104 (2025) 104594

Contents lists available at ScienceDirect

Poultry Science

journal homepage: www.elsevier.com/locate/psj

The impact of multiple exposures and movement on the fear response of poultry

Alexandra Jackson, Marcela Quino, Anusha Gautam, Melissa Gilpin, Katie Still, Denise Landers, Bethany Baker-Cook *

Department of Poultry Science, Auburn University, Office 302E, 260 Lem Morrison Dr., Auburn, Auburn, AL 36849, USA

ARTICLE INFO

Keywords:
Novel object
Voluntary human approach
Fearfulness
Broilers
Welfare assessments

ABSTRACT

Fear tests are a common research method to assess the affective state of an animal. This study aimed to assess: 1) the impact of repeated exposure to fear tests on fear response and, 2) how the addition of movement to fear stimuli during a novel object test impacts bird fear response. Over two trials, a total of 3,600 Ross 308 birds (1800 birds/trial) were raised until 42 days of age. At 23d, 30d, and 37d, three fear tests were performed, novel object, human approach, and response to observer tests. The novel object test was split into three movement treatments, stationary, intermittent, and continuous. The response to observer test was performed before and after the other tests. Data from the human approach test was analyzed for age effect by ANOVA. Novel object data was analyzed as repeated measures ANOVA for the effect of movement. Differences in response to observer were analyzed using a paired T-test. Repeated exposure to fear test within the same day decreased the fear response, with response to observer results before fear testing (66%) differing from after (42%, $P<0.01$). The fear response also decreased with multiple consecutive exposures. Both latency to human approach and latency to novel object interaction were longest on 23d, then 30d, and shortest on 37d ($P<0.01$). For both the human approach and the novel object test, at all-time points, the number of birds interested in the human or object was higher on 37d than 23d ($P<0.01$). The addition of movement to the novel object test increased the fear response at 23 days but decreased the fear response at 37 days. Overall, the repeated exposure of birds to fear tests reduced the bird's fear response, both for the repeated exposure to different fear tests on a single day and the repeated exposure to one type of fear test over the length of a flock. This impact of repeated exposure is important to recognize when designing experiments that utilize fear tests.

Introduction

Fear is an animal's reaction to the perception of danger; the fear response is the actions, either physiological or behavioral, that the animal undertakes to deal with danger. Whilst major dangers such as predators are not present in commercial poultry, there are still several aspects of poultry management that can elicit a fear response. Excessively intense fear or prolonged exposure to fear can result in chronic stress, which can lead to altered behavior and negatively affect productivity and health in birds (Jones, 1996). In poultry, fear has been found to increase mortality and injuries (Reed et al., 1993; Cransberg et al., 2000; de Haas et al., 2013), reduce growth (Cransberg et al., 2000; Jones et al., 2002; de Haas et al., 2013), worsen feed conversion (Hemsworth et al., 1994), reduce egg production, egg weight and egg quality (Vestergaard et al., 1993; Cransberg et al., 2000; de Haas et al., 2013), decrease productive performance and hatchability (Jones et al., 2002), increase in downgraded carcasses (Jones et al., 2002), and increase feather pecking (Vestergaard et al., 1993; de Haas et al., 2010). Furthermore, fearful birds are more difficult to handle and less able to adapt to changes in the environment, which can result in management problems.

Fear is a subjective state; this means it cannot be measured directly. Since bird fear levels cannot be measured directly, an indirect measure needs to be used. This can be done by measuring the bird's fear response to a situation that is supposed to elicit fear (Jones, 1987). One method used to assess the animals' fear response is via fear tests. When an animal experiences fear, this emotion will take precedence over all others and will inhibit birds from performing other behaviors (Jones, 1987). By measuring the animals' response to a mild fear-eliciting stimulus, we can infer the bird's level of fear and their mental state (Jones, 1987;

* Corresponding author.
E-mail address: bib005@auburn.edu (B. Baker-Cook).

https://doi.org/10.1016/j.psj.2024.104594
Received 29 July 2024; Accepted 25 November 2024
Available online 26 November 2024

Forkman et al., 2007). Birds that are more fearful will have a more intense fear response than those that are less fearful (Jones, 1996). In the fear tests, we present an animal with a stimulus that will elicit either an approach or avoidance response (Jones, 1996). With the approach response, birds will show interest in and try to interact with the stimulus. With the avoidance response, the birds can avoid a stimulus by ignoring the stimulus, walking a few steps away from the stimulus, or attempting to escape (Nicol, 2015). From an animal's chosen response, we can understand its fear level.

