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Can Naturalistic Environmental Enrichment Buffer Stress from Commercial Hatchery Processing in Chickens (*Gallus gallus domesticus*)?

Sandra Frödén

Examiner, Rie Henriksen
Supervisors, Per Jensen and Enya van Poucke

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Contents

1 Abstract

This study investigated the effects of environmental enrichment on the behavior and stress levels of White Leghorn chickens from a Swedish commercial hatchery. The chickens were put into two groups: control and enrichment. The control group was given standard unenriched environment, while the other group got enrichments such as UV light, plastic plants, fake grass, and peat. Behavioral observations, the novel object test, the open field test, the restraint test, and feather fault bar analysis were used to assess the effects of these enrichments. The enrichment group demonstrated more natural activities, such as perching and dust bathing, and had less severe fault bars, indicating less stress. However, no significant changes in corticosterone levels were seen across the groups during the restraint test. These findings suggest that environmental enrichment can improve poultry welfare by encouraging natural behavior and lowering stress-related outcomes. The study focuses on the potential benefits of adopting enrichment tactics into chicken farming to improve animal welfare and production.

Keywords: Corticosterone (CORT) levels, Environmental enrichment, Feather fault bars, Hatchery stress, Novel object test, Open field test, Poultry welfare, Restraint test.

2 Introduction

Commercial egg production involves the rearing of 4-5 billion laying hens worldwide, making poultry the most common agricultural animal on the planet (Nicol, 2015). Chicks in this highly industrialized industry are subjected to a variety of procedures right after hatching, many of which have been identified as severe stressors. These procedures include hatching in noisy incubators, manual sex sorting, vaccination, and transportation to rearing farms (Hedlund et al., 2019). The stress caused by these processes is not a temporary problem; it has far-reaching consequences for the chicks' immediate and long-term welfare, as well as their productivity as they grow (Hedlund et al., 2019; Hedlund et al., 2022).

Stress in animals, including chickens, is typically mediated through the activation of the hypothalamic-pituitary-adrenal (HPA) axis, a significant component of the endocrine system that controls reactions to stress and regulates many body processes. When the brain perceives a threat, it activates the adrenal glands to release glucocorticoids, primarily corticosterone (CORT) in birds (Möstl & Palme, 2002). The release of CORT is an important component of the physiological stress response and has a wide-ranging impact on the body, affecting metabolism, immune responses, and behavior. CORT is thus commonly used as a biomarker for detecting stress levels in birds, making it an important tool for assessing the influence of various husbandry practices on poultry welfare.

Stress can have a wide range of behavioral consequences in chickens. Stress can cause alterations to normal behaviors, which can act as indicators of an animal's welfare situation. For instance, chickens may exhibit changes in feeding activity, increased feather pecking, and aggressive behaviors in reaction to stress (Mason, 1991; Keeling et al., 2004; Koolhaas et al., 1999). These behavioral changes reflect not only the animal's acute discomfort or suffering, but can also have long-term consequences for its health and productivity. Moreover, stress may hinder an animal's ability to adapt to new or novel situations, which is frequently assessed using behavioral tests like the novel object test and the open field test. These tests assess an animal’s response to unfamiliar environments or objects, with altered responses indicating an underlying stress condition (Forkman et al., 2007). The measurement of CORT levels during such tests, particularly during restraint, is a well-established method for assessing stress responsiveness and general welfare in chickens (Scanes, 2016).

Hedlund et al. (2019) investigated the effects of the commercial hatchery process on the stress levels of laying hen chicks. Their study revealed that the chicks exposed to typical hatchery procedures exhibited significantly higher CORT levels compared to control groups, indicating that these processes are indeed stressful for the animals. The stress response was particularly strong during the early phases of the hatchery process, which included hatching in an environment filled with constant noise from the large fans used in commercial incubators. This noise is suspected to be a major stressor, contributing to the high CORT levels observed in the study. Although the specific effects of noise during development are not well-documented in chickens, it is known that other pre-hatch environmental factors can have significant impacts on post-hatch behavior and physiology (Riedstra & Groothuis, 2004; Yahav et al., 2004; Camm et al., 2001). Furthermore, Hedlund et al. (2019) discovered that the stress response persisted after hatching, especially during procedures such as sex sorting, vaccination, and packaging. These findings emphasize the cumulative stress that chicks experience during their first day of life, which can have lasting effects on their stress reactivity and overall well-being.

Another important indicator of stress in chickens is the presence of fault bars in feathers. Fault bars are visible malfunctions that appear as streaks of frayed or missing vane material, running perpendicular to the rachis of the feather (Bortolotti et al., 2002; Arrazola & Torrey, 2019). These bars develop during feather growth when the bird's protein deposition within the feather follicle is disrupted, most likely because of changes in muscle contraction in the follicle (Murphy et al., 1989) and blood pressure (Riddle, 1908), which is usually caused by acute stress (Jovani & Rohwer, 2017). Fault bars have been used as indicators of long-term stress in numerous bird species, as they reflect periods when the bird was unable to maintain normal feather growth due to environmental or physiological stressors (Jovani & Rohwer, 2017).

The presence of fault bars is not evenly distributed throughout all feathers. According to Pap et al. (2007), they are more common in some types of feathers, particularly those less crucial for flying, such as tail feathers. The amount and severity of fault bars can provide insights into the types of stressors the bird has experienced. For example, tail feathers are more likely to develop fault bars in response to moderate, acute stress, while wing feathers may develop them under more severe acute stress conditions (Arrazola & Torrey, 2019). Thus, fault bar evaluation provides a less invasive and reliable way for detecting birds' accumulated stress over time (Arrazola & Torrey, 2019). In commercial poultry, the presence of a high number of fault bars, particularly in tail feathers, could indicate chronic stress due to factors like feed restriction during rearing (Arrazola et al., 2017).

Given the considerable stress experienced by commercially produced chicks, researchers have investigated various of strategies to reduce these effects and improve animal welfare. One possible approach is environmental enrichment, which involves enhancing the complexity of the animals' living environment to promote natural behaviors and reduce stress (Campbell et al., 2019). Environmental enrichment can take many forms, from the provision of perches and dust baths to more complex modifications like varying the types of litter available or introducing new objects. The purpose of these modifications is to provide stimuli that encourage the animals to engage in behaviors that are necessary for their well-being but are often restricted in barren, industrial environments.

Various studies have shown that environmental enrichment can have an important effect on poultry behavior and stress levels. For example, Ross et al. (2020) discovered that enriched environments reduce the stress responses of chickens, as measured by their reactions to restraint and novel objects. The addition of enrichment items enables chickens to express a wider range of natural behaviors, which can buffer against the negative effects of stress. In particular, the presence of perches and various types of litter has been shown to reduce fearfulness and chronic stress in chicks, while also improving exploratory behaviors (Nazar et al., 2022). This is especially vital because exploratory behavior is often associated with the animal's ability to cope with new challenges, and an enriched environment may aid in learning and adaptability.

The design of enrichment programs must consider the preferences and natural behaviors of the species. For example, Skånberg et al. (2021) discovered that chicks prefer different types of materials for different activities, such as dust bathing in sand or peat and foraging with wood or hemp shavings. This shows that a diverse enrichment approach, which includes a wide range of materials and objects, may be most beneficial in supporting well-rounded development and reducing stress. Moreover, the presence of a mother hen, or some type of social enrichment, may play an important role in reducing stress in commercial settings. In natural conditions, chicks imprint on their mother immediately after hatching, and this bond plays an important role for their social development and ability to cope with stress (Hedlund et al., 2022). In commercial settings, however, chicks are often fostered without a mother figure, which may contribute to higher fear responses and stress. This makes the remaining enrichment crucial for their welfare and well-being.

In recent years, the use of ultraviolet (UV) light in poultry housing systems has received attention for its potential to improve the welfare of laying hens. UV light is part of the natural light spectrum that chickens are exposed to in outdoor environments, but it is often absent in indoor commercial settings. House et al. (2020) showed that UV light can affect a variety of behavioral and physiological factors in broilers, including growth, fear, and stress responses. The addition of UV light has been associated with reduced fearfulness and stress, likely due to its role in promoting natural behaviors that are necessary for well-being, such as foraging and exploration (Bennet & Cuthill, 1994). Moreover, UV light exposure has been associated with improved growth and bone development (Zhang et al., 2006). These findings suggest that adding UV light into the lighting regimes of poultry housing could be a simple yet effective strategy to improve the welfare of commercially produced chickens, especially in environments where they are otherwise deprived of natural sunlight.

In summary, the early life experiences of commercially produced chicks are fraught with stressors that have significant and lasting impacts on their physiology, behavior, and overall welfare. The activation of the HPA axis and consequent release of CORT are important indications of the stress response in poultry, with higher levels indicating increased stress. The potential to minimize these effects through environmental enrichment is a promising strategy for improving welfare outcomes in commercial settings. By providing a more complex and stimulating environment, enriched settings encourage the expression of natural behaviors, reduce fear and stress, and promote better coping mechanisms. Furthermore, the presence of fault bars in feathers is a useful tool for assessing the historical stress experiences of birds, providing insights into the effectiveness of welfare strategies. As the poultry industry evolves, including such welfare-enhancing practices will be crucial for ensuring the health and productivity of the large numbers of animals raised under commercial conditions.

Building upon these previous studies, the aim of this study was to investigate whether early stress experienced during commercial hatchery processing in White Leghorn chickens can be mitigated or buffered by environmental enrichment that mimics the outdoors. By evaluating various enrichment strategies and measuring relevant physiological and behavioral parameters, this research seeks to contribute to the development of effective interventions to improve the welfare and productivity of commercial poultry during this critical developmental period.

3 Materials and methods

3.1 Ethical note

The experiments were approved by the local ethical committee for animal experimentation in Linköping, license number 10492-2023.

3.2 Animals

58 already vaccinated female chickens, originated from a standardized hatchery and belonged to the White Leghorn (Lohmann LSL Lite) strain (*Gallus gallus domesticus*), were picked up at the day of hatching (2023-10-03), hereafter called day 0. After standard processing, they were brought to Linköping University's chicken facility at Vreta and placed in their home pens. Half of the chicks were randomly chosen for the control group, and the other for the experimental group, with two replicates each (Control: n=29; Enrichment: n=29. On day 1, they were all wing-tagged and weighted.

Both the control and experimental group were fed with a mixture of bird feed, chicken müsli and dried earthworms. The experimental group were also given some extra chicken müsli and dried earthworms spread out over the entire enclosure.

3.3 Home pen set-up

The chicks had access to a 60 × 120 cm large pen inside their home enclosures for the first 10 days, separated by a 40 cm high cardboard wall (figure 1). They were then granted access to the full pen, which was 240 × 120 cm and stood 160 cm tall with netting on the ceiling. They also received additional netting on the short sides and an 80 cm high plastic wall on the long sides of the pens.

All chicks had access to food, water, perches and saw dust as base material with corrugated cardboard underneath (figure 1 A). The experimental chicks also had access to plastic plants, peat for dust bathing and a UV light (Arcadia bird puresun compact lamp E27 - 2.4% 20 W) radiating both UV-A and UV-B light in the enclosure (figure 1 B). Since chicks can dust bathe in many different materials (Skånberg et al., 2021), the control groups were not given any extra material for this behavior. For the first weeks, they also had access to a heat lamp. After 10 days, when the chicks got access to the whole pen, the experimental group was introduced to fake grass laying inside a 60 × 80 × 19,5 cm pallet collar (figure 2). To get inside the pallet collar, two netted 40 × 40 cm wooden ramps were built with hooks to attach to the pallet collar. On day 15, the experimental group was introduced to another pallet collar in the same size as their new dust bathing container, and the ramps were removed (figure 3).

