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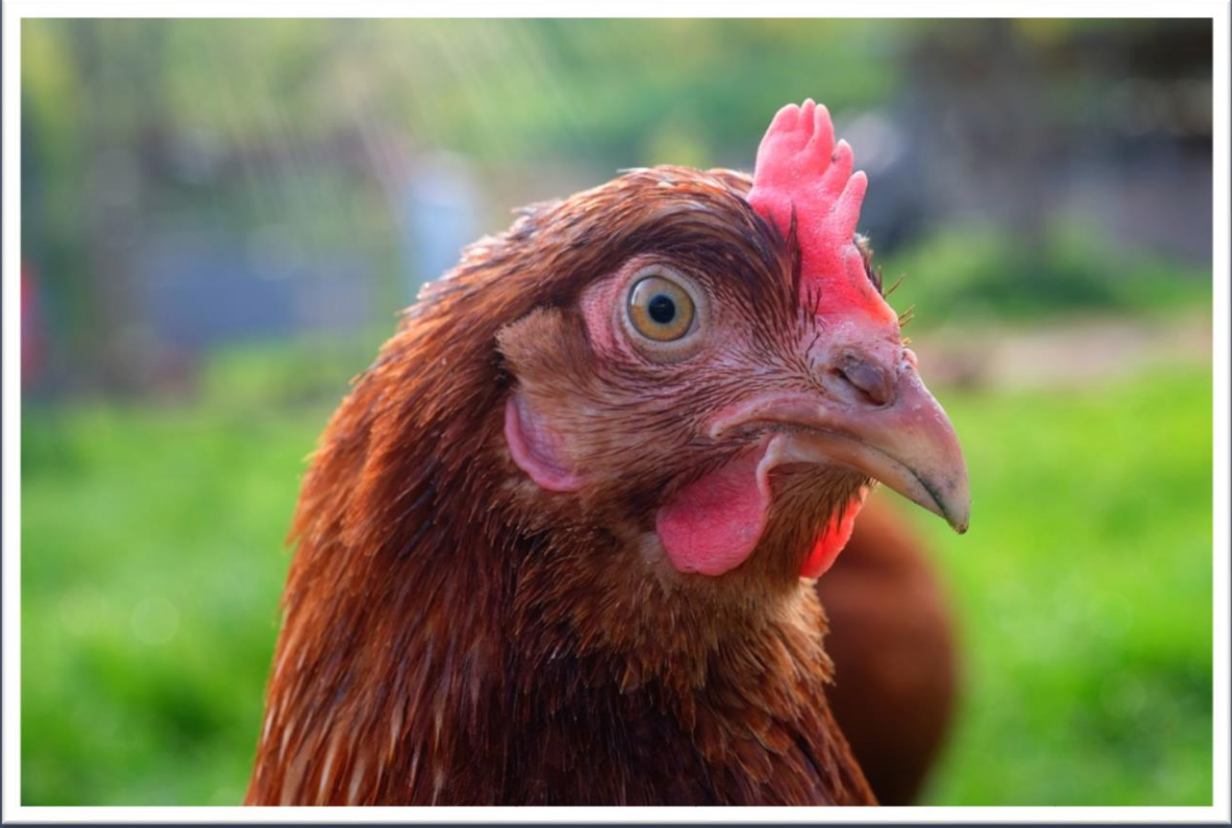

# Pilot: insect larvae as environmental enrichment

## FINDING THE BEST METHOD TO PROVIDE INSECT LARVAE AS ENVIRONMENTAL ENRICHMENT TO LAYING HENS


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DIER IN WETENSCHAP EN MAATSCHAPPIJ | Dierenwelzijn

# Inhoud

# Abstract

Feather pecking is a maladaptive behaviour caused by a restriction in behavioural repertoire. By providing environmental enrichment to chickens, they are able to perform behaviour to better adapt and cope with stressors. An example of enrichment in laying hens is supply of Black Soldier Fly (BSF) larvae. The goal of this pilot study was to compare the effects of provision of live BSF larvae to dead BSF larvae on pullets. In addition, the second goal was to compare a transparent dispenser to a non-transparent dispenser. The purpose of these comparisons was to assess which combination of dispenser type and content had the most effect on active behaviour of the pullets. The most effective combination will be used as enrichment in the main experiment to investigate the effects of BSF larvae provision on feather pecking in laying hens. Twenty-eight one day old chicks were randomly housed in four pens. During testing days two different dispenser conditions were placed in the pen. With the help of video data, observations were done on two chicks per pen for one hour after giving the dispensers. After each day the remaining larvae in the dispensers were weighed to calculate average consumption. The results showed a significant difference in larvae consumption from dispensers with live larvae compared to dispensers with dead larvae. Furthermore, the results showed significantly more active behaviour towards a transparent dispenser containing live larvae compared to a non-transparent dispenser containing dead larvae. it seems that the chickens in this experiment performed the most active behaviour towards a transparent dispenser containing live larvae. Providing live larvae in a transparent dispenser would probably have the most effect on active behaviour when used as environmental enrichment.

# 1. Introduction

The past decade there has been an increasing amount of attention towards the way we use and keep animals to provide animal products like meat, milk and eggs. People care more about the welfare of an animal and this is evident from changes in legislation (De la Fuente et al., 2017). In 2012 the European Union banned the conventional battery cage after a period to slowly phase the battery cage out since 1999. This was based on advice from the Scientific Committee on Animal Health and Animal Welfare (SCAHAW). Furthermore, a ban on beak trimming was recently implemented in the Netherlands in 2018 following the example of Norway, Finland, Sweden, Denmark and Austria. However, the public opinion plays a big and important role in this, for the Dutch people are the consumers of these products. Nowadays when we look for eggs in the supermarket, there are barn eggs, free-range eggs and organic eggs. There are also innovative new concepts to keep laying hens, for example the 'rondeel' or the 'kipster' concepts. These exceptional concepts provide laying hens with a more enriched environment to improve animal welfare. However, just as with free-range and organic farming, these concepts are troubled with welfare issues such as feather pecking and stress sensitivity. Feather pecking is observed in almost all poultry systems. Since the recent ban on beak trimming in the Netherlands, there is a serious concern for the increased damage caused by this maladaptive behaviour. Thus examining the occurrence of feather pecking is of high importance for the welfare of the chickens (Rodenburg et al., 2013).

## 1.1 Enrichment & Welfare

Production animals are often held intensively and kept in an environment that lacks stimulation. These environments are designed for maximum production, but also to decrease negative factors such as diseases. However, these systems do not consider the animal's behavioural needs. This results in limitations of their behavioural opportunities (Dixon, 2012). An environment can be made more stimulating by providing environmental enrichment. This can be defined as 'an improvement in the biological functioning of captive animals resulting from modifications to their environment', according to Newberry (1995). By providing environmental enrichment an animal has the opportunity to perform a behaviour to adapt itself and cope with a stressor, or perform highly motivated behaviour (Ninomiya, 2014).

However, how does environmental enrichment affect animal welfare? There is some discussion regarding the definition of animal welfare. Often welfare is defined as the absence of welfare problems. The five freedoms of animal welfare is such an example. These freedoms mostly focus on the absence of negative experiences, such as hunger, thirst, fear and physical pain (FAWC, 1992). At the faculty of Veterinary Medicine of the Utrecht University they maintain a different definition where '*an individual is in a state of welfare when it is able to actively adapt itself to its environment so it can reach a state it experiences as positive*' (Utrecht University, 2015; Ohl & van der Staay, 2012). With this definition environmental enrichment should positively affect animal welfare. Other goals of enrichment are reducing abnormal or maladaptive behaviour like feather pecking, and increasing the behavioural repertoire and diversity (Young, 2007). Enrichment can be divided into four types. Foraging/feed related, physical complexity, novelty, and social stimulation (Dixon, 2012). The enrichment in this study can be put under the foraging/feed related enrichments.

