

The effects of confinement on the associative learning of *Gallus gallus domesticus*

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SUMMARY

In this study, we aimed to determine if confinement affects associative learning in chickens. We assessed associative learning ability by training chickens to recognize two plates of cottage cheese: on one plate, the cottage cheese was stained green with no other additives, and on the other plate, the cottage cheese was stained pink and contained methyl anthranilate, a common chemical in bird repellent. After a training period of 7 days, we again presented each chicken with the two plates of cottage cheese and measured the amount of time before the chickens began to consume cottage cheese and the number of pecks the chickens made to each cottage cheese plate during a two minute interval. Two trials were conducted; the first was conducted before the chickens were subjected to confinement and the second was conducted after the chickens were subjected to confinement for 15 hours. We found that the difference in the time lapsed before the chickens began to consume the cottage cheese before and after confinement was significant, though the difference in the number of pecks was not. These results suggest that confinement distresses chickens, as it impairs associative learning without inducing confusion.

INTRODUCTION

For most people in the United States, though chickens are considered profitable to farm, these animals are not considered particularly intelligent (1). However, chickens are very intelligent animals (2). They engage in complicated social hierarchies and have high levels of self-awareness, numerical understanding, and visual capabilities (3, 4). Despite these high levels of intelligence, many chickens are subjected to controversial conditions in factory farms. Factory farms subject chickens to crowded, noxious living conditions; each chicken is given an average living area smaller than a sheet of paper, in which the ambient noise of surrounding chickens is deafening and the buildup of feces can lead to ammonia levels that are harmful to chickens' eyes, throats, and skin (5). Since chickens are very profitable animals to farm, are not considered particularly intelligent, and are not traditional pets in the United States, this abuse is generally unregulated. We hypothesized that chickens subjected to confinement similar to that experienced by chickens held in factory farms

would demonstrate signs of distress and struggle to complete associative learning tasks.

As a result, we designed a study to test this hypothesis, in which the independent variable was confinement and the dependent variables were the chickens' tendency to peck the more desirable, green-colored cottage cheese, the amount of time before pecking the cottage cheese, and the number of pecks of each color. The goal of this study was to determine if confinement affects chickens' abilities to learn by association. Our hypothesis was that confinement would cause either an increase in the amount of time it takes the chickens to begin the test or an inability of the chickens to accurately complete the test. After completing our experiment, we found that the amount of time it took for the chickens to begin the test increased after confinement. However, we also found that chickens were equally able to accurately participate in the test after confinement as before.

RESULTS

We hypothesized that chickens would show behavioral differences after being subjected to 15 hours of confinement by either a variation in the average number of pecks or the amount of times that they pecked at the cottage cheese. To investigate this, we compared two groups: the chickens before experiencing confinement, and the chickens after having been confined for 15 hours. Tests for both groups were conducted after the chickens had been fed cottage cheese for approximately 7 days. The median amount of time to peck in the chickens before confinement was 8.5 seconds, while the chickens after 15 hours of confinement showed a median of 35 seconds to peck. This indicated an increase in the amount of time for chickens to peck the cottage cheese after being confined (**Figure 1**). We found this increase in time to peck was significant using the Wilcoxon signed-rank test; at $p = 0.05$ $T^* = 13$ and our data obtained a measure of $T^* = 11$, since $11 < 13$ we rejected the null hypothesis. Furthermore, we anecdotally observed that, after confinement, chickens were more hesitant and cautious when presented with the plates of cottage cheese.

The control group demonstrated a median of 31.5 pecks directed towards the green cottage cheese and the treatment group demonstrated a median of 30 pecks (**Figure 2**). We found that the apparent decrease in the number of pecks by the treatment group was not significant by Wilcoxon signed-ranks test at significance level $p = 0.05$. We also hypothesized that the number of times the chickens pecked the tainted



Figure 1. Boxplot of seconds elapsed until cottage cheese was pecked.

cottage cheese (pink) would increase after the chickens were placed in confinement. However, the chickens showed absolutely no difference in the amount of times they pecked the pink cottage cheese, so we could not run a test on this data.

