How does hatchery stress affect the development of play behavior?

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1. Abstract

Play is a behavior mainly observed in young individuals that can differ greatly between species in both function and appearance. Presence of play indicates positive experiences in animals, making play a useful assessment tool for animal welfare. Commercial hatcheries expose chicks to several stressors that can affect both behavior and welfare. Unfortunately, our understanding of play behavior in chickens is still limited as only a few studies have been conducted. Therefore, the aim of this study was to describe the ontogeny of various play behaviors in modern laying hens and to investigate how stress affects play behavior in chicks. A total of 80 chicks were used whereas 40 were hatched at a hatchery and 40 were hatched under controlled conditions. The chicks were tested in groups of four in enriched test arenas twice a week during five weeks. Each test lasted 30 minutes and novel objects were placed inside the arena after 10 and 20 minutes. Behaviors were noted while analyzing videos from the experiment using one-zero sampling and later divided into the categories of solitary, social, and total play. A linear mixed model with repeated measures was used to investigate how treatment, age, and the interaction treatment*age affect play behavior. Chicks played more during certain ages and all forms of play showed a similar change with age over both treatments. The amount of play did not differ significantly between treatments but there was a numerical (non-significant) tendency for hatchery chicks to play more, which may indicate compensatory play.

Keywords: Animal behavior, Animal welfare, Gallus domesticus, Hatchery stress, Play behavior

2. Introduction

Play is a common behavior that often is observed in animals. In fact, it is very likely that most people have observed play at some point and to some extent are able to identify the occurrence of play behaviors. However, despite the prevalence and possibility of identification, the difficulty of creating a definition remains. Over the years, several attempts have been made to describe behaviors that are considered playful, which has resulted in several definitions (Bekoff, 1984). Commonly occurring definitions were then summarized by Burghardt (2005) to simplify the study of play behavior, resulting in five criteria that all must be met for a behavior to be classified as play. According to the criteria, play behavior must: (1) not contribute to current survival; (2) occur spontaneously and be self-rewarding; (3) differ from functional behaviors either in structure, context, or development; (4) occur repeatedly; (5) occur when the animal is relaxed and not undergoing stress (Burghardt, 2005).
Both young and adult individuals can engage in playful activities, but it is more commonly observed in young individuals who are still under parental care (Bekoff, 1984). The fact that it is mainly young individuals who play can be explained by young having an abundance of resources such as time and energy (Bekoff, 1984; Burghardt, 2014). Furthermore, time devoted to an activity depends on the amount of time devoted to other activities and young individuals do not yet have the necessity to perform certain behaviors important to adults, such as behaviors associated with survival or reproduction (Bekoff, 1984). The function of play may vary depending on the type of activity performed and species performing the behavior. However, occurrence of play behavior is considered beneficial as it provides social cohesion and contributes to motor and cognitive training (Bekoff, 1984; Burghardt, 2014).

Play behavior can be divided into three different categories: locomotor play (sudden and hectic movement occurring spontaneously), object play (activity involves interaction with inanimate objects), and social play (activity includes more than one individual) (Bekoff, 1984). The categories are not completely strict as they may overlap, meaning that one category does not exclude another, for instance, both social and object play can be locomotory (Bekoff, 1984). There is a consensus that play behavior indicates positive experiences in animals, which has created the possibility of using play as an indicator of animal welfare (Boissy et al., 2007; Held & Špinka, 2011). In practice, presence of play could indicate good living conditions without welfare concerns, whereas lack of play could indicate poor welfare in need of improvement (Held & Špinka, 2011). After all, play behavior occurs in relaxed animals that do not undergo stress (Burghardt, 2005). Studies examining the effect of welfare on play behavior has been performed on several livestock animals such as cattle (*Bos taurus*), pigs (*Sus scrofa domesticus*) and sheep (*Ovis aries*) (Newberry et al., 1988; Bennett & Fewell, 1987; Jensen et al., 1998). These studies have mainly been performed on young individuals when play behavior is considered most frequently occurring.