## 1.2 Aim of the study

The aim of this pilot study is to compare the provision of live larvae versus dead larvae as enrichment for chicks. In addition, the second goal is to compare dispenser types; a transparent dispenser will be compared to a non-transparent dispenser. The goal of this comparison is to assess which combination of content and dispenser type yields the most effect on active foraging behaviour. This combination will be used as enrichment in a following experiment to investigate the effects on feather pecking in laying hens. These dispensers act as kind of feeding puzzle to extend foraging times. It is hypothesised that live larvae will yield longer foraging times than dead larvae, and that a transparent dispenser will yield longer foraging times in comparison to a non-transparent dispenser. It is expected that chicks will interact more with the dispenser containing live larvae than the dispenser with dead larvae, and show more interactive behaviour towards the transparent dispenser in comparison to the non-transparent dispenser.

# 2. Materials and Methods

## 2.1 Ethical approval:

This pilot was approved by the The Animal Welfare Body Utrecht (Instantie voor Dierenwelzijn Utrecht) of Utrecht University (AVD1080020198685) . The chickens of this pilot were housed at the Faculty of Veterinary Medicine of Utrecht University.

## 2.2 Animals and materials

Twenty-eight day-old ISA Brown female layer chicks were housed in a standard housing facility at the poultry clinic of the faculty of Veterinary Medicine. Conditions of the housing were maintained at the standards of the management guide of ISA Brown pullets. This management guide shows the optimal housing conditions of ISA Brown chickens, such as temperature, humidity, light/dark cycles, weight, egg production etc. These chicks were acquired from a commercial breeder (Vepymo), which also supplies chicks to regular rearing farms. The four pens contained feed, water, a ceramic heating lamp and dark brooders that were arranged in a similar matter for every pen (Figure 1 and 2). Colouring spray, normally used on sheep, was used to mark chicks as to differentiate between the 7 chicks per group. The spray was sprayed in a container and the liquid was used to mark chicks in a different pattern to differentiate between individuals. Larvae were used for the content of the dispensers. A requirement of the larvae should be that they cannot survive in the climate of the Netherlands. This means that the larvae should not moult into flies and be able to reproduce in this climate if larvae escape. As such, Black Soldier Fly (BSF) larvae were selected. Storing all of the needed larvae at one time was not possible. Therefore the BSF larvae were acquired weekly from Circular Organics and stored at a cool temperature to prevent moulting.

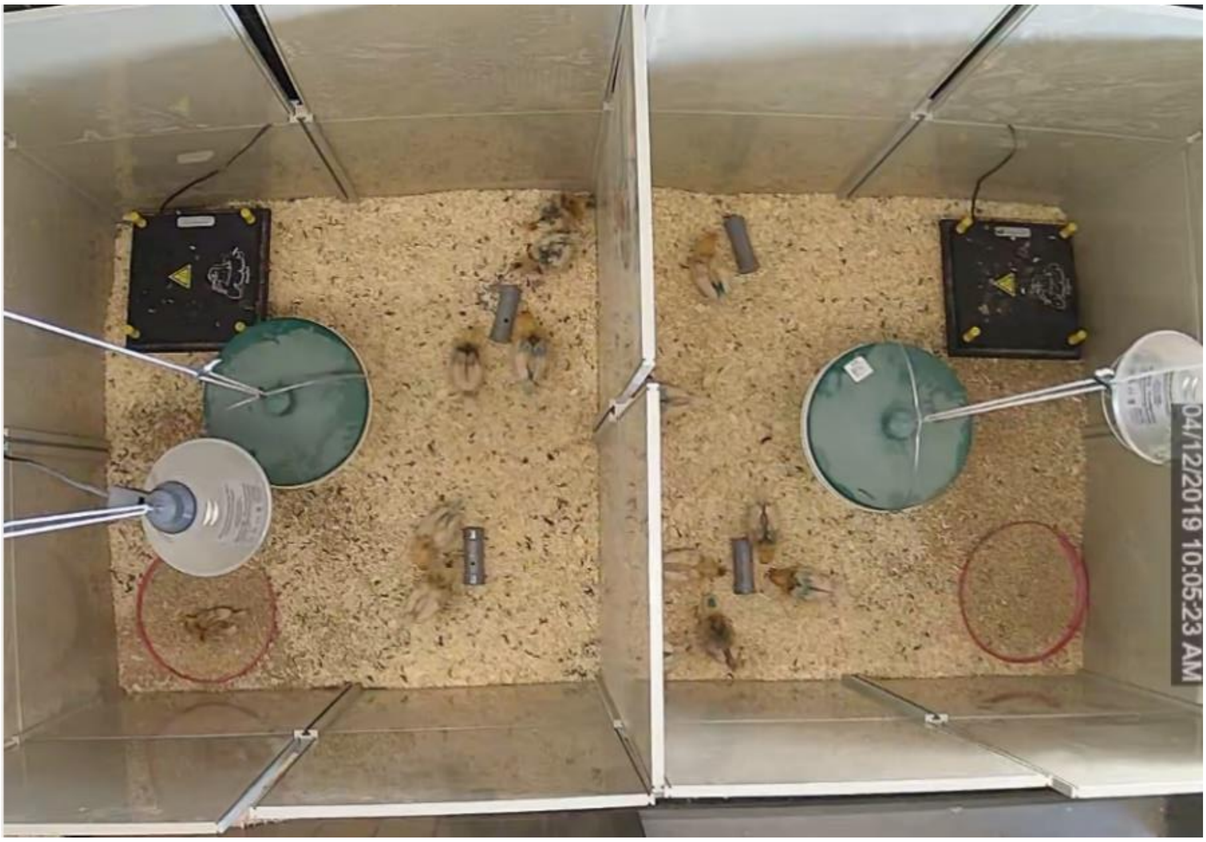


FIGURE 1: PEN 1 (LEFT) AND PEN 2 (RIGHT)

FIGURE 2: PEN 3 (LEFT) AND PEN 4 (RIGHT)

The dispensers (Figure 3) consisted of either transparent or non-transparent PVC with two removable caps to keep the larvae inside. In each tube, holes were made to act as an opening to reach the larvae inside. This experiment used short dispensers (10 cm in length with a diameter of 4 cm) with two holes measuring 7 mm in diameter to facilitate manipulation of the dispenser by young chicks. When chicks were able to easily use the short dispenser, the regular dispensers (15 cm in length with a diameter of 4 cm) with four holes measuring 7 mm in diameter were used for the following trials.

FIGURE 3: FROM LEFT TO RIGHT; SHORT TRANSPARENT DISPENSER, REGULAR TRANSPARENT DISPENSER, REGULAR NON-TRANSPARENT DISPENSER AND SHORT NON-TRANSPARENT DISPENSER

## 2.3 Methods

The 28 chicks were divided into four different groups of 7 chicks each. Birds were assigned to a pen in a randomized way with the help of a randomizer tool (Random.org). First, all the chicks were weighed to determine the mean weight. Then, the chicks were placed into 3 weight categories. The first is the uniform category which includes chicks within 90-110% of the mean weight. The other 2 categories are the below the mean weight and above the mean weight. The uniform chicks were randomly divided in four groups by using the aforementioned randomizer tool. The non-uniform chicks were distributed in a manner to keep the mean weight of the four groups as equal as possible. This was done to prevent differences in average weight per pen, which could have happened when randomly distributing such a small sample size. The chicks were weighed three times a week corresponding with the observation days to keep track of their growth. The amount of larvae provided daily was 10% of the daily nutritional need of the chicks. This information was obtained from the management guide for ISA Brown hens. This meant that the amount of larvae increased over the testing days. The amount of larvae remaining in the tubes was weighed the next day to calculate consumption of larvae. This provided an average consumption of larvae per treatment. Because the amount of larvae varied, this was calculated as a percentage of larvae consumed. Larvae were acquired alive and had to be euthanised on site to be able to provide dead larvae. Larvae were euthanised by blanching, which is a method in which you fill a container with larvae, pour boiling water onto them and leave them for 1 minute. Afterwards the larvae were drained and dried (Larouche et al., 2019). The larvae were weighed before blanching to prevent water from being added to the total weight of the larvae.

