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Lame broiler chickens respond to non-steroidal anti-inflammatory drugs with objective changes in gait function: A controlled clinical trial

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ABSTRACT

Leg health of intensively reared broiler chickens is a significant problem, yet little is known regarding the nature of lameness-associated pain. Kinematic changes in gait have been reported in naturally lame broilers following subcutaneous non-steroidal anti-inflammatory drug (NSAID) administration, compared to a placebo group. In the current study, an extensive range of gait parameters were defined using a commercial motion-capturing system to record three-dimensional temporospatial information from walking broilers pre- and post-treatment. Data analysis, performed using multi-level models, revealed gait modifications in broilers receiving NSAID, but not in those receiving saline. The effect of walking velocity was accounted for retrospectively.

An increase in velocity following NSAID treatment (carprofen: $P < 0.001$; meloxicam: $P = 0.044$) indicated improved walking ability. For several measures, the polarity of the treatment effect depended upon walking speed. At slow speeds certain parameters become more like those of non-lame broilers, which may have been indicative of improved stability: stride length and duration (both NSAIDs), transverse back displacement (meloxicam), and vertical leg displacement (carprofen). However, these same parameters also revealed that NSAID treatment caused imbalance at faster speeds, which may have signified an excessive dosage. Although doses employed were not conclusively effective, evidence was provided that factors besides body conformation influenced mobility in the test cohort. The study showed that the model would be useful in future studies to increase our understanding of pain associated with specific lameness types in broiler chickens.

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Introduction

Despite high lameness prevalence within commercial flocks (Knowles et al., 2008), investigations into whether birds experience pain as a consequence of natural lameness remain inconclusive. Complicating factors include a lack of effective validated analgesic strategies and broad heterogeneity in lameness aetiology and pathology (Bradshaw et al., 2002).

An arthritis model, in combination with pain-related behavioural change, has been successfully used to determine optimum doses of analgesics, including non-steroidal anti-inflammatory drugs (NSAIDs), in ISA Brown chicks of layer stock (Hocking et al., 2001, 2005). However, genetic factors are known to influence differential sensitivity to analgesia in the domestic fowl (Hughes, 1990) and comparable behavioural studies would appear inappropriate for broilers. Non-lame broilers spend 80% of their time 'resting' (Weeks et al., 2000), which is the key behaviour that increased post-treatment in the layer strain.

Early studies reported changes in broiler walking ability following NSAID treatment and provide evidence for lameness-associated pain. McGeown et al. (1999) recorded increased motility, while Danbury et al. (2000) observed a dose-dependent improvement in gait score during a self-selection (carprofen) feed trial. Lame broilers preferentially selected carprofen (Danbury et al., 2000) but there were pronounced variations in both feed consumption (inter-bird) and lameness severity (intra-bird), and lame birds failed to demonstrate higher carprofen plasma levels than non-lame birds. A recent study failed to corroborate evidence that lame broilers would self-select carprofen-treated food (Siegel et al., 2011), which further questions the suitability of a broiler self-selection paradigm.

A six-point 'gait scoring' scale, developed by Kestin et al. (1992), is widely used for evaluating broiler lameness. Trained personnel assign a gait score (GS) between 0 (sound) and 5 (cannot walk) based upon a broad range of criteria. Although the system is quick

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to employ and requires no specialised equipment, as a subjective methodology it lacks the capacity to discriminate between lameness type. A moderately lame 'GS 3' bird could be affected bilaterally (e.g. valgus) or unilaterally (e.g. singular hock inflammation), or lack any obvious pathology. Objective methodologies for quantitatively assessing gait are now available (Caplen et al., 2012). Kinematic analysis can accurately monitor a comprehensive range of gait components and is well suited for use in drug studies to detect subtle localised change.

The aim of this study was to assess the effect of NSAID treatment (meloxicam and carprofen) on specific gait characteristics in naturally lame broilers. It is anticipated that further broiler gait-response studies, in combination with appropriate analgesic treatments, could elucidate whether, and to what extent, pain is associated with specific lameness pathologies. In the presence of pain we would predict analgesia to facilitate positive gait improvement.

Materials and methods

Animals and husbandry

The study was carried out under the UK Animals (Scientific Procedures) Act (PPL30/2865). Local approval for the work was given by the University of Bristol Ethical Review Group.

A homogeneous group of mildly lame (GS 2), standard-reared, mixed sex, 32 day old broilers were selected from a commercial flock 4 days prior to testing. Following transport to the research facility the broilers were randomly assigned to groups of 12 and housed on shavings within 3 × 1.5 m pens. The birds were maintained on a 16 h:8 h light–dark cycle, at 20 °C, and had access to water and feed (commercial grower pellets) ad libitum, (with the exception of two 2 h periods of food withdrawal on the day of testing). All birds were weighed, examined, and gait scored on a daily basis. Only birds that became moderately lame (GS 3) were included in the study ($n = 32$). Basic pathological assessment of the test cohort (all leg joints) was conducted by trained personnel within 3 days of testing. Tests to detect the presence of infective agents were not made.