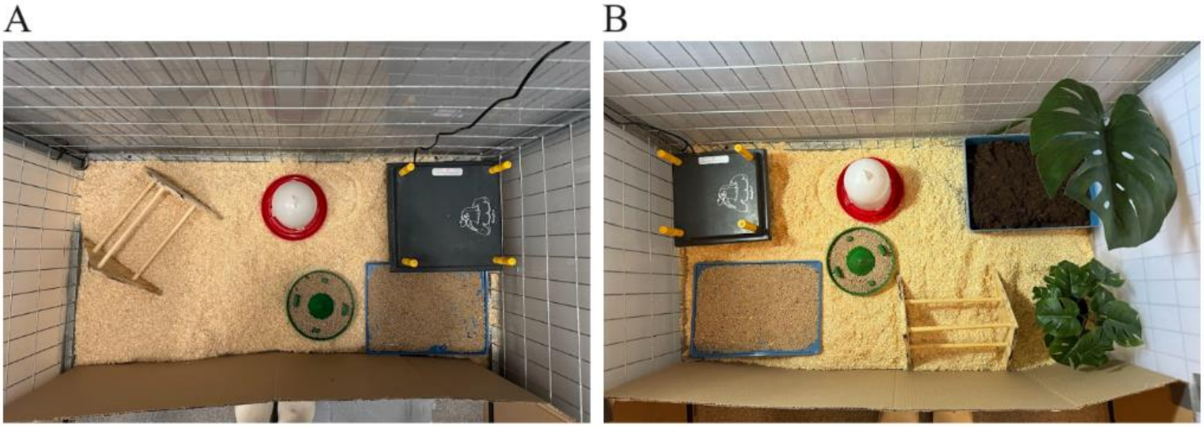

Figure 1: Home pen set-up for the first 10 days. A) Control group. B) Experimental group.

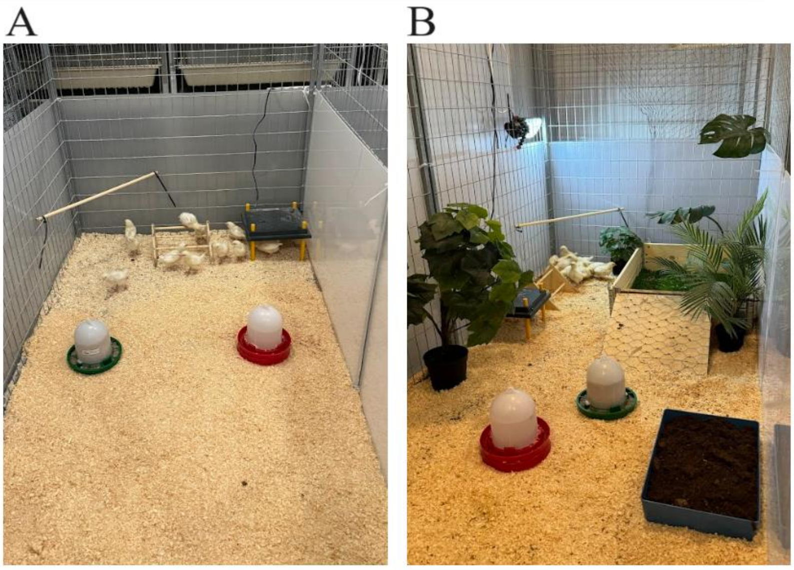

Figure 2: Home pen set-up after 10 days. A) Control group. B) Experimental group.

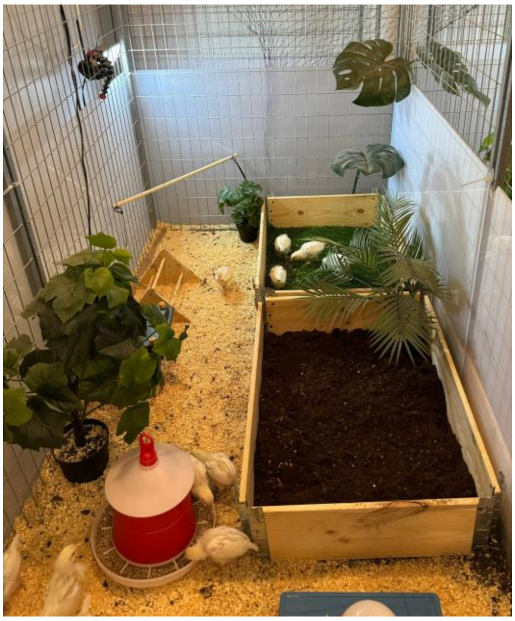

Figure 3: Home pen set-up after 15 days for the experimental group.

3.4 Experimental design

To assess whether the groups differed between treatments in relation to behavior, behavioral flexibility, fearfulness, and stress levels, several tests were conducted. Before testing for the outcome, a home pen ethological study was carried out to assess any differences in time budget between the groups. Thereafter, an open field test, a novel object test, and a restraint test were performed, along with feather sampling for feather fault bars analysis (figure 4). For each test for the outcome, the chicks were selected at random from the home pens.

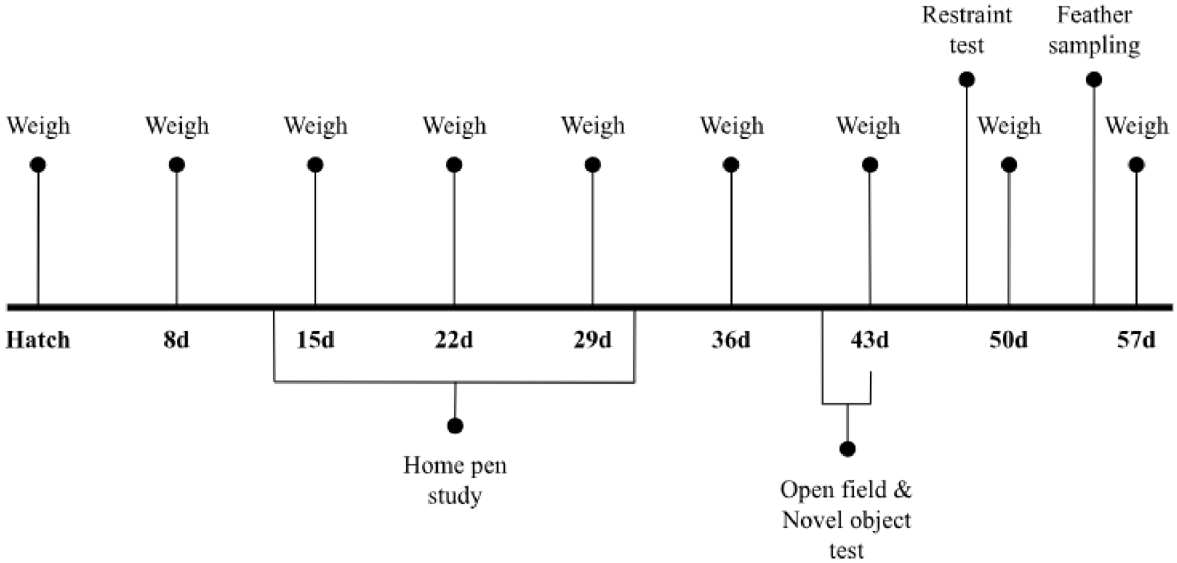

Figure 4: Timeline of the project and its contents.

3.4.1 Home pen behavioral study

Ethological tests began on day 13 to see if the chick's time budget differed between treatments. The experiment was carried out three times a week for one hour at different times of the day for three weeks. These times were from 9:00-10:00 on Mondays, 12:00-13:00 on Wednesdays, and 15:00-16:00 on Fridays. During this hour, four cameras were set up inside the room, each pointed at one pen, and the entire hour was recorded without any interruptions or anyone entering the room. These time slots were communicated to the animal technicians in order to avoid feeding the chicks shortly before the study began.

After the studies, the footage was analyzed using scan interval sampling with instantaneous recording, in which the videos pause every three minutes and the number of birds doing which behavior was recorded (table 1).

Table 1: Ethogram of recorded behaviors for the home pen behavioral analysis.

Behavior	**Description**
Forage	Scratching/digging in substrate with feet or beak
Drink or feed	Drinking from water bell or eating from food container
Exploration	Explores object or ground with eyes focusing, tries to manipulate object with beak, or picks at an object
Locomotion	Moving around the enclosure at a steady walking pace, or faster tempo. Two or more steps
Stand alert	Stands (legs erect) with open eyes, attending to the surrounding
Inactive	Laying down with eyes closed or open, neck short, no head movements, or standing still
Perch	Sits in any position on a perch
Flight	Flying or jumping exaggerated against wall or fence
Dust bathe	Sitting in the dust bath and moving soil around over parts of the body with wings, feet or beak

Comfort behavior	Shake body in standing or sitting position, stretch wings or legs, scratch body or preen feathers with beak
Gentle feather pecking	Pecking or manipulating gently at conspecific, with the receiver not responding
Severe feather pecking	Pulling feathers on a conspecific, while the receiver tries to escape or signaling discomfort
Aggressive behavior	Threat with wing flap, or walks/chase after the other bird which is walking/running/jumping/flying away, or being involved with an aggressive fight with more than just one single peck
Out of sight	Bird not visible

3.4.2 Open field test

An open field test was carried out to investigate the fearfulness of the chicks when placed in a novel environment. For this test, two 90 × 180 cm test arenas for the open field test were built using netted panels, and a covered floor with sawdust. To keep all external distractions minimized, cardboard was placed on the wall between the test arenas, as well as by the entrance to minimize the amount of light coming in from the hallway. Aside from that, the arena was vacant and unfamiliar to the chicks. Each trial began with the positioning of a chick in the center of the arena, followed by 10 minutes of recording. Afterwards, the arena was divided into eight zones (figure 5). When analyzing the videos, the number of zone crossings were counted when the back of the bird between the wings crossed a zone line.

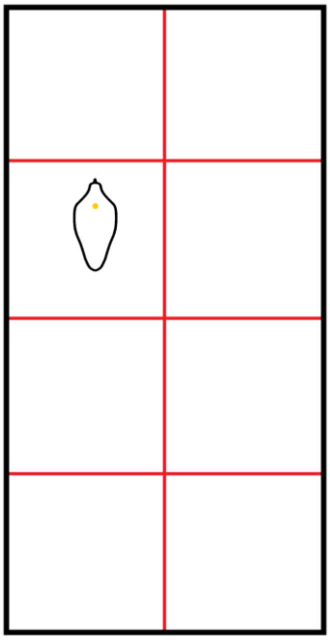

Figure 5: Illustration of the open field test set up. Each zone is divided by the red lines. A zone crossing occurred whenever the yellow dot on the chicks' back crossed one of the red lines.

3.4.3 Novel object test

A novel object test was used to investigate the chicks' fear response towards an unknown object. The same test arena as for the open field test was used for this test. The lights were turned out right after every open field test trial to place the novel object in the center of the arena, which was a cleaned and disinfected blue and silver colored can. Each novel object test trial began when the lights were switched back on and the chick was left alone for 5 minutes to be recorded. Afterwards, a circle (one chicken length in radius) was drawn around the novel object (figure 6) to display proximity to the object. When analyzing the video, the time spent outside and inside of the circle was noted, together with the latency for how long it took for the bird to enter the circle, as well as the time spent interacting with the object by physical contact (table 2).