### 2.3.1 Training

A training period was used to first let the chicks get used to the dispenser before scoring the different interactions with the two dispensers. There were five training sessions to introduce the chicks to the larvae and to reduce novelty in the first testing days (Table 1). This was done with either dead larvae or live larvae in one transparent dispenser.

TABLE 1: TRAINING DAYS SCHEDULE

| Training conditions | Pen 1 | Pen 2 | Pen 3 | Pen 4 |
|---|---|---|---|---|
| 22-11-2019 | Alive | Alive | Dead | Dead |
| 25-11-2019 | Dead | Dead | Alive | Alive |
| 27-11-2019 | Alive | Alive | Dead | Dead |
| 28-11-2019 | Dead | Dead | Alive | Alive |
| 29-11-2019 | Alive | Dead | Alive | Dead |

### 2.3.2 Testing

There were four different conditions which were introduced in eight test days. This ensured that each pen had every condition twice. These conditions consist of providing two dispensers which differed from each other. The dispenser was either transparent (T) or non-transparent (N-T) and it could be either filled with dead larvae(D) or live larvae(A). The conditions were determined before the start of the trial (Table 2). This was planned in such a manner that the order of conditions were mirrored and to prevent a learning bias.

TABLE 2: TESTING DAYS SCHEDULE

| | Pen 1 | Pen 2 | Pen 3 | Pen 4 |
|---|---|---|---|---|
| 2-12-2019 | A/D, T | A/D, T | A/D, N-T | A/D, N-T |
| 4-12-2019 | A/D, N-T | A/D, N-T | A/D, T | A/D, T |
| 9-12-2019 | D, N-T/T | D, N-T/T | A, N-T/T | A, N-T/T |
| 11-12-2019 | A, N-T/T | A, N-T/T | D, N-T/T | D, N-T/T |
| 13-12-2019 | A/D, N-T | A/D, N-T | A/D, T | A/D, T |
| 16-12-2019 | A/D, T | A/D, T | A/D, N-T | A/D, N-T |
| 18-12-2019 | A, N-T/T | A, N-T/T | D, N-T/T | D, N-T/T |
| 20-12-2019 | D, N-T/T | D, N-T/T | A, N-T/T | A, N-T/T |

| | |
|---|---|
| A | Alive |
| D | Dead |
| T | Transparent |
| N-T | Non-transparent |

On testing days the chicks were first all weighed to keep track of their weight. After the weighing, the dispenser would be prepared. In every pen two dispensers would be provided in the pen at 10:00 in the morning according to the schedule. Afterwards, entering the room was restricted to reduce factors that would affect behaviour of the chicks, which could translate into caretaker/observer bias.

## 2.4 Observations

Two cameras (Bascom BSM-XDIO-A) were positioned to record two different pens each, making four pens in total, to observe behaviour and for the possibility to prepare personnel for the main experiment with the use of footage. Due to time constraints only the video data of the first four days were used to score interaction with the dispensers. Two chicks per pen where randomly selected, their behaviour was scored for one hour after the placement of the dispenser. This was done by continuous sampling. The observer programme Boris was used for the observations (Friard & Gamba, 2016). The video data was divided and scored by three separate observers. Observer A scored video data from pen one and two, observer B scored video data from pen three and observer C scored video data from pen four. The ethogram was simplified to make scoring easier and more accurate (table 3). Running was scored because chicks often ran away when they had a larvae in their beak and other chicks would chase them. This behaviour is also known as worm-running (Cloutier et al., 2004). The behaviour around a dispenser would be scored per dispenser. This area was created by measuring the length of the dispenser and using it to make a circumference around the dispenser (Figure 4).

TABLE 3: ETHOGRAM

| Behaviour code | Behaviour type | Description | Behavioural category |
|---|---|---|---|
| RUN | State event | Running | Dispenser-related behaviour |
| ID | State event | Inactive behaviour around dispenser | Dispenser-related behaviour |
| AD | State event | Active behaviour aimed at or involving dispenser | Dispenser-related behaviour |
| NA | State event | Non-Active behaviour, like resting, sleeping or perching | Other behaviour |
| A | State event | Active behaviour like eating, drinking, walking, preening, exploring and jumping | Other behaviour |
| O | State event | Out of sight | Other behaviour |

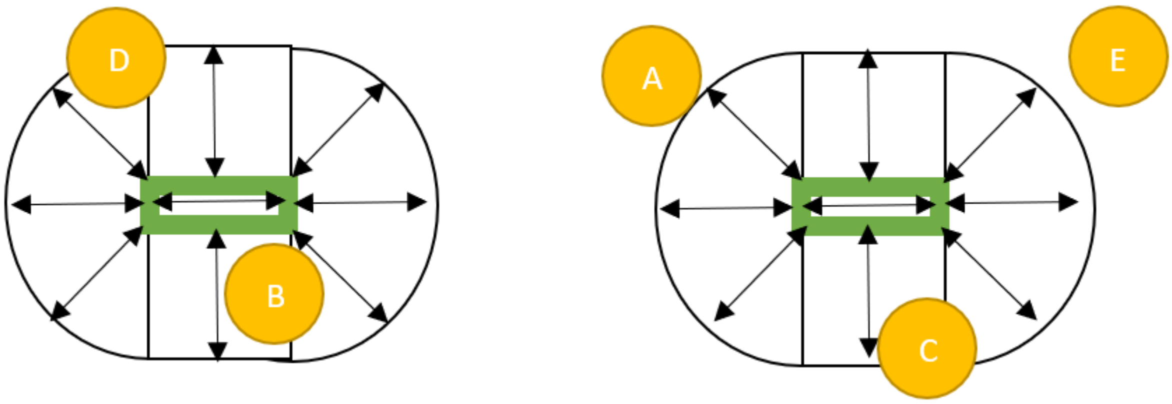


FIGURE 4: A SCHEMATIC REPRESENTATION OF THE AREA AROUND THE DISPENSER. CHICKENS A AND E ARE NOT NEARBY THE DISPENSER. CHICKENS B, C AND D ARE FULL OR PARTIALLY IN THE MARKED AREA ZO ARE NEARBY THE DISPENSER. THE GREEN LINES MARK THE DISPENSER ITSELF.

## 2.5 Statistics

The programme 'R' was used for the statistical analysis. The observations resulted in a time spent near a certain dispenser. To see if the data from different observers could be used together, inter-observer reliability was tested. Unfortunately the inter-observer reliability was poor ranging from 0,543 to 0,725. Therefore, results from the observations were analysed and compared per observer. As such, data from pen one and two were analysed together and data from pen three and four were analysed separately.  The dispensers were categorised in the four different conditions: Alive Transparent (A_T), Alive Non-Transparent (A_NT), Dead Transparent (D_T) and Dead Non-Transparent (D_NT). These conditions were all compared to each other. The amount of time chicks showed active behaviour towards a dispenser was analysed using a paired T-test, or a Wilcoxon rank-sum test in case the data was not normally distributed. To determine normality, each dataset was checked by inspecting the QC-plot, but also by performing the Shapiro-Wilk test. The same methods were used in the analysis of the average consumption of larvae. The average consumption was compared between conditions. The data was not normally distributed so a Wilcoxon rank-sum test was used. In all statistics performed in this pilot study, P-values under 0.05 were considered significant, P-Values between 0.05 and 0.10 were considered as trends and P-values above 0.10 were considered not significant. Applying a Bonferroni correction was considered, but because this experiment was an explorative pilot the correction was not applied.

# 3. Results

## 3.1 Larvae consumption

The remaining larvae in the dispenser were weighed the day after testing. This resulted in a percentage of larvae consumed. Table 4 shows the average consumption per dispenser condition from the four pens combined.

