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Effects of carprofen, meloxicam and butorphanol on broiler chickens' performance in mobility tests

Running Title: Effect of analgesic drugs on broiler mobility

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Abstract

Lame broiler chickens perform poorly in standardised mobility tests and have nociceptive thresholds that differ from those of non-lame birds, even when confounding factors such as differences in bodyweight are accounted for. This study investigated whether these altered responses could be due to pain, by comparing performance in a Group Obstacle test and a Latency to Lie (LTL) test of lame (Gait Score (GS) 2.5–4) and non-lame (GS 0–1) broilers administered analgesia or a saline control. We used exploratory subcutaneous doses of the Non-Steroidal Anti-Inflammatory Drugs meloxicam (5mg/ kg) or carprofen (35mg/kg) or the opioid butorphanol tartrate (4mg/kg). We included butorphanol to explore the possibility that NSAID drugs could improve mobility by reducing inflammation without necessarily invoking an analgesic effect. Lameness was a significant predictor in all analyses. Neither the number of obstacle crossings nor latency to cross an obstacle was significantly changed by either NSAID, but LTL was longer in lame birds given carprofen and meloxicam than in lame birds given saline. LTL was associated with foot pad dermatitis and ameliorated by both NSAIDS. Butorphanol did not affect LTL but appeared soporific in the obstacle test, increasing latency to cross and, in non-lame birds, reducing the number of crossings. Combined with data from other studies, the results suggest carprofen and meloxicam had some analgesic effect on lame birds, lending further support to concerns that lameness compromises broiler welfare. Further investigation of opioid treatments and lameness types is needed to disentangle effects on mobility and on pain.

Keywords: animal welfare, analgesia, broiler chicken, lameness, mobility, pain

Introduction

A report in 2009 by the Farm Animal Welfare Council concluded that insufficient progress was being made in addressing the problem of lameness in broiler chicken production (FAWC, 2009). Part of the difficulty in reducing lameness probably stems from its variability and multifactorial aetiology (Bradshaw *et al.*, 2002) and so far, correlations between lameness and underlying pathology have proven to be weak (McNamee *et al.*, 1998; Sandilands *et al.*, 2011; Fernandes *et al.*, 2012). Recent

work by our group has examined the influence of lameness on tests of mobility and nociceptive processing in commercially reared broiler chickens, while also modelling other factors that might explain test performance and that are often confounded with lameness (such as body mass, sex and signs of pathology). Lame broilers made fewer, later crossings than non-lame broilers in an obstacle test and had a shorter Latency to Lie (LTL) in a shallow water bath – i.e. to avoid a mildly aversive experience (Caplen *et al*, 2014). In a related study, lameness was also associated with altered thermal nociceptive threshold (Hothersall *et al*, 2014). Importantly, lameness was the most consistent predictor of performance across these tests: other factors explained less variability in results or became non-significant when lameness was included in the models. These findings suggest that lameness in commercial broilers is not simply synonymous with reduced mobility or activity and may include an element of pain or discomfort.

A relationship between lameness and pain would be confirmed if differences between lame and non-lame birds' performance on such tests were reversed or attenuated by administration of analgesic drugs. There is already some evidence to support this: data from kinematic analysis in lame broilers indicated that the NSAIDs carprofen and meloxicam caused objective changes in gait, including increased walking velocity (Caplen 2013a). Both drugs were also successful in reversing hyperalgesia (indicated by a lower nociceptive threshold) associated with induced hock inflammation (Caplen *et al*., 2013b). Finally, meloxicam elevated nociceptive threshold in lame commercial broilers, though this group of birds did not show hyperalgesia before treatment (Hothersall *et al*., 2014).

We therefore tested the efficacy of carprofen and meloxicam on the Obstacle and LTL tests described by Caplen and colleagues (2014). In addition, we included the opioid butorphanol tartrate, which is regularly used in the management of both chronic and acute pain in birds (Paul-Murphy, 2008). Unlike NSAIDs, opioid drugs act without affecting inflammation so can help to discriminate between analgesic effects and improvements to mobility that could occur independently of pain.

Evidence exists for a therapeutic action for each of these drugs in poultry and other bird species, but there has been little systematic evaluation of appropriate analgesic doses. Routes of administration and timing of post-treatment data collection also vary widely within the existing literature.