When play behavior has been assessed in open field arenas, results have indicated that calves, piglets, and lambs mainly engage in locomotor play and social play (Berger, 1980; Blackshaw et al., 1997; Hass & Jenni, 1993; Jensen et al., 1998; Mintline et al., 2012). Pen size and presence of pen mates have been found to be crucial for the occurrence of play behavior as calves, piglets and lambs play less when kept in smaller pens and become less active when lacking social contact (Anderson et al., 2015; Blackshaw et al., 1997; Bennett & Fewell, 1987; Jensen et al., 1998; Newberry et al., 1988). In addition, studies have shown that well-fed calves and piglets play more than those provided with less feed (Jensen et al., 2015; Barnes et al.,
The results of studies on livestock indicate that space allowance, social contact, and amount of food are crucial for the occurrence of play behavior and thus affects animal welfare. After all, play indicates positive experiences in animals (Boissy et al., 2007).

Studies on play behavior have for a long time been restricted to mammals as their cognitive function was considered to differ from other animal classes. However, the cognitive function of birds has several similarities with those of mammals (Jarvis et al., 2005). The similarities between mammals and birds in cognitive function emphasize the likelihood that birds, just like mammals, engage in playful behaviors to develop certain life skills (Burghardt, 2014). Unfortunately, studying and defining play in birds comes with difficulties. Unlike some species of mammals, birds lack description of play markers, which are species specific behavioral patterns that signals to surrounding individuals that the activity about to be performed is merely meant as playful (Bekoff, 2001). The lack of description of play markers makes it difficult to determine which behaviors in birds should be classified as play. Furthermore, environmental factors might make it difficult to approach and study birds in the wild, and human presence tends to be frightening for many bird species. Studying birds in captivity therefore also comes with disadvantages, as stressed individuals will not perform play behavior (Burghardt, 2005).

Despite the difficulties, play has been observed in some species of birds such as corvids and parrots (Auersperg et al., 2015). Ravens (Corvus corax), which is a type of corvid, devote time to both object and social play. More specifically, ravens tend to play by manipulating inanimate objects with a preference for novel items (Heinrich, 1995). Another play behavior that has been observed in ravens is the collecting and placing of inedible objects in caches, which is followed by interaction with other ravens over their caches (Bugnyar et al., 2007). When it comes to parrots, species such as the African grey (Psittacus erithacus) and the New Zealand kea (Nestor notabilis) have been observed devoting time to object play by combining and stacking objects and by inserting objects into other objects (Pepperberg & Shive, 2001; Gajdon et al., 2014). A type of social play that is common in both ravens and parrots is play chasing, which is when birds follow each other without a clear purpose (Diamond & Bond, 2003).

Only a limited number of studies regarding play behavior have been performed on the domestic chicken (Gallus gallus domesticus). However, there are behavioral patterns in chickens that are suggested to meet the criteria for play, such as frolicking, sparring, and food-running (Kruijt, 1964; Dawson & Siegel, 1967). During frolicking, the chicken runs or jumps around spontaneously while having its wings raised or flapping. Frolicking is considered socially contagious as individuals can start performing this behavioral pattern by observing other
frolicking individuals (Dawson & Siegel, 1967). Sparring has similarities to fighting in adults and is therefore also called play fighting. During sparring, the chickens jump and engage in physical contact, but unlike real fights, sparring does not involve any injuries (Dawson & Siegel, 1967). Just like during frolicking, wing flapping is observed in relation to sparring. Wing flapping involves the chicken rapidly moving both its wings, which can be performed both during motion and while stationary. Adult males perform wing flapping during reproduction as a courtship behavior, but the action is merely considered playful in young individuals (McGary et al., 2003). Since wing flapping is considered playful and commonly occurs during playful activities, wing flapping can also be included in play behavior studies (Vasdal et al., 2019).

Food-running, also known as worm-running, occurs when a chicken picks up an object or food item and starts running. Other chickens then often chase the individual who picked up the item, trying to obtain the item themselves (Kruijt, 1964). The age of occurrence of different play behaviors vary as some play behaviors might occur earlier than others. However, in broilers, the general amount of play behavior decreases drastically during the first five weeks of life (Liu et al., 2020). As broilers are bred to have a very rapid growth rate, the decrease of play may occur at a different time point in chickens bred for other purposes (Baxter et al., 2021).

Chickens reared for commercial egg production are during their first days of life exposed to several events that induce stress, including noise pollution, sex sorting, vaccination, and transport away from the hatchery to rearing farms (Hedlund et al., 2019). Stress during early development can affect behaviors later in life and accordingly, studies show that hatchery stress has both short- and long-term effects (Hedlund et al., 2019). Chickens exposed to hatchery stress have been found to be more fearful and pessimistic compared to chickens without stressful experiences (Hedlund et al., 2019; Hedlund et al., 2021). The question is, how is play behavior affected by hatchery stress? After all, the fact that stressed chickens have previously been shown to have a negative cognitive bias could indicate that they are in a negative emotional state, which could in turn affect the performance of play behavior.

