The relationships between age, fear responses, and walking ability of broiler chickens

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\textbf{A R T I C L E   I N F O}

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\textbf{A B S T R A C T}

Several validated fear tests rely on animals’ ability to approach or avoid a fear-provoking stimulus. However, as broiler chickens often suffer from walking impairments, inaccurate conclusions concerning their fear levels may be drawn. In the present study, the relationships between age, fear responses, and walking ability was studied in 12 fast-growing broiler chicken flocks on two conventional farms. Welfare assessments were performed when the birds were 21, 27, and 33 days old. Fear levels were assessed through two fear tests: The Stationary Person Test (SPT) and the Novel Object Test (NOT). Walking ability was assessed using the 6-point Bristol scale for gait scores. It was found that walking ability decreased with age ($P < 0.001$). Overall, the interaction between age and walking ability affected the outcome of both the SPT (SPT: $P = 0.004$) and the NOT (NOT: $P = 0.016$). The outcome of the SPT at the different ages indicated that fear levels decreased with increasing gait scores at 21 and 33 days of age, whereas the fear levels increased with increasing gait scores at 27 days of age. In comparison, the outcome of the NOT suggested that the fear response increased with increasing gait scores for ages 21 and 27. However, at age 33 days, increasing gait scores caused only a slight increase in the fear response. In conclusion, these findings suggest that the Stationary Person Test and the Novel Object Test may be influenced by poor walking ability. The results from this study suggest that walking impairment should be taken into consideration when performing the fear tests in question. The study further highlights the need to investigate other conditions, such as space limitations, that may affect the applicability of fear tests.

1. Introduction

The broiler chicken meat industry is one of the largest animal production industries in the world today (OECD/FAO, 2017). The industry seeks to meet the continuously increasing demand for broiler meat as cost-efficiently as possible, which includes genetic selection for rapid growth and a relatively high density of birds in broiler houses (Bessei, 2006). While this production strategy enables quick responses to the increasing consumer demands, the focus on cost efficiency has also resulted in substantial consequences for animal welfare (Meluzzi and Sirri, 2009).

Animal welfare can be evaluated through different criteria, such as biological functioning, naturalness, and emotional states (Fraser, 2008). Traditionally, the welfare of farm animals, such as broiler chickens, has been evaluated with an emphasis on biological functioning, e.g. the presence of walking impairment, which in broiler chickens is a condition often associated with rapid growth rate (Kestin et al., 2001).

In recent years, there has been an increased focus on animals’ emotional states in welfare assessments (Vasdal et al., 2018). Emotional states in animals cannot be measured directly (although disputed, see Demarest and Lawrence, 2001) but are usually evaluated through their physiological and behavioral components (McMillan, 2019). For example, fear can be measured through physiological measures of heart rate, and behavioral responses such as avoidance or escape behaviors (Jones et al., 1981). Fear can generally be defined as a reaction to the perception of actual danger (Forkman et al., 2007). These reactions prepare the animal through a particular pattern of adaptive behaviors to avoid or cope with threat-related stimuli (Adolphs, 2013). Although fear is considered adaptive, exaggerated fear responses may lead to unwanted consequences. In poultry, exaggerated fear responses can cause smothering or even death due to suffocation, which is both detrimental to the birds’ welfare but also constitutes an economic consequence for the farmer (Bright and Johnson, 2011).

Several tests measuring fear in poultry exist, e.g. the Avoidance...
physically move in relation to whatever potential frightening stimuli they are presented with. However, as the environment in which the birds are kept can vary tremendously, there may be several different conditions that influence their fear response, independently or in addition to the effect of the specific stimulus. For example, Gransberg et al. (2000) found a correlation between stockperson behavior and commercial broiler chickens’ fear levels, whereas Giersberg et al. (2020) found that different housing systems and age can have different effects on broiler chickens’ fear responses. Bassler et al. (2013) found that longer dark periods and increasing age decreased the broilers’ fear of humans, while Adler et al. (2020) found that partially perforated flooring systems reduced the fearfulness of the birds and that this finding was consistent with age. These studies demonstrate how the surrounding environment and management need to be taken into consideration when interpreting the results from the applied fear tests. This point becomes crucially clear in the study by Vasdal et al. (2018), who used the touch test in broiler flocks and found a relationship between the fear responses and the walking ability of the birds. They found that flocks with poor walking ability had lower scores for fearfulness; thus, high gait scores were associated with less withdrawal from humans. Therefore, they argued that poor walking ability may have influenced the outcome of the fear test as birds might not have been physically able to walk away to avoid the human, which is a central premise of the touch test. As poor walking ability is a common welfare problem of broilers, the influence of impaired walking ability might generally influence the outcome of those fear tests that rely on the birds’ ability to walk, thereby confounding walking ability and fearfulness.