Meloxicam: Cole *et al* (2009) observed that an intramuscular injection of 1.0 mg/kg meloxicam improved weight-bearing in Hispaniolan parrots (*Amazona ventralis*) with experimentally induced arthritis. Graphical data presented as part of this study indicated that the most substantial improvement occurred 2-6 h post-treatment. Baert & DeBacker (2002, 2003) described pharmacokinetic parameters of an intravenous dose of meloxicam at 0.5mg/kg in broiler chickens based on doses previously used in other species. They reported an elimination half-life of 3.21 hours, but did not include a behavioural assay. Recent work within our group using a higher subcutaneous dose of 5 mg/kg revealed objective changes in gait parameters after 3h (Caplen *et al*., 2013a) and altered nociceptive threshold (Caplen *et al*., 2013b) 6h post-treatment, both in lame broilers.

Carprofen: McGeown *et al* (1999) observed beneficial effects of carprofen on broiler chicken mobility at a dose of 1 mg/kg (subcutaneously). Danbury *et al* (2000) reported that lame chickens offered food dosed with 4mg/kg carprofen selected more drugged feed than did non-lame birds, though these findings were not replicated in a recent study (Siegel *et al*., 2011). In laying hens, Hocking *et al.*, (2005) concluded that a minimum of 30 mg/kg was required to attenuate signs of experimentally induced pain using an intra-muscular route. Caplen and colleagues (2013a,b) also reported beneficial effects on mobility and nociceptive threshold following a subcutaneous dose of carprofen at 25mg/kg, and pharmacokinetic data (Hothersall *et al.*, 2012) confirmed that plasma concentrations of carprofen were higher at 3-6 h compared with 1h following sub-cutaneous injection at 15, 25 or 35 mg/kg.

Butorphanol: Sladky *et al* (2006) state that doses of 2-4mg/kg butorphanol tartrate are widely used in psittacine birds and must be administered every 2-4 hours. Guzman *et al* (2011) concluded that for a 5 mg/kg dose in Hispaniolan parrots (*Amazona ventralis*), maintenance of plasma concentrations

"consistent with published therapeutic levels" would require dosing every 2 h intravenously or 3 h intramuscularly. A sub-cutaneous dose of 0.5mg/kg improved weight-bearing in lame turkeys in the 30 minutes after injection (Buchwalder & Uber-Eicher, 2005) 2 mg/kg was effective in improving mobility in laying hens with keel fractures measured from 30 min post-treatment (Nasr *et al*., 2012). No data detailing appropriate doses or effects of butorphanol are available for broilers but a pharmacokinetic study in broilers concluded that intravenous injection of 2mg/kg butorphanol resulted in drug levels "above the minimum effective concentration for analgesia *in mammals*" for approximately 2 hours (Singh *et al*., 2011). Surprisingly, however, subcutaneous butorphanol at 4mg/kg did not have any significant effect on nociceptive threshold in broilers up to 2 hours after administration (Hothersall *et al*., 2014)**.**

Based on these data and pilot studies, we carried out exploratory studies to examine the effect of two NSAIDs (5 mg/kg meloxicam and 35 mg/kg carprofen) and an opioid (4 mg/kg butorphanol tartrate) on performance in the Obstacle and LTL tests described in Caplen *et al* (2014). The aim was to determine whether differences in mobility between lame and non-lame birds could be reversed or reduced by analgesic treatment. If analgesic drugs were effective in relieving lameness-related pain, it was hypothesised that latency to lie and the number of crossings in the obstacle test would be increased in lame birds receiving drug treatment. For the NSAIDs, it was expected that activity in the obstacle test was most likely to be affected in the latter half of the test, whereas butorphanol was expected to act more quickly and might therefore also decrease the latency of lame birds to first cross the barrier.

