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The Old School, Brewhouse Hill, Wheathampstead,
Hertfordshire AL4 8AN, UK
www.ufaw.org.uk

Animal Welfare 2014, 23: 179-187
ISSN 0962-7286
doi: 10.7120/09627286.23.2.179

Lameness is consistently better at predicting broiler chicken performance in mobility tests than other broiler characteristics

G Caplen*, B Hothersall, CJ Nicol, RMA Parker, AE Waterman-Pearson, CA Weeks and JC Murrell

School of Veterinary Science, University of Bristol, Langford House, Langford BS40 5DU, UK
* Contact for correspondence and requests for reprints: gina.caplen@bristol.ac.uk

Abstract

To determine whether lame broilers are in pain it is necessary to compare measures of lameness and mobility before and after analgesic treatment. Such measures should not be unduly affected by other bird characteristics. This study assessed the performance of lame (gait score, GS 3–4) and non-lame (GS 0–1) broilers using two mobility tests: (i) a novel test to assess broiler ability to access resources when housed in groups (Group Obstacle test); and (ii) a Latency-to-Lie (LTL) test. Outcome test measures included number of obstacle crossings, latency to cross an obstacle, and time taken to sit in shallow water. Associations between outcome test measures and other bird characteristics (established lameness risk-factors), including strain, sex, age, mass, contact dermatitis and pathology, were also investigated. The performance of high-GS and low-GS broilers differed in both mobility tests and no other bird characteristics were as consistent a predictor as lameness. This demonstrates that mobility impairments are closely related to lameness assessed using GS, and that there is a component of lameness that cannot be explained by other bird characteristics (eg being male and heavy). This component may represent pain or discomfort. Both mobility tests are suitable for further application with analgesic testing to classify lameness-associated pain in broilers.

Keywords: *animal welfare, broiler, lameness, latency-to-lie, obstacle test, pain*

Introduction

There have long been concerns about the welfare of broiler chickens, primarily relating to their leg health (Bradshaw *et al* 2002; Gentle 2011). A recent and comprehensive survey found that by the time they reach slaughter age almost 30% of intensively reared broiler chickens in the UK are, at least moderately, lame (Knowles *et al* 2008). This conclusion was reached using the widely employed Bristol six-point Gait Score (GS) system (Kestin *et al* 1992), which utilises a qualitative, and relatively simplistic, assessment of walking style; individual birds are allocated a score on a scale ranging between GS 0 (no detectable walking abnormality) and GS 5 (unable to stand). Although the system is well suited for conducting welfare assessments on-farm it provides no direct information on broiler pain, and suffers from drawbacks as a research tool; gait scoring cannot discriminate unilateral from bilateral lameness and there is little evidence to link lameness severity (as determined by GS) with internal leg pathology (McNamee *et al* 1998; Sandilands *et al* 2011; Fernandes *et al* 2012). Lameness-associated pathologies can be infectious or non-infectious, and may involve bones, joints, ligaments and tendons (Bradshaw *et al* 2002). Since gait abnormalities are likely to arise from a combination of pathological influences, and individual adaptation to regain mobility, there is inherent difficulty in inferring whether a gait pattern is due to pain or to biomechanical factors. The enhanced body mass of modern broiler strains may reduce motivation to walk (regardless of a pain component) due to increased energy expenditure associated with locomotion; however, it is also possible that poultry, as prey species, do not display overt pain-associated behaviour as this could increase predation risk (Livingston 1994). There is a distinct possibility that some pathologies (eg those associated with inflammation and necrosis) are more likely to have a pain component than others (eg mild skeletal deformity). The welfare implications of failing to differentiate pain from other causes of gait abnormality are substantial.

Quantitative measures relating to broiler lameness have been developed, but these have not been applied in such a way as to address the potential complications summarised above. Such measures include an assessment of inactivity, which revealed that low-GS broilers spent less time lying than high-GS broilers (Weeks *et al* 2000), and a measure of latency-to-lie (LTL), which established that low-GS broilers will stand for longer to avoid contact with shallow tepid water (Weeks *et al* 2002). Neither GS nor quantitative measures provide direct evidence of pain but they can be utilised in assessments that do. The provision of analgesic drugs can provide indirect evidence for pathological pain if positive changes in behaviour or improvements in pre-defined test performance are observed following treatment. McGeown *et al* (1999) provided early evidence for lameness-associated pain when they observed a reduction in time taken for high-GS broilers to complete an obstacle course following treatment with carprofen, a non-steroidal anti-inflammatory drug (NSAID). Danbury *et al* (2000) observed that high-GS broilers preferentially ate carprofen-spiked feed in a self-selection experiment (and underwent a reduction in GS); however, such findings were not replicated in a subsequent study (Siegel *et al* 2011). Caplen *et al* (2013) recently documented objective changes in gait characteristics, including increased walking velocity, in high-GS broilers administered NSAID (carprofen and meloxicam).