There are two aims of this study, one of which is to describe the ontogeny of various play behaviors in modern laying hens. The second aim is to investigate how stress affects play behavior in chicks by comparing the development of play behavior in chickens hatched at a hatchery and chickens hatched under controlled conditions. Since chickens hatched at a hatchery are exposed to more stress and thus potentially have poorer welfare, the hypothesis is that chickens hatched at the hatchery will play less than the control-chickens.
3. Materials and methods

3.1. Ethical note
All experimental protocols required to carry out this study were approved by Linköping Council for Ethical Licensing of Animal Experiments, ethical permit no 14916-2018 (Linköping, Sweden). Experiments were conducted in accordance with the approved guidelines.

3.2. Animals and housing
A total of 80 chickens of both sexes were used, all of which originated from a commercial hatchery in Sweden. Out of these, 40 were hatched at the hatchery (hatchery chicks, HC) and 40 were hatched under controlled conditions at Linköping University (control chicks, CC). The chicks hatched in the commercial hatchery were handled according to commercial standard routines, including conveying, sex sorting by wing inspection, vaccination, and 4 hours of transport. The eggs hatched under controlled conditions were picked up at the hatchery before start of incubation and placed in an incubator at the university at the corresponding time point as the eggs in the commercial hatchery.

When the chicks from the commercial hatchery arrived, all chicks were weighed and provided with leg rings of different colors to facilitate the identification of individuals. The chicks were then placed into four different cages together with other individuals of the same treatment (either CC or HC) with 20 chicks in each cage. Each cage had a size of 68x70 cm and provided the chicks with wood shavings, a heat roof and ad libitum food and water. When the chicks reached 4 weeks of age, they were moved to larger enclosures that measured 70x140 cm. In the new enclosure, all 40 individuals in each treatment (CC, HC) were kept together. Like in the previous smaller enclosure, the chicks were provided with wood shavings, a heat lamp, and ad libitum food and water.

3.3. Procedure and experiment
The chicks were divided into groups of four consisting of both sexes prior to each experiment, which resulted in a total of 10 groups of HC, and 10 groups of CC. These smaller groups of four were then tested as a unit for 30 minutes twice a week during five weeks. The groups consisted of the same individuals each week, which was ensured by the colors of the leg rings. The test was performed in enriched arenas measuring 117x80 cm (Figure 1). The enrichment in the arenas consisted of wood shavings, a perch, a small pile of hay which was cut into about 10 cm long pieces, and a small hanging chain. In total, there were four test arenas available, i.e., four groups were tested simultaneously. Due to the chicks potentially being tired after
hatching, transportation, and handling during weighing and provision of leg rings, the first test day began when the chicks were 1 week old.

![Figure 1. The test arena used in the play study.](image)

During the tests, the four chicks with the same leg ring color were placed in each test arena. Before the testing began, the lights in the test arenas were off. When the test was ready to start, a video recording of each arena started and the lights inside the arenas were turned on. Each test lasted 30 minutes and to stimulate certain play behaviors such as food running, novel objects were placed in the test arena through a small hatch after 10 and 20 minutes of testing. After 10 minutes, the chicks were provided with a 15 cm long fake worm made of soft plastic and after 20 minutes, live mealworms were placed in the test arena in a small cardboard box. In an attempt to stimulate food running further, only three mealworms were provided to the four chicks in each arena. The chicks were then observed during the tests to examine the presence of play behaviors such as frolicking, sparring, food-running, and wing flapping. Observed behaviors were used as a basis for the creation of an ethogram that was later used during the actual data collection.

### 3.4. Data analysis

Data was collected by analyzing the videos from the experiments. Occurrence of play behaviors according to the ethogram (Table 1) was noted using one–zero sampling for every individual within a test group with intervals of 15 seconds, meaning that every 15-second period during the 30 minutes of testing, a play behavior either occurred or not for every individual inside the
test arena. Consequently, each behavior could have a maximum value of 4 during a 15-second interval and after 30 minutes, each observed behavior could result in a total of 480 data points. The observations were independent as several behaviors could occur during the same test intervals. During data collection, each test group of four individuals counted as one sampling unit.