Methods

Birds

Mixed sex broiler chickens of two commercial strains were acquired from various farms located within South West England at 25-35 days of age. On each farm visit we collected approximately equal

numbers of non-lame (Gait Score 0) and lame (Gait Score 3) birds (hereafter referred to as a flock) using the gait scoring criteria of Kestin *et al* (1992). From most flocks 14 lame and 14 non-lame birds were selected. Birds were individually colour marked using stock marker spray and housed in pens (3.05 x 1.22 m) on wood shavings at a density not exceeding the legal requirements for housing birds undergoing scientific procedures. Animal accommodation was climate-controlled at approximately 20˚C and maintained on a 16 h light: 8 h dark schedule. Birds had *ad libitum* access to water and commercial feed.

Measurement of bird characteristics

Birds were tested within the age range of 28 - 46 days. Age at testing was always balanced across test groups within any flock. Immediately pre-testing, birds were weighed and gait scored by two experienced observers, as described in Hothersall *et al* (2014). Hock burn and foot pad dermatitis (FPD) were recorded on a scale of 0-4 (Welfare Quality, 2009) and birds were assigned a score based on the mean of both legs. Sex was determined at post-mortem, within 3 days of completion of testing, at which time hock joints were dissected and any gross pathology (e.g. swelling, excessive fluid, angular deformity) recorded. This was combined additively with observations made during gait scoring to provide binary scores indicating the presence or absence of pathology (any one or more of: infection, injury, bone deformity).

Quantitative measures of mobility

Each test (LTL or obstacle test) was performed following injection of an analgesic or saline control (see below). Birds from within a single flock all received the same analgesic treatment (or saline). Gait score remained stable for only a short period following transfer to the research facility (including both improvements and substantial decline) so most flocks were only kept for four testing days. The narrow time window for testing meant it was not possible to employ a cross-over design or to subject all birds within a flock to both tests (though most birds did undergo both tests). Birds of Gait Score 0-1 were included within the category 'non-lame' and bird of Gait Score 2.5-4 were classified as 'lame'. Within these categories, exact gait score was also balanced as far as possible across treatments. Group

sizes for each treatment are provided in Table 1 and the tests themselves are described in detail within Caplen *et al* (2014).

Obstacle test

The obstacle test was conducted in the home pen using groups of 12 birds following 1 h food withdrawal. Groups contained equal numbers of lame and non-lame birds as far as possible; where necessary one or more birds of intermediate gait score were included to maintain group sizes but data associated with such individuals were not analysed. A block barrier (10cm high, 21.5 cm wide) divided the middle of the pen, creating an obstacle between the feeder and drinker. At the start of the test, all birds were placed at the drinker and continuous video footage was then recorded for 5 hours. The number of times each bird stepped up onto the obstacle (described as a 'crossing') during the test and the latency of each bird to first perform a crossing was recorded retrospectively from video footage.

Latency to Lie (LTL) test

Briefly, each bird was removed from the home pen and placed individually into a plastic box containing a 4cm depth of tepid water and a base of non-slip rubber matting. Several birds were tested simultaneously, in visual but not auditory isolation. The time taken ('latency') for the bird to sit ('lie') was recorded, up to a maximum of 15 minutes, after which the bird was dried and returned to the home pen.

Analgesic treatment

All treatments were administered subcutaneously into the dorsal neck. Control birds for all groups 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 35 mg/kg of Rimadyl (50 mg/mL injectable solution, Pfizer Animal Health). The butorphanol group received 4 mg/kg of Torbugesic (1% W/V injectable solution, Fort Dodge Animal Health). For the NSAID treatments the LTL test was performed once at 5 h post-treatment; however, for butorphanol (a faster acting drug) the LTL

test was performed three times: pre-treatment (hr 0) and then at 1 h and 2 h post-treatment. For all drug treatments the Obstacle test was conducted immediately following treatment until 5 h post-treatment, and specific intervals were also examined at the analysis stage. Birds receiving saline were tested as per the appropriate drug allocated to that flock. Some birds receiving saline for their first test (LTL or obstacle test) were also tested using the other test on a different day. For this second test they received either saline or a drug treatment.