The correlations between quantitative measures of mobility (described above) and GS is encouraging because, ideally, the comprehensive assessment of pain incidence, type and severity in commercial broilers requires evidence to be obtained via a range of diverse measures, all demonstrating tight concordance. There is, however, a potential problem. Several bird characteristics are also associated with increased GS, including body mass, growth rate, strain, sex, and foot-pad dermatitis (Julian 1997; Vestergaard & Sanotra 1999; Kestin *et al* 2001; Sanotra *et al* 2001; Berg 2004; Knowles *et al* 2008). To assess whether quantitative measures of mobility are valid pain indicators, it is important to ascertain how they are influenced by these potentially confounding bird characteristics (lameness risk factors).

To address these requirements the current study had two main objectives. The first was to devise a simple, novel test of mobility that would assess the frequency with which broilers, housed within groups, would cross an obstacle (placed between two resources: food and water) over a 5-h period (Group Obstacle test). This was loosely based upon an obstacle course but also took into account the alterations in lame broiler activity/time budgets (increased lying, fewer feeding and drinking bouts of longer duration) reported by Weeks *et al* (2000). The extended test duration and the assessment of individuals within a group-setting was designed to maximise performance-motivation and increase the likelihood that our outcome measures reflect an inability to cross the obstacle rather than a motivational-based aversion to activity. Although previous studies investigating walking ability/mobility have only tested birds individually and within a short time-frame (eg McGeown *et al* 1999; Caplen *et al* 2012) non-test birds are often used to provide an additional social cue.

The second was to investigate how performance in two quantitative tests: (i) the Group Obstacle test; and (ii) an established index of leg weakness (the LTL test), was associated with specific broiler characteristics (including strain, sex, age, body mass, contact dermatitis and joint pathology), in addition to lameness. The experimental design would inform us as to the suitability of the tests for further application, particularly in the search for evidence relating to pain via the detection of quantifiable responses to analgesic treatment. If, for example, small female broilers performed significantly better than large male broilers in the LTL test, regardless of GS, then we could conclude that this test was a better measure of body mass than of pain; as such, it would be unsuitable for the detection of analgesic-associated performance improvement. Thermal nociceptive testing was also included within our battery of measures; this study is reported elsewhere (Hothersall *et al* 2013).

Materials and methods

Study animals

Batches of mixed sex broilers from three commercial strains were acquired from farms located within south-west England at 25–35 days of age. At selection each batch (n = 18, hereafter referred to as 'flock') comprised approximately equal numbers of non-lame (Gait Score, GS 0–1) and lame (GS 3–4) birds, assessed using the criteria of Kestin *et al* (1992). Every effort was made to avoid low-GS birds that were 'abnormally' small/light and high-GS birds that were 'abnormally' large/heavy in relation to the flock as a whole.

Housing and husbandry

Following transfer to the research facility birds were individually identified, using coloured stock marker on their back and tail feathers, and housed in pens (3.1 × 1.2 m; length × width) on wood-shavings. Animal accommodation was climate-controlled at approximately 20°C and maintained on a 16:8 h light:dark schedule. Birds had *ad libitum* access to water and commercial feed.

