		>0.1	NS
3-4		0.2	NS		0.2	NS		>0.1	NS
Male vs Female									
Control	0.0096	0.9	NS						
Hatchery	2.4662	0.1	NS						

Comb and wattle damage only

	Combined			Male			Female		
	χ^2	p	sig.	χ^2	p	sig.	χ^2	p	sig.
Treatment	7.6234	0.006	**						
Pens	11.57	0.009	**	14.17	0.003	**	7.2235	0.07	T
1-2		0.6	NS		0.5	NS		>0.1	NS
1-3		0.3	NS		0.2	NS		>0.1	NS
1-4		0.008	**		0.01	**		>0.1	NS
2-3		0.7	NS		0.1	NS		>0.1	NS
2-4		0.06	T		0.005	**		>0.1	NS
3-4		0.2	NS		0.2	NS		>0.1	NS
Male vs Female									
Control	0.0003	0.9	NS						
Hatchery	10.121	0.001	**						