Statistical Analysis

Data were analysed using MLwiN v2.25 (Rasbash *et al*., 2012) to create random-intercept nested models to adjust for non-independence due to clustering within groups. For the LTL and Obstacle tests, the random part of the model comprised two levels, bird (level 1) nested within flock (level 2). Our quantitative measures (number of obstacle crossings, latency to cross the obstacle, or latency-to-lie) formed the response variables, as appropriate, and the predictors (covariates) tested included a binary score for lameness[1] (GS for the carprofen group due to lack of saline control group), age, sex, mass, binary scores for the various indicators of pathology and ordinal scores for hock burn and foot pad dermatitis. Strain was included as an additional predictor within the carprofen data set as flocks for this cohort were of two strains. These potential predictors were entered individually into the model and any that were significant ($P < 0.05$) were entered concurrently; those that became non-significant were removed. All other variables were then re-entered sequentially and any additional significant variables were retained. Standardised residuals were calculated and plotted to ensure that assumptions of normality and homoscedasticity were met. The significance of individual predictors in a model was tested using Z-tests, whereby the coefficient was divided by the standard error of the 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). Interactions between predictors were explored where there was an *a priori* reason to expect a relationship to exist. The significance of

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[1] Gait score was also modelled as an ordinal variable but in all cases the binary grouping was more parsimonious and was used in the final models.

an interaction in a model was tested using χ^2-tests and the deviance in log-likelihood between models with and without the interaction. Where more than one predictor made a significant contribution to the model, combinations of predictors were included and the model that explained the greatest amount of variance was selected.

Occasionally, a bird's data were excluded from analysis because during testing it showed signs of illness that appeared to interfere with normal behavioural responses to the test.

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

A summary of the main characteristics (lameness risk factors) of the test samples is provided within Table 1. Only lame birds were tested with carprofen as insufficient quantities of non-lame birds of an appropriate weight were present within the source flocks. Those that were present were deemed unsuitable for inclusion within the study as they were either not morphologically representative of a broiler chicken at that age, displaying stunted growth, or appeared to be underweight due to illness. No such problems were detected amongst the lame birds. Intra-individual alterations in gait meant that some birds were excluded on certain test days as their GS no longer fitted within our prescribed

range for lame and non-lame birds. For this reason, sample sizes vary slightly between tests. All data are unpaired and no attempt was made to examine relationships between the LTL and Obstacle tests.

Obstacle test

Meloxicam

Meloxicam had no significant effect on total crossings or initial latency to cross the object, in either lame or non-lame birds.

The total numbers of obstacle crossings [raw data, mean ± SD (range)] for the 5 h test period were as follows: lame + saline = 3.44 ± 3.79 (0-13); lame + meloxicam = 4.18 ± 2.81 (0-11); non-lame + saline = 14.40 ± 10.00 (0-39); non-lame + meloxicam = 14.90 ± 9.37 (0-39). *Treatment* was not a significant predictor of *total crossings*, nor was its interaction with *lameness* significant. The best model, explaining 49.9% of the variability in crossings, consisted of the predictors *lameness* (P <0.001) and *sex* (P <0.001) (Table 2): lame birds made fewer crossings than non-lame birds and male birds made fewer crossings than females. Separate models of the first and last two hours of the test produced very similar results.

Latencies to first cross the obstacle (s), mean ± SD (range), were as follows: lame + saline = 8565 ± 7321 (103-18000); lame + meloxicam = 4313 ± 5670 (9-18000); non-lame + saline = 4178 ± 5861 (47-18000); non-lame + meloxicam = 2333 ± 4398 (98-18000). *Latency* was best modelled by the predictor *lameness* ($P = 0.023$; Table 2), though the model only explained 3.7% of the variation within the data set.

Carprofen

Carprofen did not affect the number of obstacle crossings made by birds or alter latency to first cross the obstacle. The total numbers of obstacle crossings [raw data, mean ± SD (range)] for the total 5 h test period were as follows: saline = 3.84 ± 2.41 (0-8); carprofen = 3.00 ± 3.27 (0-6). *Total crossings*

was best modelled by *mass* (P = <0.001) and *GS* (P = 0.002) (Table 2), which explained 30.4% of the variation within the data.

On the basis of pharmacokinetic data suggesting that carprofen may have reached its highest concentrations in plasma serum in the later stages of the test (Hothersall *et al*., 2012) two one-hour intervals were also modelled (3-4 h, 4-5 h). In each case, drug was not a significant predictor but mass and gait score were.

The mean latency [seconds; mean ±SD (range)] to cross the obstacle for each group was as follows: saline = 5460 ± 5210 (119 - 18000); carprofen = 8870 ± 6770 (10 - 18000). No variables were found to be significant predictors of latency.