TABLE 4: AVERAGE CONSUMPTION OF LARVAE FROM THE FOUR PENS COMBINED

| | Day 1-4 | Day 5-8 | Total average |
|---|---|---|---|
| **A_T** | 90% | 100% | 95% |
| **A_NT** | 86% | 100% | 93% |
| **D_NT** | 39% | 91% | 65% |
| **D_T** | 12% | 67% | 39% |

Statistical analysis was done in three separate data sets. On all these comparisons between conditions the Wilcoxon rank-sum test was used. First the consumption of the first four days was analysed. Figure 5 shows the boxplot from day 1-4. A significant difference was found in the consumption of larvae between the conditions A_NT and D_NT (p<0.01), as well as between A_NT and D_T (p<0.001). This also was found between conditions A_T and D_NT (p<0.01), and conditions A_T and D_T (p<0.001). The second data set contained the consumption of larvae in the last four days of testing. Figure 6 shows the boxplot from the data of days 5-8. In this dataset fewer significant differences were found. Again a significant difference was found between the conditions A_T and D_NT (p<0.05), as well as between A_T and D_T (p<0.05). However, no significant difference was found between conditions A_NT and D_NT, and only a trend was found between conditions A_NT and D_T (p<0.10). The last dataset was the data from all the 8 days combined. Figure 7 shows the boxplot of the average consumption over the total experiment per condition. This data shows significant differences between the average consumption of larvae between conditions A_NT and D_NT (p<0.05), as well as A_NT and D_T (p<0.01). Furthermore, significant differences were again found between the conditions A_T and D_NT (p<0,05), and the conditions A_T and D_T (p<0.001).