In the present study, we aimed to further investigate the conditions that affect the outcome of two validated fear tests: the Stationary Person Test (SPT) and the Novel Object Test (NOT). Specifically, we investigated the relationships between age, fear responses, and walking ability in 12 conventional broiler chicken flocks. If walking ability influences the results of fear tests that rely on the animals’ ability to walk, we expected that the birds’ fear responses would decrease with increasing age and decreasing levels of walking ability. This expectation is built on the assumption that walking ability decreases with age, which has been consistently demonstrated in previous studies (Bailie et al., 2013; Bassler et al., 2013; Kestin et al., 2001; Knowles et al., 2008; Weeks et al., 2000).

2. Material and methods

2.1. Animals and housing

The study was conducted on two Danish conventional broiler chicken farms between September 2020 and June 2021. Only one broiler house, always the same, per farm was included. We studied 6 consecutive flocks on each of the two farms, i.e. 12 flocks in total. All flocks consisted of the fast-growing Ross 308 hybrid.

The broiler flocks arrived at the farms as day-old chicks. For each of the six flocks/farms, approx. 37,700 chicks were placed in the 100 m x 20 m broiler house at Farm 1, and 33,700 chicks were placed in the 109 m x 16 m broiler house at Farm 2 at the beginning of each production cycle. Both farms provided artificial light throughout the production cycle following EU regulations (Council Directive 2007/43/EC). Additionally, both broiler houses had small windows allowing for some natural light. Feeder and drinker space, feed provided, vaccinations, temperature, and humidity levels followed industry standards. No environmental enrichment was provided. The litter material consisted of wood shavings. None of the two farms practiced thinning of the flock. The target stocking density was on average 40 kg/m² at slaughter but the actual stocking density varied from 36.9 kg/m² to 42.7 kg/m² between the flocks. The birds were slaughtered between 34 and 36 days of age.

2.2. Data collection

Each flock was visited three times when the broilers were approx. 21, 27, and 33 days old. During the farm visits, assessments of fear levels (SPT, NOT) and walking ability (gait scoring) were performed. The order of the assessment of the different welfare indicators was always the same, with the SPT followed by the NOT performed when first entering the barn. After the completion of the fear tests, the gait score evaluation was performed. No changes to the light intensity were done before or during the fear tests, whereas fencing off broilers for the gait scoring was done under dimmed light conditions (see 2.3.3.). All assessments were performed by two observers per farm visit, but four observers participated in the data collection throughout the study. Before the data collection began, all observers were trained to gait score according to the Bristol scale (Kestin et al., 1992) through video material and on live animals. The observers were also trained in the performance of the two fear tests on live animals before the data collection started. During each farm visit, the observers wore dark blue overalls, black rubber boots, and facemasks.

2.3. Sampling procedures

2.3.1. Stationary person test

The Stationary Person Test (Brantsæter et al., 2017) was performed in 10 pre-determined locations evenly distributed throughout the house. The observers divided the 10 locations evenly between them such that they went to five locations each. Once observers reached a location, they stood still and counted the number of chickens within a 1-m distance every 20 s for 2 min, aided by a stopwatch. To measure the number of birds as accurately as possible, the observer always stood in the middle of a transect framed by a water and feeding line, and only counted the number of chickens within 1 m in front of them. Thus, no birds on the opposite side of the water and feeding line, relative to the observer, were counted.

2.3.2. Novel object test

The Novel Object Test (Welfare Quality, 2009) was performed in the same 10 pre-determined locations as the Stationary Person Test. Thus, after an observer had ended an SPT, the observer would immediately perform a NOT at the same location. The NOT was done by putting a novel object (in this case a colorful volleyball) down on the ground. The observer would then walk approx. 3 m away and begin to count the number of chickens within a 0.5-m distance from the ball every 10 s for 2 min.

