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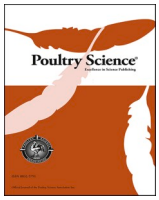

Research Note: Testing the validity of latency-to-lie tests without water for objective on-farm assessment of walking ability of broiler chickens

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ABSTRACT

The latency-to-lie (LTL) test is an objective method for assessing walking ability of broiler chickens which has traditionally consisted of placing a bird into a tub of shallow water and measuring how long it takes the standing bird to sit, with duration being negatively associated with gait score. Unfortunately, this method is impractical for use on commercial farms. Thus, the aim of this study was to assess the performance of LTL tests without the addition of water, making them more practical for use on farm. In total, 159 Ross 308 and 186 Rustic Gold broilers were assessed. Following receiving a gait score (Bristol scale), birds were placed individually into a litter lined clear plastic storage box and the LTL was conducted with a maximum test duration of 300 s. Following a 120 s period of rest, the bird was then placed on the litter near a group of flockmates, and the LTL test was repeated. This allowed for the assessment of whether containment of the bird was necessary. Latencies to lie were negatively correlated with gait score (With box: $\rho = -0.44$, $P < 0.001$; Without box: $\rho = -0.46$, $P < 0.001$). The latencies to lie (mean ± SD) when using a box were 129.0 ± 82.0, 114.0 ± 78.8, 71.9 ± 54.9, 45.8 ± 35.8, and 7.9 ± 14.0 s and without a box were 104.0 ± 97.2, 52.2 ± 53.8, 27.9 ± 29.9, 22.8 ± 27.0, and 14.0 ± 19.4 s for gait scores 0, 1, 2, 3, and 4, respectively. There was no effect of hybrid on the performance of either of the tests. Results suggest that the LTL tests without water could serve as an objective and valid measure of walking ability on farm, with the LTL test with a box showing better ability at distinguishing between specific gait scores compared to the LTL test without a box, though possible impacts of fatigue due to study design should be considered.

Introduction

Poor walking ability in broilers presents welfare and production efficiency challenges to the poultry industry. Birds with poor walking ability may struggle to reach or compete for necessary resources like feed and water (EFSA AHAW Panel, 2023), leading to slower growth rates and reduced slaughter weights (Gocsik et al., 2014). Birds may be hindered in their ability to perform behaviours such as dustbathing, foraging, walking, and preening, leading to frustration and poor health outcomes (EFSA AHAW Panel, 2023). Those with poor walking ability spend more time in a sitting or lying position, placing them at heightened risk for developing contact dermatitis if litter quality is poor (EFSA AHAW Panel, 2023). Additionally, evidence suggests that poor walking ability may be associated with the experience of pain (e.g., Danbury et al., 2000; McGeown et al., 1999; Riber et al., 2021). Mortality (Wideman Jr et al., 2012) and condemnations at slaughter (Granquist et al., 2019) have also been linked with poor walking ability.

Walking ability can be influenced by a number of factors including genetics, nutrition, and management practices which fluctuate over time. Monitoring walking ability across different contexts and over time is important to ensure positive welfare and good production efficiency. Currently, the most widely used tool for assessing broiler walking ability is a 6-point ordinal scale where birds are visually assessed and given a score from 0, where the bird walks with no detectable abnormality, up to a score of 5, where the bird is unable to walk at all (Kestin et al., 1992). This method is regarded as the gold standard for assessing walking ability and is often used in the validation of novel or alternative assessment methods.

Challenges associated with traditional gait scoring, such as its subjective nature and required training and regular calibration, have led to

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the development of more objective assessment methods, reviewed by Wurtz et al. (2024). One such method is the "Latency to Lie (LTL)" test. The test consists of measuring the duration that birds can remain standing, with longer durations being associated with better walking ability. The initially developed test placed birds in groups or individually into a test pen or tub containing a shallow layer of water (Berg and Sanotra, 2003; Weeks et al., 2002), with the presumption that birds find sitting in water to be aversive and thus would encourage them to stay standing for as long as they could. However, this method is impractical for on-farm assessment of birds due to the equipment requirements and the need to maintain a consistent water temperature. Therefore, additional tests have been developed that do not use a layer of water (e.g., Bailie et al., 2013; Norring et al., 2019), though in these studies, direct comparisons between latencies to lie and gait scores were not made. Therefore, validation, especially under commercial farm settings, is needed to determine if the LTL test without water is a suitable alternative to traditional gait scoring. Thus, the aim of this study was to validate the use of LTL tests without water, both with and without a box to contain birds, for the assessment of walking ability of broilers on farm. A sub aim was to assess the performance of these methods on different broiler hybrids.