Kinematic data collection

Broilers were tested in one of two batches, at either 36 or 43 days of age. Within the first batch, birds were allocated to a control ($n = 5$), meloxicam ($n = 6$), or carprofen ($n = 6$), group (balanced according to body mass). Birds in the second batch were allocated to groups as before ($n = 5$ per group). Three-dimensional kinematic data were collected pre- and post-treatment using an infra-red camera ProReflex motion-capturing system and Qualysis Track manager Software (Qualysis AB); the training and testing methodologies are described in Caplen et al. (2012). Briefly, following a 2 h period of food deprivation, birds were fitted with a cloth poultry saddle (incorporating a retro-reflective back marker) and two retro-reflective leg markers (attached to the posterior aspect of the metatarsal bone immediately above each foot). Birds were filmed (15 s at 120 Hz) as they walked along a 3 m runway towards a food reward and social cue. Each continuous passage along at least half of the runway was termed a 'run'. Data were collected from approximately six runs for each bird (both pre- and post-treatment).

Analgesic treatment

Birds were used as their own controls, receiving a SC treatment immediately following baseline data collection. The control group received 1.5 mL saline, the meloxicam group received 5 mg/kg of Metacam (5 mg/mL injectable solution, Boehringer Ingelheim), and the carprofen group received 25 mg/kg of Rimadyl (50 mg/mL injectable solution, Pfizer Animal Health). These doses were selected on the basis that they maintained baseline thermal sensitivity (via antinociception) in a study of induced lameness (Caplen et al., 2011). Once treated, each bird was returned to its home pen for 3 h and then re-tested (same order) to generate post-treatment data. Timings for data collection were based upon pharmacokinetics data (Baert and De Backer, 2003; Hothersall et al., 2012). Birds had access to food during the first hour of the intermediate rest period.

Data processing

The following kinematic gait parameters were calculated for each stride (contact of one foot to ipsilateral contact): stride duration (SD), stride length (SL), stance (ST), double-leg support (DS), vertical leg displacement (VL), lateral back displacement (LB), vertical back displacement (VB), and velocity (VEL). Stance was calculated as the percentage of the stride duration spent in the stance phase. Measurements were collected in paired overlapping strides (one right and one left) and each pair used to generate mean values (see Caplen et al., 2012).

Statistics

Multilevel modelling software (MLwiN v2.22) was used to create random-intercept nested models reflecting the hierarchical structure of the data set; gait parameter was the response variable, and the nested hierarchy comprised three levels (stride within run, within bird). To investigate the effect of velocity on gait parameters pre-treatment data were modelled using VEL as a single predictor, best-fitted as a linear (1), 2nd (2), or 3rd (3) degree polynomial (VEL_{poly}). Variance partitioning coefficients (VPC) were calculated to assign the percentage variation unaccounted for by VEL to each of the three levels. Models were also constructed to detect inter-group differences in gait characteristics pre-treatment.

Treatment effect (pre- vs. post-) was investigated using group-specific data sets and modelled in a similar manner to above. For those gait parameters where VEL was included within the model an interaction between 'group' and VEL was also incorporated. The significance of individual predictors in a model was tested using *Z*-tests, 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). The significance of interactions in a model were tested using χ^2-tests and the deviance in log-likelihood between models with and without the interaction (whereby d.f. = VEL_{poly}). The percentage variation in the data set accounted for by a model was calculated as the difference between the variation of the response variable before and after the addition of explanatory variables to the model.

Results

The influence of velocity on gait characteristics

Mean (±SD) pre-treatment gait parameter values are summarised in Table 1. Pre-treatment data analysis (birds: $n = 32$) revealed positive associations between velocity and SL, VL, and VB, and negative associations between velocity and SD, ST, and DS ($P < 0.001$ in all cases). Models accounted for 87.0 (SL), 60.0 (VL), 18.5 (VB), 83.2 (SD), 63.9 (ST) and 51.3% (DS) of the variation present within the data sets respectively (Fig. 1E). Rapid changes in gait parameter with velocity at lower speed ranges, followed by reduced rates of change and eventual plateau at higher speeds (e.g. SL, SD, DS, and VL) were indicative of the measures approaching their physical limits. These results imply that without controlling for speed direct gait comparison is not possible. A positive linear relationship was evident between VL and SL ($y = 0.0964x + 46.64$, $r^2 = 0.69$) pre-treatment.

After accounting for velocity, the calculation of VPC (%) for each of the nested levels pre-treatment (580–600 strides) revealed that most unexplained variation (VPC: stride; run; bird) for SD (40; 18; 42), SL (36; 7; 57), ST (21; 21; 58) and VL (22; 12; 66) occurred between birds. The majority of variation in VB (34; 56; 10) occurred between runs, whilst DS (59; 17; 24) demonstrated high variance

Table 1
Mean[a] (±SD) gait parameter values for moderately lame broilers ($n = 32$) pre-treatment.

Gait parameter[b]	Mean value
SD, s	0.66 (±0.15)
SL, mm	352.91 (±83.44)
ST, %	63.21(±6.04)
DS, %	30.10 (±10.34)
VL, mm	80.69 (±9.67)
LB, mm	68.14 (±8.57)
VB, mm	9.90 (±3.17)
VEL, mm/s	611.06 (±266.84)

[a] Calculated using individual bird averages.
[b] SD, stride duration; SL, stride length; ST, stance; DS, double-leg support; VL, vertical leg displacement; LB, lateral back displacement; VB, vertical back displacement; VEL, velocity.

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