2.3.3. Gait score

After the two fear tests, approx. 120 birds from six different pre-determined locations were gait scored using the Bristol scale (Kestin et al., 1992). At each location, approx. 20 birds were carefully fenced in using a mobile catching pen. Before the birds were fenced in, the light intensity in the barn was dimmed to avoid panic in the flock. After the birds were fenced in, the light intensity was changed back to normal. Hereafter, one of the observers would encourage each bird to walk either inside or outside of the catching pen until all birds had been gait scored. At age 21 and 27 days, when the chickens were less mobile and taking up too much space to walk properly inside the catching pen, they were let out of the pen during the gait scoring. The two observers scored the gait of each bird based on the following criteria given by Kestin et al. (1992): 0 = the bird walked normally with no detectable abnormality, 1 = the bird had a...
slight defect which was difficult to define precisely, \(2 = \text{the bird had a definite and identifiable defect in its gait, but the lesion did not hinder it from moving,} \) \(3 = \text{identifiable abnormality, e.g. in the form of a limping, jerky or unsteady walk that hindered movement,} \) \(4 = \text{the bird had a severe gait defect and sat down at the first available opportunity, but it was still capable of walking,} \) \(5 = \text{the bird was completely incapable of walking on its feet and any movement was achieved with the assistance of the wings or by crawling on shanks.} \)

2.4. Ethics statement

The study was carried out according to the guidelines of the Danish Animal Experiments Inspectorate with respect to animal experimentation and care of animals under study.

2.5. Statistical analysis

All data analyses were performed in R (Team and R Core Team, 2018).

2.5.1. Calculation of gait score

The number of observations of each gait score for all flocks at all ages was calculated. The observations for gait scores between 3 and 5 were combined into one category because of a low number of observations for each of these gait scores. Thus, we analyzed gait scores for the following score categories: 0, 1, 2, and 3–5. To examine the influence of age on gait score, we fitted a multinomial log-linear model with gait score as the discrete response variable and age as a discrete fixed effect (Venables and Ripley, 2002). As this model does not allow for the inclusion of random effects, we included flock id as a fixed effect.

The degree of agreement among and between the four observers was determined through Fleiss Kappa (Fleiss et al., 2003).

2.5.2. Calculation of stationary person test score

A data frame was created in R containing the farm id, flock id, age, average SPT score for each location (i.e. average number of birds within 1 m from the observer during the 2-min sampling time), logarithmic average SPT score, and the average gait score for each flock at each age.

To investigate the relationship between age, gait score, and the SPT score, we formulated a GLMM with a Gamma distribution, as an inspection for normality of the residuals revealed that the residuals were not normally distributed (Bates et al., 2015). The model was formulated as follows: Log SPT score = gait score + age + gait score:age + flock id. The dependent log SPT score was included as a continuous variable, whereas all fixed effects (gait score, age, and the interaction between gait score and age) were included as discrete variables. Flock id was included as a random effect. To investigate differences between all ages, we performed pairwise comparisons with the Tukey Test which was performed on the log scale.

2.5.3. Calculation of novel object test score

We created a new data frame in R containing the farm id, flock id, age, average NOT score in each location (i.e. average number of birds within 0.5 m from the novel object within the 2-min sampling time), logarithmic average NOT score, and the average gait score for each flock at each age.

The effect of age and the interaction between gait score and age on the NOT score were investigated through the same analyses as described for the SPT. The GLMM model with a Gamma distribution was formulated as follows: Log NOT score = gait score + age + gait score:age + flock id.

3. Results

3.1. Gait score

At the commencement of the study, all four observers were found to have good levels of agreement in gait scoring between each other (Fleiss kappa = 0.63) and excellent levels of agreement with themselves across time (Fleiss kappa > 0.75).

The distribution of gait scores at the three ages can be seen in Fig. 1. At 21 and 27 days of age, gait score 0 was the most frequent score, whereas the most frequent score for age 33 was 1.

Age had a highly significant effect on the walking abilities of the birds (\(\chi^2 = 1080.30, P < 0.001\)). The odds ratios for the distribution of gait scores between different ages are provided in Table 1. Overall, the smallest difference was observed between ages 21 and 27, where the highest odds were for GS2, which was an odds ratio of 9.70 for a 27-day-old bird compared to a 21-day-old bird, meaning that broilers at 27 days of age were 9.70 times more likely to have a gait score of 2 than a gait score of 0, compared to broilers at 21 days of age.

A slightly higher difference was observed between 27-day-old birds and 33-day-old birds. Also here, the highest odds were observed for GS2. Thus, between the two ages, the highest odds for a change in gait score were observed for GS2.