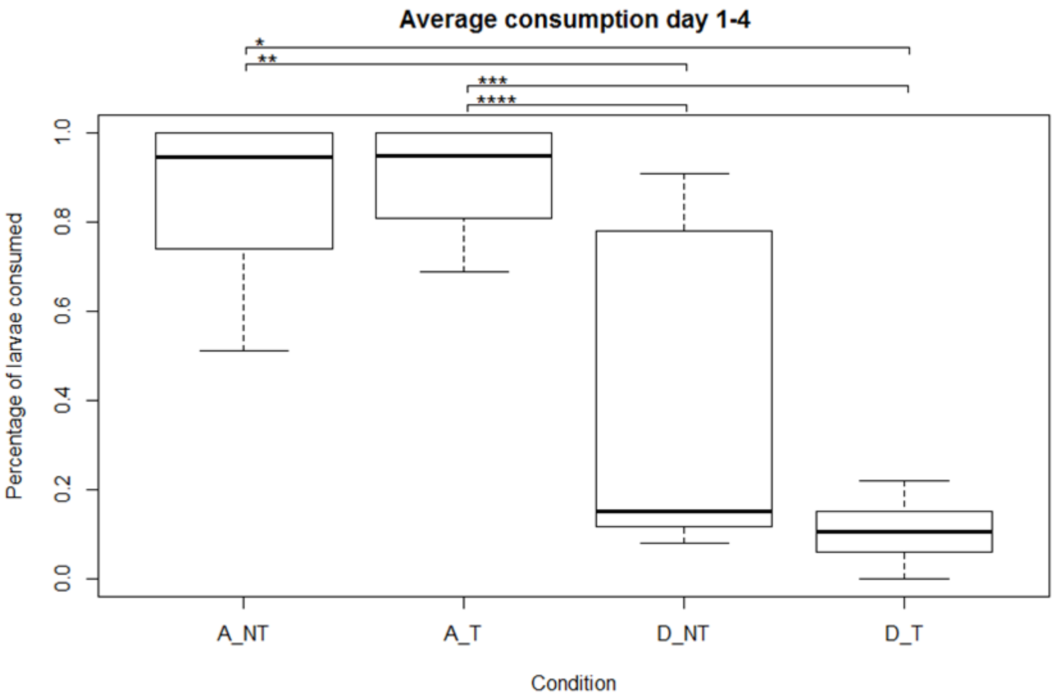


FIGURE 5: AVERAGE CONSUMPTION ON THE FIRST FOUR DAYS OF TESTING. *: P<0.001, **: P<0.01, ***: P<0.001, ****: P<0.01

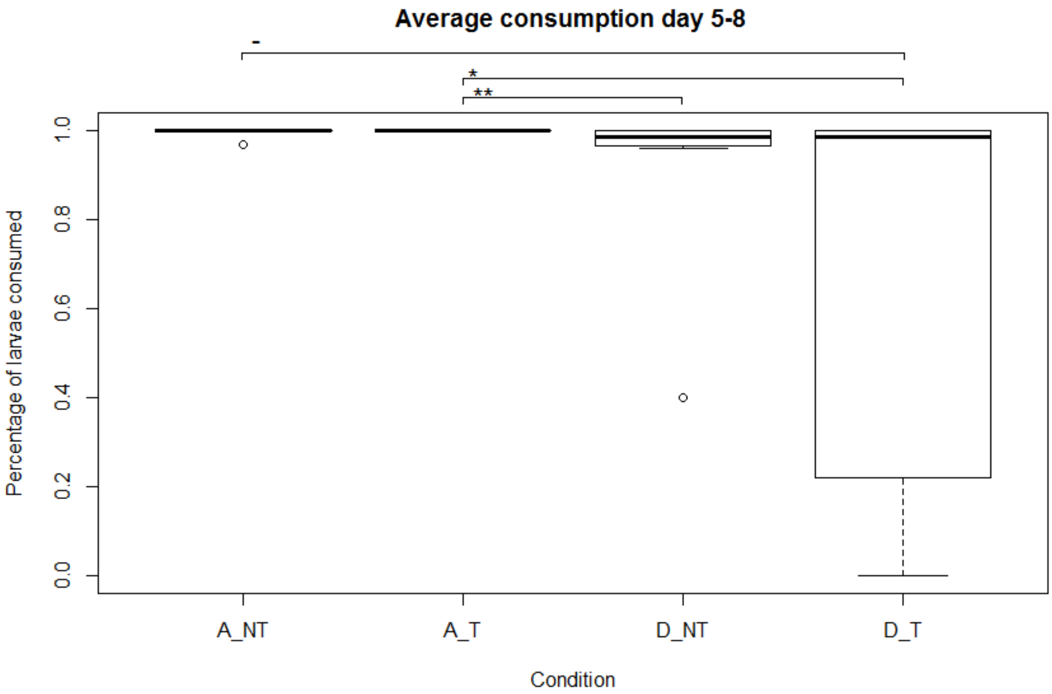


FIGURE 6: AVERAGE CONSUMPTION ON THE LAST FOUR DAYS OF TESTING.*: P<0.05, **: P<0.05, -: P<0.10

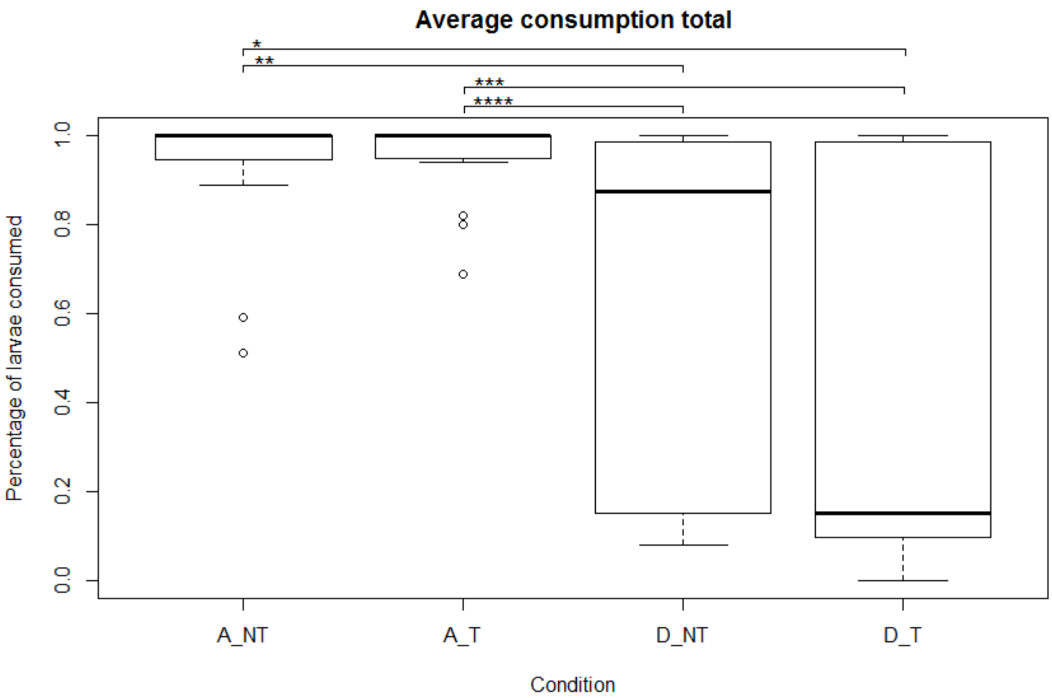


FIGURE 7: AVERAGE CONSUMPTION FROM ALL THE EIGHT DAYS TOTAL.*: P<0.01, **: P<0.05, ***: P<0.001, ****: P<0.05

## 3.2 Chicken weight

De weight of the chickens was measured per individual and can be found in table 5 (see appendix). It shows the weight of the chickens during the testing days. The weight of the chickens was uniform at start. However during the testing days weights scattered and the uniformity decreased to 50% at one point. This means that 50% of the chickens were between 90% and 110% of the average weight, with the outliers being the lightest chicken of 151 gram and the heaviest chicken of 378 gram at the end of the pilot.

## 3.3 Active time with the dispensers

The time of active behaviour towards a dispenser are shown in the following boxplots. Because of the low inter-observer reliability the four datasets were first analysed separately before analysing the combined datasets. Figure 8 shows the data from pen 1 where two chicks were observed for two hours for four days. For this dataset T-tests were used as the data was normally distributed. There is a significant difference between time performing active behaviour towards an A_T dispenser in comparison to a D_NT dispenser ($p<0.01$) or a D_T dispenser ($p<0.05$). Figure 9 is from pen 1 and pen 2, and shows data from two chicks of each pen observed for one hour. The data in this dataset was not normally distributed so instead of the T-test the Wilcoxon rank-sum test was used. The data shows a significant difference in time performing active behaviour towards a A_T dispenser than a D_NT dispenser ($p<0.001$). Also significantly more time was spent performing active behaviour towards a D_T dispenser in comparison to a D_NT dispenser ($p<0.05$). Figure 10 and Figure 11 show the data from pen 3 and pen 4. Data from pen 3 was normally distributed so the T-test was used. However, data from pen 4 was not normally distributed so the Wilcoxon rank-sum test was used instead. Although there were no significant differences in the boxplots of pen 3 and 4. Both the plots seem to show the highest amount of time performing active behaviour towards a A_T dispenser. After analysing the datasets separately, the datasets from chickens observed for one hour were combined into one dataset. Figure 12 shows the boxplot of the combined datasets. Again significant differences were found between A_T dispensers and D_T dispensers as well as D_NT dispensers. However, there is also a significant difference between the A_T dispenser and the A_NT dispenser. Significantly more time is spent performing active behaviour towards A_T dispensers in comparison to the other dispenser types.

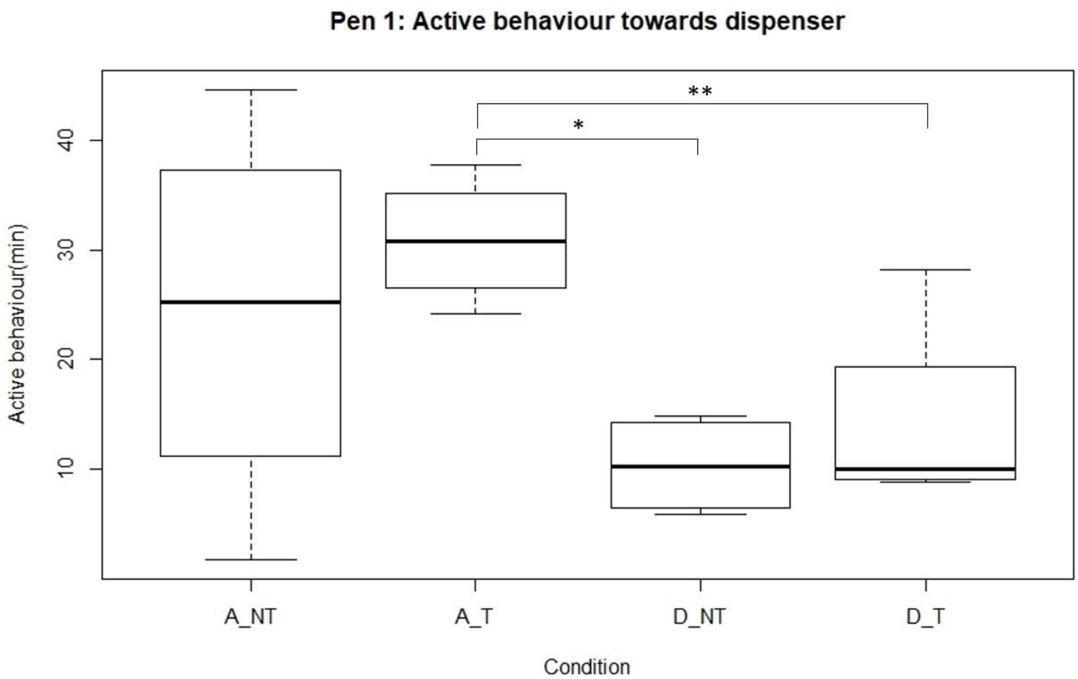


FIGURE 8: PEN 1 TWO CHICKS OBSERVED FOR TWO HOURS. *: P-VALUE = 0,00969, **: P-VALUE = 0,04651.

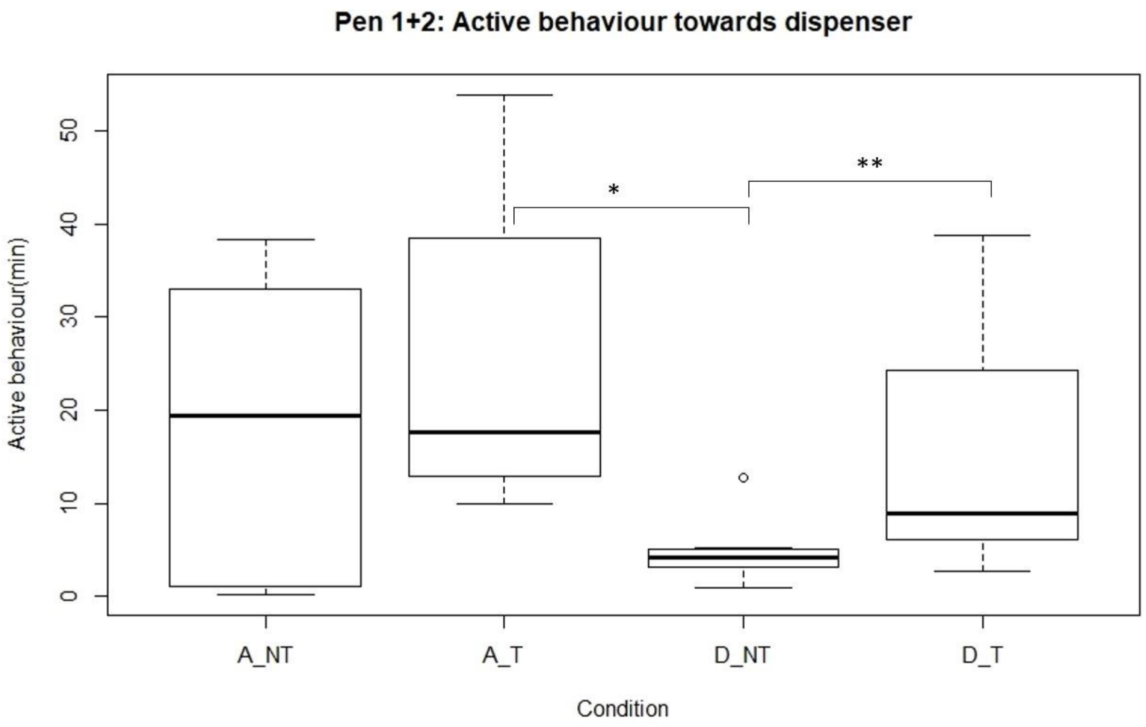


FIGURE 9: PEN 1+2 TOTAL OF FOUR CHICKS OBSERVED FOR 1 HOUR. *: P-VALUE = 0,0006216, **: P-VALUE = 0,03792.