After data collection was completed, behaviors were divided into two different categories, called “solitary” and “social”, depending on whether the behavior was performed alone or together with another individual. All observations for behaviors belonging to either solitary or social play were then summarized for each sampling unit, test day and treatment prior to statistical tests. In addition, “total play” was also tested, which was the sum of all observed behaviors. Statistical tests were performed in SPSS Statistics version 27.0 to compare the frequency of different behaviors over time between HC and CC. A linear mixed model with repeated measures (MMRM) was used with play behaviors (solitary, social, or total play) as dependent variables, and age and treatment (HC or CC) as factors. In addition, charts were created in excel to illustrate the change in frequency of play over time as a comparison between HC and CC.

**Table 1.** Ethogram for all observed behaviors inside the test arenas divided into the corresponding categories of solitary and social play. Definitions have been adapted from Dawson and Siegel (1967), Cloutier et al. (2004), Baxter et al. (2019), and Liu et al. (2020).

<table>
<thead>
<tr>
<th>Category</th>
<th>Behavior</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solitary</td>
<td>Running</td>
<td>Spontaneous forward movement with at least twice the normal walking pace, often including rapid direction changes. Can start either while walking or being stationary. Wing flapping does not occur simultaneously.</td>
</tr>
<tr>
<td></td>
<td>Frolicking</td>
<td>Spontaneous and rapid running and/or jumping while wings either flapping or raised. Often with rapid direction changes. A frolicking bout ends when the bird resumes another activity.</td>
</tr>
<tr>
<td>Wing flapping</td>
<td>Rapid vertical movement with both wings while stationary or walking up to 2 steps. Excludes wing flaps performed by a bird to balance itself or correct its feathers.</td>
<td></td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Spinning</td>
<td>Circling movement around the birds own axis with at least twice the normal walking pace. Wing flapping does not occur simultaneously.</td>
<td></td>
</tr>
<tr>
<td>Spinning while wing flapping</td>
<td>Circling movement around the birds own axis with at least twice the normal walking pace. Wing flapping occurs simultaneously.</td>
<td></td>
</tr>
<tr>
<td>Worm running</td>
<td>A bird picks up an object in its beak and starts either walking or running. Rapid changes of pace or direction might occur. Other birds may begin to chase the bird carrying the object.</td>
<td></td>
</tr>
<tr>
<td>Worm running - mealworm</td>
<td>A bird picks up a mealworm in its beak and starts either walking or running. Rapid changes of pace or direction might occur. Other birds may begin to chase the bird carrying the mealworm.</td>
<td></td>
</tr>
<tr>
<td>Worm pecking</td>
<td>Bird pecks at fake worm or mealworm on the ground. The worm may be lifted off the ground and/or shaken but not carried.</td>
<td></td>
</tr>
<tr>
<td><strong>Social</strong></td>
<td><strong>Sparring jumping - no contact</strong></td>
<td>Simulation of fighting behavior with no obvious aggression. Two birds jumping while standing close facing one another. Jumping may also be performed by one bird while the other bird is passive or displays light avoidance. Wings flapping, extended, or kept to the sides. May include light kicking. No physical contact involved.</td>
</tr>
</tbody>
</table>
Sparring jumping - contact  Simulation of fighting behavior with no obvious aggression. Two birds jumping while standing close facing one another. Jumping may also be performed by one bird while the other bird is passive or displays light avoidance. Wings flapping, extended, or kept to the sides. May include light kicking. Involves physical contact.

Sparring stand-off – no contact  Simulation of fighting behavior (threats) with no obvious aggression. The following behaviors may occur during a bout: birds face each other briefly, stepping close and backing off from one another. While stepping close, necks and feathers around the neck are often raised. Pecking towards neck, head, or beak of the receiving bird. Wings are either flapping, extended or kept to the sides. Involves no physical contact.

Sparring stand-off – contact  Simulation of fighting behavior (threats) with no obvious aggression. The following behaviors may occur during a bout: birds face each other briefly, stepping close and backing off from one another. While stepping close, necks and feathers around the neck are often raised. Pecking towards neck, head, or beak of the receiving bird. Wings are either flapping, extended or kept to the sides. Involves physical contact.

Worm chasing  A bird chases after an individual performing worm-running and may try to obtain the carried object.
Worm exchange
An object is obtained by one bird from another bird’s beak. The object can be obtained either by a worm chasing bird from a worm running bird, or from a stationary bird with an object in its beak.

