THE EFFECTS OF EARLY LIFE TREATMENTS ON FEARFULNESS AND FEATHER PECKING IN LAYING HENS

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Abstract

Feather pecking (FP) is a major welfare issue in laying hen farming, therefore it is necessary to find out which factors could play a role in this. There are three main factors that play a role in the development of feather pecking; not being able to properly express the motivation to show foraging and feeding behaviour, the influence of maternal hormones and the ability to cope with fear and stress. When the chick has enough opportunities to show foraging behaviour from an early age, FP behaviour decreases. Feather pecking could be related to fearfulness in laying hens. In this research, this looked into the effects of early life treatments on feather pecking behaviour and fearfulness in laying hens. Two early life treatments were applied; incubation of the eggs under a 12:12 light dark-cycle (LD-cycle) with a green light and feeding live larvae in a food puzzle. Early life treatments were expected to cause a reduction in feather pecking behaviour and fearfulness. By providing the chick with a food puzzle with live larvae, and therefore more foraging opportunities, it was expected to cause a decrease in feather pecking behaviour or even prevent it from developing this behaviour. Half of the eggs were incubated in a 12 hour light-12 hour dark cycle (12:12 LD-cycle) and the other half was incubated in the dark. There were 20 pens with ten chicks each. Half of the pens received the larvae daily and the other pens did not. In total there were four treatment groups; dark/enriched (D/E, n=7), light dark/enriched (12:12 LD cycle with green light) (LD/E, n=3), dark/not enriched (D/NE, n=3), light dark/not enriched (LD/NE, n=7), in which D/NE is the control group. During home pen observations feather pecking behaviour was scored. A social reinstatement test was used to look at fear responses of the chicks. From the results, an assumption could be made. It seems that larvae treatment caused a decrease in GFP behaviour. But because there were no significant outcomes, this could not be confirmed. We also could not confirm the effectiveness of the treatments on SFP behaviour. The effects of the treatments on the fear responses in the social reinstatement test also could not be confirmed. Thus more research is required to see if early life treatments could reduce feather pecking and fearfulness in laying hens. If research could be done with bigger sample size and more home pen observations, there might be a significant outcome found.

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Introduction

Background of the study

In today's society, there is more demand for organic products and products with a natural origin. In the past, we tried to adapt the animal to fit into our way of housing them. Nowadays it is more common to try to adjust the environment of the animal to increase the welfare of the animals used for the production of food. For some time now breeding organisations are researching how they can efficiently breed an animal that combines good production with little feather pecking behaviour and low susceptibility to diseases (van Niekerk, de Jong, van Krimpen, & Veldkamp, 2011). While breeding measures can make a positive contribution, breeding a chicken that shows less feather pecking as the only measure is not enough to solve the problem of excessive feather pecking and cannibalism. A package of husbandry and management measures will have to be developed, which ensures that this animal is kept in the best possible environment so that the threshold for pecking is as high as possible(van Niekerk et al., 2011). The consumers want more products that come from animals that can show their natural behaviour, and therefore increase their welfare. This is also the case for poultry farming. The eggs from organic and free-range farms have the highest price. This agrees with the expectations of the consumers that these eggs come from the most welfare-friendly systems(Knierim, 2006). Although more and more chickens have gained access to outdoor areas, this does not solve all welfare problems(Knierim, 2006). Several studies have concluded that access to an outdoor area and good use of the outdoor run has a preventative effect on feather pecking(Bestman & Wagenaar, 2003; Green, Lewis, Kimpton, & Nicol, 2000; Mahboub, Müller, & Borell, 2004; Nicol, Pötzsch, Lewis, & Green, 2003). In both conventional and organic poultry farming, feather pecking is one of the most urgent welfare problems. Trimming the upper beak of the chickens was used to reduce the damage caused by feather pecking, but this has been prohibited in the Netherlands since 2019. So it is important to find out which factors play a role in the development of feather pecking behaviour and what kind of environmental changes can be made to reduce feather pecking.

What is feather pecking?

Feather pecking is the behaviour when birds peck the feathers of conspecifics (de Haas & van der Eijk, 2018). Feather pecking can be divided into gentle feather pecking and severe feather pecking (Rodenburg, et al., 2013; Savory, 1995). Gentle FP (GFP) is a behaviour that is common in birds and can be related to social discrimination and exploration (Riedstra, & Groothuis, 2002). Severe FP (SFP) can lead to injuries in birds like feather damage, bald areas and in extreme cases to cannibalistic pecking (Savory, 1995). The different types of FP (gentle, severe and cannibalistic) are presumed to have different underlying motivations, and therefore show differences in behaviour (Buitenhuis & Kjaer, 2008; Savory, Mann, & MaCleod, 1999). Aggressive pecking is a behaviour that is shown to maintain the dominance hierarchy (Rodenburg et al., 2013). Aggressive pecking is more directed towards the head and comb while FP usually is directed toward the back area of the chicken (Rodenburg et al., 2013).

Causes of feather pecking

Feather pecking has different causes and is, therefore, a multifactorial problem (Rodenburg et al., 2013). In the study by Rodenburg et al. (2013) is concluded that there are three main factors that play a role in the development of feather pecking; not being able to properly express the motivation to show foraging and feeding behaviour, the influence of maternal hormones and the ability to cope with fear and stress. The most important factor in causing feather pecking is the limitation of foraging behaviour, mainly from an early age. Providing chicks with enough litter from an early age is important because it is shown that the absence of litter has consequences later in life (Rodenburg et al., 2013).

Foraging behaviour

The study by Rodenburg et al. (2013) shows the importance of early access to litter for foraging: provision of wood shavings or straw reduces FP later in life (Rodenburg et al., 2013). If FP is the result of the lack of foraging opportunities, providing enough litter for foraging would reduce FP. This is mostly true when enough litter is provided and foraging behaviour increases, FP decreases but does not completely stop (Rodenburg et al., 2013; Blokhuis & van der Haar, 1992; Nicol et al., 2001).

Feeding live larvae

Chickens in free-range farming spend about 37% of their time foraging for live insects (Star et al., 2020). The inability to express this natural foraging behaviour can increase pecking behaviour (Blokhuis & Wiepkema, 1998). Feeding the chicks live larvae would stimulate this natural foraging behaviour (Carr, 2016). Live larvae are not only a moving visual stimulus but are also of nutritional value (Clara, Regolin, Vallortigara, & Rogers, 2009; Paul et al., 2016; Paul et al., 2017). In material and methods is an explanation of what role the nutritional value plays in our study. In the study by Star et al. (2020) was concluded that feeding live larvae to older laying hens with intact beaks resulted in the chickens having a better feather condition.

Light conditions during incubation

Chickens are commercially incubated in the dark. But in natural circumstances, the chicks would receive some light during incubation. This happens when the hen leaves the nest to feed(Archer, Shivaprasad, & Mench, 2009). Feather pecking in the early life of a chick is a form of social exploration. Social recognition is important for social exploration. Social recognition is a lateralized function and this can be influenced by light conditions during the incubation(Riedstra & Groothuis, 2004). An earlier study on incubation shows that light during incubation causes lateralisation of the visual pathways. Lateralisation of the visual pathways affects some post-hatch behaviours, like the ones that are related to fear and learning(Archer & Mench, 2014; Rogers, 1995). .

The study by Archer et al. (2009) showed that broiler chicks that were incubated in the dark or with constant light had a greater bilateral physical asymmetry than chicks that received 12h of light during incubation. From this can be said that not sufficient light during incubation causes problems with the development of the chick (Archer, Gregory & Mench, 2013). Therefore bilateral physical asymmetry can be considered an indication of stress during development (Møller, Sanotra, & Vestergaard, 1995). The study by Archer et al. (2013) showed that incubation in a 12:12 LD cycle caused a decrease in bilateral physical asymmetry and this can reduce the stress susceptibility after hatching.

Fearfulness and feather pecking

Feather pecking can be related to certain behavioural characteristics that the chickens may have, such as fearfulness. Fearfulness is the likelihood of an individual to be frightened easily (Jones, 1996). FP can increase fearfulness (Blokhuis,& Beutler, 1992; Vestergaard, Kruijt, & Hogan, 1993) but on the other hand, fear can be a predictor to develop FP. Chickens that are fearful show less movement in the open field test (Rodenburg, de Haas, Nielsen, & Buitenhuis, 2010). Research by Rodenburg et al. (2004) shows that chicks that were less active in the open field test were more likely to develop FP later in life than active chicks (Rodenburg et al., 2004). Various studies (Hughes & Duncan, 1972; Vestergaard et al., 1993) showed that FP can also cause an increase in fearful behaviour in the victims.