The absolute largest difference was observed between ages 21 and 33. Again, the highest difference in odds was for GS2 where the odds of having this gait score increased dramatically between a 33-day-old bird compared to a 21-day-old bird.

3.2. Stationary person test

The average number of birds within 1 m of the SPT, performed at the three different ages, are provided in Table 2. When investigating the influence of gait score on the fear response to the stationary person, an interaction between age and gait score was found to affect the outcome of the SPT (\(\chi^2 = 11.134, P = 0.004\)). The estimated relationship from our model between SPT score, age, and gait score is depicted in Fig. 2. Here it can be seen how the outcome of the SPT increased with increasing gait scores for age 21 and increased slightly for age 33. Thus, the fear response decreased with increasing gait scores, i.e. more birds were found within 1 m of the stationary person. However, for age 27 the opposite was true, as higher gait scores resulted in a lower score in the SPT and, thereby, a higher fear response.

3.3. Novel object test

The average number of birds within 0.5 m of the NOT, performed at the three different ages, are provided in Table 3. Overall, the interaction between age and gait score had a significant effect on the outcome of the NOT (\(\chi^2 = 8.2954, P = 0.016\)). The relationship between NOT score, age, and gait score is depicted in Fig. 3. Here it can be seen how the number of birds in proximity to the novel object decreased (i.e. the fear response increased) with increasing gait scores for ages 21 and 27. However, for age 33, the outcome of the fear tests was almost constant, with increasing gait scores causing only a slight increase in the fear response.

4. Discussion

The objective of the present study was to investigate the effects of age and walking ability on fear responses, measured with the Stationary Person Test and the Novel Object Test, in broiler chickens. We found a relationship between the birds’ walking abilities and fear responses, as decreased locomotor abilities led to an overall decrease in fear responses when measured with the SPT but an increase in fear responses when measured with the NOT.
We found that the birds’ walking ability decreased with increasing age, which agrees with our expectation and the results of several previous studies (Bailie et al., 2013; Bassler et al., 2013; Kestin et al., 2001; Knowles et al., 2008; Weeks et al., 2000). Such decrease in walking ability with age has previously been linked to an increase in live weight and stocking density (Kestin et al., 2001; Sanotra et al., 2003; Sorensen et al., 2000; Thomas et al., 2004). Previous research has also demonstrated other factors which affect broiler walking ability, such as diet, sex, and lighting schedules (Brickett et al., 2007; Sanotra et al., 2002; Sun et al., 2013). However, as our flocks were all mixed-sex groups with similar diets and lighting schedules, these conditions do not seem likely explanations for the observed differences in walking ability. Although the birds’ walking ability decreased with age, we observed lower gait scores in our study compared to other recent studies investigating walking ability in broiler chickens using the same method (Granquist et al., 2019; Kittelsen et al., 2017; Vasdal et al., 2018). We cannot know for certain why this difference exists but one explanation might be possible country specific differences in feeding and management procedures.

Previous research has found conflicting results regarding the relationship between walking ability and fear levels in broiler chickens (Vasdal et al., 2018; Vestergaard and Sanotra, 1999); however, results may depend on the type of fear test used. Vestergaard and Sanotra (1999) found that higher scores for walking impairment increased fear levels, as measured by tonic immobility (TI), in broiler chickens. They argued that the increased fear level in lame birds was unlikely to be directly due to physical difficulties, as TI was recorded to end at the bird’s first attempt to get up, i.e. lame birds appeared to be more fearful in general. For the outcome of the NOT in our study, we observed similar results as those reported by Vestergaard and Sanotra (1999) as increasing gait scores resulted in increased fear levels. In contrast, Vasdal et al. (2018) found that lameness was associated with decreased fear levels, as measured by the touch test (TT), in broiler chickens. The opposite effects presented here, concerning the effect of walking impairment on fear levels, may be explained by the differences between the fear tests used. The TI test, used by Vestergaard and Sanotra (1999), attempts to mimic the capture of a predator and is one of the few fear tests that is supposed to be independent of the birds’ walking ability. The test builds on the assumption that the birds will show “death feigning” to be able to escape when the “predator” loses its grip (Forkman et al., 2007). Therefore, the result that impaired walking ability leads to increased fear responses may be caused by an indirect effect of walking ability because of the combination of a physical inability to get up and the potential panic the birds may experience due to such physical inabilities. Thus, when a bird is restrained and restricted in its’ ability to escape (e.g. due to walking impairments), this may result in increased fear levels. Unlike the TI test, the NOT, which measures the fear of novelty, does directly depend on the birds’ walking abilities as it depends on their ability to approach a novel object. Our results stating that impaired walking ability was associated with increased fear responses may therefore be explained more directly by poor walking ability.