4. Results
There was no significant effect of treatment on solitary, social, nor total play. Furthermore, there was no significant interaction between treatment and age for neither solitary, social, nor total play. Removing the non-significant interaction terms from the models did not change the significance level of neither treatment, nor age. However, there was a significant effect of age on all dependent variables, as the amount of play behavior tended to gradually increase from 8 days of age to then reach its peak on day 29. After 29 days of age, the amount of play started to decrease (Figure 2 – 3). For statistics, see table 2.

Table 2. Results from linear mixed model (MMRM) illustrating the tests of fixed effects for the dependent variables solitary, social, and total play.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Source</th>
<th>Numerator df</th>
<th>Denominator df</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solitary play</td>
<td>Treatment</td>
<td>1</td>
<td>142.220</td>
<td>2.039</td>
<td>0.155</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>9</td>
<td>35.149</td>
<td>85.919</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Treatment*Age</td>
<td>9</td>
<td>35.149</td>
<td>1.111</td>
<td>0.380</td>
</tr>
<tr>
<td>Social play</td>
<td>Treatment</td>
<td>1</td>
<td>133.814</td>
<td>0.850</td>
<td>0.358</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>9</td>
<td>31.161</td>
<td>26.807</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Treatment*Age</td>
<td>9</td>
<td>31.161</td>
<td>1.233</td>
<td>0.311</td>
</tr>
<tr>
<td>Total play</td>
<td>Treatment</td>
<td>1</td>
<td>136.816</td>
<td>1.818</td>
<td>0.180</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>9</td>
<td>35.874</td>
<td>79.458</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Treatment*Age</td>
<td>9</td>
<td>35.874</td>
<td>1.407</td>
<td>0.222</td>
</tr>
</tbody>
</table>
The change in play frequency over time for each individually observed behavior followed a similar pattern as the summarized categories solitary, social, and total play (Figure 4). Most behaviors were consistent with a gradual increase in frequency after test day 8, which continued until a peak was reached. The peak was then followed by a decrease in frequency of observed play behavior. Compared to the graphs for solitary, social, and total play where the peak was quite clear on day 29, the individual graphs for each behavior varied regarding when the peak was reached. However, most behaviors had their peak around day 25 (Figure 4).

There were some exceptions where the behavior developed differently from the otherwise commonly occurring pattern. The exceptions apply to spinning (Figure 4d), spinning while wing flapping (Figure 4e), all sparring related behaviors (jumping no contact, jumping contact, stand-off no contact, stand-off contact) (Figure 4f–4i), worm exchange (Figure 4m), and worm pecking (Figure 4n). In comparison to other behaviors, the onset of behaviors related to spinning
and sparring did not occur until later with the first observations as late as day 15 for spinning (Figure 4d, 4e) and day 18 for sparring (Figure 4f – 4i). Furthermore, sparring related behaviors did not have a clear peak of observations at a certain time point and lacked a clear decrease in frequency as the chickens got older (Figure 4f – 4i). Like sparring, worm exchange and worm pecking also lacked a clear decrease of observations after a certain age (Figure 4m, 4n).
Figure 4. Mean of sum for all observed behaviors (±SE) over time, illustrating the change in frequency of play as a comparison between HC and CC. The behaviors shown in the graphs are
as follows: a: Running, b: Frolicking, c: Wing flapping, d: Spinning, e: Spinning while wing flapping, f: Jumping – no contact, g: Jumping – contact, h: Stand-off – no contact, i: Stand-off – contact, j: Worm running, k: Worm running – mealworm, l: Worm chasing, m: Worm exchange, n: Worm pecking. The scale of the y-axis differs between graphs to better illustrate behaviors with few observations.

5. Discussion
All forms of play had a similar developmental pattern and thus showed a similar change with age. The results indicate that the amount of play did not differ significantly between HC and CC, which does not align with the hypothesis that chickens hatched at the hatchery will play less than the control-chickens. However, there was a consistent numerical difference between treatments in the amount of play performed where HC generally performed more playing. We think this possibly can be explained by compensatory play.

The results showed that there was a significant effect of age, as the chicks played more during certain ages than others. More specifically, the amount of play gradually increased until the peak was reached at somewhere between 25 and 29 days of age, after which a decrease in play behavior could be seen. However, the presence of play behavior was almost non-existent when the chicks were 8 respectively 10 days old, as only a few observations of play were registered. It was not until test day 3, at 15 days of age, that the amount of play reached a more substantial increase. The same pattern of change in play behavior over age could be seen in most observed behaviors and in all dependent variables, that is solitary, social, and total play. A very likely reason why the chicks barely engaged in play during the first two test days is that the chicks were not given any opportunity to get used to the test arena before the first test occasion.