DISCUSSION

The goal of our research was to measure chickens' color recognition and association capabilities and determine if factory farm conditions, such as confinement, affect these capabilities. We attempted to measure these attributes using various approaches throughout the course of our research. Originally, we planned to condition each chicken to recognize the color red as "good" and the color green as "bad" using a reward system. In this planned experiment, chickens would demonstrate this association by pecking one of two small, colored squares of paper: one green and one red. In order to train them, they would receive a treat (a dried mealworm, or a click from a dog training clicker) when they pecked the red piece of paper, and they would not receive a reward when they pecked the green piece of paper. This set-up was inspired by an experiment we read about in which chicks were tested on their color preference through the use of colored circles laid on the floor (6). This study was able to effectively condition chickens to show color preference, so we were confident that a similar set up would work for our chickens as well.

We would then conduct our test by offering each chicken the two pieces of paper, and measuring how many times they pecked the red colored paper (in hopes of getting a treat) and the amount of times they pecked the green colored paper (incorrectly assuming they would get a treat). The pecks directed towards the red paper would be labeled as "correct" and pecks directed toward the green paper would be labeled as "incorrect." The chickens' scores would be calculated as the number of correct pecks over the total number of pecks. After confining the chickens, we would repeat the test and compare the changes in scores.

This approach was attempted repeatedly with six different chickens throughout the course of six weeks, yet after this time, it became apparent that our approach was not

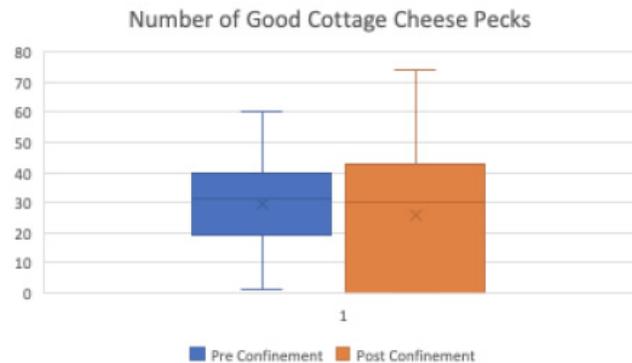


Figure 2. Boxplot of the number of times each chicken pecked the green cottage cheese.

appropriate. We struggled to entice the skittish chickens to peck the paper even once, because they did not find the task appealing. Some of the more intelligent chickens appeared to pick up on the pattern, but even those chickens were unreliable. Ultimately, we could not encourage almost any of the chickens to peck either of the paper squares or even show interest in the test. After running one test run where a chicken did not peck either color for the entirety of the test, we decided to amend our approach. After reaching out to a researcher who worked with our class and has done a lot of biology research with live animals, we decided that using colored foods would be a more promising way for us to study chickens' abilities to learn by association.

We first attempted to use green- and pink-dyed yogurt, and supplemented the pink yogurt with methyl anthranilate, which is commonly used as bird repellent (7). However, on the first day we attempted our new method, the chickens would not even touch the yogurt. After a second failed attempt the following day, we decided we needed to use a different food product. We decided to use cottage cheese, because we thought that cottage cheese would be easier for the chickens to eat due to the curds. Fortunately, the first day the chickens tried the cottage cheese, they ate it without hesitation.

With our new experiment, we began to rethink how our hypothesis would translate to our results. We suspected that placing the chickens into confinement would induce distress and make them less willing to taste the cottage cheese. We also thought that it might lead to increased confusion, causing them to peck both types of cottage cheese even though they had already been trained that the pink cottage cheese would taste bad. We hypothesized that, after confinement, the amount of time before the chickens pecked the cottage cheese would increase, the amount of times the chickens pecked the green cottage cheese would decrease, and the amount of time the chickens pecked the pink cottage cheese would increase.

Our first hypothesis, that the time before pecking the cottage cheese would increase, was supported by our experiment. The previously confined chickens were wholly unwilling to try the cottage cheese until they had investigated

it long enough, unlike in the control test, where they ate the cottage cheese very quickly and without hesitation. The next two hypotheses, that the amount of times the chickens pecked the green would change and that the amount of times they pecked the red would increase, were not supported by our experiments. The first reason that we believe the number of green cottage cheese pecks did not change is due to the fact that several times, the chickens spilled the contents of their provided food and water while they were in confinement. Because the food got spilled, it is likely that when they were tested immediately after being brought out of confinement, they were quite hungry. Although they were more hesitant to eat, once they tasted the untainted green cottage cheese, they were hungry enough to let their hunger override their potential skittishness. We also suspect that we may have flavored the pink cottage cheese with methyl anthranilate too strongly. This would have made it harder for the chickens to show signs of confusion; if it were very strongly ingrained in their memory that the pink cottage cheese tasted terrible, they would be less likely to make any mistakes in the future than they might have been if we had flavored it slightly more moderately, perhaps skewing our data by making it harder for them to demonstrate any confusion.