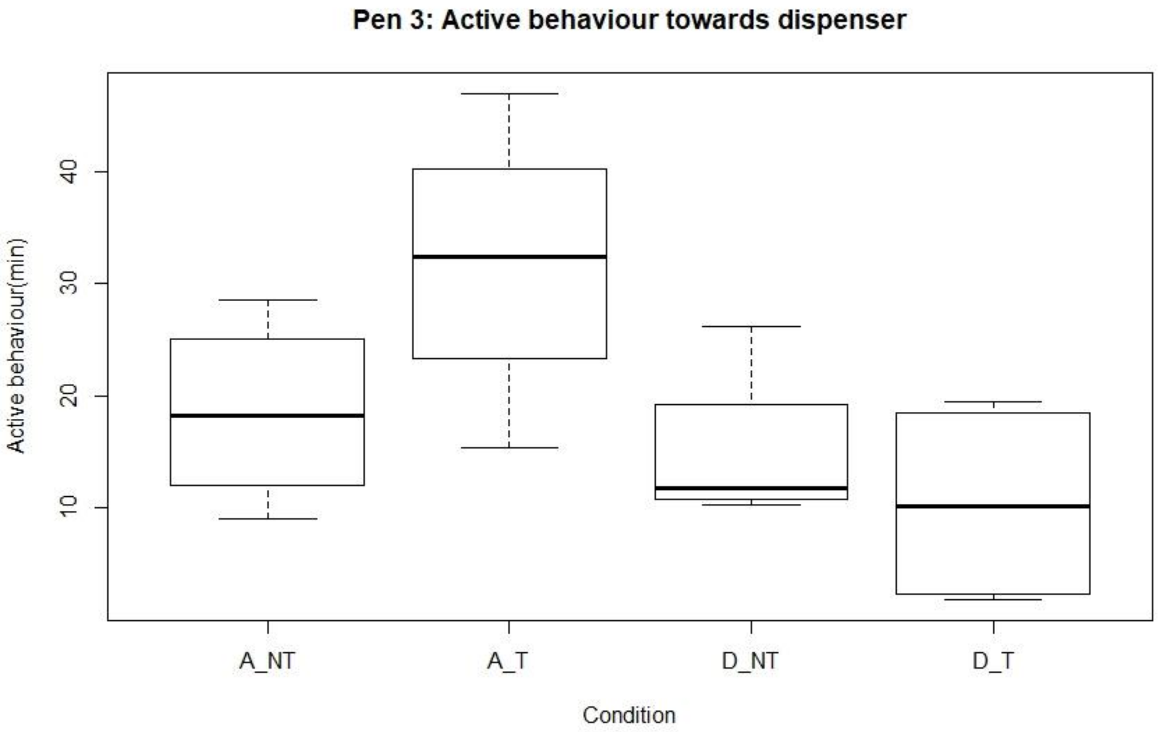


FIGURE 10: PEN 3 TWO CHICKS OBSERVED FOR 1 HOUR.