During analysis of the video recordings, one could see that the chicks, especially during the first test days, tended to stay close together and devote minimal time to all types of movement. In addition, the chicks seemed to strongly avoid the center of the arena, which resulted in the chicks spending most of the time along the walls. The fact that the chicks did not have the courage to leave the group to wander around inside the arena on their own suggests that the chicks may have experienced fear. After all, staying close to other individuals gives a sense of security, as safety can be found in numbers (Park & Hinsz, 2006). The probability that the chickens may have experienced fear is reinforced by results from previous studies, showing that stressed individuals who experience anxiety tend to be less active and stick to the walls of test arenas, which was also the case in this study (Kraeuter et al., 2019). After all, the center of
the arena can be perceived as threatening for the chickens as open spaces lack protection against potential dangers such as predators. Furthermore, the behaviors that occurred during the first days of testing were performed with caution and seemed to be mainly explorative. This observation is in accordance with previously mentioned thoughts as play occurs in relaxed animals not undergoing stress, such as anxiety or fear (Burghardt, 2005). The increase in observed play at test day 3 could be explained by the chicks, at this time, have been accustomed to the test arena and no longer feel threatened by the new environment.

A decrease in play frequency around 25 and 29 days of age could be explained by the fact that this age coincides with the end of maternal care. Mother hen often starts to distance herself from her chicks at 4 – 8 weeks of age, but the exact timing may vary greatly as some hens continue to provide maternal care until the chicks are 12 weeks old (McBride et al., 1969). The ceasing of maternal care forces the chicks to become more independent and thus, there will not be as much time available that can be devoted to play. This is in accordance with previous studies indicating that young individuals under parental care play more than others (Bekoff, 1984).

The fact that the amount of play did not significantly differ between the potentially stressed chicks from the hatchery and control chicks is not consistent with previous studies on other species, as several other livestock animals have shown significantly less play in stressed individuals (Newberry et al., 1988; Bennett & Fewell, 1987; Jensen et al., 1998). There are several potential reasons why the amount of play did not differ significantly between stressed and non-stressed individuals of chickens. In part, the result may be due to a lack of knowledge regarding play behavior in chickens and birds in general, as behaviors may have been assessed incorrectly. Furthermore, there is a possibility that the hatching, test environment, or the handling of chicks has induced stress in both treatment groups, which could have led to a loss of differences between treatments. There could also be a genetic explanation for the result, as stress resistance is highly individual and largely depends on the stress resistance and environment to which the parentals are exposed (Henriksen et al., 2011). Therefore, there is a possibility that other chicks from the same hatchery with other parentals will be more affected by the hatchery stress and thus play less than the chickens tested in this study. To rule out that the result is not due to lack of differences in stress between treatments, it would have been necessary to test the CORT-levels of chickens in the different treatment groups.

Even though there were no significant differences between HC and CC in amount of play performed, there was a numerical (non-significant) tendency for HC to play more than CC. It
is important to note that previous studies showing reduced play in stressed individuals are generally about animals that experience poor welfare during testing. The potentially stressed chicks in this study have been exposed to stress in the past but were kept in the same environment as the CC during all test days. Possibly, play is not affected by previous stress to the same degree, or in the same way, as ongoing stress. The stressed chicks may have been more motivated to play as a compensation for previous stress, which could be the reason why HC seemed to play slightly more than CC.

The results indicated no significant interaction between treatment and age, meaning that the change of play frequency over time was the same in HC and CC. However, even though there was no significant difference, CC tended to play slightly more than HC until day 18. After day 18, it was the other way around with a higher occurrence of play in HC than CC. This could be due to HC displaying a greater extent of fearful behaviors during the first test days compared to CC, such as staying close to other individuals and avoiding the arena center. Potentially, it could mean that HC has been affected by hatchery stress in the sense that it takes longer to calm down in new situations. This is not entirely surprising, as stressed individuals have been found to be more fearful in novel arena tests compared to non-stressed individuals (Hedlund et al., 2019). Consequently, it takes longer for HC to get used to the test arena and start playing. After HC has managed to get used to the arena, more play occurs in HC than CC, which could be due to HC experiencing greater contrast effects. Both HC and CC should, to some extent, be affected by the contrast between the cages where they are usually kept and the test arenas as the arenas provides more space and enrichment. However, the early stress to which HC has been exposed may have caused depression which makes the contrast between the cages and the arena greater (Hammen, 2015). This could cause more play in HC than CC, as HC may experience even more joy inside the arena compared to CC.