Fear tests are becoming more prevalent in poultry research as a method to assess bird welfare. Several different fear tests are currently used which involve exposure to a fear-inducing stimulus. This stimulus can be a novel object (the novel object test), a predator (voluntary human approach test or response to observer test), a new environment (the novel arena test), or restraint (tonic immobility or inversion test) (Forkman et al., 2007). It is also becoming more common for fear tests to be repeated multiple times throughout an experiment. This means that the birds are exposed to either the same fear test multiple times throughout the experiment (Tahamtani and Riber, 2020; Campbell et al., 2022; Manet et al., 2023 Hammond et al., 2024) or are exposed to multiple fear tests on the same day (Huth and Archer, 2015; Franco, et al., 2022; Edgar et al., 2023). One of the important qualities of the fear-eliciting stimuli used in many of these tests is novelty (either a novel arena or a novel object). While there is some concern that the repetition of these tests may reduce their novelty, the impact that this has on the fear response is currently unknown.

The nature and extent of the fear response to a possible fear-eliciting event can be influenced by several qualities of the event: novelty, intensity, duration, suddenness, and movement (Gray, 1987). The addition of movement to the novel object test adds another dimension to the fear stimulus. This additional dimension may provide us with an improved understanding of the level of fear and the mental well-being of the bird. Furthermore, a prior study suggested that the number of birds able to interact with a novel object during a novel object test may influence the results, making it seem as though birds are more fearful as fewer birds want to interact with the novel object (Rasmussen et al., 2022). As birds age, they become larger, meaning that fewer birds are able to fit around the object, and thus, fewer birds can interact with it. When tests such as the novel object test are done in large pens or in commercial barns, only the birds that are in the vicinity of the object are able to participate in the test. Moving the object around the pen or barn may allow for a greater number of birds to have the opportunity to interact with the object and participate in the test, providing a better understanding of the whole flock's fear level and welfare.

This experiment aims to see if repeated exposure to different fear tests on the same day and repeated exposure to the same fear test multiple times throughout the duration of a flock can impact the bird's fear response. Additionally, it aims to see if the addition of movement to the novel object test changes the bird's fear response.

Materials and methods

Experimental design

To test the impact of movement on the novel object test, each pen was assigned one of three novel object treatments: traditional stationary novel object test, intermittent movement novel object test, and continuous movement novel object test. The study was a complete randomized design and each treatment had 8 replicates, with 75 birds per replicate. To test the impact of repeated exposure on the fear response, all the birds were exposed to a total of four fear tests on each test day (response to observer, voluntary human approach, novel object, and response to observer), with three test days per flock.

Animal husbandry

Over two flocks, a total of 3600 mixed-sex Ross 308 chicks (1,800 chicks/trial) were randomly distributed into 24-floor pens (9 m^2). Birds were housed at a stocking density of 75 birds/pen and raised until 42 days of age. Pens were equipped with three hanging feeders and an automatic nipple water line. Birds had *ad libitum* access to feed and water throughout the trial and were fed a standard corn-soy-based. The lightening regime was 23 hours of continuous light and 1 hour of dark from placement to 7 days of age, then modified to 18 hours of continuous light and 6 hours of darkness until the trial period was over, in accordance with primary breeder recommendations. The study was conducted with the approval of Auburn University's Institutional Animal Care and Use Committee.

Behavioral tests

Behavioral tests were performed at 23, 30, and 37 days of age to assess fear response on a pen level: novel object test, voluntary human approach test, and response to observer test. On test days, the response to the observer test was done first. The birds were then subjected to either the voluntary human approach test or the novel object test. Half of the pens received the novel object test first, followed by the voluntary human approach test, and the other half received the voluntary human approach test first, followed by the novel object test. This was done to ensure the order of testing did not bias results with all pens being randomly assigned prior to each day of testing. After all the pens were tested for novel object and voluntary human approach, the response to observer test was performed a second time. All pens underwent novel object and voluntary human approach tests once and response to observer test twice.

Response to observer test

To determine if exposure to the fear assessments influenced subsequent fear responses, the response to observer test (adapted from Schwean-Lardner, et al., 2012) was performed both before and after the other fear tests. This was done by one observer walking quietly along the long-side length of each pen on the outside (Fig. 1) and recording the birds with a handheld video camera (MINOLTA MN220NV, Japan). During this time, only a single observer recording bird responses was present in the barn. The number of birds within the field of view that physically moved away from the observer was recorded.

Voluntary human approach test

The voluntary human approach test was performed as described by Gierberg et al. (2020). For this test, an observer located themselves within the pen against the center of long-side edge, as shown in Fig. 1, and maintained a squat position with their knees drawn up to their chest for three minutes. Latency to interaction and the number of birds within 25 cm of the observer every 30 seconds were recorded. For latency to interaction, the time until a bird made any form of physical interaction with the observer, such as pecking at the observer's boots or sleeves was measured. The observer wore a white lab coat to ensure the attire

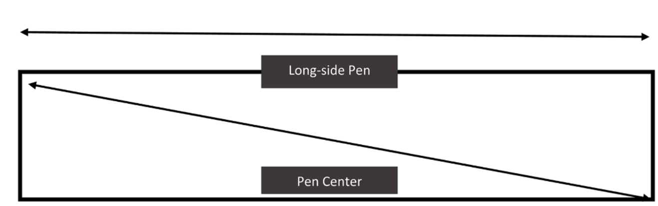

Fig. 1. Orientation of pen housing broilers (75/pen) subjected to behavior tests.