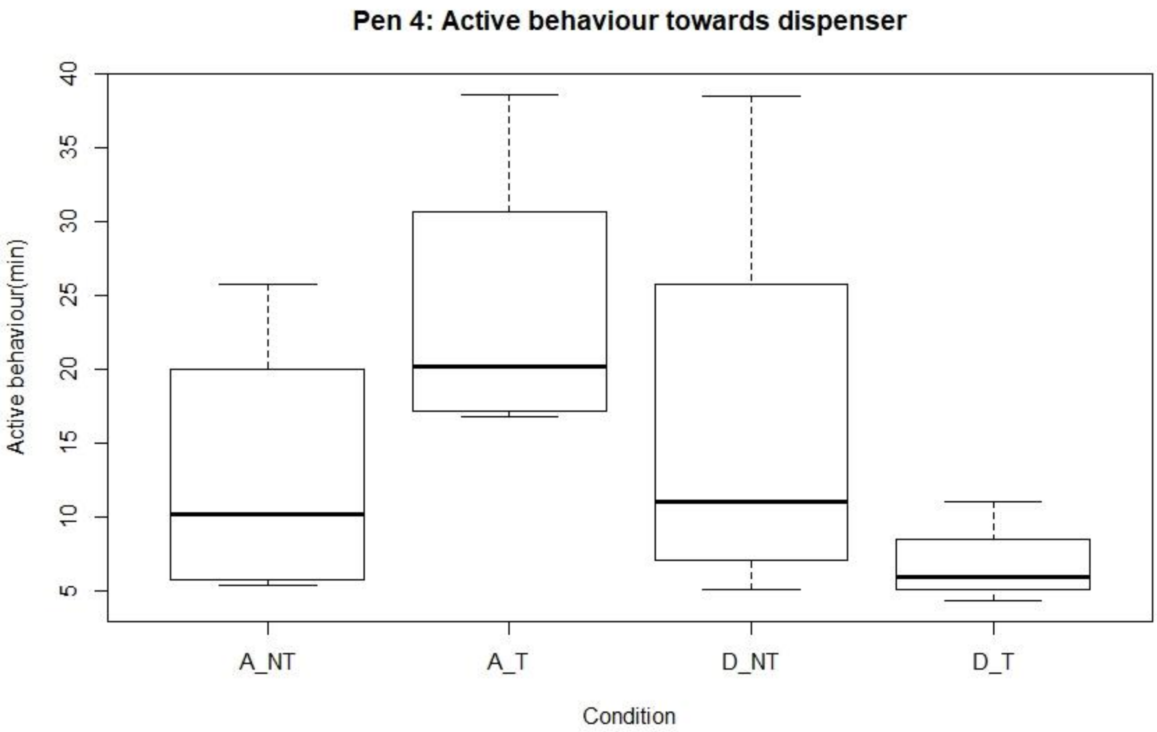


**FIGURE 11: PEN 4 TWO CHICKS OBSERVED FOR 1 HOUR.**

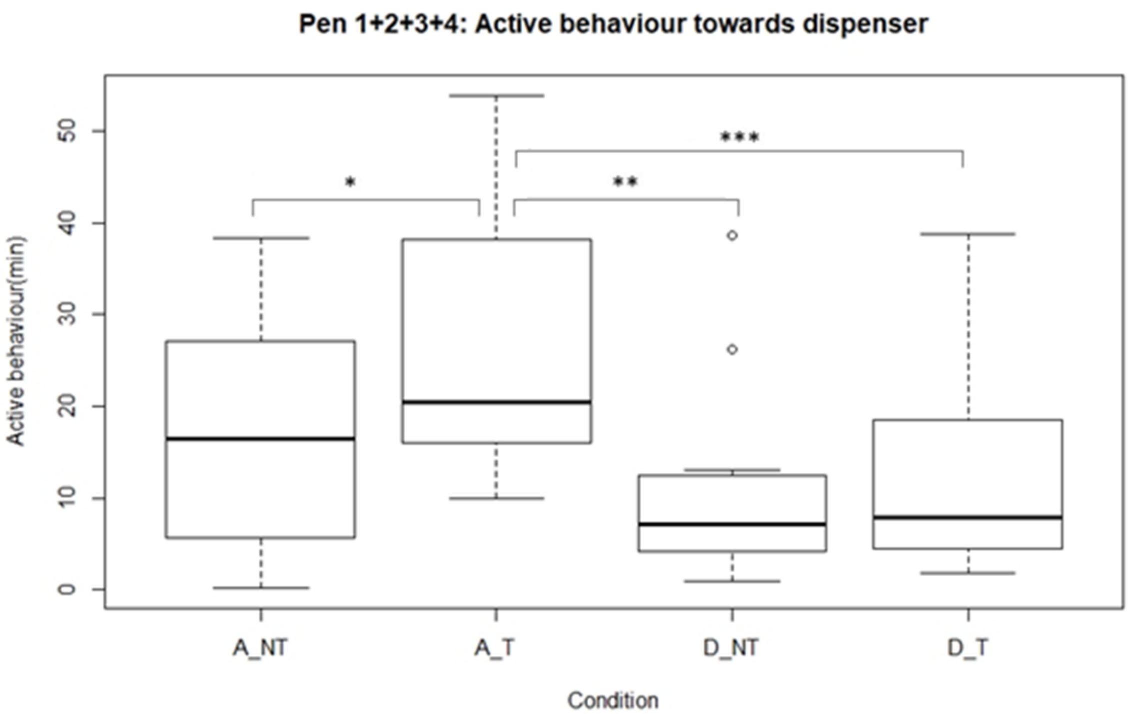


**FIGURE 12: PEN 1+2+3+4 TWO CHICKS OBSERVED FOR 1 HOUR.**

# 4. Discussion

The aim of this pilot study was to compare provision of live larvae versus dead larvae and to compare dispenser types. The goal of the comparison was to assess which combination of content and type of dispenser had the most effect on active behaviour towards the dispenser. This was achieved by looking at time spent performing active behaviour towards different dispenser conditions and from the amount of larvae consumed from each dispenser. Looking at the results, a preference towards the condition alive larvae and transparent dispenser has been noticed. It is possible that this preference can be replicated on a larger scale, such as in the main experiment. Whether the preference for a transparent dispenser containing live larvae affects the behavioural welfare of chickens, will be determined in the main study. In this main experiment, the goal is to investigate the effects of providing a dispenser filled with larvae as an enrichment to prevent feather pecking behaviour.

## 4.1 Larvae consumption

The consumption of the larvae was measured to detect differences in active behaviour towards the different dispensers. However, comparing these percentages is difficult and drawing conclusions from them even more so. Dispensers with dead larvae showed less larvae consumption compared to dispensers with live larvae. However, no account was made for the fact that live larvae would crawl out of the dispenser. Therefore, the lower consumption of dead larvae does not necessarily equal to more active behaviour towards dispensers containing live larvae. The dispensers with live larvae seemed to function more like a slow feeding device, where larvae would crawl out of the holes, rather than a device that needed to be manipulated by using the beak to reach larvae inside the dispenser. One could argue that the chickens even showed less active behaviour; because the larvae were easily obtained, the dispenser quickly emptied and active behaviour towards the dispenser would stop. Or perhaps chickens spent more active behaviour towards a dispenser with dead larvae, because obtaining the larvae was more difficult. The significant differences in the first four days are greatly reduced in the last four days. There was no significant difference in larvae consumption between the non-transparent dispenser containing live larvae, and the transparent and non-transparent dispensers holding dead larvae. This could indicate a learning effect, in which the chickens learned how to reach the dead larvae inside the dispensers more easily.

## 4.2 Chicken body weight

The weight of every chicken was measured to keep track of growth and uniformity of the groups. These results are not of importance for the aim of the study, however they are of importance for securing the welfare of an animal. The uniformity quickly lowered after two weeks with chicks that weighed 10% above the average as well as chicks weighing below 10% of the average. This led to performing tests on faecal samples to rule out diseases such as coccidiosis. The tests were all negative. To look further for a possible explanation of the decrease in uniformity, a strict examination of the environment was done. This showed that daylight hours were not properly maintained during the first 2 weeks because of a computer malfunction. The lighting schedule was maintained at 23 hours light against one hour of darkness, which was the light cycle for one-day-old chicks. As soon as the malfunction was discovered, the lighting schedule was quickly adjusted, because in the second week it should have already been 20 hours of light and four hours of dark. The loss of uniformity could probably be assigned to the lighting being faulty. This could also have caused a bias in the results, as this malfunction could have influenced the behaviour of the chicks. Another possible explanation of the loss of uniformity could be the treatment with larvae itself. As there was no control group without larvae, it is difficult to say what the effects of larvae consumption are on the uniformity of chicken body weight.

## 4.3 Active time with the dispensers

The separate datasets results show twice that there is significantly more active behaviour towards a transparent dispenser containing live larvae compared to a non-transparent dispenser containing dead larvae. This could mean the chicks had a preference to live contents of the dispenser, as well as a preference towards the transparent dispenser. However, no significant differences were found between different dispenser types when they both contained live larvae. On the other hand, there was significantly less active behaviour towards a non-transparent dispenser containing dead larvae in comparison to a transparent dispenser also containing dead larvae. It could be stated that when the dispenser content was live, the dispenser type had no or very little effect on active behaviour, and when the dispenser content was dead, dispenser type did have a higher effect on active behaviour. Chickens have shown a preference to moving objects, so maybe live BSF larvae are more attractive than dead BSF larvae (Jones et al., 1998). This could also be explained from an instinctual view of the chicken, because a live larvae would be fresh and therefore more appealing than a dead larvae that could be spoiled. Perhaps the stimulation of live larvae crawling out of the dispenser would diminish the effect of visually seeing the contents of the dispenser, or seeing the dead larvae inside the transparent dispenser had a negative effect on active behaviour towards the dispenser. When looking at the combined dataset, the same significant differences were found between the transparent dispenser containing live larvae and both dispenser types containing dead larvae. However, the combined boxplot also shows a significant difference between the dispenser types both containing live larvae. Significantly more time is spent performing active behaviour towards the transparent dispenser in comparison to the non-transparent dispenser. However, the low inter-observer reliability should be taken into account. The datasets were nevertheless combined to show a clearer picture of the results, because the boxplots from the separate datasets seemed to show similar results.

A literature search for similar experiments yielded hardly any results. However, providing larvae to broilers has been examined. These studies focus primarily on the performance of the broilers for meat production, or the safety of feeding larvae for the broilers themselves and for public health. Only one very recent study also looked at activity and foraging behaviour of broilers. This study showed an increase in foraging behaviour and general activity when broilers where fed BSF larvae. This effect was the highest with the group that received larvae twice a day and the largest amount in comparison to lesser amount and one a day (Ipema et al., 2020).

## 4.4 Conclusion

When looking at the data, there can be a lot of discussion. It is unfortunate that the data from all the pens could not be compared to each other, because of the low inter-observer reliability. This is the reason why the datasets were first analysed separately before eventually combining them to create a better picture of the results. The boxplots from the separate datasets seem to show similar results. However, from pen 3 and pen 4 there seem to be no significant differences in active behaviour towards dispenser conditions. In the results from pen 1, and from pen 1 and pen 2 combined, there do seem to be significant differences. Perhaps this could be caused by an observer bias. The observers each observed one pen without switching. This could have created a positive or negative effect on the results.

The results from the larvae consumption do show a difference between the dispenser types. However, these significant differences between live larvae consumption and dead larvae consumption could be explained by live larvae escaping the dispenser, and even so these differences seem to diminish after time. Next to the small sample size of subjects and the disturbed light/dark cycle this makes it difficult to draw hard conclusions. However with everything discussed above, it seems that the chickens in this experiment performed the most active behaviour towards a transparent dispenser containing live larvae. Providing live larvae in a transparent dispenser would probably have the most effect on active behaviour when used as an enrichment.

## 4.5 Future research

The data from this study is very limited. Because of time constraints only a small portion of the video data could be observed. One hour after providing the dispenser is a very small window, and because of this short window a bias may have occurred in active behaviour. Using the video data to observe for a longer amount of time could give a better view of the effects of the different dispenser conditions.

As was found during the earlier literature search, the provision of insect larvae as enrichment to laying hens has not yet been widely researched. This may be due to the fact that providing larvae is a rather new method of creating environmental enrichment. Given the positive effects of BSF larvae on broiler behaviour found by Ipema et al. (2020), it seems that doing more research on the provision of larvae to laying hens would be beneficial.