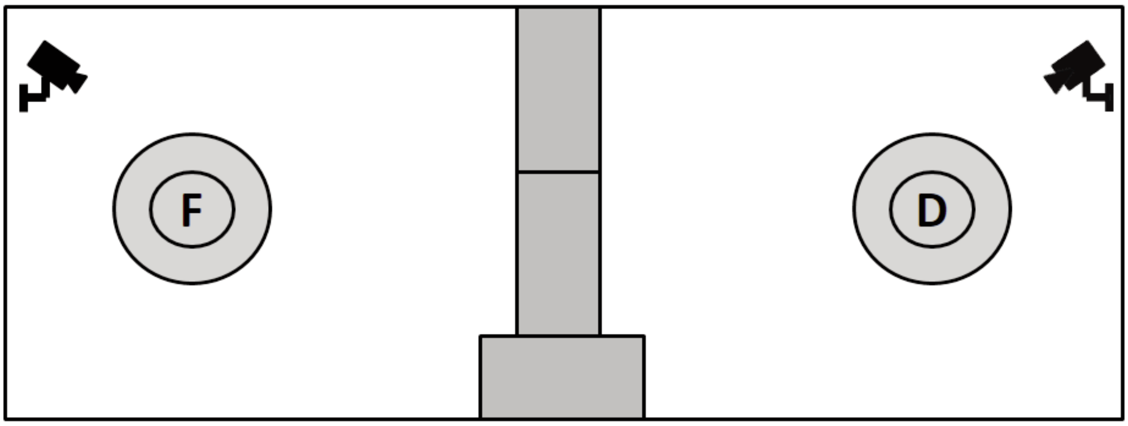
Measurement of bird characteristics

Broilers were tested within an age-range of 28–43 days ('age'). Immediately prior to testing all birds were gait scored by two experienced experimenters. Birds of GS 0–1 were allocated to the non-lame group and birds of GS 3–4 were allocated to the lame group; this formed the binary explanatory variable 'lameness'. Individuals allocated a GS outside of this range were not used for data collection. Broilers were also weighed ('mass'), and hock burn ('HB') and foot-pad dermatitis ('FPD') were assessed using a scale of 0–4, where 0 = none, 4 = severe open ulcers (Welfare Quality® 2009); final scores were based on the mean of both legs for each type of dermatitis. 'Sex' was determined retrospectively at post mortem, within three days of completion of testing, at which time hock joints were dissected and any gross pathology recorded, ie injury, infection (tissue inflammation, excessive or discoloured joint fluid), and bone deformity. Birds were awarded a binary score for presence (1) or absence (0) of internal leg 'pathology' on the basis of this visual examination. No swabs were taken so we were unable to ascertain whether a bacterial or viral infection was present.

Group Obstacle test

The Group Obstacle test was conducted in the home pen using groups of 12 individually marked birds. A barrier consisting of three aerated concrete blocks (44 × 21.5 × 10 cm; length × width × height) was placed across the middle of the pen. This created an obstacle between the feeder at one end and the drinker at the other (Figure 1). The spacing ensured that birds could not feed or

Figure 1



Typical layout of a pen used in the Group Obstacle test (not to scale) demonstrating the position of the feeder (F), drinker (D), the three-block obstacle and the two video cameras.

drink while perched on the blocks but had to step over the obstacle to gain access to the alternative resource. Birds were habituated to the presence of the blocks, positioned to allow access to both resources, within the pen for 24 h before the test. The feeder was removed from the pen for 1 h prior to starting the test, at which point the feeder was returned, all birds were manoeuvred to the end of the pen containing the drinker, and the obstacle placed in the test position. The experimenter then left the room immediately and continuous video footage was recorded using two cameras located at either end of the pen (approximately 1 m above the floor) linked directly to a PC. Video data were analysed retrospectively using The Observer® XT 10 software (Noldus, The Netherlands) to count the number of times each bird stepped up onto the obstacle with both feet within hourly intervals of a 5-h test period. Occurrences when birds did not completely traverse the obstacle but stepped up onto the block and then stepped back down to re-access the side of the pen in which they had previously been located were also included. For the analysis, each combination of a step-up and a step-down will be notionally described as a 'crossing'. The latency (s) of each bird to first perform a crossing (usually to access the feeder) was also recorded. Test groups were balanced to contain equal numbers of high-GS and low-GS birds and, where necessary, birds of GS 2 were also included to maintain group sizes (although data for these intermediate birds were not analysed). The position of the feeder and drinker on the left- and right-hand side of the pen were balanced within our experimental design.