Materials and methods

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

Animals and housing

Four visits to two commercial broiler facilities in Denmark were conducted in May and July 2023 for data collection. In total, 159 Ross 308 (flock 1: 58, flock 2: 101) and 186 Rustic Gold (flock 1: 84, flock 2: 102) broilers were assessed a few days prior to slaughter. The flocks of Ross 308 broilers were mixed sex groups and housed under conventional conditions with a growth rate of 62-63 g/day and a stocking density of around 40 kg/m^2. The flocks of Rustic Gold broilers were mixed sex groups housed in a welfare labelled system ("Level 1") with a growth rate of 50-51 g/day and a stocking density of around 38 kg/m^2.

The breakdown of the number of birds within each gait score category per flock is presented in Table 1.

Gait scoring and LTL tests

Birds were first gait scored by a single trained observer using the six-point Bristol scale where scores range from 0 (no detectable abnormality) to 5 (unable to walk) (Kestin et al., 1992). The observer was trained using video and live observations alongside two experienced observers. Prior to the study, the observer had experience in scoring over 2000 broilers and received an intra-observer reliability score of 0.97 (Kendal's coefficient of Concordance). Birds were selected nonrandomly leading to overall counts of 29, 84, 183, 36, 13, and 0 birds with gait scores 0, 1, 2, 3, 4, and 5 respectively. Birds with a gait score of 5 were not included in this study due to welfare concerns and the fact that they do not meet the criteria for the LTL test due to their inability to stand.

Table 1
Number of birds within each gait score category which were tested during visits to two conventional flocks (Ross 308) and two Level 1 flocks (Rustic Gold).

		Gait Score				
Farm	Flock	0	1	2	3	4
Conventional	Flock 1	4	11	30	8	5
	Flock 2	14	24	54	6	3
	Total	18	35	84	14	8
Level 1	Flock 1	4	22	45	12	1
	Flock 2	7	27	54	10	4
	Total	11	49	99	22	5
All	Total	29	84	183	36	13

Three clear plastic storage boxes (smart store™, 59 × 39 × 43 cm) were used to contain the birds for the LTL test conducted with a box. The boxes were lined with approximately 3 cm of litter from the floor of the broiler house. Boxes were placed on the ground within the house near the entrance and were spaced apart from one another to avoid disturbing the birds in the neighbouring boxes. After receiving a gait score, the bird was placed individually into a box in a standing position and a timekeeper (one per box) started their stopwatch. The timekeeper stopped the stopwatch when the bird sat down (i.e., the hocks and breast came in contact with the litter). If the bird did not sit down within 300 s, the test was ended.

After completion of the test (i.e., the bird sat down or 300 s was reached) the bird was allowed to remain undisturbed in the box for a period of 120 s to rest. The bird was allowed to sit or stand during this rest period. This rest period was determined based on the duration of typical daytime resting bouts observed in broilers (Forslind et al., 2021). As birds were not forced to remain standing for durations longer than they would under normal, undisturbed conditions, we considered the duration of 120 s rest as sufficient before conducting the second LTL test, this time without the aid of a box.

Following the resting period, the timekeeper gently placed the bird on the ground near a group of flockmates. The latency to lie was determined, with the timer starting once the bird was placed on the floor and ending once the bird assumed a sitting position. The observer maintained visual contact with the bird throughout the test while remaining at a distance outside of the bird's flight zone. The reason for placing the bird near flockmates was to reduce the likelihood that the bird would walk towards the comfort of other birds during the test.

Statistical analyses

Statistics were conducted using R Statistical Software v4.3.3 (R Core Team, 2024). Spearman's rank correlation coefficients were obtained to assess the relationship between gait scores and latencies to lie (s) when using a box and when not using a box. To assess whether individual latencies to lie with or without the box were different, a Friedman test, which is a non-parametric test with repeated measures, was used. To assess if there were significant differences in latencies to lie between gait scores, a Kruskal-Wallis Rank Sum test was used. Post hoc pairwise Dunn's tests with a Benjamini-Hochberg correction for multiple tests were used to compare latencies to lie between each pair of gait scores. To assess if latencies to lie were impacted by hybrid, a Kruskal-Wallis Rank Sum test was conducted within each gait score. As very few birds remained standing for the maximum 300 s of the test (with box: $n = 7$; without box: $n = 5$), these data were included in the analysis.