Research goal(s)

In this research, we want to find out how feather pecking can be reduced in laying hens. This is done by ensuring that rearing conditions better reflect their natural environment and by offering enrichment. The enrichment that is given is incubation in a light-dark cycle and feeding the chicks live larvae. The larvae are given in a food puzzle, this stimulates natural foraging behaviour like pecking and scratching. The aim of the research is to find out if early life treatments, like incubation in an LD-cycle and feeding live larvae, reduce fearfulness and FP.

Another goal is to look at the individual pecking behaviour (pecker and victim) and to find out if there is a correlation in the amount of fearfulness it shows in the runway test. We expect that the chickens will be less fearful and show more natural foraging behaviour and will be likely to show less feather pecking.

Hypothesis

Do feeding live larvae in a food puzzle and incubation in a light-dark cycle reduce fearfulness and feather pecking in the early life of laying hens?

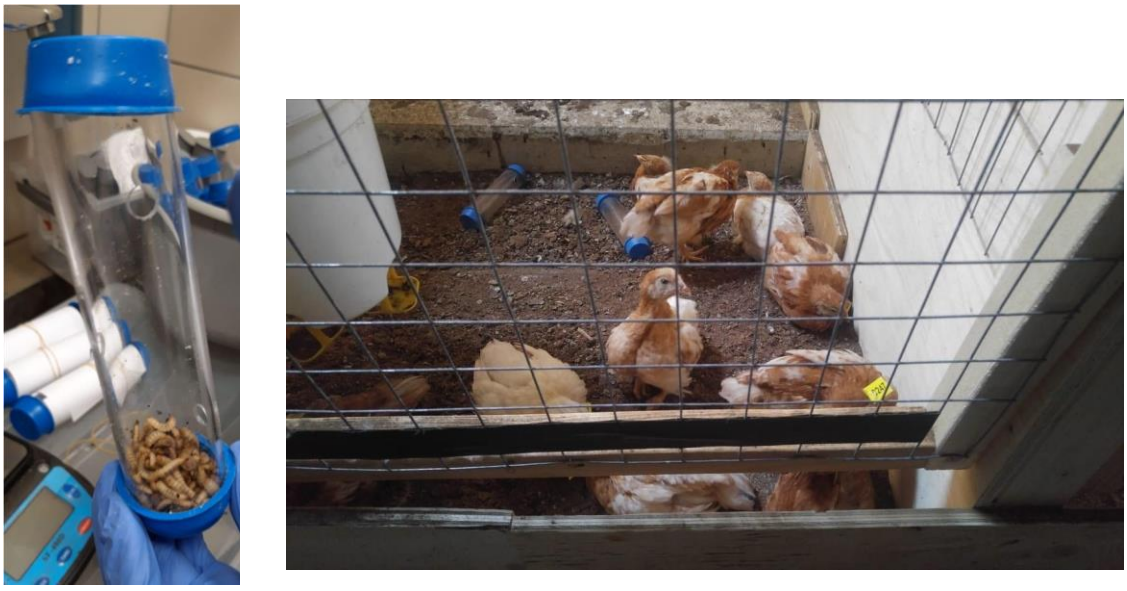
H0: Feeding live larvae in a food puzzle and incubation in a light-dark cycle does not reduce fearfulness and feather pecking in the early life of laying hens.

H1: Feeding live larvae in a food puzzle and incubation in a light-dark cycle reduces fearfulness and feather pecking in the early life of laying hens.

Material and methods

Animals and early life treatments

In this study, 200 ISA Brown laying hens were used. Before hatching the eggs (n=600) were incubated in two different conditions. Half of the eggs (n=300) were incubated in the dark and the other half (n=300) was incubated under a 12:12 LD-cycle with a green light. After hatching, 200 female chicks were housed in groups of 10 per pen (20 pens in total). The chicks were monitored for 18 weeks in total. 18 weeks is the age where chickens usually move to the laying farm to start laying eggs, thus marking the end of the rearing phase. During these 18 weeks half of the pens (n=10) was fed with live larvae and the other half (n=10) was not. In this research, the larvae that were given were the larvae of the black soldier fly, which is already approved for poultry feeding in Europe (Commission regulation (EU) no 68/2013 on the catalogue of feed materials text with EEA relevance, 2013) In this study, we did not want the nutritional value of the larvae to play a role, so the chicks that did not get the live larvae got additional feed to compensate for the possible nutritional values of the larvae. All chicks received standard rearing feed for laying hens and the chickens that didn't receive larvae got a custom-made additive to their feed, which had the same composition as that of the black soldier fly (BSF-replacer). This was 10% of the total feed since the chickens who received larvae got 10% of their daily feed intake in grams of larvae. The live larvae are given in a food puzzle (see Figure 1) and therefore stimulate natural foraging behaviour (pecking and scratching). In total there are four different groups; dark/enriched (D/E, n=7), light dark/enriched (12:12 LD cycle with green light) (LD/E, n=3), dark/not enriched (D/NE, n=3), light dark/not enriched (LD/NE, n=7), in which D/NE is the control group.



A *B*

Figure 1: A; The design of the tubes in which the live larvae were fed. The tubes are closed at the top and at the bottom. It had several holes in the transparent part for the chick to get to the larvae. B; The pen got 2 tubes once a day and were placed the way it shows in the picture.

Home pen observations

The home pen observations occurred in week 5, this age was chosen because of the amount of FP behaviour shown on this age(de Haas, Bolhuis, Kemp, Groothuis, & Rodenburg, 2014). The observations were done by three observers, each observing another pen. The pen was observed one time in the early morning (EM), one time in the late morning (LM) and one time in the afternoon (AF). There were 60 observations in total spread over 4 days. 2 observers did 24 observations and one observer did 12 observations. The feather pecking protocol was based on the research by Van der Eijk et al. (2018) so that the data could be compared if necessary. Feather pecking was recorded during 30-minute observations using behaviour sampling (samples all occurrences of some specific behaviours). FP was scored as gentle FP or severe FP (see Table 1). Aggressive pecks were not scored because these have a different motivation than gentle FP and severe FP. We also scored who pecked and who received. Individual pecking behaviour was also recorded. The phenotypes of the individual pecking behaviour were determined afterwards based on the data of the home pen observations (see Table 2). Order of pen observations was randomized over time, treatment and observer. The observers sat in front of the pen for the observations. The observations started with five minutes of habituation time, followed by 25 minutes of observations. Every five minutes, the observer scored the behaviours that were seen for the time budget scoring. For the time budget scoring the following behaviours were scored: resting, preening, foraging, foraging with larvae tubes, eating, drinking, dust bathing, active, feather pecking and if the bird was out of sight (see Table 3). In between the time budget scoring, the observer scored the feather pecking behaviour according to the ethogram. If a bird ate feathers off the ground, this was also recorded.

Table 1: Ethogram feather pecking (derived from van der Eijk, Lammers, Li, Kjaer, & Rodenburg, 2018)

Behaviour	**Description**
Gentle Feather Peck	Bird makes gentle beak contact with the feathers of another bird without visibly altering the position of the feathers. The recipient makes no apparent response. Each peck is recorded (if it is not stereotyped). Each peck was recorded as given or received.
Severe Feather Peck	Bird grips and pulls or tears vigorously at a feather of another bird with her beak, causing the feather to lift up, break or be pulled out. The recipient reacts to the peck by vocalising, moving away or turning towards the pecking bird. Each peck is recorded. Each peck was recorded as given or received.

Table 2: Individual pecking behaviour scoring, based on the data of the home pen observations. (derived from van der Eijk, Lammers, Li, Kjaer, & Rodenburg, 2018)

Type of pecker	Description
Pecker (P)	If a bird gives more than one SFP, it is defined as a pecker
Victim (V)	If a bird receives more than one SFP, it is defined as a victim
Victim-Pecker (V-P)	If a bird gave and received more than one SFP it is defined a Victim-Pecker
Neutral (N)	If a bird gave and received zero or one SFP it was defined as a neutral

Table 3: Ethogram time budget scoring

Behaviour	**Description**
Resting	Lying on the floor or sitting on the perch.
Preen	Grooming themselves, with beak manipulating their feathers.
Forage	Pecking at the ground or the environment. Scratching with their feet. *Also when they forage the larvae tubes.
Eating	Eating while sitting on the yellow feeding tray.
Drinking	Drinking from their drinking bucket.
Dust bathing	Combined preening and scratching behaviour during which the chick pecks and scratches at the dust bath area, then squats down and follows an organized sequence of behaviour patterns such as head rubbing and vertical wing shaking.
Active	Running, standing, flying or jumping
Feather pecking	See ethogram feather pecking
Out of sight	The chick is out of sight so the behaviour can not be scored.