Table 2: Ethogram of recorded behaviors for the novel object test.

Behavior	**Description**
Latency to enter the circle	Time taken to enter the circle from the test started

Time spent in circle	Time spent in the circle in proximity to the novel object. When the center of the chick's body was inside the circle, it was regarded as being inside of the circle
Time spent outside of circle	Time spent outside of the circle
Manipulate novel object	Number of times the novel object were manipulated/touched

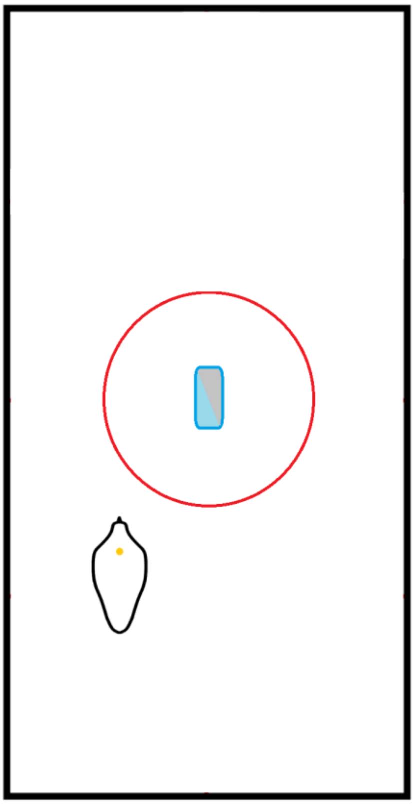

Figure 6: Illustration of the novel object test set up. Proximity zone is illustrated by the red circle, and the novel object is illustrated by the blue and silver rectangle. Proximity occurred whenever the yellow dot on the chicks' back crossed the red line.

3.4.4 Restraint test

To evaluate the chick's reactions and stress levels when they are gently restrained, a restraint test was conducted at day 48, where blood samples were collected to gather physiological data of the response. During the test, each chicken was carefully placed inside a netted bag with restricted mobility, which functioned as a restraint method. The netting bag was secured to prevent escape while allowing for enough airflow. Chickens were restrained for a period of three minutes. Blood samples were taken from each chicken immediately before and after the restraint test to measure corticosterone (CORT) levels in Pg cort/mL plasma. Sampling was performed by puncturing the brachial vein with a sterile needle and syringe, and the blood collected in 200 µL Li-Hep tubes. The blood samples were then centrifuged for 10 minutes at 3000 rpm to separate plasma from other cellular components and stored in a -80°C freezer until analyzed. CORT levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit by following the manufacturer's instructions, which enabled quantitative assessments of CORT concentrations in plasma samples.

3.4.5 Feather fault bars

At 55 days of age, six feathers were obtained from each chicken: two wing feathers (P8, third outer wing feather), two cover feathers (SC1, longest scapular feather), and two tail feathers (R1, mid tail feather) (figure 7). All feathers were cut above the growing follicle. The number of fault bars, and their condition, were then analyzed using a stereo microscope. The fault bars were classified according to length and visibility as severe (≥5 mm, visible without microscope), moderate (<5 mm, visible without microscope), and faint (<5 mm, only visible through microscope) (figure 8).

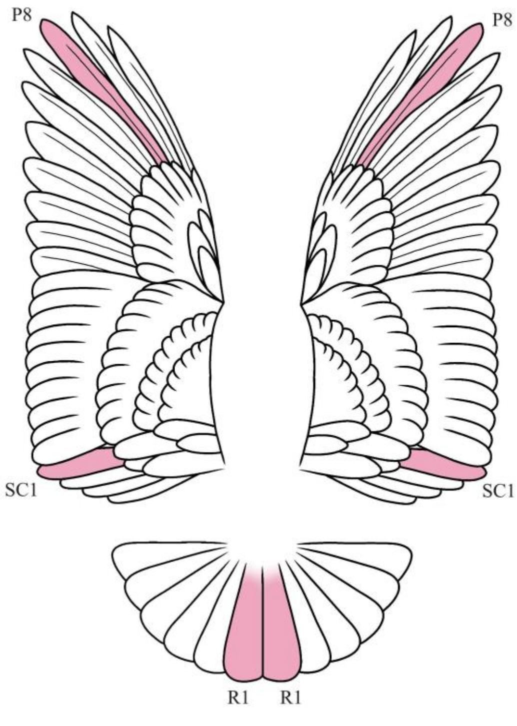

Figure 7: Illustration of the placement of feathers used for the fault bars analysis. P8 is the third of the outer wing feathers, SC1 is the longest scapular cover feather of the wing, and R1 is the mid tail feather.

Figure 8: Example of a severe feather fault bar.

3.4.6 Weight

All chicks were weighed once a week from the day after hatching until 8 weeks of age.

3.5 Statistical analysis

All statistical tests were performed using IBM SPSS Statistics (version 28). A generalized linear model, with link function normal, was applied to test each behavioral variable for the home pen behavioral study, the open field test and the novel object test using a Wald Chi-Squared (Waldχ^2) test to establish significance. As the model treated the data as if every observation occation were independent observations, no conclusions can be drawn outside of these four pens. Hence, they could be considered as pseudoreplicates, since the prodcedure provides analysis of variance when the same measurement is made several times on each subject or case. For the restraint test, non-parametric tests were applied by using the Mann-Whitney U test for comparison between treatments, and Wilcoxon signed-rank test for comparisons between before and after restraint test. The number of fault bars were also analyzed using the Mann-Whitney U test. Means $\pm$ standard error are presented in the results. Differences were considered significant at p-value < 0.05.

4 Results

4.1 Home pen behavioral study

Each behavioral variable was looked at with regard to treatment (figure 9) and week, as the results differed between observation weeks.

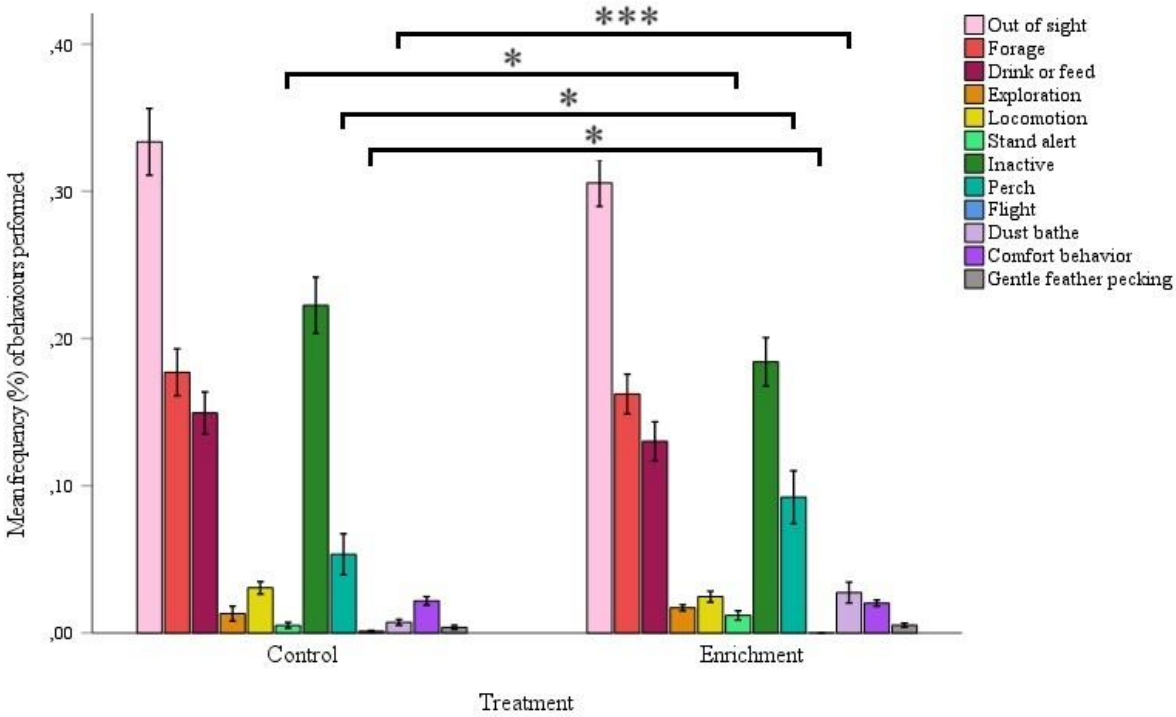

Figure 9: Frequency of each behavior in control and enrichment groups. *P<0.05 ***P<0.001

In terms of foraging behavior, there was no significant difference between the treatments or weeks (Wald$\chi^2_{(treatment)}$=0.646; $P_{(treatment)}$=0.421; Wald$\chi^2_{(week)}$=5.546; $P_{(week)}$=0.062; figure 10). However, a tendency can be argued regarding the difference between the weeks, where the behavior seems to increase over time for both groups.

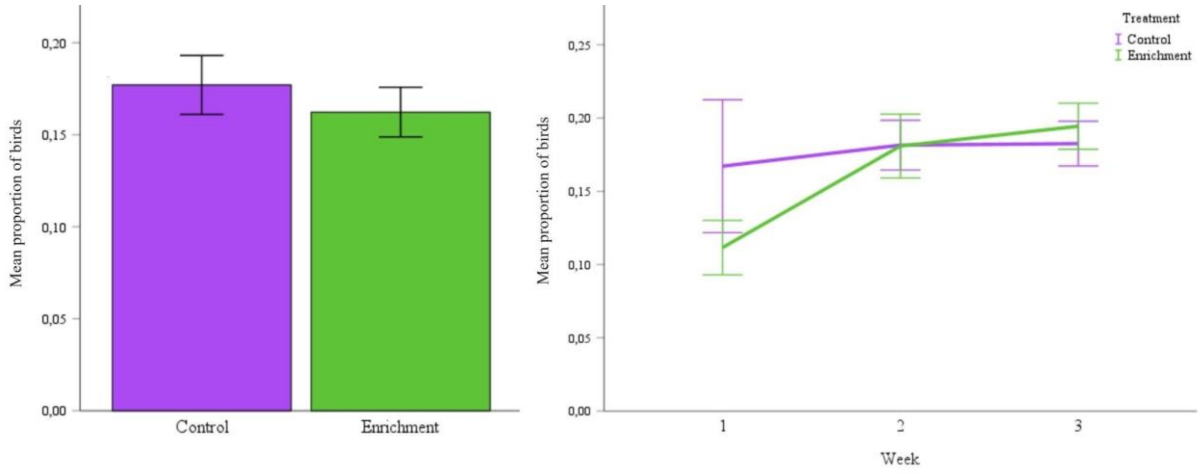

Figure 10: The number of birds performing foraging behavior in control and enrichment groups overall and across weeks.

There was a significant difference between the weeks in regards to drinking or feeding behavior, where the behavior seems to decrease over time (Wald$\chi^2_{(treatment)}$=1.872; $P_{(treatment)}$=0.171; Wald$\chi^2_{(week)}$=27.062; $P_{(week)}$<0.001; figure 11). However, no differences were found between the treatments.