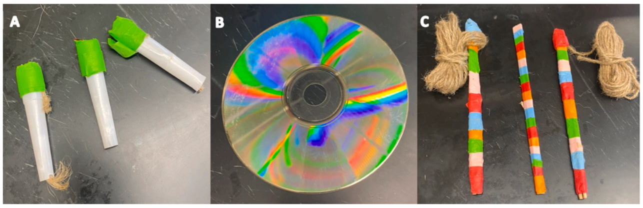

Fig. 2. Objects used for novel object testing A) Kazoo, B) CD, and C) Stick wrapped in Multicolor tape. The order of presentation for the objects for trial 1 was B at 23 days, A at 30 days, and C at 37 days. The order for trial two was C at 23 days, B at 30 days, and A at 37 days.

differed from that usually worn by the animal caretakers.

Novel object test

A standard novel object test and two modified novel object tests, intermittent movement and continuous movement were adapted from Welfare Quality (2009). For the standard treatment, the object was stationary and placed in the center of the pen, which initiated the three-minute observation period. In both modified novel object tests, the novel object was placed in the far corner of the pen and manually moved diagonally across the floor of the pen via an attached string. To ensure consistency, observers were trained prior to testing to move the object in the same manner. For the intermittent treatment, the novel object alternated between movement and stationary phases in 30 second increments over a 3-minute observational period. In the continuous movement treatment, the novel object was slowly pulled across the floor of the pen continuously for three minutes. For all three treatments, the latency to the first approach (bird within 25 cm of the novel object), the latency to the first interaction with the novel object, and the number of birds within 25 cm of the object every thirty seconds were recorded. To maintain object novelty and avoid habituation, different novel objects were used at each age. The order in which the objects were presented was balanced across trials to prevent bias from the object used. The three novel objects were chopsticks wrapped in color tape, kazoos, and CDS (Fig. 2). The person recording the response and the one handling the novel object remained at least two feet from outside the pen. This setup was consistent across all treatments, with the same number of people outside the pen for each test, ensuring that all birds experienced the same conditions.

Statistical analysis

Data was tested for normality and transformed when necessary. The data for response to observer test was analyzed using a paired T-test (Proc TTest) in SAS 9.4. The voluntary human approach data were analyzed for the main effect of age one-way ANOVA, respectively, with poisson distribution (Proc Glimmix) in SAS 9.4. The novel object data was analyzed with repeated measures ANOVA (Proc Mixed) in SAS 9.4 to determine the differences across the three ages. Means were separated *via* the Tukey-Kramer method. Proc Corr was used to correlate the results of the different novel object treatments with the voluntary human approach results. Statistical significance was defined as $P \leq 0.05$.

Results

Response to observer

There was a difference in the fear response as measured by response to observer tests before and after the birds were exposed to the voluntary human approach and novel object tests (Fig. 3). With the response to observer test before, 66% of birds moved away from the observer, and 42% of birds moved away after they were exposed to the different fear tests (t value = 12.85, $P<0.01$). At each of the individual ages, the same effect was found with fewer birds moving away from the observer after fear test exposure compared to before fear test exposure. At 23 days of age, 60% of birds moved away from the observer before the fear tests and 50% of birds moved away after fear tests ($P<0.01$), at 30 days of age 68% of birds responded to pre-fear tests, and 43% responded post fear test ($P<0.01$), and at 37 days of age, 69% responded before fear tests and 33% responded after the fear tests ($P<0.01$).

Voluntary human approach test

Latency to approach the human was shortest at 37d (80s), then 30d (110s), and longest at 23d (162s) (Fig. 4). When looking at the number of birds around the human, throughout all time points, the least number of birds were found around the human observer at 23 days of age, and the highest was at 37 days ($P<0.01$, for all time points) (Fig. 5).

Novel object test

An interaction effect was found for latency to interact with the novel object (Table 1). The longest latency to interaction was found for continuous movement at 23 days of age (139s), then intermediate movement at 23 days of age (113s). Whilst the shortest latency to interaction was found with intermediate movement and then continuous movement at 37 days of age (4 and 12s, respectively).

The movement treatment impacted both the latency to approach and interact with the novel object. The shortest latency to interaction was found for the stationary object (36s), followed by the intermediate movement object (55s), and the longest for the continuous movement object (75s) (Table 1). The latency to approach was shorter for the stationary object (10s) than either intermittent or continuous movement (15 and 24s, respectively). The number of birds approaching the novel object was the highest for the stationary object, at 30, 90, 120, 150, and 180s after the object was placed (Fig. 6).