Social reinstatement test

A runway test was used to measure social reinstatement behaviour in chickens. The runway test is a test where the chick has to move through a corridor to get to the pen mates on the other side(Marin, Freytes, Guzman, & Jones, 2001). The test was conducted at three and four weeks of age. The set-up that was used, was a square apparatus with a Plexiglas panel in the middle (see Figure 2). On one side there was a mirror and on the other side, there was not. On the side where the mirror was, there was also a recording of the sounds of the home pen. The test was a social reinstatement test because the mirror and sound recordings were used as a social stimulus(Marin et al., 2001). This simulation was to motivate the chick to move past the Plexiglas panel. The longer it took for the chick to move, the more fearful it was. The chick was individually transported from the home pen to the experimental room in a transport box. The chick was placed in the apparatus by one experimenter (experimenter A), who walked out of sight of the chick after placing it in the apparatus. The other experimenter (experimenter B) was situated in front of a monitor and looked into the apparatus with the camera. Both the experimenters started the stopwatch at the same time and experimenter A scored latency to vocalise, the number of vocalisations and experimenter B scored latency to approach the mirror. This test contains some events that are scary for the chicks; capture by the experimenter, exposure to a novel object and also social stress (separation from their pen mates). The responses the chick shows in the test depend on the amount of fearfulness of the chick had during the test and therefore the test can be used to determine the fearfulness in the chicks (Marin, Freytes, Guzman, & Jones, 2001).

Figure 2: Test set up social reinstatement. It was a square apparatus with a Plexiglas barrier in the middle. There was a mirror on the other side (bottom right photo) of where the chick was placed (top right photo).

Statistical analysis

For the analysis of the home pen observations the data of the GFP given and SFP given were used. This was chosen because if the totals (given and received added up) were used it was possible to use a score twice. The pens (experimental unit) were divided into the treatment groups according to which treatment the pen had received. The data of GFP given was normally distributed, this was tested with the Shapiro-Wilk normality test (W>0,90). The data of the SFP given was not normally distributed, this was tested with the Shapiro-Wilk normality test (W<0,90). For the analysis in Rstudio mixed linear models were used. The GFP given and SFP given were used as repeated measurements. Generalized linear models were used for the social reinstatement test. The variables were: the number of vocalisations, latency to vocalise, latency to approach the mirror, SFP given and SFP received. None of the variables was normally distributed (W<0,90). To test if there was a correlation between SFP given, SFP received and latency to approach the mirror, latency to vocalise and number of vocalisations, a Spearman's rank correlation test was used. To see if there was a difference in the variables latency to approach the mirror, latency to vocalise and the number of vocalisations between the different treatment groups, a Kruskal-Wallis test was used. For the analysis to see if the types of peckers are significantly different in each treatment group, Goodman and Kruskal's tau test was used. For a detailed overview of the statistical tests and models that were used, see Table 5 in the appendix.

Results

Home pen observations

The pens were divided into four treatment groups: D/E(n=7), D/NE (n=3), LD/E (n=3) and LD/NE (n=7). This unequal distribution of the pens was due to an error in the randomization process. In the groups with the larvae treatment was less GFP seen (p=0.0543). This tendency can be seen in figure 3. The outcome for the effect of the incubation treatment on GFP was not significant (p=0.2250). In figure 4 can be seen that in the control group (D/NE) there is almost no SFP. The LD/NE group has the most SFP seen compared to the other groups. The LD/NE group has more SFP than the D/NE group. From this, you could say that in the group with light incubation is more SFP behaviour. The group D/E has more SFP than the D/NE group. From this could be said that the larvae enrichment could cause more SFP behaviour. There was no significant outcome on the effects of the incubation treatment (p=0.4900) and the larvae treatment (p=0.4508) on SFP found, so none of it can be confirmed.

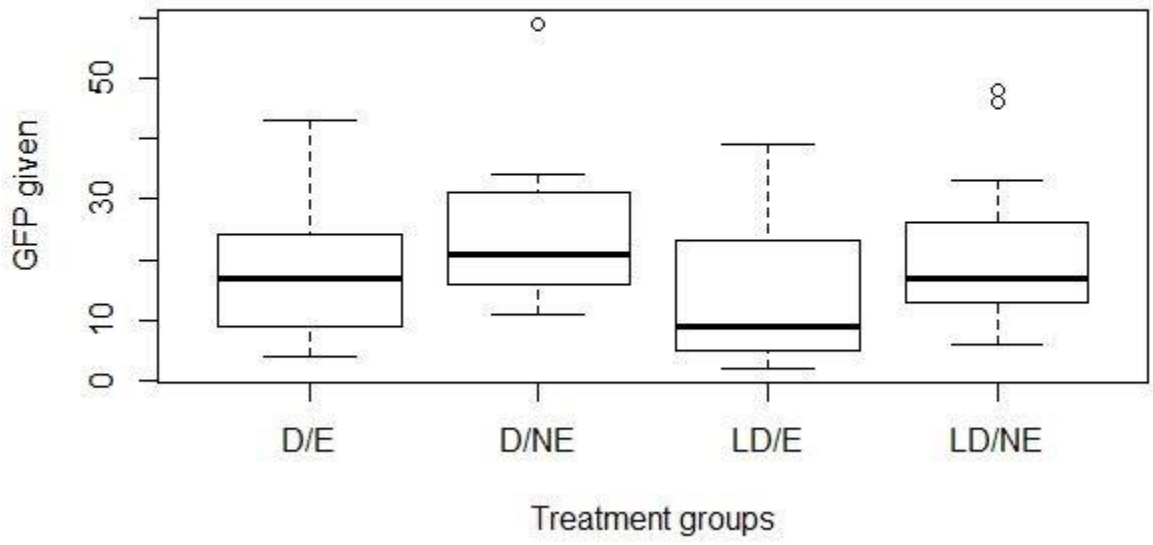


Figure 3: GFP given per treatment group. D/E(n=7), D/NE (n=3), LD/E (n=3) and LD/NE (n=7).

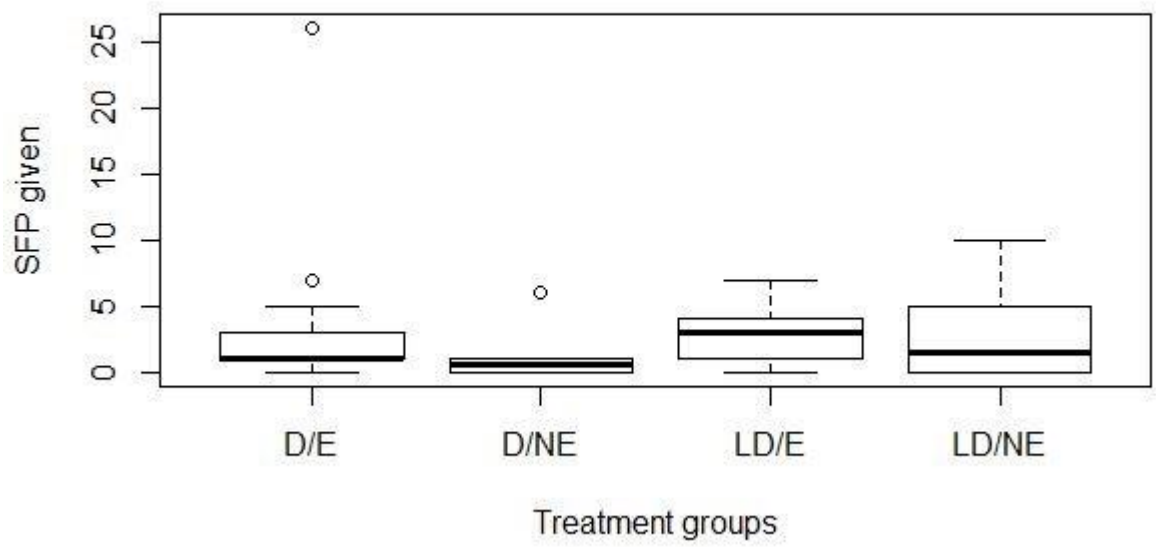


Figure 4: SFP given per treatment group. D/E(n=7), D/NE (n=3), LD/E (n=3) and LD/NE (n=7).