Results and discussion

Validity with and without the use of a box

Latencies to lie were moderately negatively correlated with gait score (with box: $\rho = -0.44$, $P < 0.001$; without box: $\rho = -0.46$, $P < 0.001$) meaning that birds with lower (better) gait scores remained standing for longer periods of time than those with higher (worse) gait scores. Latencies to lie for each gait score category for the test conducted with and without a box are presented in Fig. 1. Latencies to lie were lower when the test was conducted without the box, compared to with the box ($\chi^2 = 127.25$, df = 1, $P < 0.001$). Other studies, such as Norring et al. (2019) and Bailie et al. (2013), have also reported shorter latencies to lie in tests conducted without water and without a box, compared to studies using water in a tub, such as done by Berg and Sanotra (2003). This could be explained by the fact that the box was a novel stimulus that put the birds into a more alert state, encouraging them to stand as long

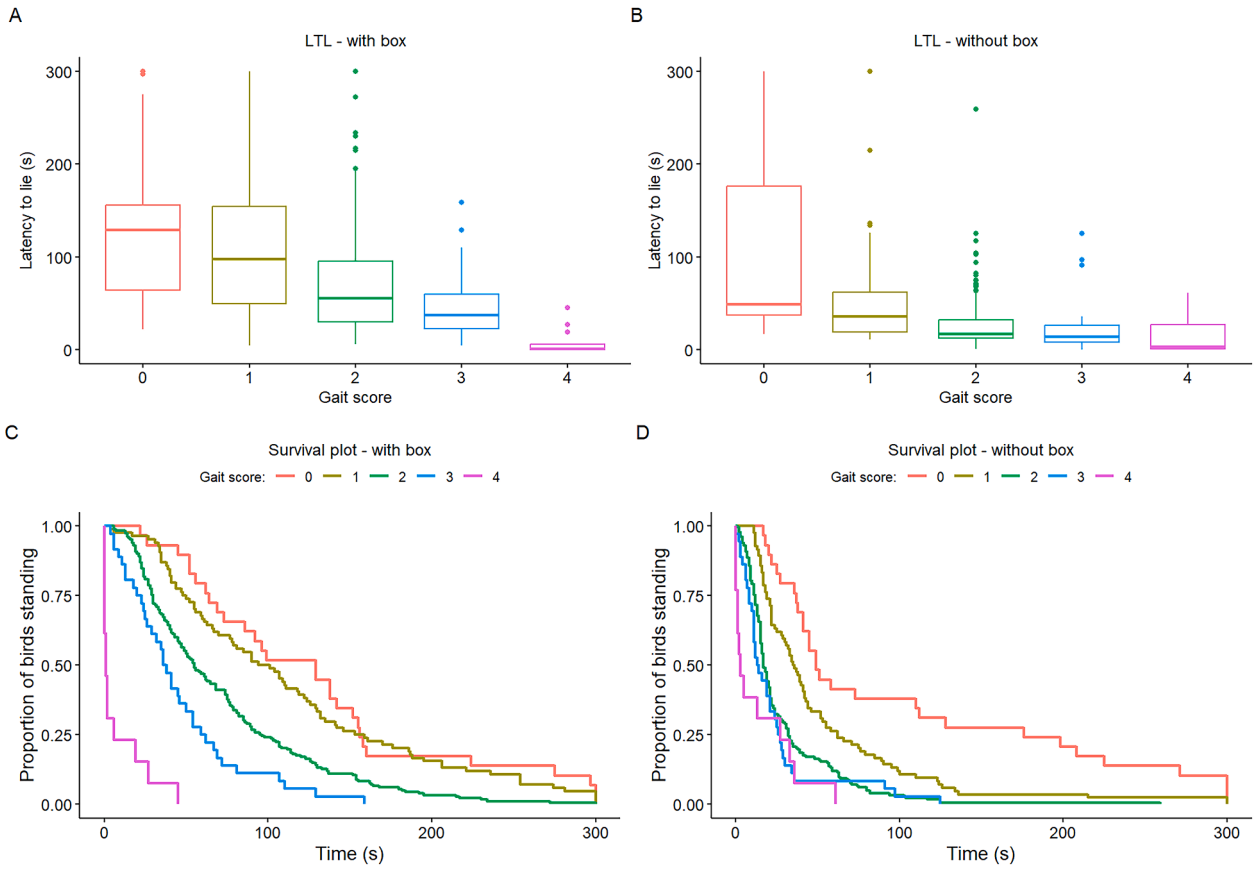

Fig. 1. Latencies to lie (s) by gait score (0 to 4) when conducting the test using a box (A) and without a box (B). Survival plots showing the proportion of birds remaining standing across the test duration (300 s) when the test was conducted with (C) and without (D) a box.