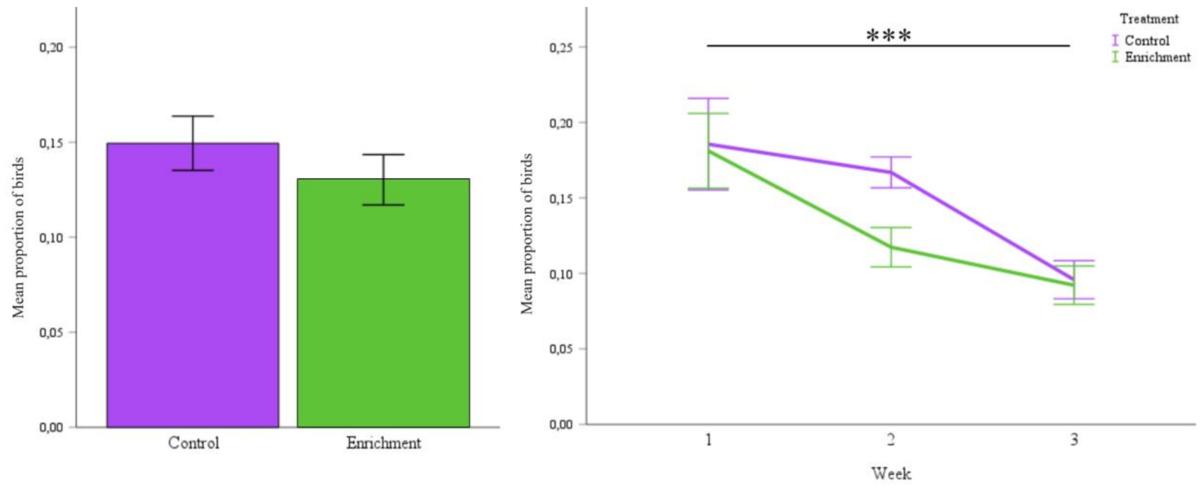

Figure 11: The number of birds performing drinking or feeding behavior in control and enrichment groups overall and across weeks. ***P<0.001

No differences were found regarding the explorative behavior (Wald$\chi^2_{(treatment)}$=0.607; $P_{(treatment)}$=0.436; Wald$\chi^2_{(week)}$=2.014; $P_{(week)}$=0.365; figure 12).

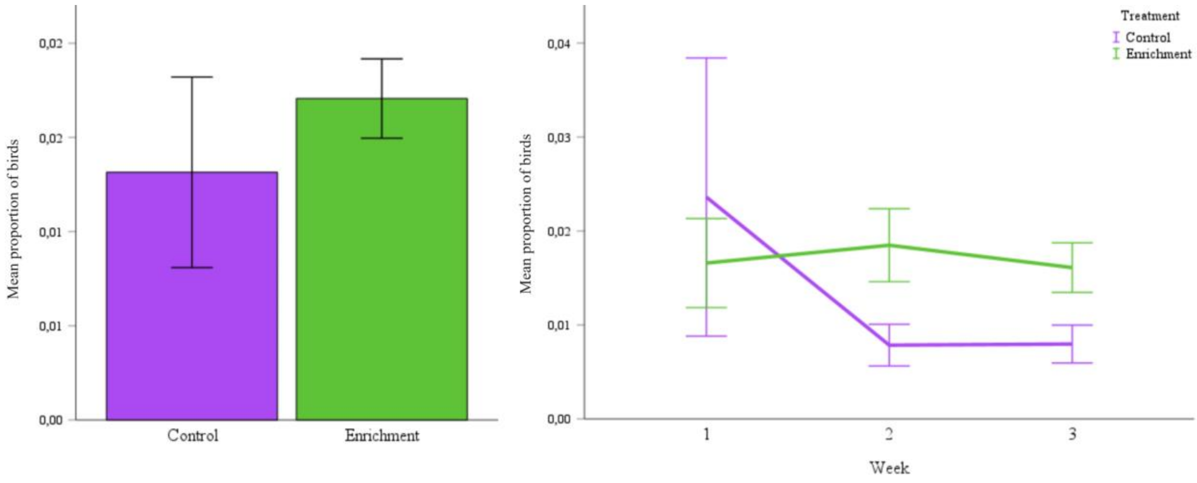

Figure 12: The number of birds performing explorative behavior in control and enrichment groups overall and across weeks.

In locomotive behavior, a significant difference were found between the weeks, as it decreases over time, but no difference between treatments (Wald$\chi^2_{(treatment)}$=2.346; $P_{(treatment)}$=0.126; Wald$\chi^2_{(week)}$=32.484; $P_{(week)}$<0.001; figure 13).

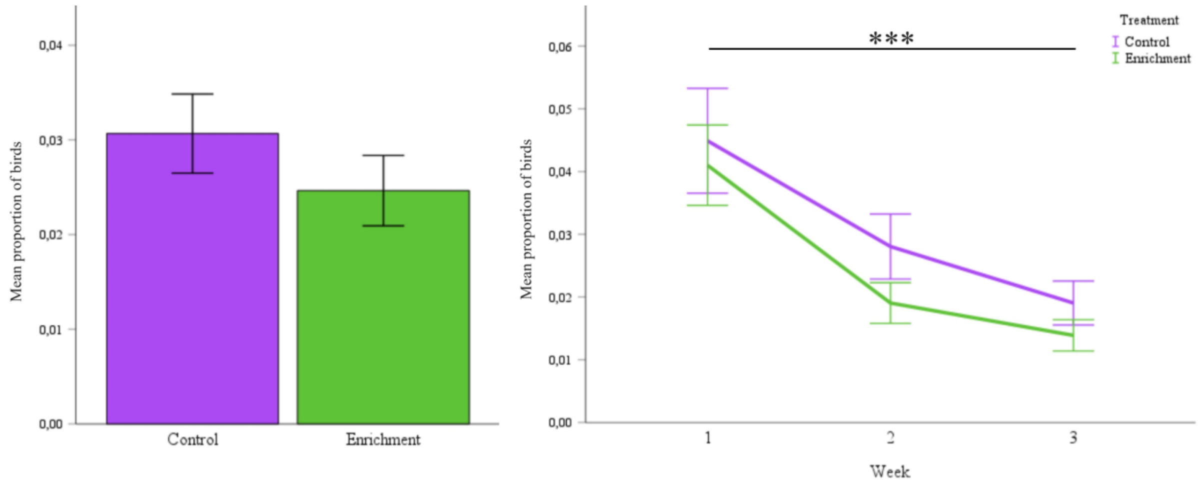

Figure 13: The number of birds performing locomotive behavior in control and enrichment groups overall and across weeks. ***P<0.001

A significant difference were found between treatments in regard of stand alert behavior, where the enrichment group performed the behavior more (Wald$\chi^2_{(treatment)}$=4.211; $P_{(treatment)}$=0.040; Wald$\chi^2_{(week)}$=3.521; $P_{(week)}$=0.172; figure 14). However, no differences were found between the weeks.

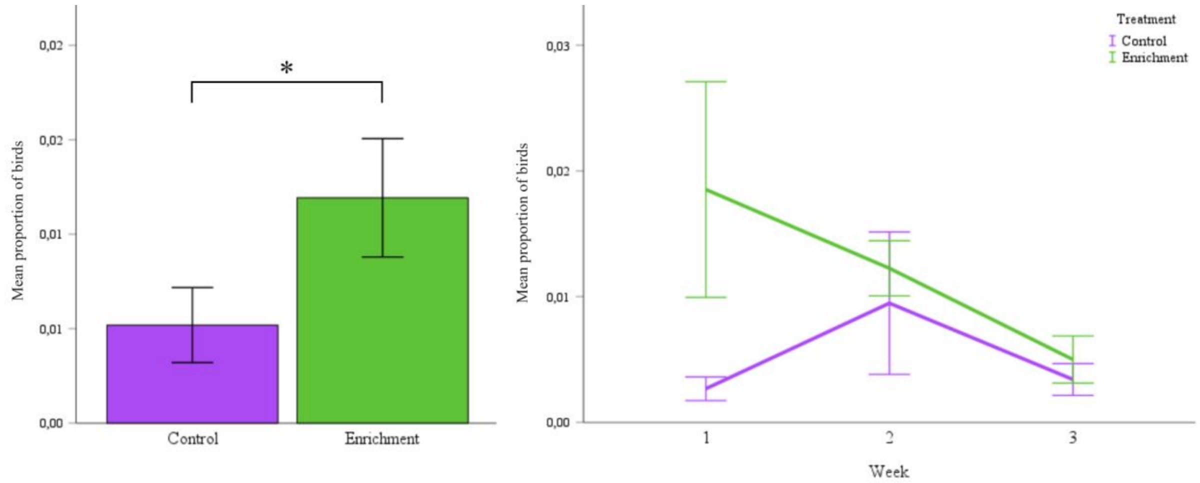

Figure 14: The number of birds performing stand alert behavior in control and enrichment groups overall and across weeks. *P<0.05

In regard of being inactive, no differences were found between the treatments or weeks (Wald$\chi^2_{(treatment)}$=2.823; $P_{(treatment)}$=0.093; Wald$\chi^2_{(week)}$=1.473; $P_{(week)}$=0.479; figure 15).

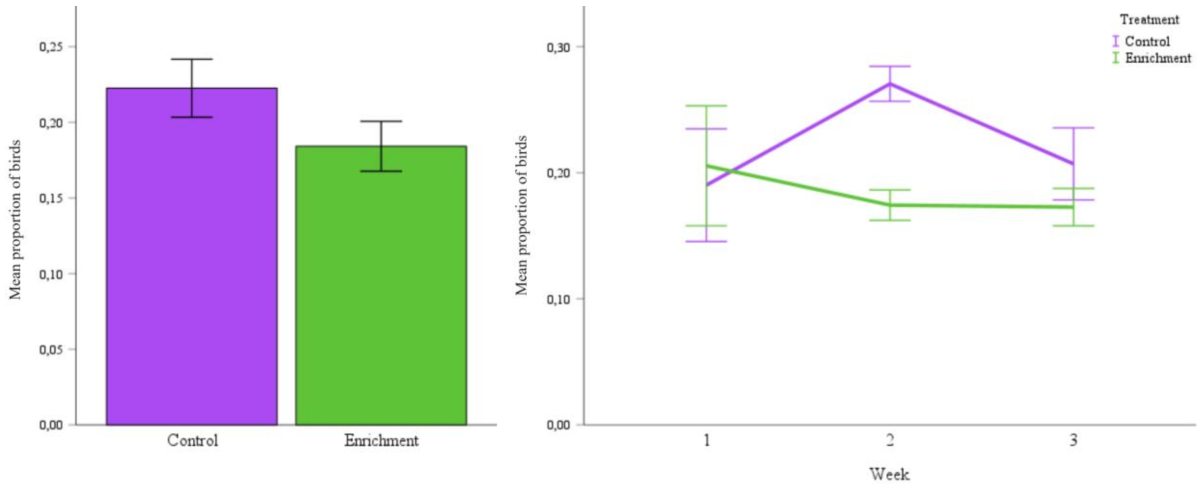

Figure 15: The number of birds being inactive in control and enrichment groups overall and across weeks.

For perching behavior, there was a difference between the treatments as well as between the weeks (Wald$\chi^2_{(treatment)}$=6.946; $P_{(treatment)}$=0.008; Wald$\chi^2_{(week)}$=42.904; $P_{(week)}$<0.001; figure 16). The behavior was performed more by the enrichment group, and it increases in frequency over time for both treatments.