Social reinstatement test

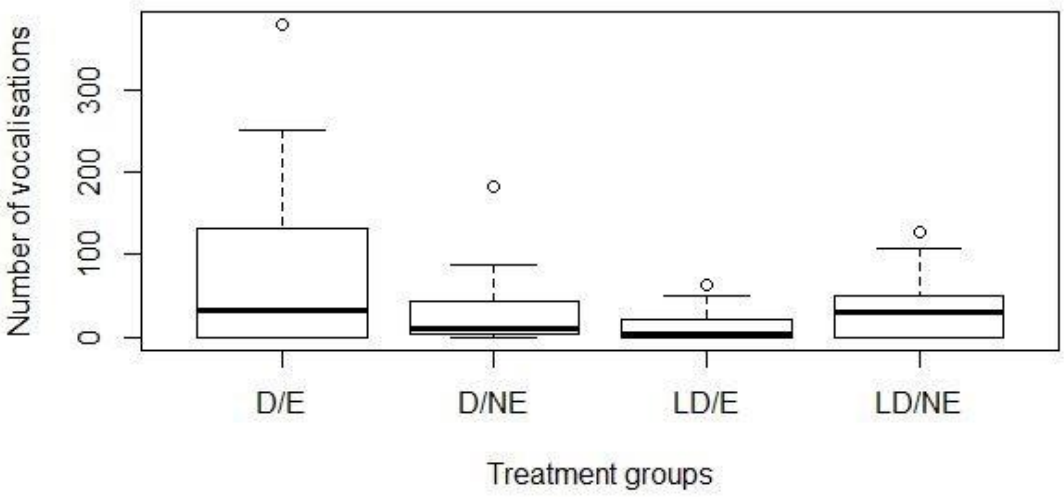


Figure 5: Number of vocalisations measured during the social reinstatement test per treatment group. D/E(n=71), D/NE (n=28), LD/E (n=34) and LD/NE (n=77).

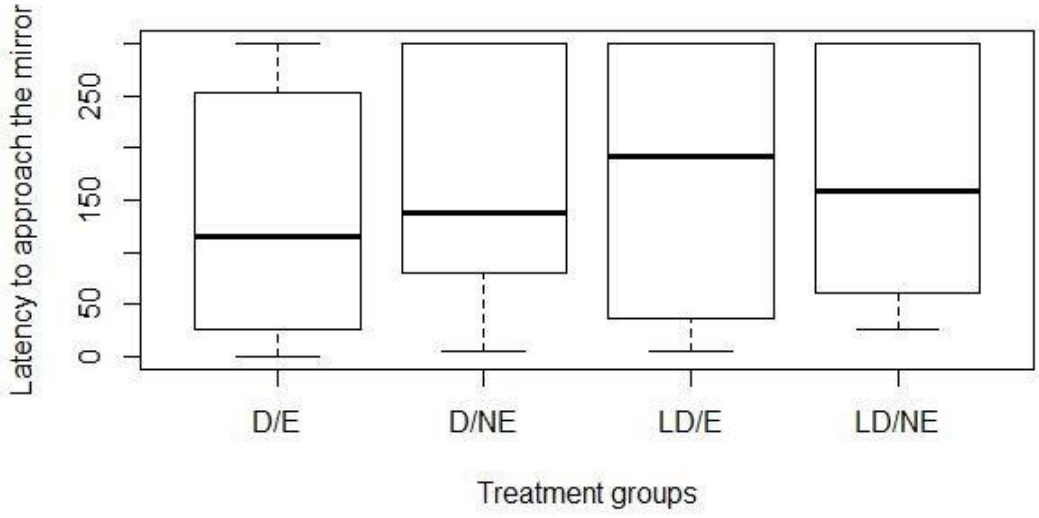


Figure 6: Latency to approach the mirror measured during the social reinstatement test per treatment group. D/E(n=71), D/NE (n=28), LD/E (n=34) and LD/NE (n=77).

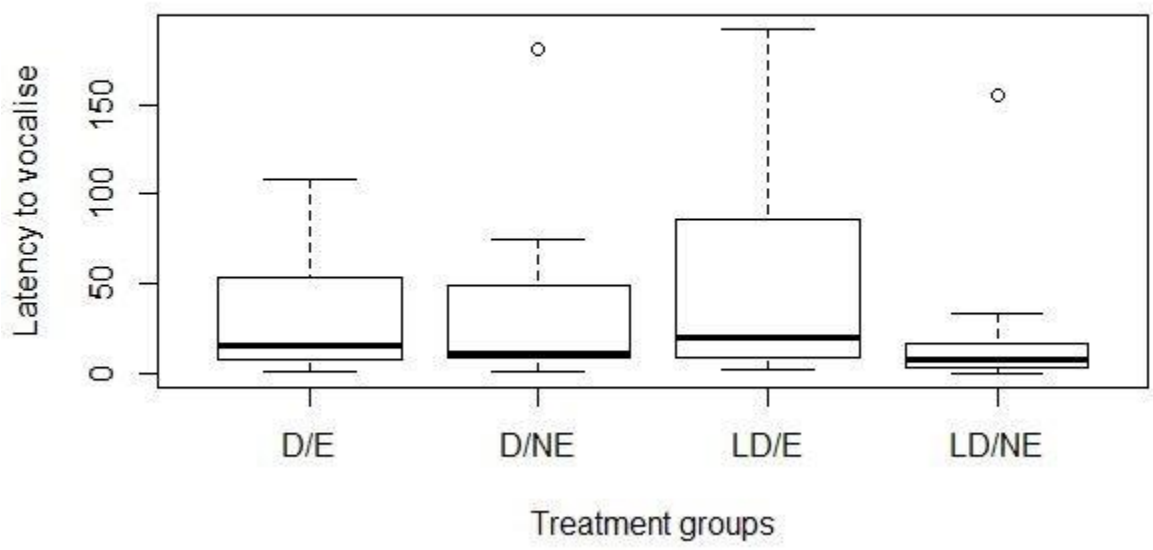


Figure 7: Latency to vocalise measured during the social reinstatement test per treatment group. D/E(n=71), D/NE (n=28), LD/E (n=34) and LD/NE (n=77).

For the social reinstatement test, the variables were: latency to approach the mirror, latency to vocalise and number of vocalisations. The variables were not considered paired data. For the social reinstatement test, the chicks were individually tested. There were four treatment groups: D/E(n=71), D/NE (n=28), LD/E (n=34) and LD/NE (n=77). In Figure 5 it seems that in the LD/E group there were fewer vocalisations than in the D/E group. A Kruskal-Wallis test was performed and showed no significant outcome (p-value = 0.2123). There is no significant difference in latency to approach the mirror between the different treatment groups (p-value = 0.3905, see Figure 6). In Figure 7 it seems that the LD/NE group had the shortest latency to vocalise, but there was no significant difference found in between the treatment groups (p-value = 0.2798).To compare the data of the social reinstatement test with the feather pecking behaviour, the data SFP given and SFP received of the home pen observations were used.

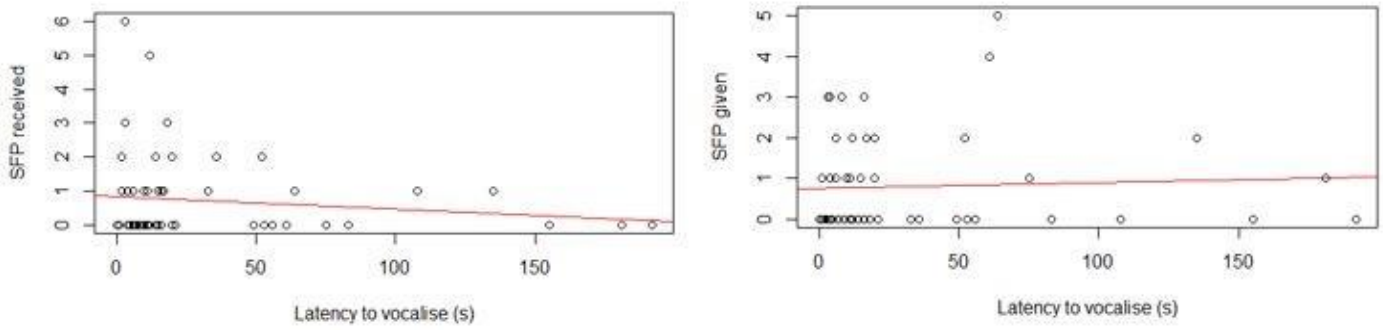


Figure 8: Latency to vocalise compared to SFP received and SPF given. D/E(n=71), D/NE (n=28), LD/E (n=34) and LD/NE (n=77)

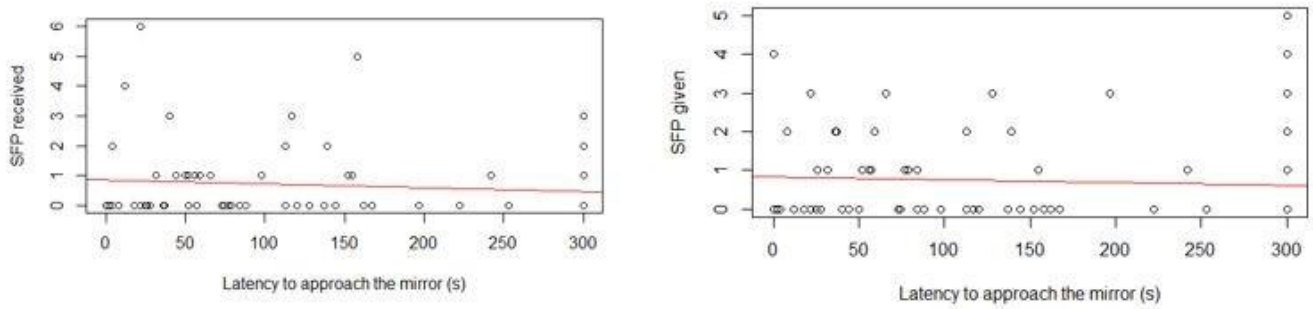


Figure 9: Latency to approach the mirror compared to SFP given and SFP received. D/E(n=71), D/NE (n=28), LD/E (n=34) and LD/NE (n=77).