Latency-to-Lie (LTL) test

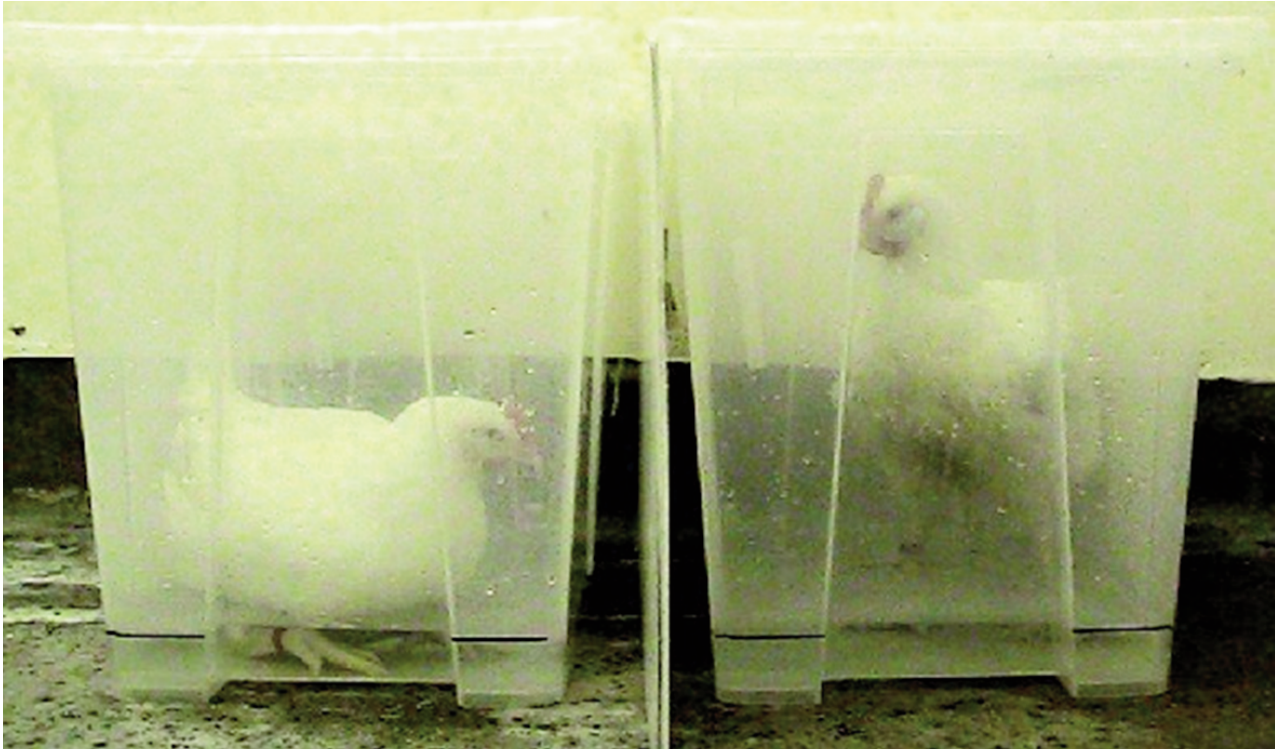
The Latency-to-Lie (LTL) test closely followed the methodology of Berg and Sanotra (2003). Briefly, each bird was removed from the home pen and placed individually into a transparent plastic box (dimensions: 57 × 39 × 42 cm; Figure 2) containing a piece of rubber matting affixed to the internal base (to provide a non-slip surface) and shallow water (30°C at a depth of 4 cm). Up to six birds were tested simultaneously in adjacent boxes in visual, but not auditory, isolation. The time at which each bird: (i) was placed standing into the water and; (ii) sat, was recorded, and the LTL calculated (as the difference between the two). As soon as a bird sat, or if it remained standing after 900 s (the cut-off period for the test), it was dried and returned to the home pen. A number of birds began to sit, appearing unaware of the water, but immediately returned to standing once their breast feathers made contact with the water surface. Such 'dips' were not considered to reflect the bird's ability to remain standing, unless three dips were made within 10 s (in which case the third dip was taken as the LTL).

The two tests (Group Obstacle and LTL) were conducted on different days. Due to variability in intra-individual GS over time (some broilers demonstrated improved walking ability, while others deteriorated) it was not possible to use exactly the same group of birds for both tests.

Statistical analysis

Descriptive statistics were generated using SPSS Version 19. Data were analysed using the multilevel modelling software MLwiN v2.25 (Rasbash *et al* 2012) to create random-intercept models to adjust for non-independence due to clustering within groups. For both tests, the random part of the model comprised two levels: 'bird' (level 1) nested within 'flock' (level 2). LTL, number of crossings, and latency-to-cross formed our response variables. To examine the effects of bird characteristics upon each outcome measure (response variable) the explanatory variables ('lameness', 'strain', 'age', 'sex', 'mass', 'HB', 'FPD', 'pathology') were entered into the relevant model as individual predictors. *Z*-tests were used to examine the significance of each, whereby the coefficient was divided by the standard error of coefficient to generate respective *Z*-values. *P*-values were calculated as the area of the Normal distribution greater than or equal to the *Z*-value, multiplied by two (two-tailed analysis).

Figure 2



Broilers undergoing the latency-to-Lie (LTL) test. The bird on the left is sitting in 4 cm warm water, demonstrating the endpoint of the test.