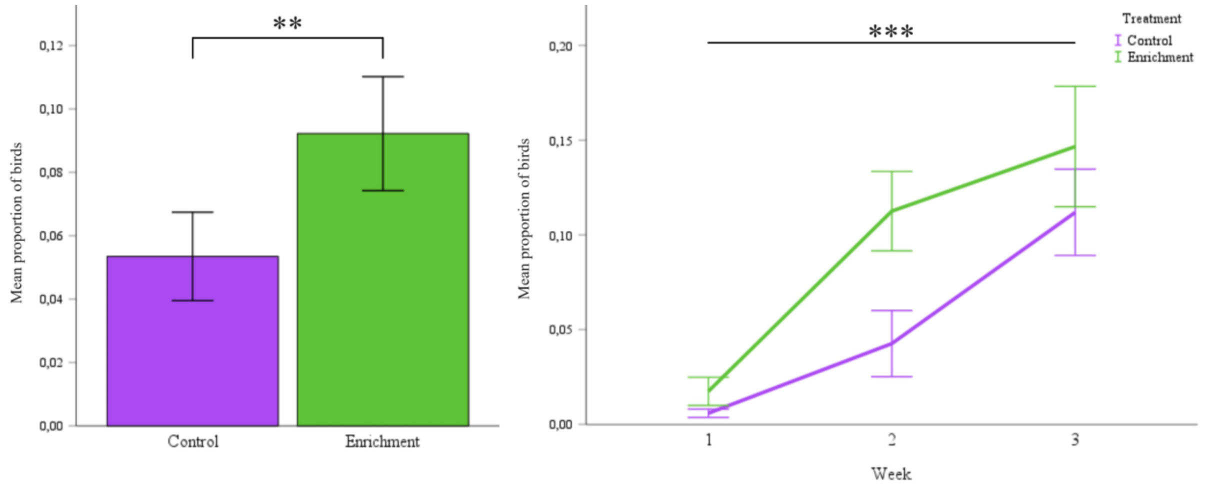

Figure 16: The number of birds performing perching behavior in control and enrichment groups overall and across weeks. **P<0.01, ***P<0.001.

A difference between the treatments were found regarding flight behavior, where the control group performed the behavior more (Wald$\chi^2_{(treatment)}$=5.007; $P_{(treatment)}$=0.025; Wald$\chi^2_{(week)}$=2.503; $P_{(week)}$=0.286; figure 17). However, no difference was found between the weeks.

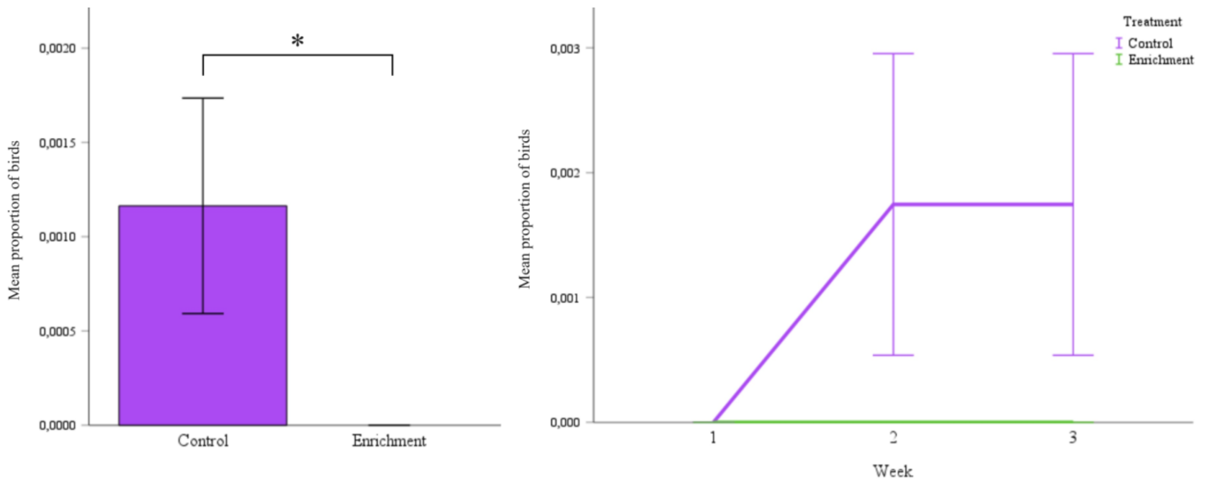

Figure 17: The number of birds performing flying behavior in control and enrichment groups overall and across weeks. *P<0.05

For dust bathing behavior, there was a difference between the treatments and the weeks, where the enrichment group performed the behavior more often (Wald$\chi^2_{(treatment)}$=10.506; $P_{(treatment)}$=0.001; Wald$\chi^2_{(week)}$=0.025; $P_{(week)}$=0.025; figure 18). The frequency of the behavior also changed over the weeks.

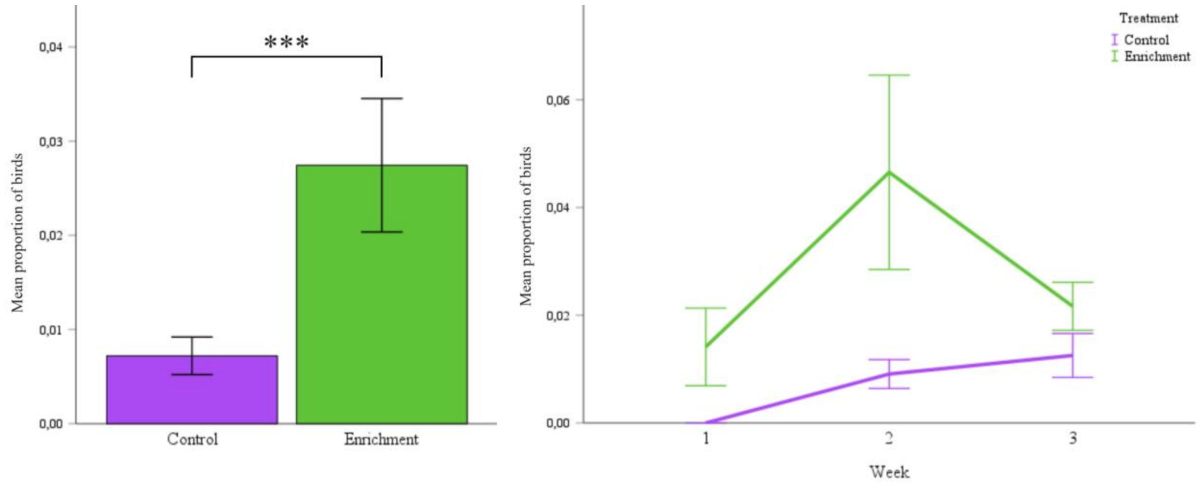

Figure 18: The number of birds performing dust bathing behavior in control and. enrichment groups overall and across weeks. ***P<0.001

No differences were found in regard of comfort behaviors (Wald$\chi^2_{(treatment)}$=0.238; $P_{(treatment)}$=0.625; Wald$\chi^2_{(week)}$=3.690; $P_{(week)}$=0.158; figure 19).

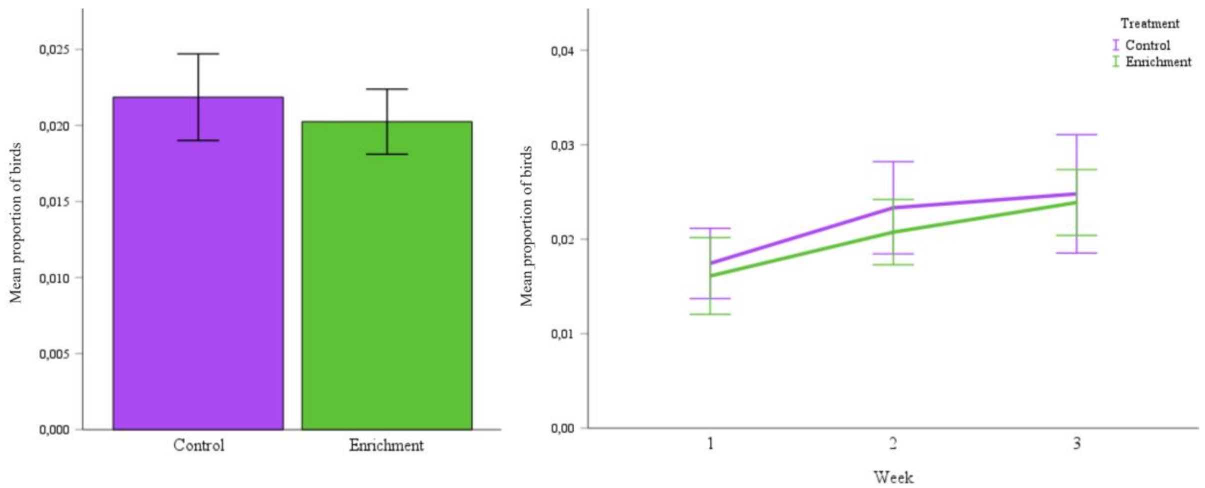

Figure 19: The number of birds performing comfort behaviors in control and enrichment groups overall and across weeks.

In regard of gentle feather pecking behavior, there was a difference between the weeks, where the behavior generally increased over time (Wald$\chi^2_{(treatment)}$=0.846; $P_{(treatment)}$=0.358; Wald$\chi^2_{(week)}$=9.704; $P_{(week)}$=0.008; figure 20). However, no differences were found between the treatments.

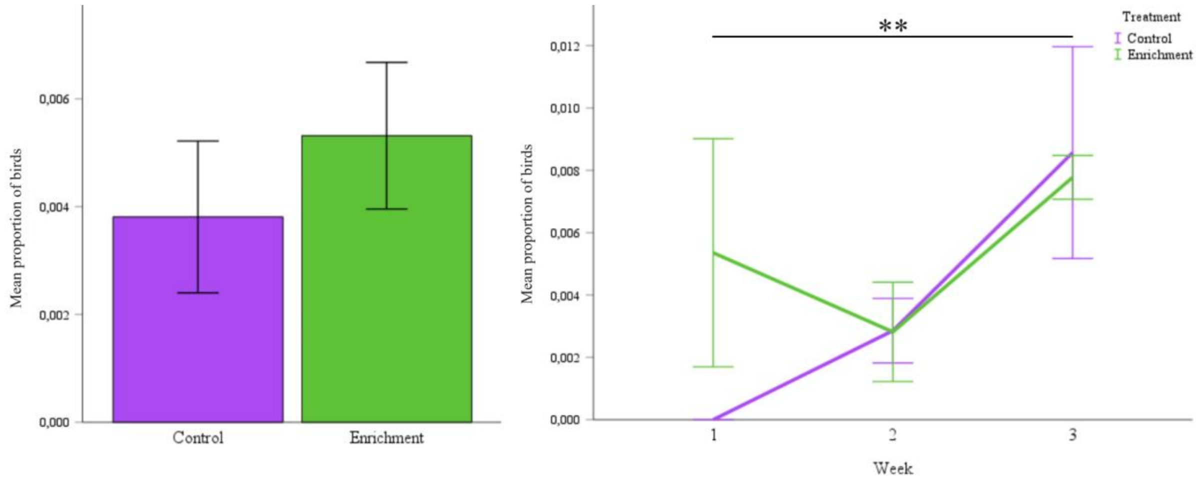

Figure 20: The number of birds performing gentle feather pecking behavior in control and enrichment groups overall and across weeks. **P<0.01

4.2 Open field test

There was so significance between the treatments regarding the amount of zone crossings in the open field test (Waldχ^2=2.198; P=0.138; figure 21).