as they could before assuming a sitting position, a position that makes them more vulnerable. In the current study, when testing was conducted without a box, birds were placed near conspecifics to prevent them from walking long distances during the test. However, this may have contributed to the short latencies to lie observed as birds were more comfortable. An additional possible contributing factor to the shorter latencies to lie when testing without a box was that this test was conducted after the birds were gait scored and LTL tested with the box. This was done due to the practical constraints of working in a commercial facility, where recatching the birds after conducting the LTL test without the box would not have been possible without causing unnecessary stress and disruptions to the flock. For this reason, we incorporated a resting period to minimize potential fatigue effects.

While the Spearman's rank correlation observed in this study was not strong (i.e., $-0.8 \geq \rho > -1$), there were significant differences in latencies to lie between the various gait scores (with box: $\chi^2 = 75.35$, $P < 0.001$, df = 4; without box: $\chi^2 = 75.00$, $P < 0.001$, df = 4). Post hoc pairwise tests revealed that latencies to lie when using a box were significantly different ($P < 0.05$) between all pairs of gait scores except for between 0 and 1 ($P = 0.166$). When not using a box, significant differences ($P < 0.05$) in latencies to lie were found for all pairs of gait scores except for between 2 and 3 (tendency, $P = 0.082$), and 3 and 4 ($P = 0.111$). This demonstrates the potential for using latency to lie durations to classify birds with low or high gait scores. This is further illustrated by the survival plots presented in Fig. 1, where it is clear that birds that sat after a short duration were much more likely to have a high (poor) gait score than those that remained standing for longer periods.

Validity of the test for assessing two hybrids

Within each gait score category, there were not differences in latencies to lie between the two hybrids, except for gait score 1 when conducting the test without a box, where the Ross 308 hybrid tended to have longer latencies to lie than the Rustic Gold hybrid ($P = 0.088$).

While broiler hybrid would be confounded with management practices on each farm, the fact that no significant differences between the two commercial farms/hybrids suggests that LTL tests without water are sufficiently robust to be used on a variety of broiler hybrids and production systems. Past studies of LTL tests conducted with water also proved to be robust to farm and hybrid effects (Berg and Sanotra, 2003; Weeks et al., 2002).

Performance of the LTL test without water

Previous studies conducting LTL tests with water found clear negative correlations between latencies to lie and gait scores (Berg and Sanotra, 2003; Weeks et al., 2002). Similar relationships were observed in this study when the test was conducted without water. One notable difference between tests conducted with and without water is that the observed latencies to lie are shorter when the test is conducted without water. For example, the latencies to lie when using a box in this study without water were roughly half of those reported by Berg and Sanotra (2003) where water was used. This is beneficial in terms of time required to conduct the test, especially given that latencies to lie remain strongly correlated with gait scores as demonstrated in our study. This also suggests that water is not a necessary element to conduct robust LTL tests, making these tests much more practical to conduct.

Results from our study are promising for the use of a modified LTL test without water as an objective alternative to traditional gait scoring. This method has several advantages including not requiring extensive observer training. The test is relatively quick to conduct, and a single observer has the option of scoring multiple birds simultaneously when

boxes are used. Another advantage of LTL tests is that birds that may be experiencing pain do not have to be forced to walk, which is a necessity during most other methods used for assessing walking ability. Further, birds are not exposed to water which could be perceived as aversive or could dampen their plumage hindering thermoregulation. Additional research is needed to determine how many birds need to be tested in order to achieve a representative sample of the flock. Until this is formally assessed, we expect that the current available protocols for gait scoring would be appropriate.

Results suggest that the LTL test without water could serve as an objective and valid measure of walking ability on farm, with the test conducted with a box showing improved ability to distinguish between specific gait scores compared to the test conducted without a box. Having an objective, low tech test which requires no training could greatly facilitate assessment of walking ability and would allow for direct comparisons to be made between birds or flocks scored by different observers.

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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