Additional models were then created by including all variables remaining after backwards and forwards regressions initially, and removing non-significant terms until all were significant. All other variables were then included one-by-one. The most significant model was retained and the process repeated until no more variables were significant. Interactions between predictors were explored where there was an *a priori* reason to expect a relationship to exist. The significance of complete models, and specific interactions within a model, were tested using χ^2-tests and the deviance in log-likelihood between models with and without the explanatory variable(s) or interaction. Data were transformed as necessary and standardised residuals were calculated and plotted to ensure that assumptions of normality and heteroscedasticity (homogeneity of variance) were met.

Ethical considerations

This study was carried out under Home Office Licence (PPL30/2865) and approved by the University of Bristol Ethical Review Group. The Home Office Code of Recommendations for the Housing of Poultry was met or exceeded at all times. Birds were euthanised by a pre-2013 approved Schedule One method (dislocation of the neck or barbiturate anaesthetic overdose) within three days of final data collection. Additional pre-determined humane endpoints used in this study were as follows: (i) birds that became excessively lame (> GS 4); (ii) any bird that demonstrated obvious signs of distress or sickness.

Results

Population characteristics

See Table 1 for a summary of the main characteristics (lameness risk factors) of the test populations. There were considerably fewer low-GS (than high-GS) broilers due to frequent changes in GS that occurred between selection and testing, and difficulties in finding sufficient numbers of low-GS birds on-farm that did not exhibit substantially restricted growth. In the majority of cases this 'stunting' appeared to be due to other forms of illness and, as such, these individuals were deemed unsuitable for use within this study.

Determination of whether the Group Obstacle test is a suitable novel measure of mobility

As the number of crossings per hour was very low for the high-GS birds it was not possible to transform this variable to achieve a normal distribution, so instead we report results for 'total crossings' during the 5-h test. The best model was a square-root transformation of this response variable with inclusion of 'lameness' as a single predictor (explanatory variable). 'Lameness' reduced the number of crossings ($z = -11.95$, $P < 0.001$); the back-transformed mean (with 95% CI) number of total crossings predicted by the model was 12.2 (10.7, 13.8) for low-GS birds and 3.1 (2.5, 3.8) for high-GS birds. This model explained 47.9% of the variance within the dataset and all of the remaining variance existed at the individual ('bird') level. The ability to detect pronounced differences in the number of obstacle crossings made by the two groups of birds (of disparate GS) to access resources confirmed that this test was a successful, and potentially useful, novel test of mobility.

Table 1 Characteristics of the test broiler population (with associated descriptive statistics) used to investigate two quantitative measures of leg health: the Group Obstacle test and the Latency-to-Lie (LTL) test.

Characteristic	Obstacle test			Latency-to-Lie test		
	Lame (GS 3–4)	Non-lame (GS 0–1)	Significant difference	Lame (GS 3–4)	Non-lame (GS 0–1)	Significant difference
Population size (n)	85	65		87	67	
Age (days)†	35 (± 3)	34 (± 3)	ns	35 (± 7)	35 (± 5)	ns
Mass (kg)‡	190 (± 0.04)	1.54 (± 0.03)	$t = -7.19$, $P < 0.001$	1.93 (± 0.34)	1.57 (± 0.26)	$t = -7.20$, $P < 0.001$
Sex	M: n = 63 F: n = 22	M: n = 21 F: n = 44	$\chi^2 = 30.0$, df = 1, $P < 0.001$	M: n = 64 F: n = 23	M: n = 19 F: n = 48	$\chi^2 = 30.1$, df = 1, $P < 0.001$
Hock burn, HB†§ (% with score > 0)	0.0 (± 0.3), (34.1)	0.0 (± 0.0), (18.5)	$Z = -1.98$, $P = 0.048$	0.0 (± 0.5), 46.0	0.0 (± 0.0), (17.9)	$Z = -3.61$, $P < 0.001$
Foot-pad dermatitis, FPD^{1,3} (% with score > 0)	0.5 (± 2.0), (60.0)	0.4 (± 1.0), 60.0	ns	0.5 (± 1.5), (66.7)	0.25 (1.0), (± 58.2)	$Z = -2.21$, $P = 0.027$
Pathology (%)	28.2	7.7	$Z = 3.97$, $P < 0.001$	33.3	7.5	$Z = 4.37$, $P < 0.001$
Type$^{\#}$ (%): Infection						
Deformity	29.2	80.0		27.6	80.0	
Injury	83.3	20.0		89.7	20.0	
	8.3	0.0		10.3	20.0	

[†] Median (± inter-quartile range), IQR;

[‡] Mean (± SEM);

[§] Value assigned according to a severity scale of 0–4, where 0 = none, 4 = severe open ulcers (Welfare Quality® 2009). A score of 3 was the maximum seen within our test population.