Our data suggests that the confinement experienced by chickens in most factory farms has the potential to be distressing. The effects of stress on the chickens influenced them to regard the cottage cheese hesitantly, when they had previously considered it harmless. These results led us to recognize the importance of improving conditions in factory farms for chickens. However, this data is only applicable to female egg-laying strains of chickens. Since we were only using egg-laying chickens in our experiment, the results could not be used to represent the response of any broiler type chicken or male chickens.

If we were able to replicate this experiment with more time and resources, we would have introduced controls for noise and light. Factory farms are extremely loud and have bright lights on all year round, which are not natural living conditions for chickens. Chickens lay eggs based on the season, which is signaled to their body by light changes (i.e., lengthening days in spring and shortening days in fall). Therefore, the presence of constant light can put a stress on a chicken's body by constantly signaling to it to lay; we are curious as to how this variable might affect chicken performance on our experimental task. We also noticed that loud noises seriously distressed the chickens, which made us wonder how constant noise might affect them. We also would have introduced more colors to our test to see if the chickens had potential color preferences that could have altered the results.

MATERIALS AND METHODS

In this experiment, we tested chickens' ability to learn by association before and after confinement. Their level of intelligence was determined by using a simple, yet effective, associative learning test: color recognition. For this test,

chickens were presented with two different plates of cottage cheese. We decided to use cottage cheese, because it is easy for us to mix with other substances and because it is safe and easy for chickens to eat. One of the plates had unflavored cottage cheese that had been dyed a bright green, while the other plate had a portion of cottage cheese that had been dyed bright pink and flavored with methyl anthranilate (half a teaspoon of methyl anthranilate per cup of cottage cheese). The chemical we mixed in, methyl anthranilate, is a harmless chemical that is often used as bird-repellent due to its taste. We decided to use the colors green and pink because both are vivid colors that have been shown to be of equal preference to chicks (5) In this way, choosing the "good" green cottage cheese granted the chickens a snack, while choosing the "bad" pink cottage cheese gave the chickens a foul taste in their mouth.

We split the chickens into two randomly selected groups of five and one group of four. To accurately simulate the confinement used in factory farms, we wanted the chickens to be confined with multiple other chickens. We only had access to two cages, so we could not do them all together. We decided on three groups because each group was an appropriate size for confinement and because we could monitor each chicken to measure their understanding of the color association. For approximately 7 days before we conducted the control test, we fed the chickens the green and pink cottage cheeses to condition them with the mindset that the green would taste good and pink would taste bad, effectively training them to always choose to eat the green and avoid the pink. We gave them seven days, because we observed that after this time period the chickens consistently peck the green cottage cheese and avoid the pink.

After training, we then tested each chicken individually, wherein each chicken was given a cup each of the green and pink cottage cheese. We then recorded their behavior for two minutes, which started immediately after the chicken was placed on the table. We recorded the time at which the first peck occurred, as well as how many times the chicken pecked each color. Following the initial test, we placed each group in confinement, giving each chicken the spatial limitations of approximately 8.5 inches by 11 inches. These limitations were based off the amount of space that chickens are generally given in factory farms, which is around 66 square inches. We did not choose to use the exact size limitations because we did not want to the chickens so crowded that they would overheat. The increment (15 hours) was chosen due to our schedule. We confined the chickens at night, when all of the chickens would usually be checked on, and returned the next day shortly after 2 pm, which resulted in a 15-hour period of confinement. After 15 hours in these conditions, we repeated the testing again on each chicken individually.

After collecting the data, we used a Wilcoxon signed-rank test to determine if confinement had a significant effect on the amount of time it took the chickens to first peck the cottage cheese and the number of times they pecked each

type of cottage cheese. We used this test because we wanted to compare the before and after confinement data and were unable to determine that our data was normally distributed.

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