Regarding the choice of observed behaviors, there is some doubt whether certain behaviors should really be classified as play. Most observed behaviors followed a similar developmental pattern as they underwent an increase in frequency during the first weeks of life followed by a decrease after a certain age. This is in accordance with previous studies that have examined play behavior in chickens (Liu et al., 2020). However, there were behaviors observed in this study that deviated greatly from the expected pattern and therefore should probably not be classified as play. The deviant behaviors are spinning, spinning while wing flapping, worm exchange, and worm pecking. Both spinning and spinning while wing flapping had a very low occurrence with first observations as late as 22 days of age. After 22 days of age the number of
observations was continuously low, and occurrences seemed to rather be a coincidence than a behavioral pattern. When it comes to worm exchange, there was a lack of pattern as the behavior had a somewhat even occurrence during all test days. Furthermore, there were very few observations of worm exchange which gives an unreliable result. Worm pecking had, compared to the other dubious behaviors, an adequate number of observations and a steady increase in frequency as the chicks got older. However, the occurrence of worm pecking did not start to decrease after a certain age as the frequency of the behavior continued to increase. Based on the result, it is very likely that worm pecking should be classified as a foraging behavior rather than a play behavior. As spinning, spinning while wing flapping, worm exchange, and worm pecking did not follow the expected pattern and thus most likely were not play behaviors, they should be excluded from future studies on play behavior in chickens.

Other behaviors that developed differently from what was expected were all sparring related behaviors (jumping no contact, jumping contact, stand-off no contact, stand-off contact). However, sparring or some other sort of play fighting is a common play behavior that occurs in many species (Hass & Jenni, 1993; Blackshaw et al., 1997; Jensen et al., 1998). Sparring has also previously been described and is already widely accepted as play in chickens (Dawson & Siegel, 1967; Liu et al., 2020). It is therefore more likely that these behaviors have been assessed incorrectly rather than not being play behaviors at all. Sparring started to occur more frequently from day 18 onwards and had no clear decrease after a certain age like most other behaviors. The late onset of sparring related behaviors does not necessarily have to be strange, as the age at which different behaviors begin can vary. In other species, play fighting such as sparring, tends to start later than other play behaviors (Napolitano et al., 2009). However, the lack of frequency reduction raises the question of whether the behavior was still considered playful during the later test days. In this case, play markers would have been particularly useful in determining the purpose of performed behaviors as sparring has similarities to real fighting and aggression in adults (Dawson & Siegel, 1967). Unfortunately, there are no described play markers in chickens as they have not yet been identified. A reliable result was instead attempted by avoiding the registration of sparring behaviors where any of the involved individuals showed signs of wanting to escape. Another possible explanation is that sparring reaches its peak later than other observed behaviors as similar observations have been made in other species (Gomendio, 1988). Consequently, the peak of observations may occur after the last test day.

When it comes to the test arena, there is room for improvement and some adjustments should be considered. Primarily, there is a concern regarding the size of the test arena as the chicks
were quite large during the last test days. This raises the question of whether the test arena was large enough to allow play in older chicks. Therefore, the size of the test arena should potentially be reconsidered and made larger in future studies. The enrichment inside the test arena seemed for the most part appropriate to stimulate play. However, the enrichment should, to some extent, also be reconsidered as the perch did not seem optimal for the purpose of the study. Frankly, the perch seemed to be in the way during a play bout rather than contributing to the occurrence of play behavior. The use of a smaller perch would have been preferable with a placement that does not hinder chicks from playing, such as in one of the corners of the test arena. More cameras inside the test arena would also have been preferable as some behaviors may be difficult to observe when only viewing the chickens from above. In addition, recording with audio could have been beneficial as sound recordings may make it easier to determine which behaviors that are playful.

The sampling method used has both advantages and disadvantages. Something that is advantageous with the sampling method is the simplicity to observe over longer periods of time without any major risk of mistakes. Short observation intervals might also give a more reliable result. However, it is conceivable that the method used may give an incorrect impression as play can be ongoing over the course of multiple intervals. It may be that some individuals play more often than others, while others play for a longer time. To be able to see these differences, continuous sampling would potentially have been a better suited choice. Continuous sampling would however imply a higher risk of missing the occurrence of behaviors and the observation time would probably have needed to be shortened. Hence, both continuous sampling and one-zero sampling that was used in this study comes with advantages and disadvantages that should be considered.