[#] Of those individuals with an identified pathology the prevalence of each pathological 'type' was also calculated. The different types were accounted for separately even if an individual had more than one (percentages may, therefore, total ≥ 100%).

Table 2 A summary of univariate models associated with each significant population characteristic ('predictor' or explanatory variable) for the three outcome measures (response variables) for comparison with the final models described within the manuscript. The percentage of the variance within the dataset explained by each significant model is denoted by '$\%_{Var}$'.

Predictor	Obstacle test: total crossings†			Obstacle test: latency to cross‡			LTL†		
	Z	*P*-value	$\%_{Var}$	*Z*	*P*-value	$\%_{Var}$	*Z*	*P*-value	$\%_{Var}$
Lameness	−11.95	< 0.001	47.9	4.33	< 0.001	9.0	−16.77	< 0.001	63.1
Strain		ns			ns			ns	
Sex	2.65	0.008	0.7		ns		4.97	< 0.001	13.3
Age		ns		−2.06	0.04	6.2		ns	
Mass	−4.82	< 0.001	13.7		ns		−4.12	< 0.001	6.2
FPD		ns			ns		−3.99	< 0.001	6.1
HB		ns		−3.81	< 0.001	18.6	−3.08	0.002	0.8
Pathology	−3.03	0.002	1.6		ns		−3.98	< 0.001	12.4

[†] Square-root transformed;

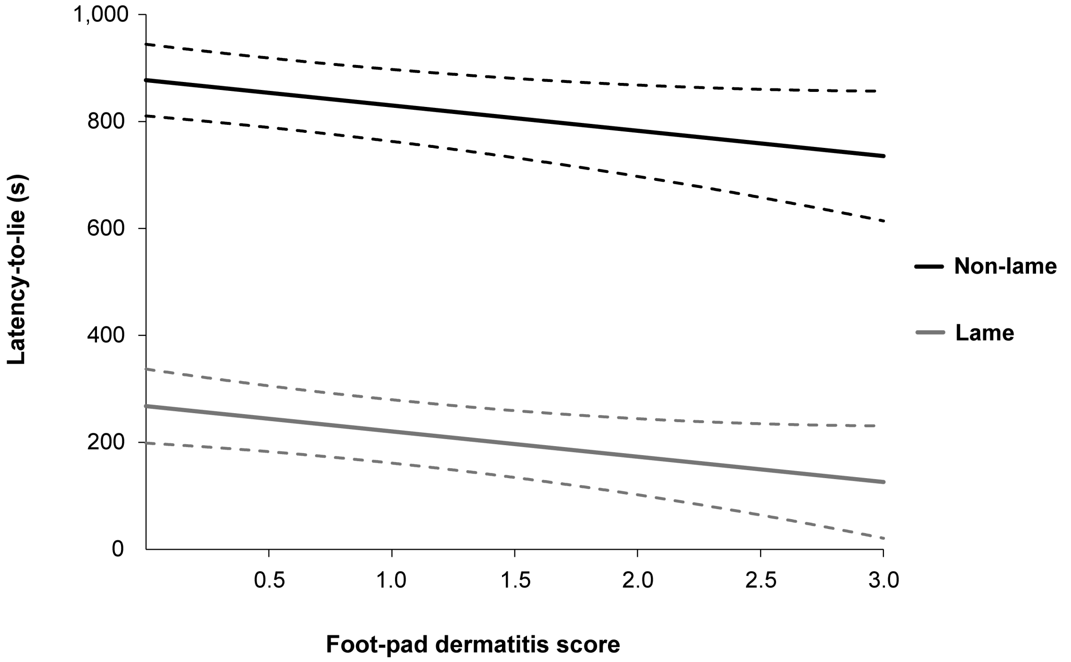
[‡] Natural log-transformed.

The influence of bird characteristics upon test measure outcomes

Group Obstacle test

The best model for total obstacle crossings (described above) comprised 'lameness' as a single, highly significant, predictor variable ($P < 0.001$). Other population characteristics that demonstrated a significant univariate relationship with 'total crossings' (when modelled as a square-root transformed response variable) included 'sex' ($P = 0.008$), 'mass' ($P < 0.001$) and 'pathology' ($P = 0.002$) (Table 2). Although these predictors were all individually significant they did not remain significant when modelled as covariates, in combination with 'lameness'. The amount of

Figure 3



Modelled predictions (± 95% CI) of the reduction in latency-to-lie associated with increasing foot-pad dermatitis severity in groups of non-lame (GS 0–1) and lame (GS 3–4) broilers.

variance within the data set accounted for by the 'lameness' model could not be improved by the inclusion of any other population characteristic, nor by modelling any combination of characteristics.