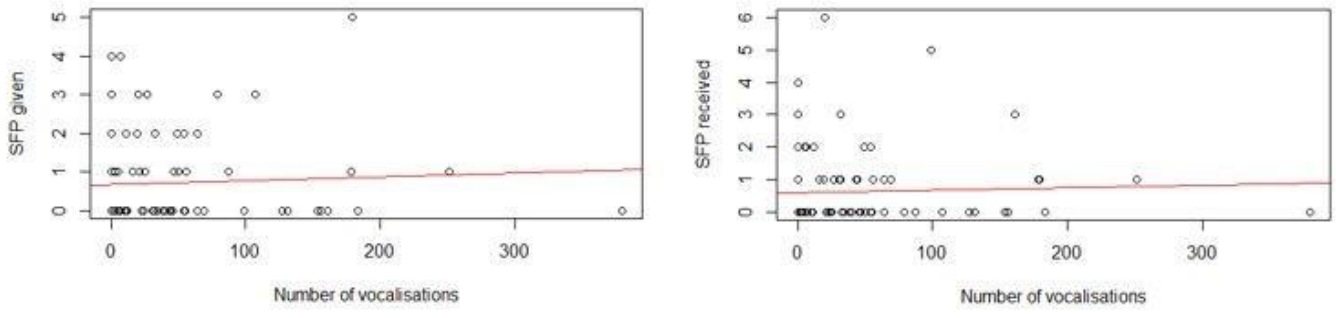


Figure 10: Number of vocalisations compared to SFP given and SFP received. D/E(n=71), D/NE (n=28), LD/E (n=34) and LD/NE (n=77).

Figure 8 shows the latency to vocalise had no effect on the SFP given and SFP received. The correlation between the latency to vocalise and the SFP given was not significant (p=0.9631) and the correlation was very weak (R= 0.1502267). The correlation between SFP received and the latency to vocalise was not significant (p= 0.29678) and the correlation was very weak (R=0.03632461). In Figure 10 can be seen if the number of vocalisations had an effect on the SFP given and SFP received. The correlation between SFP given and the number of vocalisations was not significant (p=0.9099) and the correlation was very weak (R=0.1408296).

The correlation between SFP received and the number of vocalisations was not significant (p=0.64401) and the correlation was weak (R=0.1988008). In Figure 9 can be seen if the latency to approach the mirror had an effect on the SFP given and SFP received. The correlation between the latency to approach the mirror and SFP given was not significant (p=0.6711) and the correlation was very weak (R=-0.1314202). The correlation between SFP received and the latency to approach the mirror was not significant (p= 0.64668) and the correlation was very weak (R=-0.06655757).

Table 4: Number of chicks per phenotype in the treatment groups (see Table 2 for an explanation of the abbreviations of the type of peckers).

Group	D/E	D/NE	LD/E	LD/NE
Phenotype				
N	22	11	6	17
P	2	0	3	3
V	2	0	2	5
V-P	4	0	1	2

In Figure 11 has been displayed the effect of the early life treatments on individual feather pecking behaviour (types of pecker/phenotype) and the number of vocalisations, latency to vocalise and the latency to approach the mirror. In Figure 11 can be seen that in the control group (D/NE) there were only neutrals, so no victims, peckers or V-P's, compared to the D/E, LD/NE and LD/E groups. So this could mean that the treatments had an effect on the feather pecking behaviour. What stands out in Figure 11 is that all the neutrals had the longest latency to approach the mirror. Something else that stands out is that the group that received both the treatments (LD/E) had the least amount of vocalisations, the longest latency to vocalise and a high latency to approach the mirror. But nothing cannot really be said about this because there were so few SFP seen, that the phenotypes (types of peckers) could change really easily. In Table 4 are the number of chicks per type of pecker/phenotype in the different treatment groups. What stands out in Figure 11 and Table 4 is that in the control group (D/NE) there are only neutrals. The phenotypes are not significantly different in each treatment group (p= 0.067).

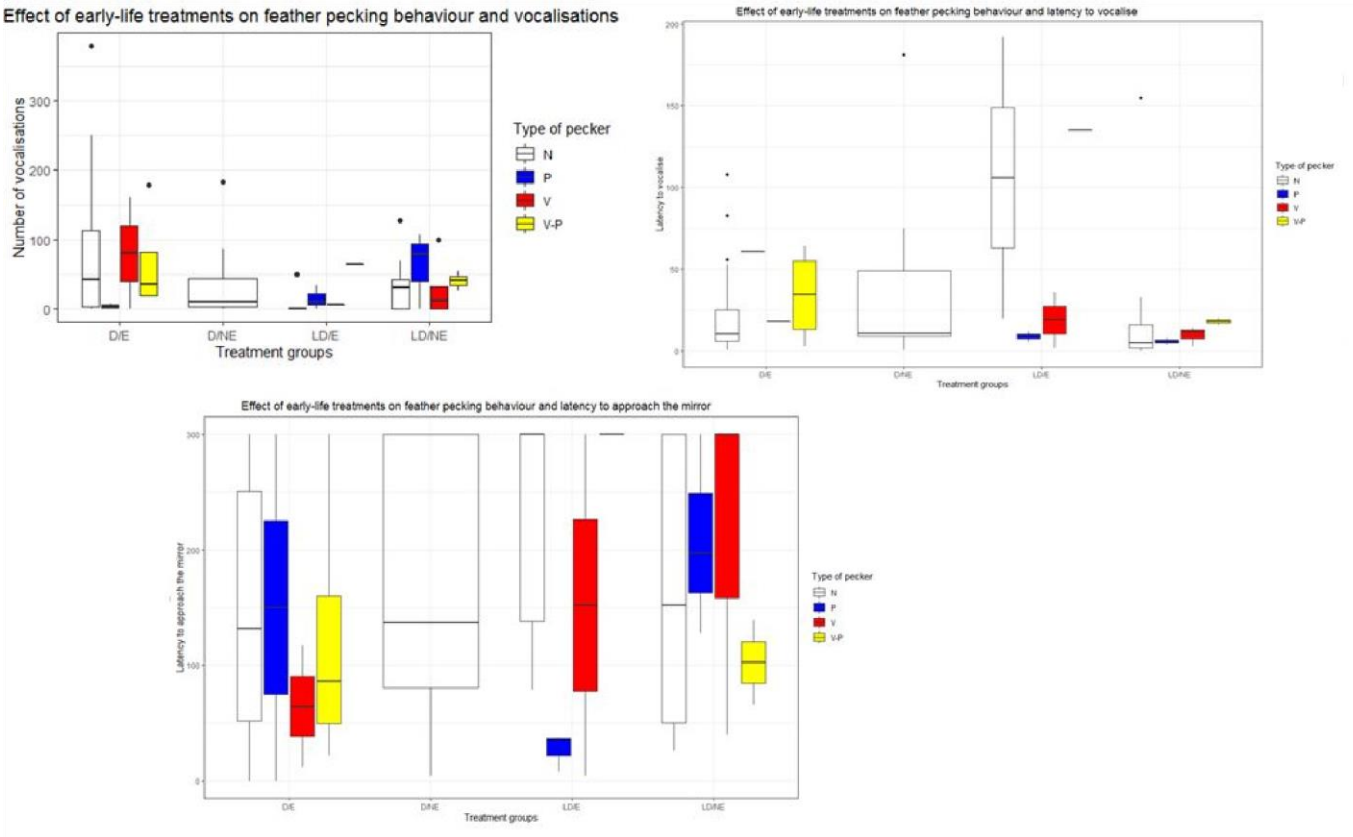


Figure 11: Effect of early life treatments on feather pecking behaviour and fear responses. Comparisons of the treatment groups, type of peckers and number of vocalisations, latency to vocalise and latency to approach the mirror.