Both the latency to approach and interact with the novel decreased

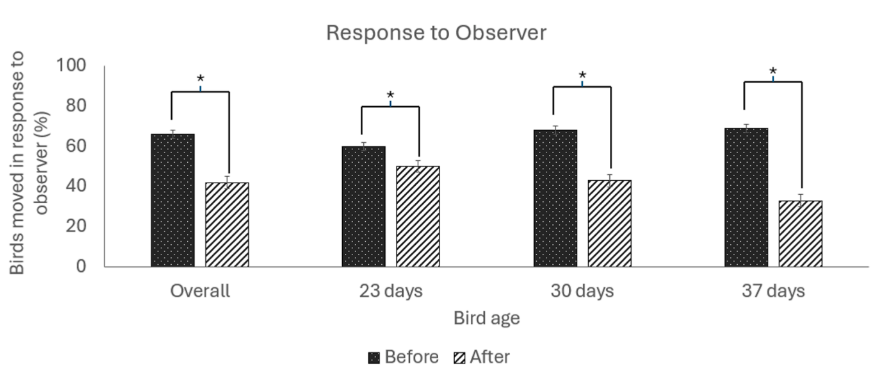

Fig. 3. The percentage of birds that moved away from the observer overall and at 23, 30, and 37 days of age.

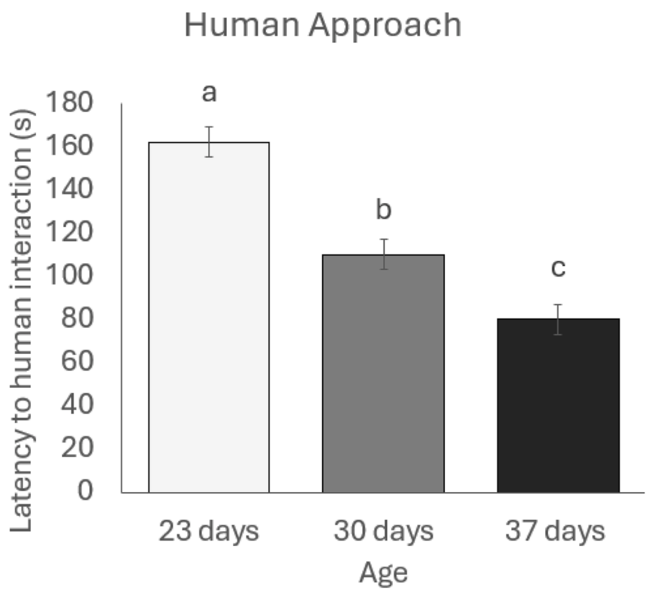

Fig. 4. The time taken for broilers to interact with the human in the voluntary human approach test at 23, 30 and 37 days of age.

with age (Table 1). The longest latency to interaction was found on 23d (105s), then 30d (49s), and finally at 37 days (12s). Similarly, the longest latency to approach was found at 23d (29s), then 30d (14s) and 37d (6s). The number of birds approaching the novel object was lowest on 23d at all time points (Fig. 7).

Novel object and voluntary approach correlation

A significant moderate correlation was found for latency to voluntary human approach interaction and latency to novel object approach and interaction for continuous movement (Table 2). For the two other treatments, intermittent and stationary, low correlations were found. When correlating the number of birds approaching the object to the number of birds approaching the human observer at different time points. The intermittent movement treatment had the highest correlation between the number of birds approaching the object and approaching the human at 30s (moderate correlation 0.54), at 60s (moderate correlation 0.60), and at 180s (moderate correlation 0.42). Continuous movement treatment had the highest correlation with the human approach in the 90s (moderate correlation 0.53) and 150s (0.52). The stationary novel object had the highest correlation with the human approach at 120s (moderate correlation 0.44). No correlation was found between the stationary novel object treatment results, and the human approach results at 90 and 180s. All the other correlations were moderate or low.

Discussion

The performance of multiple fear tests on one day impacts the bird's response to the observer. At all ages, a greater percentage of birds moved away from the responder in the first test, which occurred before any other test took place, and then after the final test, after all the fear tests had been performed. This suggests that when birds are exposed to multiple fear tests on the same day, their response diminishes over time. This decrease in response could either be because the birds are habituated to humans, their activity, and the fear testing. Therefore, they are less fearful and show less of a fear response (Jones, 1996). Repeated testing may have induced fatigue, despite intervals allowing the bird to rest. This fatigue likely reduced the bird response to fear stimuli, as increased fatigue makes it more energy-intensive for birds to react. Consequently, a stronger fear stimulus may be necessary to observe behavioral changes.