Latency to first cross the barrier was best modelled as a natural log-transformed response variable and included 'lameness' (z = 5.91, P < 0.001), 'mass' (z = –2.85, P = 0.004) and 'HB' (z = –4.68, P < 0.001) as covariates. This model explained 36.5% of the variance within the dataset. The estimated percentage change (with 95% CI) in latency-to-cross was as follows: 1,007.8% (398.9%, 2,359.9%) increase for high-GS over low-GS birds, –81.3% (–90.7%, –62.3%) decrease per one-unit increase in HB severity, and –86.1% (–96.4%, –46.1%) decrease per 1 kg increase in body mass. When more than one predictor was included within a model, the magnitude of the parameter estimates could not be used to rank the importance of a specific predictor in influencing the response variable. This was due to the magnitudes being specific to a unit of measurement, and any judgment regarding weighting would have inevitably been subjective. The majority of the 63.5% variance unexplained by the model existed at the individual ('bird') level (bird: 89.4%; flock: 10.6%). All three explanatory variables were also significant when modelled as single predictors but, individually, each explained far less variation within the data set than when modelled in combination (Table 2).

Latency-to-Lie (LTL) test

The final model for LTL contained the predictors 'lameness' (z = –17.53, P < 0.001) and 'FPD' (z = –2.25, P = 0.024), and collectively accounted for 69.9% of the variation in the LTL dataset (Figure 3). High-GS broilers sat 610 s (95% CI: 541-678 s) sooner than low-GS broilers, and the model predicted a one-unit increase in FPD score to decrease LTL by 47 s (95% CI: 6–88 s). After fitting the model, the majority of the remaining 30.1% unexplained variance existed at the individual ('bird') level (bird: 84.6%; flock: 15.4%).

As a large proportion of birds either sat very early in the test, or remained standing at the maximum cut-off time, it was not possible to transform the data to achieve satisfactory normality and heteroscedasticity of residual plots. We, therefore, cross-referenced our findings with those from a logistic model in which the response variable was a binary recoding of the original: '0' (did not lie; latency > 900 s) versus '1' (latency < 900 s). As with the continuous response model, a binary model indicated both 'lameness' (z = –6.63, P < 0.001) and 'FPD' (z = –2.78, P = 0.005) to be significant. 'Lameness' increased the odds of lying by 261.4× and a 1-unit increase in 'FPD' increased the odds of lying by 3.8×.

Other population characteristics that demonstrated a significant univariate relationship with LTL (when modelled as a square-root transformed response variable) included 'sex' (P < 0.001), 'mass' (P < 0.001), 'pathology' (P < 0.001), and 'HB' (P = 0.002) (Table 2).

Discussion

The low-GS broilers made a significantly greater number of crossings than the high-GS broilers in the Group Obstacle test. Considering this and the relative ease with which this test could be performed (although the actual test lasted several hours the video data were analysed retrospectively with relative rapidity), we considered the Group Obstacle test to provide a good indication of leg health. Fewer crossings made by the high-GS birds concur with the study of Weeks *et al* (2000), who reported that high-GS broilers rescheduled behaviour by feeding and drinking in fewer bouts of longer duration. This was consistent with increased costs associated with standing/walking. Although broilers have a basal motivation to walk for a food reward we standardised motivation further using a short period of feed withdrawal (to remove the possibility that some individuals had only just finished a feeding bout prior to testing). The obstacle was low enough to allow the birds to retain visual contact with the rest of the group even when sitting. When testing poultry in groups individual behaviour will be influenced by social facilitation (Clayton 1978), meaning that the frequency of certain behaviours will increase due to the sight of others performing them. Since test groups were balanced as far as possible for the two GS-categories, we consider social facilitation to have been beneficial in this instance. The test measured the physical ability of motivated birds to cross the obstacle and the sight of other birds feeding/drinking provided an additional stimulus. A lack of motivation to perform a physical effort would have had consequences for the interpretation of any immobility observed in terms of compromised welfare. Although motivation to walk can improve locomotive ability (Bokkers & Koene 2004), and attentional mechanisms have been shown to override pain-related behaviour (Gentle 2001), not all birds crossed the obstacle in the 5-h test period. Some high-GS birds were observed to consider crossing (stood looking over the obstacle) but motivation to feed was not great enough for them to attempt the manoeuvre, suggesting that these individuals were experiencing discomfort.