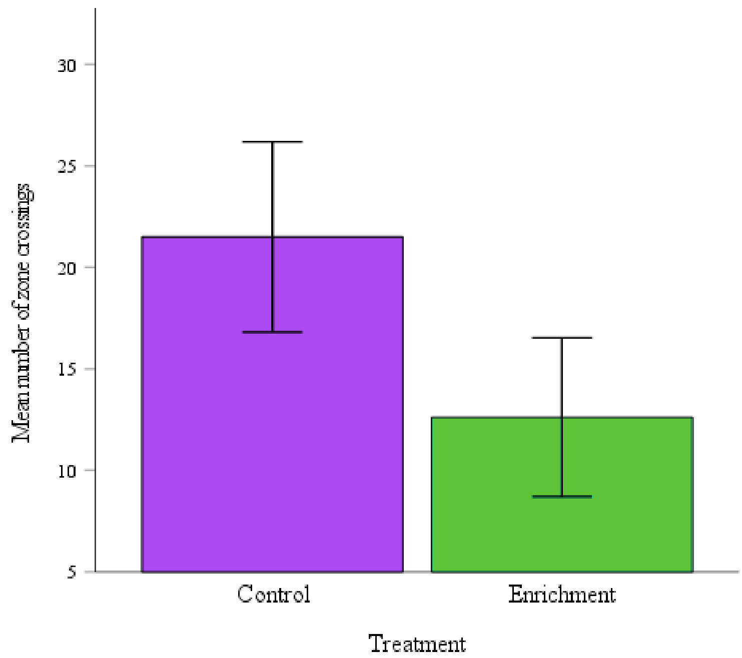

Figure 21: The number of zone crossings in the open field test between control and enrichment groups.

4.3 Novel object test

There was a significant difference between the treatments in terms of the amount spent remote of the novel object, where the enrichment group spent less time remote of the object than the control group (Waldχ^2=3.912; P=0.048; figure 22).

However, no significant difference were found regarding the latency before the chicks entered proximity (Waldχ^2=1.821; P=0.177; figure 22).

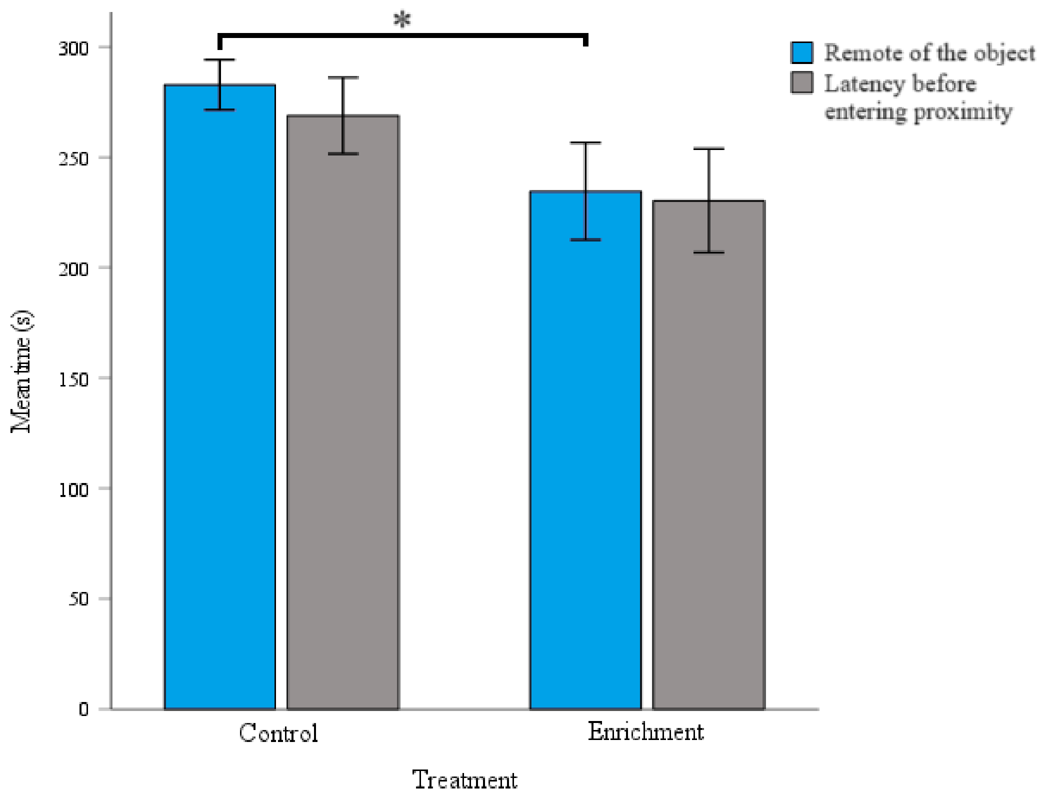

Figure 22: The mean time the chickens spent remote of the novel object, and the latency of them entering proximity to the object, in the novel object test between control and enrichment groups. *P<0.05.

4.4 Restraint test

There was no difference between treatments regarding the amount of CORT-levels in the plasma ($U_{\text{Before restraint}}$=46.5; $P_{\text{Before restraint}}$=0.791; $U_{\text{After restraint}}$=118; $P_{\text{After restraint}}$=0.539; U_{Overall}=319.5; P_{Overall}=0.587; figure 23). However, there was a difference between before and after restraint, where the amount increased significantly after the test (Z_{Control}=-3.921; P_{Control}=<0.001; $Z_{\text{Enrichment}}$=-5.012; $P_{\text{Enrichment}}$=<0.001; Z_{Overall}=-6.334; P_{Overall}=<0.001; figure 23).

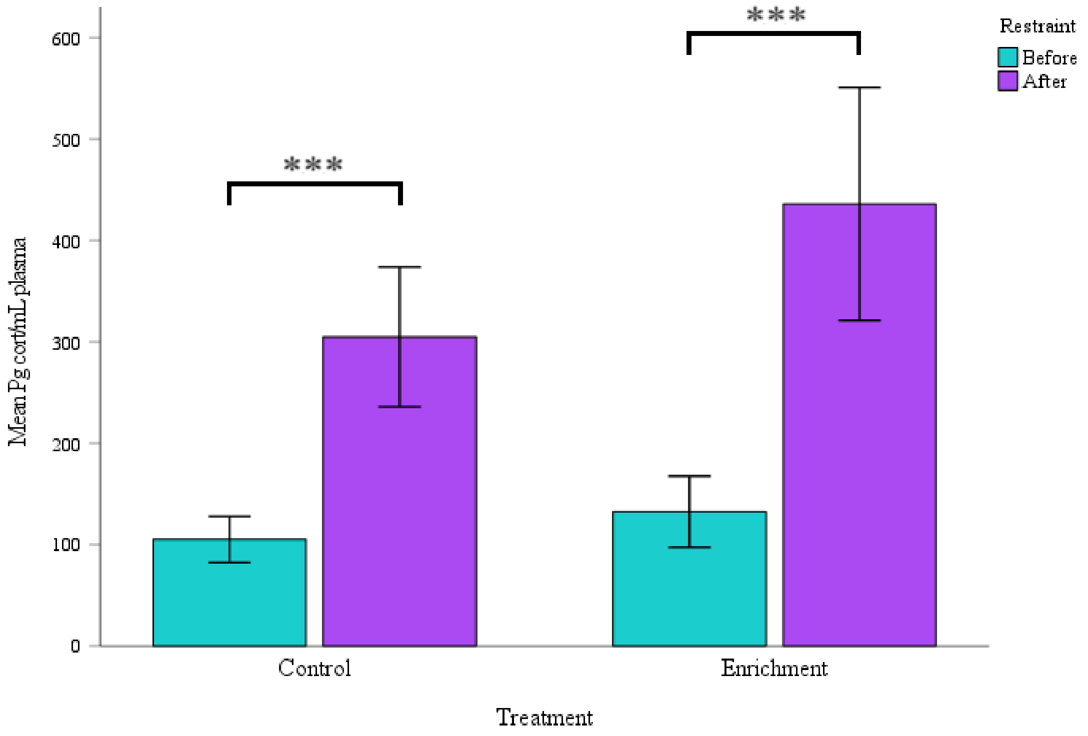

Figure 23: The corticosterone (CORT) levels before and after restraint test between control and enrichment groups. ***P=<0.001.

4.5 Feather fault bars

For the P8 feathers, there was a significant difference between the treatments regarding severe and faint fault bars, where the control group had significantly more severe fault bars, and the enrichment group had more faint fault bars, but no difference regarding moderate fault bars (U_{Severe}=333.5; P_{Severe}=0.01; $U_{Moderate}$=420.5; $P_{Moderate}$=1.000; U_{Faint}=284; P_{Faint}=0.028). A difference was also found between the treatments in the SC1 feathers regarding faint fault bars, where the enrichment group had more fault bars than the control group (U_{Severe}=407; P_{Severe}=0.710; $U_{Moderate}$=358; $P_{Moderate}$=0.225; U_{Faint}=230.5; P_{Faint}=0.003). In the R1 feathers, differences were found in all categories of fault bars, where the control group had significantly more fault bars (U_{Severe}=198; P_{Severe}=<0.001; $U_{Moderate}$=198; $P_{Moderate}$=<0.001; U_{Faint}=168.5; P_{Faint}=<0.001) (figure 24-26). When adding up all types of fault bars, a significant difference between the group was seen in the SC1 and R1 feathers, where the control group had more fault bars in the R1 feathers, but fewer in the SC1 feathers (U_{P8}=329; P_{P8}=0.146; U_{SC1}=237; P_{SC1}=0.004; U_{R1}=159; P_{R1}=<0.001) (figure 27).

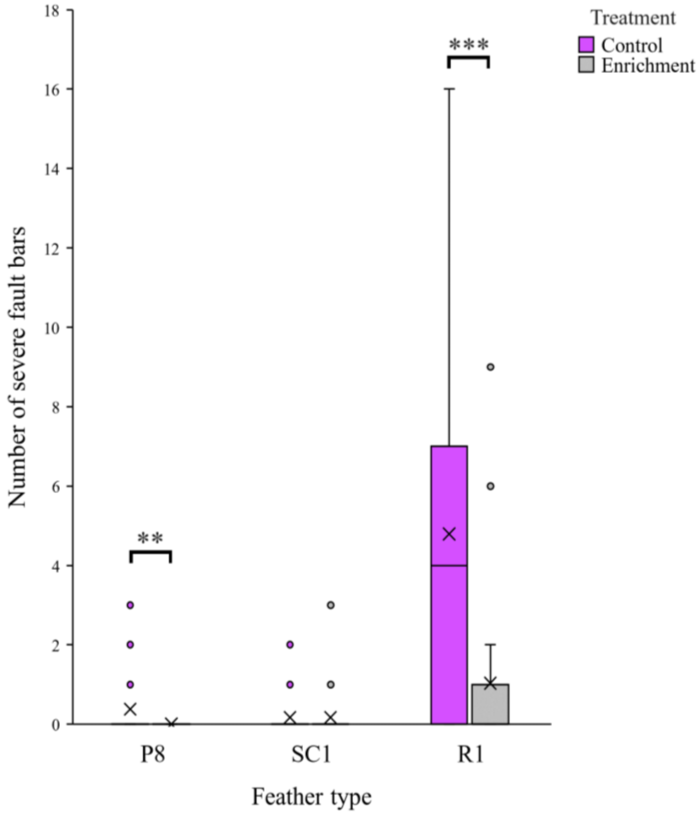

Figure 24: Severe fault bar occurrence across different feather types (P8, SC1, R1) in control and enrichment groups. **P=<0.01, **P=<0.001