Repeated exposure to multiple successive fear tests seems to reduce the fear response, indicating an increase in fearlessness in broilers. This was seen both for the voluntary human approach and the novel object test. With the latency to the first interaction becoming shorter and more birds interacting with the human or object, as the weeks went on. The response to the observer also showed a similar pattern within the post-test test results. The number of birds moving away from the observer in the post-test decreased from 50% in the first week to 43% in the second week and 33% in the final week. Indicating that as the weeks progressed, birds were less fearful. The pre-test response stayed relatively consistent throughout the weeks, ranging from 60% in the first week to 69% in the last week. Erasmus and Swanson (2014), and Miller et al., (2005) had previously seen that repeated exposure to humans in consecutive fear tests also reduced fearfulness in turkeys and quails. The reduction in bird response to tests when exposed to the test multiple times could be indicative that fear reduces with age, which has been suggested by Rasmussen et al. (2022). However, this decrease in response, particularly with the novel object test, could indicate that the birds are habituating to the test situation (Jones, 1988; Forkman et al., 2007). Although the object used in the test was always novel, the test situation itself was not. Each time, a person would come and place a novel object into the pen (in the moving treatments, the object would then start moving). So, it is possible that they became habituated to the test situation and thus were less fearful of the object. Notably, on day 30, the birds started to use the objects for worm-running, a type of play (Cloutier et al., 2004); this behavior became more prominent on day 37. The presence of a play response to the novel object confirms a reduction in fear response, as the birds would not play with an object that elicits fear.

Overall, the addition of movement to the novel object increased the fear response. Fewer birds interacted with the moving novel objects, and

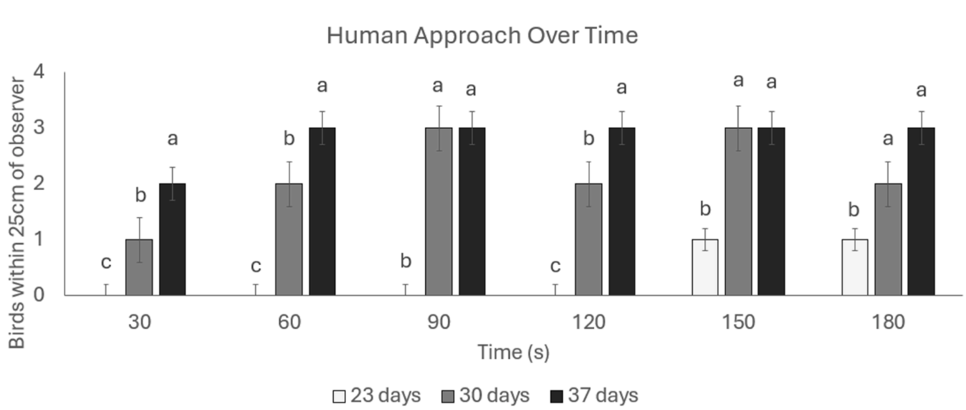

Fig. 5. The number of broilers within 25 cm of the human observer at 30, 60, 90, 120, 150, 180s during the voluntary human approach test on day 23, 30 and 37.

Table 1
The effect of bird age and the addition of movement to novel object test on the latencies of broilers to interact and approach the novel object.

	Latency to interaction with object (s)	Latency to approach of object (s)
Treatment		
Stationary	36[b]	10[b]
Intermittent	55[ab]	15[ab]
Continuous	75[a]	24[a]
P value	<0.01	<0.01
SEM	8.6	3.5
Age		
23	105[a]	29[a]
30	49[b]	14[b]
37	12[c]	6[b]
P value	<0.01	<0.01
SEM	6.7	3.3
*Age * Treatment*		
S23	63[cd]	14
S30	25[cde]	5
S37	21[de]	10
I23	113[ab]	34
I30	49[cde]	10
I37	4[e]	2
C23	139[a]	39
C30	74[bc]	26
C37	12[de]	6
P value	<0.01	NS
SEM	5.2	2.2

it took them longer to approach the moving object. When looking at the responses over time, the birds initially took longer to interact with the moving novel objects compared to stationary ones, this pattern changed as they aged. By day 37, the latency to interact with the novel object was shortest for the moving objects. This change in latency to interact with age aligns with how the play behavior involving the object developed. The play behavior started at day 30 days, and its performance increased substantially on day 37. Newberry (1995) found that moving stimuli stimulate more play than stationary stimuli. The authors hypothesize that with the initial test at 23 days, the addition of movement to the

Table 2
The correlation between the human approach (HA) and novel object (NO) results for the different novel object movement treatment