'Lameness' explained more variation in obstacle crossings than any other predictor(s). We did not formally examine temporal patterns but the high-GS birds performed fewer crossings than low-GS birds over all 5 h of the test rather than delaying their activity. We expected females to perform better in this test due to their lower body mass; however, 'sex' was not significant when modelled in combination with 'lameness'. McGeown *et al* (1999) found that high-GS birds took longer to complete a course containing two obstacles. Similarly, our high-GS group displayed an increased latency-to-cross the obstacle compared to the low-GS group. The best model for latency-to-cross included covariates ('mass' and 'HB') in addition to 'lameness', yet only explained < 40% variation within the data set. A decrease in latency with increased mass (once 'lameness' had been accounted for) was initially unexpected since heavier birds would require greater physical exertion to traverse the obstacle; however, the heavier birds may have had a greater motivation to feed. Interestingly, Bokkers and Koene (2002) found that male broilers walked a runway (with and without obstacles) to access a mealworm reward at faster speeds than females, despite their greater weight. The observation that more high-GS, than low-GS, birds had HB is likely due to these compromised birds spending more time sitting on wet litter; however, the effect of increasing HB severity upon decreasing latency was unexpected, especially since HB was confounded with lameness.

As per Weeks *et al* (2002) 'lameness' appeared to overcome the broilers' innate aversion to body contact with water by significantly decreasing LTL. Since 'lameness' was a more effective predictor than 'mass' it suggests that we measured a response that was directly related to a sensation of discomfort rather than the effect of fatigue linked to heavy body mass. Allowing for a series of exploratory 'dips' within the test duration allowed the birds to experience the aversive stimulus (chest touching water), and choose whether they were prepared to continue taking avoidance action (remain standing), without taking weight off their legs. The observation that LTL decreased with increased FPD severity may suggest that the presence of foot ulcers produced discomfort during this test; however, Berg and Sanotra (2003) failed to find similar evidence. In future studies concerned with assessing pain relating to specific leg pathologies, it may be appropriate to use the Group Obstacle test in preference to the LTL test if the presence of FPD is unbalanced within the test population (due to its influence upon the test outcome measure).

The majority of any remaining variance, unexplained by the final model, was at the individual ('bird') level in all tests, and similar findings were recorded for gait parameters measured as part of a kinematics study (Caplen *et al* 2012). Although we were careful to select birds that, externally, were as homogeneous as possible, we still observed a wide range of different abnormalities internally, amongst birds assigned the same gait score, eg GS 3 (moderately lame). Although a highly significant association between 'GS' and 'pathology' ($z = -5.21$, $P < 0.001$) was evident within the test population, the observation that 'lameness' was a better explanatory variable than 'pathology' for all outcome measures suggests that a farm-selected group of GS 3 broilers will not experience homogeneous pain. The majority of the high-GS birds had no obvious pathology at post mortem yet the group as a whole demonstrated significantly reduced performance in comparison to the low-GS birds. This provides further impetus for using quantitative test measures to target specific pathologies or identify subsets for future assessment (eg analgesic drug response studies). A means to achieve prior selection of a test cohort (in addition to GS) may prove particularly useful since comprehensive pathological assessments can only be performed retrospectively and treatment with anti-inflammatory drugs may alter visual diagnosis.

Conclusion

This study demonstrated that mobility impairments encompassed within the broad term 'lameness', (determined qualitatively via visual assessment of walking ability and allocation of gait score, GS), are closely associated with a broader range of quantitative measures designed to assess leg weakness. High-GS and low-GS broiler performance significantly differed in both the LTL and Group Obstacle test, and no other population characteristic ('age', 'sex', 'strain', 'mass', 'FPD', 'HB', 'pathology') associated with leg weakness, were as consistent a predictor as 'lameness' for our three outcome test measures (LTL, total obstacle crossings and latency-to-cross). Although a group of birds assigned a high-GS will undoubtedly display heterogeneity in their leg health, we provide evidence that there is a component of 'lameness' that is additional to being eg male and heavy, and may thus represent 'discomfort'. On the basis of our findings we consider the outcome measures of both quantitative tests to be directly relevant to welfare assessment and we envisage that they could provide a basis for future studies via the selection of homogeneous test populations and, when used in combination with analgesic testing, could gainfully assist the investigation into lameness-associated pathology-specific pain in broiler chickens.

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