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# Appendix

TABLE 5: WEIGHTS OF THE CHICKENS

| Weights in grams | 18-nov | 20-nov | 22-nov | 25-nov | 27-nov | 29-nov | 2-dec | 4-dec | 6-dec | 9-dec | 11-dec | 13-dec | 16-dec | 18-dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pen 1 | | | | | | | | | | | | | | |
| Brown | 38 | 44 | 57,0 | 74,0 | 92,0 | 109,0 | 136,0 | 158,0 | 188,0 | 215,0 | 238,0 | 266,0 | 307,0 | 332,0 |
| Bue | 38 | 45 | 60,0 | 74,0 | 89,0 | 108,0 | 136,0 | 161,0 | 185,0 | 218,0 | 243,0 | 271,0 | 314,0 | 342,0 |
| Black | 38 | 45 | 55,0 | 69,0 | 86,0 | 102,0 | 126,0 | 141,0 | 180,0 | 202,0 | 222,0 | 247,0 | 287,0 | 307,0 |
| silver | 39 | 45 | 53,0 | 66,0 | 82,0 | 94,0 | 125,0 | 153,0 | 187,0 | 219,0 | 249,0 | 280,0 | 330,0 | 360,0 |
| Purple | 39 | 45 | 56,0 | 64,0 | 79,0 | 96,0 | 118,0 | 136,0 | 167,0 | 191,0 | 213,0 | 238,0 | 276,0 | 301,0 |
| Green | 38 | 44 | 57,0 | 71,0 | 87,0 | 101,0 | 118,0 | 135,0 | 158,0 | 176,0 | 198,0 | 225,0 | 269,0 | 288,0 |
| Yellow | 36 | 41 | 49,0 | 60,0 | 72,0 | 88,0 | 113,0 | 134,0 | 153,0 | 177,0 | 199,0 | 227,0 | 270,0 | 286,0 |
| **Average** | 38,0 | 44,1 | 55,3 | 68,3 | 83,9 | 99,7 | 124,6 | 145,4 | 174,0 | 199,7 | 223,1 | 250,6 | 293,3 | 316,6 |
| Pen 2 | | | | | | | | | | | | | | |
| Brown | 36 | 41 | 52,0 | 62,0 | 70,0 | 83,0 | **101,0** | 112,0 | 138,0 | 156,0 | 176,0 | 206,0 | 248,0 | 269,0 |
| Blue | 34 | 39 | 46,0 | 60,0 | 71,0 | 80,0 | **100,0** | 109,0 | 141,0 | 158,0 | 172,0 | 190,0 | 216,0 | 228,0 |
| Gray | 38 | 44 | 54,0 | 68,0 | 81,0 | 90,0 | 113,0 | 132,0 | 158,0 | 181,0 | 201,0 | 230,0 | 273,0 | 297,0 |
| silver | 40 | 44 | 57,0 | 69,0 | 82,0 | 98,0 | 127,0 | 145,0 | 177,0 | 202,0 | 225,0 | 254,0 | 299,0 | 319,0 |
| Purple | 40 | 43 | 52,0 | 62,0 | 75,0 | 83,0 | **104,0** | 113,0 | 144,0 | 169,0 | 186,0 | 207,0 | 242,0 | 259,0 |
| Green | 38 | 43 | 51,0 | 63,0 | 76,0 | 94,0 | 129,0 | 154,0 | 195,0 | 222,0 | 254,0 | 285,0 | 335,0 | 360,0 |
| Yellow | 39 | 42 | 51,0 | 69,0 | 84,0 | 96,0 | 125,0 | 144,0 | 182,0 | 205,0 | 229,0 | 262,0 | 312,0 | 344,0 |
| **Average** | 37,9 | 42,3 | 51,9 | 64,7 | 77,0 | 89,1 | 114,1 | 129,9 | 162,1 | 184,7 | 206,1 | 233,4 | 275,0 | 296,6 |
| Pen 3 | | | | | | | | | | | | | | |
| Brown | 38 | 39 | 47,0 | 58,0 | 72,0 | 78,0 | **85,0** | **99,0** | **103,0** | **106,0** | **116,0** | **122,0** | **136,0** | **151,0** |
| Blue | 31 | 38 | 47,0 | 61,0 | 75,0 | 91,0 | 117,0 | 137,0 | 166,0 | 194,0 | 217,0 | 242,0 | 288,0 | 312,0 |
| Black | 41 | 46 | 56,0 | 70,0 | 87,0 | 102,0 | 126,0 | 142,0 | 170,0 | 184,0 | 210,0 | 232,0 | 274,0 | 302,0 |
| silver | 41 | 46 | 57,0 | 73,0 | 90,0 | 103,0 | 132,0 | 152,0 | 186,0 | 211,0 | 236,0 | 267,0 | 315,0 | 347,0 |
| Purple | 35 | 44 | 53,0 | 65,0 | 80,0 | 92,0 | 118,0 | 138,0 | 165,0 | 188,0 | 213,0 | 238,0 | 276,0 | 299,0 |
| Green | 41 | 46 | 59,0 | 73,0 | 86,0 | 96,0 | 119,0 | 143,0 | 167,0 | 186,0 | 207,0 | 240,0 | 283,0 | 304,0 |
| Yellow | 36 | 42 | 52,0 | 60,0 | 67,0 | 71,0 | **80,0** | **89,0** | **107,0** | **126,0** | **153,0** | 180,0 | 225,0 | 261,0 |
| **Average** | 37,6 | 43,0 | 53,0 | 65,7 | 79,6 | 90,4 | 111,0 | 128,6 | 152,0 | 170,7 | 193,1 | 217,3 | 256,7 | 282,3 |
| Pen 4 | | | | | | | | | | | | | | |
| Brown | 39 | 47 | 58,0 | 74,0 | 90,0 | 104,0 | 133,0 | 155,0 | 180,0 | 205,0 | 232,0 | 264,0 | 312,0 | 339,0 |
| Blue | 40 | 47 | 60,0 | 76,0 | 93,0 | 111,0 | 138,0 | 158,0 | 189,0 | 215,0 | 237,0 | 264,0 | 311,0 | 328,0 |
| Black | 40 | 43 | 50,0 | 64,0 | 79,0 | 91,0 | 115,0 | 132,0 | 159,0 | 177,0 | 194,0 | 213,0 | 242,0 | 259,0 |
| silver | 37 | 45 | 59,0 | 78,0 | 96,0 | 115,0 | 146,0 | 169,0 | 195,0 | 223,0 | 253,0 | 282,0 | 329,0 | 354,0 |
| Purple | 36 | 41 | 56,0 | 75,0 | 91,0 | 103,0 | 132,0 | 150,0 | 185,0 | 208,0 | 235,0 | 265,0 | 314,0 | 344,0 |
| Green | 35 | 39 | 47,0 | 52,0 | 62,0 | 75,0 | **101,0** | 119,0 | 147,0 | 168,0 | 191,0 | 216,0 | 255,0 | 278,0 |
| Yellow | 40 | 46 | 58,0 | 75,0 | 90,0 | 107,0 | 133,0 | 153,0 | 179,0 | 205,0 | 232,0 | 260,0 | 305,0 | 328,0 |
| **Average** | 38,1 | 44,0 | 55,4 | 70,6 | 85,9 | 100,9 | 128,3 | 148,0 | 176,3 | 200,1 | 224,9 | 252,0 | 295,4 | 318,6 |
| Reference: | 38 | | | 64-67 | | | 132-139 | | | 211-222 | | | 296-312 | |
| Total average | 37,9 | 43,4 | 53,9 | 67,3 | 81,6 | 95,0 | 119,5 | 138,0 | 166,1 | 188,8 | 211,8 | 238,3 | 280,1 | 303,5 |
| % Uniform | 96,4 | 96,4 | 78,6 | 78,6 | 64,3 | 60,7 | 53,6 | 53,6 | 53,6 | 53,6 | 53,6 | 46,4 | 50,0 | 53,6 |