	Novel object (NO) movement treatment		
Variables Correlated	Stationary	Intermittent	Continuous
NO at 30s v HA* at 30s**	0.34	0.54	0.43
	0.02	<.01	<.01
NO at 60s v HA at 60s	0.40	0.60	0.52
	0.01	<.01	<.01
NO at 90s v HA at 90s	0.21	0.44	0.53
	0.16	<.01	<.01
NO at 120s v HA at 120s	0.44	0.39	0.42
	<.01	<.01	<.01
NO at 150s v HA at 150s	0.31	0.43	0.52
	0.04	<.01	<.01
NO at 180s v HA at 180s	0.23	0.42	0.39
	0.11	<.01	<.01
NO Interaction v HA Interaction	0.33	0.37	0.43
	0.02	0.01	<.01
NO Approach v HA Interaction	0.32	0.31	0.42
	0.02	0.03	<.01

* NO = Novel object HA = Voluntary human approach
** Number of birds within 25cm novel object at 30s v number of birds within 25cm human at 30s

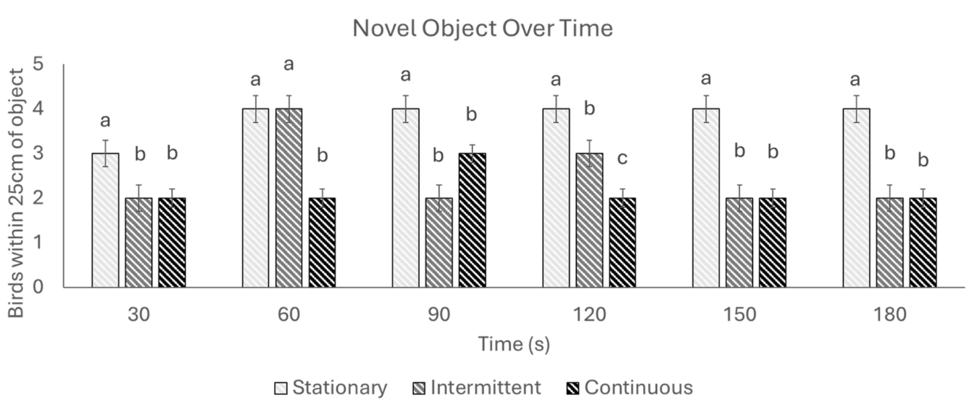

Fig. 6. The number of broilers within 25 cm of the novel object at 30, 60, 90, 120, 150 and 180s, when the object was stationary, moving intermittently or moving continuously during the novel object test.

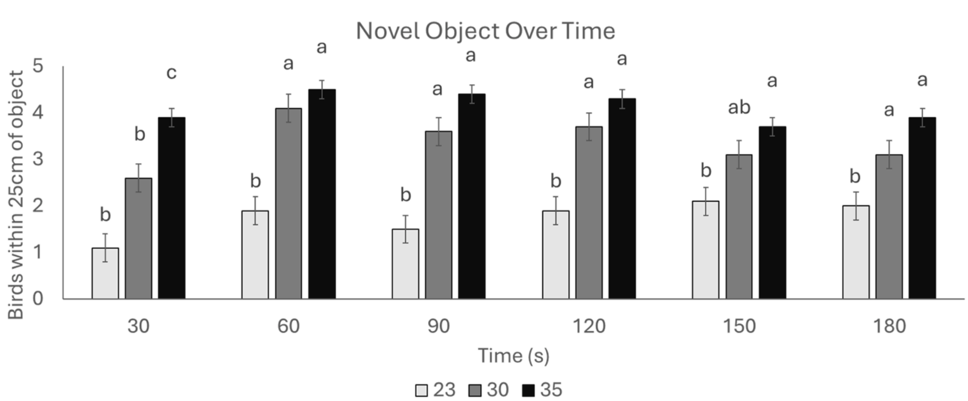

Fig. 7. The number of broilers within 25 cm of the human observer at 30, 60, 90, 120, 150, 180s during the novel object test on day 23, 30 and 37.

novel object test increased neophobia. However, by the second exposure to the test on day 30, some birds were accustomed to the testing situation and were no longer fearful of the testing situation despite a novel object being used, and instead of the movement stimulating a fear response, it stimulated a play response. This theory was also postulated in the review by Forkman et al. (2007) and is supported by findings from studies conducted by Jones, (1988), Erasmus et al. (2014) and Miller et al. (2005), which examined the effect and validity of repeated measurements of fear in poultry. With the third exposure, at 37 days, this pattern continued with more birds responding to objects with a play response than a fear response. This also suggests that the whole testing situation, rather than the specific novel object itself, may be what causes the fear response. This is important to acknowledge, as it may mean that when repeating the novel object test with a different object, the bird may not experience it as a novel situation and have a reduced fear response, potentially skewing the resulting inferences about underlying fear levels.

Although the total number of birds interacting with the novel object during movement treatments was lower, more birds within the pen could interact with the object for these treatments. The authors also observed that in treatments involving a moving novel object, birds that were not close to the novel object often interacted with the string used to move it. It was decided that these interactions should not be included in the novel object interaction count, as the string would not have been novel to the birds on days 30 and 37.