Table 1
The odds ratios of the different gait scores between all ages. GS0 is used as reference. The confidence intervals are provided in parentheses. df = 3 for all odds ratios.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>GS1</th>
<th>GS2</th>
<th>GS3-5</th>
<th>LRT</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 / 27</td>
<td>2.46 (2.07–2.93)</td>
<td>9.70 (5.35–17.57)</td>
<td>2.37 (1.07–5.26)</td>
<td>168.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>21 / 33</td>
<td>11.74 (9.61–14.33)</td>
<td>102.63 (57.54–183.07)</td>
<td>14.24 (6.82–29.70)</td>
<td>1048.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>27 / 33</td>
<td>4.76 (3.95–5.75)</td>
<td>10.58 (7.91–14.15)</td>
<td>6.01 (3.21–11.24)</td>
<td>425.1</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Table 2
Average number of birds (raw data) within 1 m of the observer during the stationary person test (SPT) at 21, 27, and 33 days of age.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Number of birds</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>5.48</td>
<td>5.60</td>
</tr>
<tr>
<td>27</td>
<td>16.65</td>
<td>9.56</td>
</tr>
<tr>
<td>33</td>
<td>17.26</td>
<td>9.10</td>
</tr>
</tbody>
</table>

Fig. 1. The proportion of birds (raw data) given the different gait scores for each of the three ages.
TT, which measures the fear of humans, used by Vasdal (2018) also depends on walking ability, but, unlike the NOT, this test relies more on the birds’ ability to avoid rather than approach. The difference between approach and avoidance may explain the opposite effects that walking ability seems to have on fear responses in this case. If impaired walking ability makes it difficult for broilers to approach the novel object, less effort may be expressed by the broiler to reach the NO (i.e. broilers do not actively need to avoid a fear-inducing situation whether they are physically able to or not), which would be interpreted as an increased fear response of the NOT. On the other hand, if impaired walking ability makes it difficult for broilers to avoid the person in the TT, this will result in an interpreted decreased fear response according to the TT as broilers need to actively avoid the person but cannot due to physical impairment.

Similar to Vasdal et al. (2018), we also found that increasing gait scores overall led to decreased fear levels when measured with the SPT. Like the TT, the SPT aims to measure the fear of humans and is

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Number of birds</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>0.89</td>
<td>1.62</td>
</tr>
<tr>
<td>27</td>
<td>4.22</td>
<td>4.01</td>
</tr>
<tr>
<td>33</td>
<td>1.92</td>
<td>1.87</td>
</tr>
</tbody>
</table>

Fig. 2. The interaction between the log-transformed SPT score and gait score for each age.

Fig. 3. The interaction between the log-transformed NOT scores and gait score for each age.
dependent on the birds’ walking abilities. Our result stating that decreasing fear responses were observed for both ages 21 and 33 days was in contrast to age 27 days where fear responses increased with increasing gait scores. However, as gait scores rarely exceeded GS1 for age 21 days, these results might not be completely reliable or relevant when discussing the effect of gait scores on fear levels. Thus, in the following, we will focus on discussing the difference in the effect of increasing gait scores between ages 27 and 33 days. The contrasting results between ages 27 and 33 days may be explained based on the birds’ immediate placement in relation to the stationary person at the start of the SPT. At age 27, there were usually not many birds present in proximity to the observer (within 1 m) at the commencement of the test. Higher gait scores may therefore have prevented the birds from approaching the stationary person, categorizing them as more fearful. However, for age 33, there were often birds present in proximity to the observer at the start of the fear test, probably due to the increase in stocking density with age, leaving limited space to keep a distance from an approaching person. Increasing gait scores may therefore have made it difficult for the birds to walk away from the stationary person, categorizing them as less fearful. This apparent effect of impaired walking ability on the result of the SPT is leaning against the point made by Vasdal et al. (2018): An impact of walking ability on measured fear response may lead to incorrect conclusions regarding birds’ fear responses, since lame birds are simply not capable of showing behaviors (e.g. avoiding or approaching) that would be interpreted as a fear response. However, for the walking ability to have such an effect, we must assume that the walking ability of the birds has reached a level where gait has deteriorated to such an extent that it affects the birds’ ability to move around, thus from GS3 and onwards (Kestin et al., 1992). As these gait scores only accounted for a very small proportion of the birds in our study, this does not seem to be a likely explanation for our results. This suggests that the change in fear response that we observed with age may be due to other causes.