Discussion

In this study, it was investigated if feather pecking in laying hens could be reduced or to prevent the FP behaviour from developing. It was also investigated if the treatments that were given would cause a reduction in fearfulness in the chicks. We wanted to find out if incubating the eggs in a 12:12 LD cycle and feeding the chicks live larvae in a food puzzle would reduce feather pecking and reduce fearfulness. This was investigated through the home pen observations and the social reinstatement test. Our expectations were that early life treatments would reduce feather pecking behaviour and fearfulness. We found that larvae treatment could have a decreasing effect on GFP. There was a tendency found of effect so it could not yet be confirmed. We could not confirm the effect of the treatments on the SFP behaviour. We could not find an effect of the treatments on the latency to approach the mirror, latency to vocalise and the number of vocalisations shown in the social reinstatement test. We could not find a correlation between the latency to approach the mirror, latency to vocalise, number of vocalisations and the SFP. There was no difference found in phenotypes between the different groups.

Home pen observations

In Figure 3 can be seen that in the groups LD/E and D/E the amount of GFP was less than in the groups without the larvae enrichment. However, variation in larvae groups is much higher than in non-larvae groups, making such predictions less reliable. Though the greater variation displayed might also be a product of uneven sample sizes (3 samples compared to 7 samples). We found that there could be a decreasing effect of GFP as a result of the larvae treatment, but could not yet confirm this.

Feather pecking

Like mentioned earlier, it is important for chicks to have early access to litter for foraging because this reduces FP later in life. When enough litter is provided and foraging behaviour increases, FP behaviour decreases but does not stop completely (Blokhuis & van der Haar, 1992; Nicol et al., 2001; Rodenburg et al., 2013). In this study, this was also seen. In the pens that received the larvae treatment was less GFP seen.

Larvae treatment

The study by Carr (2016) showed that feeding live larvae would stimulate natural foraging behaviour. In our research, the larvae were given in a food puzzle. This food puzzle was designed to stimulate natural foraging behaviour even more. When the larvae were given we saw that the chicks interacted with the tubes (where the larvae were in). They pecked at the tubes and showed scratching behaviour.

Light during incubation

As mentioned before, GFP is a form of social exploration in the early life of a chick and that social recognition is important for that (Riedstra & Groothuis, 2004). The study by Riedstra et al. (2004) shows that social recognition is a lateralized function and that it can be influenced by light during incubation. Light during incubation causes lateralisation of the visual pathways and this affects some post-hatch behaviours, like the ones that are related to fear and learning(Archer & Mench, 2014; Rogers, 1995). The study by Archer et al. (2013) showed that chicks that were incubated in a 12:12 LD cycle were less susceptible to stress. We expected to see in this study that the chicks that were incubated in the 12:12 LD cycle would also be less susceptible to stress and show fewer stress responses in the social reinstatement test. Unfortunately, we could not find an effect of the incubation treatment.

Issues on a larger scale

During this research, some problems were encountered with the randomization of the pens for the treatment groups. There were supposed to be five pens per treatment group but now it is D/E(n=7), D/NE (n=3), LD/E (n=3) and LD/NE (n=7). This resulted in that the treatment groups were not equal and that made it hard to compare them. We also found out that there were males in some pens (1,3,4,5 and 7) before the home pen observations. They were euthanized after the home pen observations. The males were not tested during the social reinstatement test. Due to practical constraints concerning the COVID-19 pandemic, it was not possible to do some experiments like they were planned. An open field test was planned to test the chicks for their fearfulness, but this test was cancelled. We also wanted to repeat the home pen observations again at a later age, but this was also not possible. Due to the COVID-19 pandemic, I had to change my main question and research proposal when I had already started writing the report. We had to use the data we already collected because all further experiments were cancelled. Therefore, and because of the unequal distribution of pens, there was not much data and a small sample size. Maybe with a bigger sample size, there would have been a significant outcome of the effect of the larvae treatment on the GFP behaviour.

Although there were no significant outcomes from this research, there were some effects found. It seems that the larvae treatment had a decreasing effect on GFP. The results found in this study could contribute to improving the welfare of laying hens. Further research could be conducted to determine the effectiveness of early life treatments on feather pecking behaviour. Within the PPILOW project, which this research was a part of, the research team will be doing more research on this. This research was part of round 1 of the PPILOW project, there will be a round 2 with 200 more chicks. Further research should be carried out to establish the effect of early life treatments on SFP. This is necessary to confirm if early life treatments contribute to the decrease in GFP and increase of SFP that was seen in this study. This could be done by using the same treatments and doing more home pen observations and behaviour scoring. If this is done, with also a bigger sample size, there might be a significant outcome.

Conclusion

In this research, we looked at the effects of incubation in a light-dark cycle and feeding live larvae in a food puzzle to reduce fearfulness and feather pecking in the early life of laying hens. Our expectations were that early life treatments would reduce feather pecking behaviour and fearfulness. From the results, an assumption could be made. It seems that larvae treatment caused a decrease in GFP behaviour. But because there were no significant outcomes, this could not be confirmed. We also could not confirm the effectiveness of the treatments on SFP behaviour. The effects of the treatments on the fear responses in the social reinstatement test also could not be confirmed. Thus more research is required to see if early life treatments could reduce feather pecking and fearfulness in laying hens.

References

Archer, G. S., & Mench, J. A. (2014). The effects of the duration and onset of light stimulation during incubation on the behavior, plasma melatonin levels, and productivity of broiler chickens. *Journal of Animal Science, 92*(4), 1753-1758. doi:10.2527/jas.2013-7129

Archer, G. S., Shivaprasad, H. L., & Mench, J. A. (2009). Effect of providing light during incubation on the health, productivity, and behavior of broiler chickens. *Poultry Science, 88*(1), 29-37. doi:https://doi.org/10.3382/ps.2008-00221

Archer, G. S., & Mench, J. A. (2013). The effects of light stimulation during incubation on indicators of stress susceptibility in broilers. *Poultry Science, 92*(12), 3103-3108. doi:https://doi.org/10.3382/ps.2013-03434

Bestman, M. W. P., & Wagenaar, J. P. (2003). Farm level factors associated with feather pecking in organic laying hens. *Livestock Production Science, 80*(1), 133-140. doi:10.1016/S0301-6226(02)00314-7

Blokhuis, H. J., & Haar, J. W. Van der. (1992). Effects of pecking incentives during rearing on feather pecking of laying hens. *British Poultry Science, 33*(1), 17-24. doi:10.1080/00071669208417440

Blokhuis, H.J., Beutler, A. (1992). Feather pecking damage and tonic immobility response in two lines of white leghorn hens. *Journal of Animal Science,* (70), 170.

Blokhuis, H. J., & Wiepkema, P. R. (1998). Studies of feather pecking in poultry. *Veterinary Quarterly, 20*(1), 6-9. doi:10.1080/01652176.1998.9694825

Buitenhuis, A. J., & Kjaer, J. B. (2008). Long term selection for reduced or increased pecking behaviour in laying hens. *World's Poultry Science Journal, 64*(4), 477-487. doi:10.1017/S0043933908000159

Carr, N. (2016). *Domestic animals and leisure; : Berlin, Germany, 2016;* . Berlin, Germany: Springer.

Clara, E., Regolin, L., Vallortigara, G., & Rogers, L. J. (2009). Chicks prefer to peck at insect-like elongated stimuli moving in a direction orthogonal to their longer axis. *Animal Cognition, 12*(6), 755-765. doi:10.1007/s10071-009-0235-y

Coleman, M. A. (1979). Effect of light during incubation and egg size on post hatch weights of broilers. *Poulty. Science., 58*, 1045.