Although the different fear tests rely on different fear stimuli to elicit stimulus-specific responses, the tests all measure the same underlying, non-specific fearfulness (Jones, 1987). This is indicated by their correlation to each other (Jones, 1987). The addition of movement seems to have increased the correlation between the novel object's results and the human approach's results. The highest and strongest correlations were found for the intermittent and then continuous movement treatments. This may indicate that the addition of movement improves the novel object test's ability to measure the underlying, non-specific fear response. However, no single novel object treatment was more highly correlated with the results of the voluntary human approach than the others. It is important to note that correlations should not be over-interpreted and that a correlation between these results may not be biologically significant. Further research is needed to clarify how adding movement to fear tests can help interpret the underlying fear response.

It has been suggested that different objects may be perceived differently and thus elicit different fear responses (Erasmus and Swanson, 2014; Rasmussen et al., 2022), and that this may make it hard to differentiate whether the changes in the fear response are due to age, repeated exposure, or if they are due to one object eliciting more fear than another. To ensure object novelty, and prevent this impacting the results, the order in which the objects were presented to the birds was changed for each trial. Analysis was performed for the effect of the object used, and order of presentation, and no effect was found (P=0.24). Indicating that the object used did not impact the fear response. This may be because the novel object tests measure the fearfulness of the test situation in its entirety, rather than solely the novel object used.

Previous research has highlighted that broiler leg health and their ability to walk can have a large impact on fear tests (Bassler et al., 2013; Rassmussen et al., 2022). In fear tests like the voluntary human approach, novel object, and response to the observer, the birds need to actively approach or withdraw from the stimuli. Walking impairments may prevent chickens from being able to respond to the stimuli as desired (Vasdal et al., 2018), and may lead to misinterpretation of fear level. In the current study, we do not believe that walking ability had a major impact on the fear test results, as the number of birds that chose to approach the human and the novel object increased with age. If the birds were lame and unable to move, there would have been a decrease in the number of birds approaching the observer and the object. Gait scores, a measure of bird, walkability can influence fear test outcomes (Vasdal et al., 2018). In a study using the touch test, birds with higher scores were unable to avoid the fear stimuli in a touch test (Vasdal et al., 2018). The bird's walking ability is an important consideration when choosing and interpreting fear tests in broilers, especially at older ages, as it may influence the accuracy of results.

Conclusion

To conclude, when broilers are repeatedly exposed to fear tests their fear response decreases. This occurs both when tests are repeated multiple times throughout the flock's duration and when multiple different fear tests are repeated on the same day. The addition of movement to the novel object test resulted in an increased fear response on the day that the birds were exposed to the test. However, when the test was repeated, the birds were less fearful of the novel objects with movement than the stationary novel objects. Considering the potential impact of repeated exposure to fear tests on the fear response is crucial to consider this when designing experiments to evaluate bird welfare. Repeated testing can alter bird behavior, leading to habituation and affecting the validity of the experimental results. Interpretation of these results may lead to incorrect conclusions that could negatively impact bird welfare. Therefore, it is essential to design testing procedures that ensure an accurate measurement of bird welfare.

Disclosures

The authors of this manuscript declare no conflicts of interest.

References

Bassler, A.W., Arnould, C., Butterworth, A., Colin, L., De Jong, I.C., Ferrante, V., Ferrari, P., Haslam, S., Wemelsfelder, F., Blokhuis, H.J., 2013. Potential risk factors associated with contact dermatitis, lameness, negative emotional state, and fear of humans in broiler chicken flocks. Poult. Sci. 92, 2811–2826.

Campbell, A.M., Johnson, A.M., Persia, M.E., Jacobs, L., 2022. Effects of Housing System on Anxiety, Chronic Stress, Fear, and Immune Function in Bovan Brown Laying Hens. Animals 12, 1803.

Cloutier, S., Newberry, R.C., Honda, K., 2004. Comparison of social ranks based on worm-running and aggressive behaviour in young domestic fowl. Behav. Proc. 65, 79–86.

Cransberg, P.H., Hemsworth, P.H., Coleman, G.J., 2000. Human factors affecting the behavior and productivity of commercial broiler chickens. Br. Poult. Sci. 41, 272–279.

de Haas, E.N., Nielsen, B., Buitenhuis, A.J., Rodenburg, T.B., 2010. Selection on feather pecking affects response to novelty and foraging behavior in laying hens. Appl. Anim. Behav. Sci. 124, 90–96.

de Haas, E.N., Kemp, B., Bolhuis, J.E., Groothuis, T., Rodenburg, T.B., 2013. Fear, stress, and feather pecking in commercial white and brown laying hen parent-stock flocks and their relationships with production parameters. Poult. Sci. 92, 2259–2269.