It might be that the behavioral response that we interpret as fear behavior, i.e. either approach or avoidance behavior, is influenced by a motivation to explore rather than a lack of fear (Forkman et al., 2007), and this motivation may change with age. In the present study, the birds may have developed an increasing motivation to explore from age 21 to age 27 days, which would explain the increase in the number of birds within proximity to both fear-inducing stimuli. A study by Bokkers and Koene (2004) suggests that motivation is the dominant determinative factor for walking in broilers with low body weight, whereas physical ability is the dominant determinative factor for walking in broilers with high body weight. The latter point, that physical ability affects the performance of behaviors that the birds are otherwise motivated to do, has been proposed by several other studies (Bokkers and Koene, 2003; Norring et al., 2016; Weeks et al., 2000). Therefore, our observations of the rather small change in the fear response from age 27 to age 33 days for the SPT and an increase in the fear response for NOT may be explained by the increase in walking impairment affecting the birds such that they engage less with either the stationary person or the novel object. Indeed, it has been found that although broilers with only slight gait impairments (GS2) are physically capable of walking, their performance of motivated active behaviors is negatively affected (Riber et al., 2021). Thus, rather than simply measuring a lack of fear, we may be measuring the lack of motivation to perform any locomotor behavior as the birds get heavier and experience more challenges with their locomotor abilities.

The difference in the response between SPT and NOT may also stem from the differences between the two tests. In the application of the tests, we had different criteria for when the birds were considered to be in proximity (1 m for SPT; 0.5 m for NOT) and, thereby, when they were considered fearful. Thus, the differences in applied distances potentially made it more likely for the birds to be counted as fearful in the NOT as compared to the SPT since they had to walk rather close to the novel object to not be interpreted as fearful. Furthermore, the birds may have had different levels of “habituation” to these repeated tests. Since the birds were exposed to barn staff every day, they may have become more habituated to humans in an SPT (and thereby less fearful) as animals can generalize their experiences from one person to another. The novel object used was the same at all ages, but we consider that the risk of the birds’ habituating to it was rather small because of the high number of birds in the barn and the relatively low number of birds being able to see and interact with the novel object during the tests. The decision to use the same object was based on our aim to investigate the development in fear responses with ages. Different objects might have elicited different fear responses, making it difficult to tease apart whether a potential change in fear response was due to age or due to the birds being more fearful of one object compared to another.

Finally, the decrease in the birds’ fear response with age may be caused by changes in the birds’ environment. One rather obvious consequence of the increase in age is the concurrent increase in body size and, thereby, the decrease in space availability. Thus, the overall decrease in fear responses that we observe may be influenced by the continuous decrease of available space with age, which may give the false impression that the birds are less fearful when they in reality do not have a free choice due to space limitations. The apparent increase in fear levels at age 33 in the NOT may also be explained by a lack of space availability as there is room for fewer broilers around the novel object at this age, simply because of their size. As previously mentioned, the difference in proximity criteria between the NOT and SPT may explain why a similar decrease is not observed for the SPT. Previous studies have investigated the effect of space availability on fear levels measured through the tonic immobility test (Anderson et al., 2021; Buïjs et al., 2009; Estevez, 2007; Onbasilar et al., 2008), but, to our knowledge, no studies have investigated the effect of space availability on the outcome of fear tests that relies on the birds’ ability approach or withdraw.

5. Conclusion

Our results suggest that broiler chickens’ fear responses are influenced by age and walking ability but also the type of test used. This may lead to false assumptions about fear levels in broiler flocks with walking impairments. In addition, there seem to be other possible conditions that may affect the outcome of the type of fear tests that rely on the animals’ ability to walk, e.g. motivation and space availability. Therefore, we recommend that such conditions be taken into account when performing fear tests. Future studies should look further into how motivation to explore, size of the birds, and space availability influence the outcome of fear tests to help determine what conditions should be present to ensure the applicability of a range of fear tests.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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