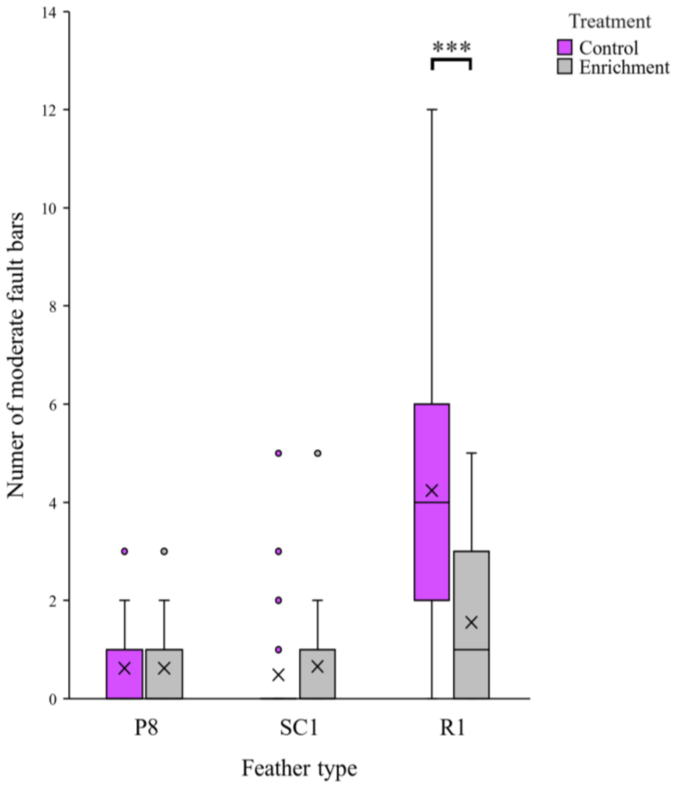

Figure 25: Moderate fault bar occurrence across different feather types (P8, SC1, R1) in control and enrichment groups. ***P=<0.001

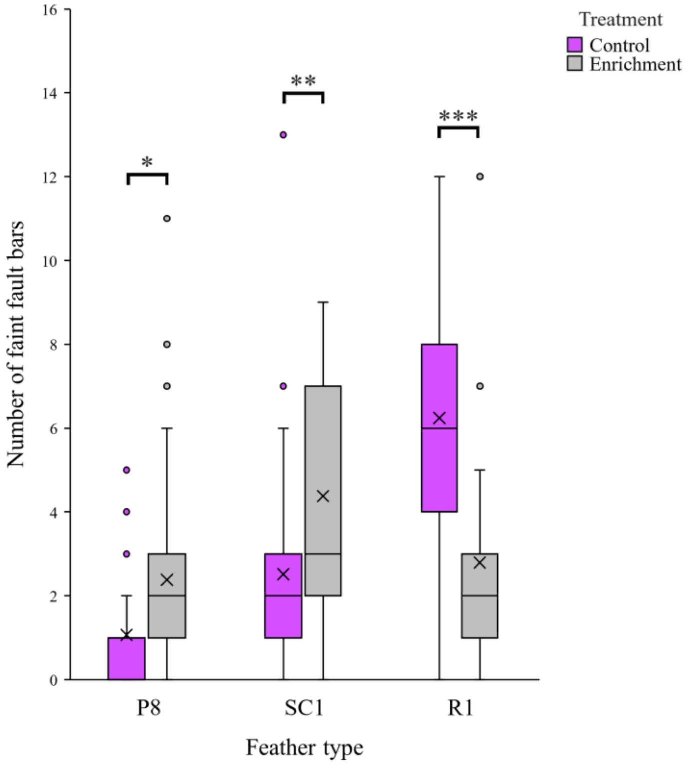

Figure 26: Faint fault bar occurrence across different feather types (P8, SC1, R1) in control and enrichment groups. *P=<0.05, **P<0.01, ***P=<0.001

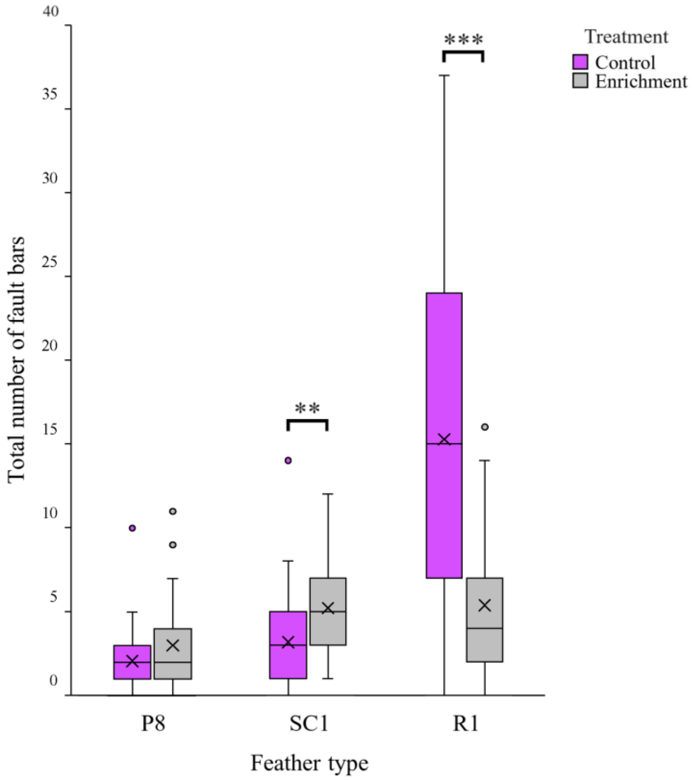

Figure 27: Total number of fault bar occurrence across different feather types (P8, SC1, R1) in control and enrichment groups. **P<0.01, ***P=<0.001

When adding all types of fault bars together, a significant difference was found between the treatments, where the control group had over all more fault bars than the enrichment group (U=229; P=0.003; figure 28).

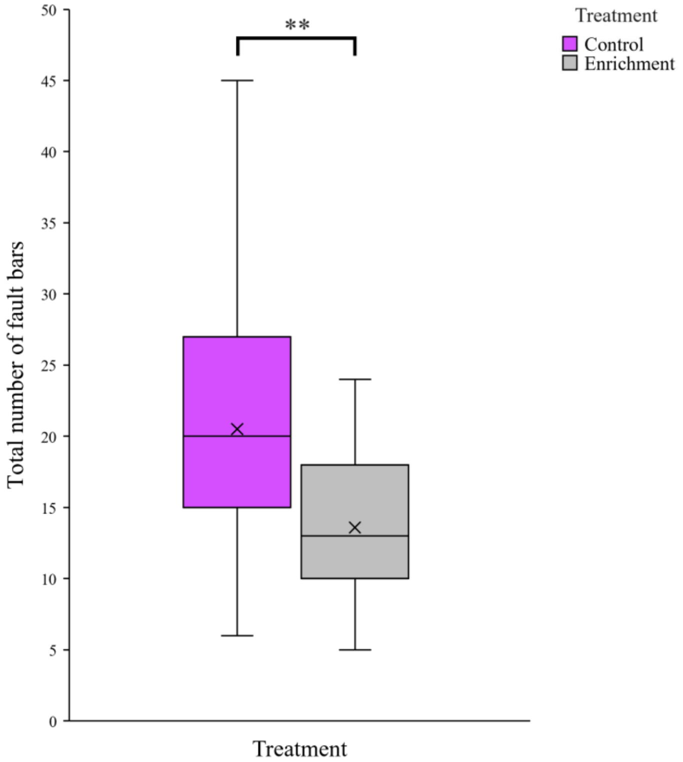

Figure 28: Total number of fault bar occurrences in control and enrichment groups.
**P=<0.01

4.6 Weight

Regarding the weight, there was no significant difference between the treatments ($Wald\chi^2_{(treatment)}$=1.218; $P_{(treatment)}$=0.270; figure 29). The chicken's weight did, however, progressively increased over time, consistent with its natural body growth.

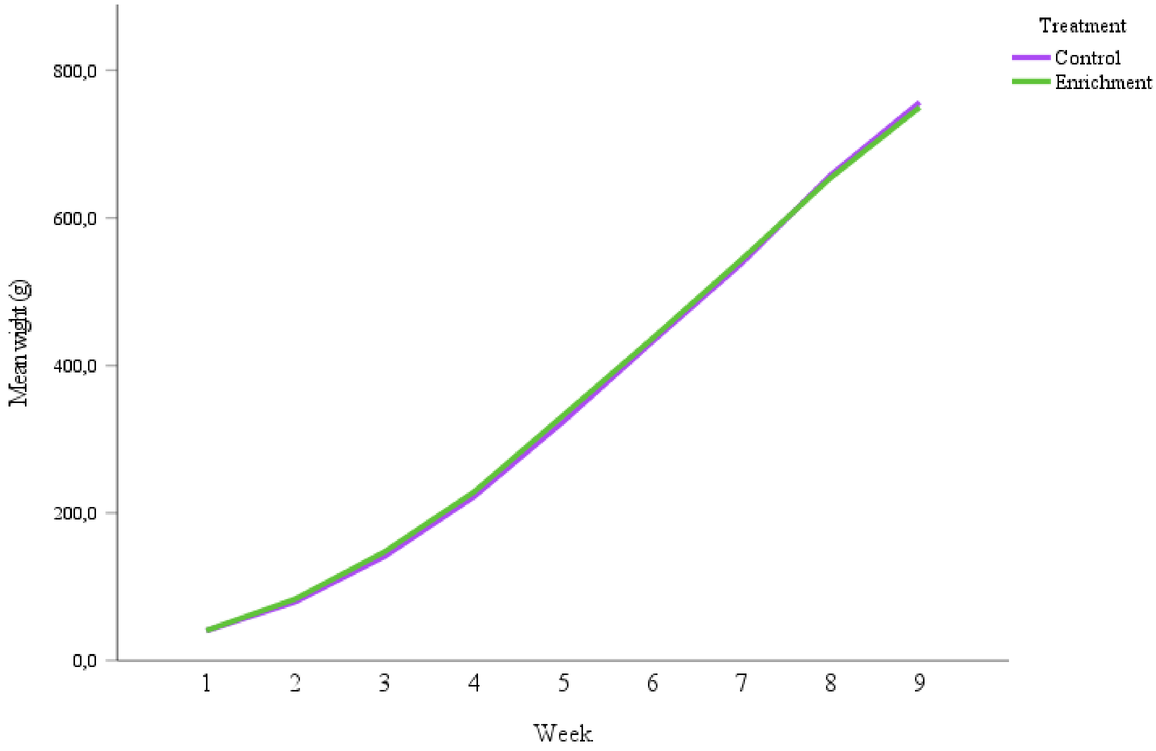

Figure 29: Changes in body weight over time in control and enrichment groups.

5 Discussion

The purpose of this study was to investigate the effects of environmental enrichment on the behavior and stress levels of female White Leghorn chickens. The hypothesis was that enriched environments would result in behavioral differences and lower stress levels compared to control conditions. These findings provided partial support for the hypothesis. As an indicator of lower stress-levels, more perching and dust bathing behavior were found in the enriched group, as well as less fearfulness of a novel object, which indicates a more calm and relatex state. They also exhibited decreased flight behavior, which is typically linked to fear and perceived threats. Furthermore, the enriched group had overall fewer fault bars, suggesting that environmental enrichment can minimize stress-related feather malformations.

However, some findings were slightly contradictory compared to the hypothesis and previous research. For example, the enriched group exhibit more stand altert behavior, which is typically related with anxiety. Still, the enrichment may have improved the chicks sensory and cognitive engagement, resulting in heightened awareness to their surrounding and curiosity (Newberry, 1995; Campbell et al. 2019). Furthermore, no differences were observed across the groups in the open field test, which is interesting given that recent research has demonstrated that an increased environmental complexity can reduce the fear of novel environments (Campbell et al., 2019).