Edgar, J.L., Omi, Y., Booth, F., Mackie, N., Richards, G., 2023. Fear, anxiety, and production in laying hens with healed keel bone fractures. Poult. Sci. 102, 102514.

Erasmus, M., Swanson, J., 2014. Temperamental turkeys: reliability of behavioral responses to four tests of fear. Appl. Anim. Behav. Sci. 157, 100–108.

Forkman, B., Boissy, A., Meunier-Salaün, M.C., Canali, E., Jones, R.B., 2007. A critical review of fear tests used on cattle, pigs, sheep, poultry, and horses. Physiol. Behav. 92, 240–374.

Franco, B.R., Shynkaruk, T., Crowe, T., Fancher, B., French, N., Gillingham, S., Schwean-Lardner, K., 2022. Light color and the commercial broiler: effect on behavior, fear, and stress. Poult. Sci. 101, 102052.

Giersberg, M.F., Poolen, I., de Baere, K., Gunnink, H., van Hattum, T., van Riel, J.W., de Jong, I.C., 2020. Comparative assessment of general behavior and fear-related responses in hatchery-hatched and on-farm hatched broiler chickens. Appl. Anim. Behav. Sci. 232, 105100.

Gray, J.A., 1987. The psychology of fear and stress, 2nd Ed. Cambridge University Press, Cambridge, UK.

Hammond, A., K. Buchynski, T. Shynkaruk, J. Brown, T. Crowe, K. Schwean-Lardner. 2024. Do flickering lights impact turkey hen behavior, stress, and fear?. Poult. Sci. 103:103699.

Hemsworth, P.E., Coleman, G.J., Barnett, J.L., Jones, R.B., 1994. Behavioral responses to humans and the productivity of commercial broiler chickens. Appl. Anim. Behav. Sci. 41, 101–114.

Huth, J.C., Archer, G.S., 2015. Comparison of Two LED Light Bulbs to a Dimmable CFL and their Effects on Broiler Chicken Growth. Stress, and Fear. Poult. Sci 94, 2027–2036.

Jones, R.B., 1987. Assessment of fear in adult laying hens: correlational analysis of methods and measures. Br. Poult. Sci. 28, 319–326.

Jones, R.B., 1988. Repeatability of Fear Ranks among Adult Laying Hens. Appl. Anim. Behav. Sci. 19, 287–304.

Jones, R.B., 1996. Fear and adaptability in poultry: insights, implications, and imperatives. World's. Poult. Sci. J. 52, 131–174.
Jones, R.B., 2002. Role of comparative psychology in the development of effective environmental enrichment strategies to improve poultry welfare. Int. J. Comp. Psychol. 15, 77–106.
Manet, M.W.E., Kliphuis, S., Nordquist, R.E., Goerlich, V.C., Tuyttens, F.A.M., Rodenburg, T.B., 2023. Brown and white layer pullet hybrids show different fear responses towards humans, but what role does light during incubation play in that? Appl. Anim. Behav. Sci 267, 106056.
Miller, K.A., Garner, J.P., Mench, J.A., 2005. The test–retest reliability of four behavioural tests of fearfulness for quail: a critical evaluation. Appl. Anim. Behav. Sci 92, 113–127.
Newberry, R.C., 1995. Environmental enrichment: increasing the biological relevance of captive environments. Appl. Anim. Behav. Sci. 44, 229–243.
Nicol, C.J., 2015. Behavioral Contributions to Welfare Assessment. Pages 58-78 in The Behavioural Biology of Chickens. CABI, Wallingford, UK.
Rasmussen, S., Erasmus, M., Riber, A.B., 2022. The relationships between age, fearresponses, and walking ability of broiler chickens. Appl. Anim. Behav. Sci. 254, 105713.
Reed, H.J., Wilkins, L.J., Austin, S.D., Gregory, N.G., 1993. The effect of environmental enrichment during rearing on fear reactions and depopulation trauma in adult caged hens. Appl. Anim. Behav. Sci. 36, 39–46.
Schwean-Lardner, K., Fancher, B.I., Classen, H.L., 2012. Impact of daylength on behavioral output in commercial broilers. Appl. Anim. Behav. Sci. 137, 24–52.
Tahamtani, F.M., Riber, A.B., 2020. The effect of qualitative feed restriction in broiler breeder pullets on fear and motivation to explore. Appl. Anim. Behav. Sci. 228, 105009.
Vasdal, G., Moe, R.O., de Jong, I.C., Granquist, E.G., 2018. The relationship between measures of fear of humans and lameness in broiler chicken flocks. Animal. 12, 334–339.
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. Anim. Behav. 45, 1127–1140.
Welfare Quality, 2009. The Welfare Quality Assessment Protocol for Broiler Chickens and Laying Hens.