Commission regulation (EU) no 68/2013 of 16 January 2013 on the catalogue of feed materials text with EEA relevance, RegulationU.S.C. (2013). Retrieved from https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32013R0068

de Haas, E. N., Bolhuis, J. E., Kemp, B., Groothuis, T. G. G., & Rodenburg, T. B. (2014). Parents and early life environment affect behavioral development of laying hen chickens. *PLoS ONE, 9*(3), e90577. doi:10.1371/journal.pone.0090577

de Haas, E. N., & van der Eijk, Jerine A. J. (2018). Where in the serotonergic system does it go wrong? Unravelling the route by which the serotonergic system affects feather pecking in chickens. *Neuroscience & Biobehavioral Reviews, 95*, 170-188. doi:https://doi.org/10.1016/j.neubiorev.2018.07.007

Green, L. E., Lewis, K., Kimpton, A., & Nicol, C. J. (2000). Cross-sectional study of the prevalence of feather pecking in laying hens in alternative systems and its associations with management and disease. *Veterinary Record, 147*(9), 233. doi:10.1136/vr.147.9.233

Hughes, B. O., & Duncan, I. J. H. (1972). The influence of strain and environmental factors upon feather pecking and cannibalism in fowls. *British Poultry Science, 13*(6), 525-547. doi:10.1080/00071667208415981

Jones, R. B. (1996). Fear and adaptability in poultry: Insights, implications and imperatives. *World's Poultry Science Journal, 52*(2), 131-174. doi:10.1079/WPS19960013

Knierim, U. (2006). Animal welfare aspects of outdoor runs for laying hens: A review. *NJAS - Wageningen Journal of Life Sciences, 54*(2), 133-145. doi:https://doi.org/10.1016/S1573-5214(06)80017-5

Mahboub, H. D. H., Müller, J., & Borell, E. V. (2004). Outdoor use, tonic immobility, heterophil/lymphocyte ratio and feather condition in free-range laying hens of different genotype. *British Poultry Science, 45*(6), 738-744. doi:10.1080/00071660400014267

Marin, R. H., Freytes, P., Guzman, D., & Jones, B. R. (2001). Effects of an acute stressor on fear and on the social reinstatement responses of domestic chicks to cagemates and strangers. *Applied Animal Behaviour Science, 71*(1), 57-66. doi:10.1016/S0168-1591(00)00167-2

Møller, A. P., Sanotra, G. S., & Vestergaard, K. S. (1995). Developmental stability in relation to population density and breed of chickens gallus gallus. *Poultry Science, 74*(11), 1761-1771. doi:https://doi.org/10.3382/ps.0741761

Nicol, C. J., Lindberg, A. C., Phillips, A. J., Pope, S. J., Wilkins, L. J., & Green, L. E. (2001). Influence of prior exposure to wood shavings on feather pecking, dustbathing and foraging in adult laying hens. *Applied Animal Behaviour Science, 73*(2), 141-155. doi:10.1016/S0168-1591(01)00126-5

Nicol, C. J., Pötzsch, C., Lewis, K., & Green, L. E. (2003). Matched concurrent case-control study of risk factors for feather pecking in hens on free-range commercial farms in the UK. *British Poultry Science, 44*(4), 515-523. doi:10.1080/00071660310001616255

Paul, A., Frederich, M., Megido, R. C., Alabi, T., Malik, P., Uyttenbroeck, R., Danthine, S. (2017). Insect fatty acids: A comparison of lipids from three orthopterans and tenebrio molitor L. larvae. *Journal of Asia-Pacific Entomology, 20*(2), 337-340. doi:10.1016/j.aspen.2017.02.001

Paul, A., Frederich, M., Uyttenbroeck, R., Malik, P., Filocco, S., Richel, A., Danthine, S. (2016). Nutritional composition and rearing potential of the meadow grasshopper (chorthippus parallelus zetterstedt). *Journal of Asia-Pacific Entomology, 19*(4), 1111-1116. doi:10.1016/j.aspen.2016.09.012

Riedstra, B., & Groothuis, T. G. G. (2004). Prenatal light exposure affects early feather-pecking behaviour in the domestic chick. *Animal Behaviour, 67*(6), 1037-1042. doi:10.1016/j.anbehav.2003.10.009

Riedstra, B., & Groothuis, T. G. G. (2002). Early feather pecking as a form of social exploration: The effect of group stability on feather pecking and tonic immobility in domestic chicks. *Applied Animal Behaviour Science, 77*(2), 127-138. doi:https://doi.org/10.1016/S0168-1591(02)00031-X

Rodenburg, T. B., de Haas, E. N., Nielsen, B. L., & Buitenhuis, A. J. (2010). Fearfulness and feather damage in laying hens divergently selected for high and low feather pecking. *Applied Animal Behaviour Science, 128*(1), 91-96. doi:https://doi.org/10.1016/j.applanim.2010.09.017

Rodenburg, T. B., van Krimpen, M. M., de Jong, I. C., de Haas, E. N., Kops, M. S., Riedstra, B. J., Nicol, C. J. (2013). The prevention and control of feather pecking in laying hens: Identifying the underlying principles. *World's Poultry Science Journal, 69*(2), 361-374. doi:10.1017/S0043933913000354

Rodenburg, T., Buitenhuis, A., Ask, B., Uitdehaag, K., Koene, P., van der Poel, J., Bovenhuis, H. (2004). Genetic and phenotypic correlations between feather pecking and open-field response in laying hens at two different ages. *Behavior Genetics, 34*(4), 407-415. doi:10.1023/B:BEGE.0000023646.46940.2d

Rogers, L. J. (1995). The development of brain and behaviour in the chicken. *The Development of Brain and Behaviour in the Chicken.,* Retrieved from https://www.cabdirect.org/cabdirect/abstract/19952206567

Savory, C. J. (1995). Feather pecking and cannibalism. *World's Poultry Science Journal, 51*(2), 215-219. doi:10.1079/WPS19950016

Savory, C. J., Mann, J. S., & MaCleod, M. G. (1999). Incidence of pecking damage in growing bantams in relation to food form, group size, stocking density, dietary tryptophan concentration and dietary protein source. *British Poultry Science, 40*(5), 579-584. doi:10.1080/00071669986936

Star, L., Arsiwalla, T., Molist, F., Leushuis, R., Dalim, M., & Paul, A. (2020). Gradual provision of live black soldier fly (hermetia illucens) larvae to older laying hens: Effect on production performance, egg quality, feather condition and behavior. *Animals : An Open Access Journal from MDPI, 10*(2), 216. doi:10.3390/ani10020216

van der Eijk, Jerine A. J., Lammers, A., Kjaer, J. B., & Rodenburg, T. B. (2019). Stress response, peripheral serotonin and natural antibodies in feather pecking genotypes and phenotypes and their relation with coping style. *Physiology .Behaviour* doi:https://doi.org/10.1016/j.physbeh.2018.10.021

van der Eijk, Jerine A. J., Lammers, A., Li, P., Kjaer, J. B., & Rodenburg, T. B. (2018). Feather pecking genotype and phenotype affect behavioural responses of laying hens. *Applied Animal Behaviour Science, 205*, 141-150. doi:https://doi.org/10.1016/j.applanim.2018.05.027

van Niekerk, T. G. C. M., de Jong, I. C., van Krimpen, M. M., & Veldkamp, T. (2011). *Wageningen UR livestock research. Partner in livestock innovations.* Lelystad: Wageningen UR Livestock Research.

Vestergaard, K. S., Kruijt, J. P., & Hogan, J. A. (1993). Feather pecking and chronic fear in groups of red junglefowl: Their relations to dustbathing, rearing environment and social status. *Animal Behaviour, 45*(6), 1127-1140. doi:https://doi.org/10.1006/anbe.1993.1137