5.1 Home pen behavioral study

The home pen behavioral study revealed significant differences between the control and enrichment groups in several behaviors. Notably, enriched chickens engaged in more perching and dust bathing behaviors than control chickens. Perching is an important behavior for chickens, as it is directly related to their natural instincts for roosting and avoiding predators. Appropriate perching opportunities have been proven to be important to the well-being of laying hens since they directly affect their ability to engage in natural behaviors. The enrichment group in the study displayed more perching behavior than the control group, implying that the more realistic environment provided in the enrichment condition better encouraged this important behavior. Perching is not just for comfort, it also helps to reduce stress and improve overall health. According to Anderson et al. (2024), chickens housed on elevated perches have fewer bone defects and are in better overall physical condition, underscoring the importance of perches in intensive farming systems. Furthermore, Nicol (2015) notes that perches allow chickens to engage in natural roosting activity, which is critical to their psychological well-being. As a result, the greater perching behavior found in the enrichment group implies that this group had a more positive welfare state since they were better able to engage in this essential behavior. However, the result of this study could be due to the enrichment group having more perching options, as they frequently used pallet collars as perches. A different outcome could have been attained if the control group had access to pallet collars as perching areas as well.

The increased frequency of dust bathing in the enriched group could be due to the availability of dust bathing material, as chicks usually prefer sand or peat to dust bathe in (Skånberg et al., 2021). The enriched group had access to peat for dust bathing, while the control group only had wood shavings available. However, during the home pen study, none of the chicks were found to dust bathe in the peat, with all incidents occurring in the wood shavings. The observed increase in dust bathing behavior in the enrichment group compared to the control group demonstrates the importance of environmental enrichment in encouraging natural behaviors in laying hens. Dust bathing is an important behavior for chickens, providing both hygienic and psychological purposes. It keeps chickens' feathers healthy by removing excess oil and parasites, while also providing stress relief and relaxation (Olsson & Keeling, 2005).

The introduction of more naturalistic enrichment materials, such as peat and plastic plants, most likely provided the enriched group with a better substrate and environment to support this natural behavior. Furthermore, the capacity to engage in dust bathing may reduce stress levels since it meets a fundamental behavioral demand, contributing to improved overall welfare in the enriched group (Olsson & Keeling, 2005). As a result, the more frequent dust bathing found in the enriched group not only shows a better living environment, but it also implies that offering such enrichment could be a critical strategy for enhancing welfare outcomes in commercial chicken production.

In this study, the enrichment group displayed significantly more stand alert behavior than the control group. This discovery is rather inconsequential since stand alert behavior is frequently related with increased vigilance and anxiety, which would normally be predicted to decrease with environmental enrichment. However, the enriched group's higher stand alert behavior may indicate a different element of their behavioral repertoire. Environmental enrichment can improve animals' sensory and cognitive engagement with their surroundings, resulting in enhanced awareness and reaction to stimuli (Newberry, 1995; Campbell et al. 2019). The richer environment gave the birds more opportunity to interact with and monitor their surroundings, which may have caused them to display a more alert posture as part of their curious and environmental scanning behavior. Furthermore, the enriched group's frequent stand alert response could imply a greater sense of curiosity and alertness than fear or worry. Previous research has indicated that animals in enriched habitats tend to exhibit more curious and curiosity-driven behaviors when exposed to novel or complicated situations. Thus, the higher stand alert behavior in the enrichment group could be viewed as an indication of increased environmental engagement, in which the birds actively observe and interact with their surroundings. This implies that, whereas stand alert behavior is often associated with alertness, in an enriched environment, it may reflect a good feature of environmental awareness and cognitive engagement rather than a sign of stress.

Flight behavior, which is usually triggered by fear or perceived threats, was significantly more common in the control group than in the enrichment group. This shows that the enriched environment created a more secure and engaging environment, minimizing the urge for escape behaviors. Previous study has demonstrated that enriched surroundings reduce fear and anxiety in poultry by permitting natural behaviors like foraging and dust bathing, which are critical for stress reduction (Campbell et al., 2019). The reduced flight behavior in the enriched group suggests more comfort and security, most likely because of improved emotional regulation and stress resistance created by the complex environment (Ross et al., 2020). However, since the control group only performed the behavior six times and the enrichment group never performed it, I can not draw any conclusions regarding this finding.

One important limitation of the statistical model used in this study is that each observation occasion is treated as if it were independent measures. This raises the possibility of pseudoreplication, in which the model understates variability while overestimating degrees of freedom. As a result, the statistical analysis cannot be generalized beyond the 58 chickens studied, and the findings should be interpreted with caution. While the research provides insight into whether environmental enrichment promotes more natural and varied behavior, the findings should be viewed with the caution that the model does not account for repeated measurements on the same chickens. Despite these statistical restrictions, the descriptive statistics, combined with the analysis, support the conclusion that the enrichment had the expected effect. Although the enrichment appears to have encouraged more natural behaviors in the chickens, future studies should account for the non-independence of observations using more advanced statistical models to properly address the structure of the data.

5.2 Open field test

The open field test is commonly used to assess fear responses, exploration and activity levels. This test is a usual method to assess an animal's reaction to a new environment, and higher exploration is generally regarded as less anxiety or fear. Contrary to predictions, there were not any significant differences between the groups during the test. These findings are rather surprising, given that earlier research has repeatedly demonstrated that environmental enrichment can lower fearfulness and responses to stress in chickens, as birds raised in richer environments exhibit lower stress levels and more curiosity-driven behavior in novel situations (Campbell et al., 2019). This lack of significant findings could be due to the short duration of these tests or to the chicken's potential adaptation to their environments over time. It is also possible that the enrichment provided was not sufficient to generate significant behavioral changes detectable in these short-term testing. These findings emphasize the complexities of animal behavior and the need for further studies to identify the conditions under which environmental enrichment might effectively reduce anxiety and promote welfare in chickens.

5.3 Novel object test

The novel object test results showed that the enrichment group spent more time in proximity to the novel object than the control group. This behavior demonstrates that the enriched chickens are more comfortable and less afraid of novel stimuli. In contrast, the control group remained further away from the novel object, indicating increased neophobia—fear of unfamiliar objects. This behavior is consistent with observations in other species, where neophobic responses cause a slower approach to food when novel things are present, as observed in studies by Fischer et al. (2016). The observed behavioral difference between the groups could be related to stress affecting their ability to cope with unfamiliar stimuli. Forkman et al. (2007) previously stated that stress could prevent an animal's ability to adjust to new additions in their environment, which could explain why the control group demonstrated increased avoidance behavior. This supports evidence to the theory that the control group was under more overall stress than the enrichment group. The enriched habitat, with its increased complexity and stimulus, most likely encouraged the enrichment hens in developing stronger coping mechanisms, lowering fear and helping them to engage more easily with novel objects in their surroundings.

5.4 Restraint test

The restraint rest, which measures stress response using corticosterone (CORT) levels, found no significant difference between the control and enrichment groups. The lack of significant data could indicate that the environmental enrichment offered was insufficient to cause any physiological changes visible by this test. The findings align with previous research, which found mixed results regarding the impact of environmental enrichment on stress hormone levels. For example, Hedlund et al. (2019) found that the effects of enrichment on CORT levels are not always constant, possibly due to differences in the type, length, and degree of enrichment offered. Furthermore, it is possible that the stress experienced by the chickens during the restraint test was a high enough common stressor that enrichment was insufficient to buffer against it, resulting in similar CORT levels in both groups.

5.5 Feather fault bars

The enrichment group's feathers had overall fewer fault bars than the control group, which supports the theory that environmental complexity reduces stress-related feather malformations in events of acute stress (Arrazola & Torrey, 2019; Jovani & Rohwer, 2017). This finding further supports the theory of using fault bars as an indicator of stress (Bortolotti et al., 2002; Romero et al., 2005; Strochlic & Romero, 2008; DesRochers et al., 2009), and not only for measuring body condition and nutritional status (Riddle, 1908; Murphy et al., 1988; Arrazola et al., 2017). The majority of the fault bars were found in the tail (R1) feathers, which is in line with previous research the results, where tail feathers had significantly more fault bars than wing feathers (Arrazola & Torrey, 2019; Bonnekamp et al., 2017), supporting the theory that fault bars usually appears on feathers not as important for flight (Bonnekamp et al., 2017; Jovani & Blas, 2004; Pap et al., 2007; Jovani et al., 2010).

Interestingly, the enriched group had more faint fault bars in the wing (P8 and SC1) feathers, but fewer in the tail feathers. According to Arrazola & Torrey (2019), wing or tail feathers might be sensitive indications of negative experiences, depending on the degree of the stressor. They suggest that tail feathers can act as good indicators for moderate acute stress, while wing feathers can indicate severe acute stress. Looking at the faint fault bars, one could conclude that the enrichment group had more severe acute stress than the control group. However, this only applies to faint fault bars, since there were no differences between the groups in terms of moderate fault bars on either of the wing feathers, or severe fault bars on the SC1 wing feather. However, in the P8 wing feather, the control group showed considerably more severe fault bars, indicating a contradicting finding within this study. When the three types of fault bars were combined, the control group had more fault bars on the tail feathers but less on the SC1 wing feathers, highlighting the contradictory findings of prior research by Arrazola and Torrey (2019) on the role of feather type.

5.6 Conclusion

This study demonstrated that environmental enrichment had significant effects on the behavior and stress levels of White Leghorn chickens. Enrichment strategies that mimic natural settings resulted in more natural behaviors like perching and dust bathing while decreasing stress symptoms like flight behavior and severe feather fault bars. These findings emphasize the need of including environmental enrichment in industrial chicken farming to increase animal comfort. While other behavioral measures demonstrated no significant differences, the overall trend supports the idea that richer surroundings can improve poultry wellbeing by encouraging natural behaviors and lowering stress. These findings offer vital insights into designing more humane and productive poultry raising strategies.

5.7 Implications and future research

The findings of this study have important implications for chicken farming techniques, particularly in terms of improving animal wellbeing through environmental enrichment. The observed behavioral changes, such as more dust bathing and perching in enriched environments, highlight the potential of such tactics to encourage natural behaviors while reducing stress-related reactions in chicken. This is significant not only for ethical reasons, but also for economic benefits, as less stressed and healthier birds are more productive and require fewer medical interventions. Future research should look into the long-term impact of various types and combinations of environmental enrichment on behavior and physiological stress responses. Furthermore, future research should look into the genetic and epigenetic variables that contribute to individual diversity in enrichment responses, which could help guide selective breeding programs targeted at improving welfare qualities in poultry. Expanding research to include different strains and ages of chickens would also provide an increased understanding of how enrichment influences welfare in different settings.

6 Societal and ethical considerations

Throughout this study, care was taken to ensure that the chickens' welfare was prioritized, in accordance with ethical animal research norms. The study followed the 3Rs principles (Replacement, Reduction, and Refinement), limiting the number of animals used and ensuring that they were not exposed to unnecessary stress. Recovery periods were provided between experiments to allow the birds to recover. Understanding and enhancing animal welfare in poultry production has important societal effects. Better welfare is not only consistent with ethical norms, but it can also affect public perception and market demand for ethically produced animal products. However, it is essential to assess the cost and practicality of implementing enrichment tactics on a wide scale, and to address these issues through future research and policy development.

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