Based on the previously discussed aspects, future studies on play behavior in chicks should consider a different ethogram. More specifically, spinning, spinning while wing flapping, worm exchange, and worm pecking should be excluded from future studies. Furthermore, due to few observations, the behaviors of jumping no contact, jumping contact, stand-off no contact, and stand-off contact should be bundled together into one behavior called sparring. The enrichment and size of the test arena should also undergo some changes to allow as much play as possible. Future studies should also consider using more cameras to be able to observe the chicks from several angles, preferably with audio recordings available. It would also have been beneficial to allow the chicks to habituate to the test arena before the first day of testing.
5.1. Conclusions
There was a consistent, non-significant, numerical difference between treatments in the amount of play performed, suggesting that there may have been compensatory play in HC. This is contradictory from previous studies on other animal species suggesting a lower occurrence of play in stressed individuals. The results indicate that age is a large factor for play behavior as chicks play more during certain ages than others. More specifically, all play behaviors follow a similar developmental pattern as the behavior undergoes an increase in frequency during the first weeks of life followed by a decrease after a certain age. Some behaviors might be more commonly occurring during certain ages than others. The difficulty of assessing play behaviors remains, as studies on play in chickens are still insufficient. Further studies are necessary to get a better understanding of play in chickens and to get clarity in how hatchery stress affects welfare.

6. Societal and ethical considerations
This study includes live animals, which may cause some concern as animal research entails an almost inevitable risk that the animals may be exposed to suffering. To prevent suffering to the greatest extent possible, all methods underwent a review and were approved prior to the study which was then conducted in accordance to approved guidelines. However, despite an approved method, it is impossible to rule out the risk that the animals may still, to some extent, experience suffering. The suffering was tried to be kept to a minimum by providing pens with all the necessities for performing natural behaviors without hinderance. Furthermore, overcrowding of the pens was avoided by ensuring that all chicks had an appropriate amount of space. In addition, the study was only observational, which should cause the animals a minimal amount of suffering. Studies referred to in this study may, however, have used methods that caused more suffering as animals have been exposed to living conditions that are expected to cause some extent of discomfort. Despite this, previous studies are of great importance as they have contributed to the knowledge we have today.

Animal testing is sometimes necessary to acquire knowledge of certain subjects and can thus not always be avoided. For example, studies on chickens are required to gain an increased understanding of chicken behavior. When considering conducting a study that includes animals, it is important to weigh the benefits of the study against the disadvantages such as potential suffering of involved animals. If the benefits of a study do not exceed the potential suffering to which the animals are exposed, the method should be reconsidered. Animal testing should not be avoided simply because it is animal testing as research involving animal experiments are not
necessarily worse than other processes involving animals. In fact, it is possible that chicks in this study suffer less than chicks raised for poultry farming as chicks in poultry often are kept in large groups where overcrowding is not particularly uncommon. However, whenever possible the use of animals in studies should be avoided.

This study can contribute with several benefits both in the short and long term. First and foremost, it can contribute to an increased knowledge of chicken behavior that may be important for both farmers and researchers. Future research may benefit from the knowledge gained in this study as it can contribute with new research ideas and be useful when planning methodology. The study can also lead to an increased understanding of play and its significance for welfare, which is not only important for chickens but for all types of animals kept in captivity. Studies that increase the understanding of animal behavior and welfare are important as they raise awareness towards animals and their living conditions. In the long run, this can lead to improved welfare for animals as more people are taking animal welfare seriously. Studies on chickens are of particular importance as it is primarily the welfare of chickens that is currently often neglected. The fact that no major consideration is given to chickens raises a concern as chickens are our most common livestock animal. Today, intensive animal farming is common to provide the continuously growing human population with food at the lowest possible cost. It is therefore important that we can provide animals with as good living conditions as possible. Knowledge about play and its relation to welfare can facilitate the assessment of welfare in the future and make it easier to improve living conditions. Not only would that be beneficial for the lives of animals as it would also provide human and economic benefits. After all, an improved welfare can lead to a more sustainable industry, as it may lead to animals producing more and potentially better products. In turn, this can eventually lead to increased economic profit for farmers.

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