Appendix

Table 5: Script Rstudio used for statistical analysis. Sidenote: when it says 'latency to approach conspecifics' this is the same data as 'latency to approach the mirror'.

```
attach(Data_FP_pen_totals)
names(Data_FP_pen_totals)

summary(Data_FP_pen_totals)


plot(factor(Group),GFP_given,xl

ab="Treatment groups",ylab="GFP

given") shapiro.test('SFP

given') shapiro.test('SFP

received') shapiro.test('GFP

given') shapiro.test('GFP

received') library(nlme)

?lme

model2=lme(GFP_given
~Incubation*Larvae,
data=Data_FP_pen_totals, random=
~1|Pen, na.action=na.exclude)

model4=lme(GFP_given ~
Incubation+Larvae,
data=Data_FP_pen_totals, random=
~1|Pen, na.action=na.exclude)

model5=lme(GFP_given ~ Larvae,

data=Data_FP_pen_totals,

random= ~1|Pen,

na.action=na.exclude)

summary(model2) summary(model4)

summary(model5)

modelSFP1=lme(SFP_given ~
Incubation*Larvae,
plot(factor(Group),SFP_given,xl
ab="Treatment groups",ylab="SFP
given")


data=Data_FP_pen_totals, random=
~1|Pen, na.action=na.exclude)
summary(modelSFP1)

modelSFP2=lme(SFP_given ~
Incubation+Larvae,

data=Data_FP_pen_totals, random=

~1|Pen, na.action=na.exclude)

summary(modelSFP2)

require(ggplot2)

ggplot(data=Data_FP_pen_totals,
aes(x=Larvae, y=GFP_given)) +
geom_boxplot(aes(fill=Incubatio
n), coef = 100) + labs(x =
"Larvae condition", y = "Number
of gentle feather pecks given",
fill = "Incubation condition")
+ theme_bw() +
scale_fill_manual(values=c("dar
k grey", "blue")) +
ggtitle("Effect of early-life
treatments on gentle feather
pecking behavior") +
theme(plot.title =
element_text(hjust = 0.3))

ggplot(data=Data_FP_pen_totals,
aes(x=Larvae, y=SFP_given)) +
geom_boxplot(aes(fill=Incubatio
n), coef = 120) + labs(x =
"Larvae condition", y = "Number
```

```
of severe feather pecks given",
fill = "Incubation condition")
+ theme_bw() +
scale_fill_manual(values=c("dar
k grey", "blue")) +
ggtitle("Effect of early-life
treatments on severe feather
pecking behavior") +
theme(plot.title =
element_text(hjust = 0.3))
detach(Data_FP_pen_totals)

attach(Data_social_reinstatemen
t_test_ALL)

plot(`Latency_to_vocalise(s)`,S
FP_given,xlab="Latency to


vocalise (s)",ylab="SFP given",
abline(lm(SFP_given ~
`Latency_to_vocalise(s)`, data =
Data_social_reinstatement_test_
ALL), col= "red"))

plot(`Latency_to_vocalise(s)`,S
FP_received,xlab="Latency to
vocalise (s)",ylab="SFP
received",
abline(lm(SFP_received ~
`Latency_to_vocalise(s)`, data =
Data_social_reinstatement_test_
ALL), col= "red"))

plot(Number_of_vocalisations,SF
P_given,xlab="Number of
vocalisations",ylab="SFP given",
abline(reg, col=
"red"))

plot(Number_of_vocalisations,SF
P_received,xlab="Number of
vocalisations",ylab="SFP
received",
abline(lm(SFP_received ~
Number_of_vocalisations, data =
Data_social_reinstatement_test_
ALL), col= "red"))

plot(`Latency_to_approach_the_m
irror(s)`,SFP_received,xlab="La
tency to approach the mirror
(s)",ylab="SFP received",
abline(lm(SFP_received ~
`Latency_to_approach_the_mirror
(s)`, data =
Data_social_reinstatement_test_
ALL), col= "red"))

plot(`Latency_to_approach_the_m
irror(s)`,SFP_given,xlab="Laten
cy to approach the mirror
(s)",ylab="SFP given",
abline(lm(SFP_given ~
`Latency_to_approach_the_mirror
(s)`, data =


Data_social_reinstatement_test_
ALL), col= "red"))

shapiro.test(`Latency_to_approa
ch_conspecifics(s)`)

shapiro.test(`Latency_to_vocali
se(s)`)

shapiro.test(Number_of_vocalisa

tions) shapiro.test(SFP_given)

shapiro.test(SFP_received)

library(nlme)

modelSR1=lme(SFP_given ~
`Latency_to_approach_conspecifi
cs(s)`*`Latency_to_vocalise(s)`
*Number_of_vocalisations)

model1=lme(SFP_given ~
Latency_to_approach_conspecific
s(s)* Latency_to_vocalise(s)*
Number_of_vocalisations,
data=Data_social_reinstatement_
test_ALL, random= ~1|Pen,
na.action=na.exclude)
```

```
modelSR1 <-glm(SFP_given ~
`Latency_to_approach_conspecifi
cs(s)`*`Latency_to_vocalise(s)`
*Number_of_vocalisations,
data=Data_social_reinstatement_
test_ALL) summary(modelSR1)

modelSR2 <-glm(SFP_given ~
`Latency_to_approach_conspecifi
cs(s)`+`Latency_to_vocalise(s)`
+Number_of_vocalisations,
data=Data_social_reinstatement_
test_ALL) summary(modelSR2)

modelSR3 <- glm(SFP_received
~`Latency_to_approach_conspecif
ics(s)`*`Latency_to_vocalise(s)
`*Number_of_vocalisations, data
=
Data_social_reinstatement_test_
ALL) summary(modelSR3)

modelSR4 <- glm(SFP_received ~
`Latency_to_approach_conspecifi
cs(s)`+`Latency_to_vocalise(s)`
+ Number_of_vocalisations, data
=
Data_social_reinstatement_test_
ALL) summary(modelSR4)

cor.test(`Latency_to_vocalise(s
)`,SFP_given, method =
"spearman")

cor.test(`Latency_to_vocalise(s
)`, SFP_received, method =
"spearman")

cor.test(Number_of_vocalisation
s, SFP_given, method=
"spearman")

cor.test(Number_of_vocalisation
s, SFP_received, method =
"spearman")

cor.test(`Latency_to_approach_c
onspecifics(s)`, SFP_given,
method = "spearman")

cor.test(`Latency_to_approach_c
onspecifics(s)`,SFP_received,
method = "spearman")

names(Data_social_reinstatement
_test_ALL)

summary(Data_social_reinstateme

nt_test_ALL) table(Type,Group)

library(ggplot2)

ggplot(data=Data_social_reinsta
tement_test_ALL, aes(x=Group,
y=Number_of_vocalisations)) +

  geom_boxplot(aes(fill=Type))
+

  labs(x = "Treatment groups", y
= "Number of vocalisations",
fill = "Type of pecker") +

  theme_bw() +
scale_fill_manual(values=c("dar
k grey", "blue", "red",
"yellow")) +

  ggtitle("Effect of early-life
treatments on feather pecking
behaviour and vocalisations") +

  theme(plot.title =
element_text(hjust = 0.3))

ggplot(data=Data_social_reinsta
tement_test_ALL, aes(x=Group,
y=`Latency_to_vocalise(s)`)) +

  geom_boxplot(aes(fill=Type))
+
```

```
  labs(x = "Treatment groups", y = "Latency to vocalise", fill = "Type of pecker") +

  theme_bw() + scale_fill_manual(values=c("dark grey", "blue", "red", "yellow")) +

  ggtitle("Effect of early-life treatments on feather pecking behaviour and latency to vocalise") +

  theme(plot.title = element_text(hjust = 0.3))

ggplot(data=Data_social_reinstatement_test_ALL, aes(x=Group, y=`Latency_to_approach_the_mirror(s)`) + geom_boxplot(aes(fill=Type)) + labs(x = "Treatment groups", y = "Latency to approach the mirror", fill = "Type of pecker") + theme_bw() + scale_fill_manual(values=c("dark grey", "blue", "red", "yellow")) + ggtitle("Effect of early-life treatments on feather pecking behaviour and latency to approach the mirror") + theme(plot.title = element_text(hjust = 0.3))

detach(Data_social_reinstatement_test_ALL)

attach(Number_of_chicks_per_pen_and_group) table(`Chick ID`, Group)

newdata<-aggregate(Number_of_chicks_per_pen_and_group$`Chick ID`, by = list(Category = Number_of_chicks_per_pen_and_group$Group, Number_of_chicks_per_pen_and_group$Pen), FUN = sum)

summary(newdata)

names(newdata)[1]<-"Group"

names(newdata)[2]<-"Pen"

table(newdata)

table(Pen, Group)

detach(Number_of_chicks_per_pen_and_group)

attach(Data_social_reinstatement_test_ALL) table(Type, Group)

install.packages("GoodmanKruskal") library(GoodmanKruskal)

GKtau(Group, Type)

detach(Data_social_reinstatement_test_ALL)

attach(Data_social_reinstatement_test_ALL)

mean(`Latency_to_approach_the_mirror(s)`)

mean(`Latency_to_vocalise(s)`, useNA = excludeNA)

mean(Number_of_vocalisations)

tapply(`Latency_to_approach_the_mirror(s)`,Group,mean)

tapply(`Latency_to_vocalise(s)`,Group,mean)
```

```
tapply(Number_of_vocalisations,
Group,mean)

tapply(`Latency_to_approach_the
_mirror(s)`, Incubation,mean)

tapply(Number_of_vocalisations,
Incubation,mean)

tapply(`Latency_to_approach_the
_mirror(s)`, Larvae,mean)
tapply(Number_of_vocalisations,
Larvae,mean)


kruskal.test(`Latency_to_vocali
se(s)`,Group)

plot(factor(Group),Number_of_vo
calisations)

kruskal.test(`Latency_to_approa
ch_the_mirror(s)`,Group)

kruskal.test(Number_of_vocalisa
tions,Group) plot(factor(Group),
`Latency_to_approach_the_mirror
(s)`)

plot(factor(Group),`Latency_to_
vocalise(s)`)
```