Butorphanol

Butorphanol had no significant effect on total crossings, but was associated with a reduction in crossings within the first two hours of the test in non-lame birds. It was also predicted to increase latency to first cross in all birds.

The total numbers of crossings [raw data, mean ± SD (range)] for the 5 h test period were as follows: lame + saline = 2.96 ± 2.27 (0-8); lame + butorphanol = 2.48 ± 1.90 (0-8); non-lame + saline = 12.12 ± 6.45 (3-30); non-lame + butorphanol = 10.29 ± 6.42 (1-31). *Total crossings* was best modelled using *lameness* (P <0.001) as the only predictor, explaining 50.9% of the variation in crossings. There was no significant main effect of treatment, nor any interaction between *treatment* and *lameness*.

Sub-cutaneous injection of butorphanol was recently found to be relatively fast acting in laying hens (Nasr *et al*., 2012), so we also modelled data from the first 2 h of the test. The number of crossings, mean ± SD, for this interval were as follows: lame + saline = 1.17 ± 1.31 (range: 0-6); lame + butorphanol = 0.67 ± 0.84 (range: 0-2); non-lame + saline = 4.88 ± 2.51 (range: 0-9); non-lame +

butorphanol = 2.50 ± 3.01 (range: 0-13). For the *0-2 h crossings* results the best model consisted of *treatment*, *lameness*, and the interaction between the two variables (P <0.001) (Table 2, Figure 1). This model explained 54.5% of the variation. Thus within the first two hours of the test butorphanol decreased activity of non-lame birds such that the number of crossings made did not differ significantly between lame and non-lame birds.

Latencies to cross the obstacle [s; (mean ± SD (range)] were as follows: lame + saline = 6097 ± 6834 (78-18000); lame + butorphanol = 7563 ± 5491 (56-18000); non-lame + saline = 1268 ± 1386 (1-4173); non-lame + butorphanol = 2556 ± 2772 (8-8040).

Latency was best modelled by *lameness* (P <0.001), *treatment* (P = 0.046), *mass* (P = 0.023) and *hock burn* (P = 0.022) (Table 2), which together explained 35.7% of variation in latency. Being lame and receiving butorphanol increased *latency* by 5619.0 and 953.8 s respectively. In contrast, *mass* and *hock burn* decreased *latency* (by 490.6 s per 1 kg increase in body mass and by 474.6 s per one-unit increase in hock burn score).

The Latency to Lie (LTL) test

Meloxicam

Modelling showed that meloxicam increased LTL in lame birds.

LTL results (s), mean ± SD (range), were as follows (raw data): lame + saline = 140.8 ± 186.2 (5-780); lame + meloxicam = 326.9 ± 332.8 (14-900); non-lame + saline = 844.0 ± 168.8 (245-900); non-lame + meloxicam = 826.5 ± 204.0 (214-900). The final model accounted for 69.6% of variation in latency to lie and contained an interaction between *lameness* and *treatment* (P = 0.017, Table 3). Meloxicam increased *LTL* in lame but not in non-lame birds (Figure 2).

An additional model containing a significant interaction between *FPD* and *treatment* (χ^2 = 5.69, p = 0.034) indicated that LTL reduced with increasing FPD severity in those birds receiving saline treatment, while LTL was maintained, regardless of FPD severity, in those that received meloxicam (Figure 3). This model accounted for 18.7% of variation in the LTL dataset**.**

Carprofen

Carprofen was found to increase LTL across the range of gait scores tested.

LTL results (s), mean ± SD, were as follows (raw data): saline = 83.4 ± 107.6 (range: 0-451); carprofen = 193.9 ± 247.6 (range: 0-900). The final model contained the predictors *GS* (P = 0.001) and *treatment* (P = 0.024, Table 3). Latency to lie decreased as gait score increased, but was higher in birds treated with carprofen (Figure 4). The model accounted for 31.9% of the variation in latency to lie.

An additional model containing a significant interaction between *FPD* and *treatment* (χ^2 = 9.631, p = 0.004) indicated that LTL decreased with increasing FPD severity in those birds receiving saline treatment, while LTL increased with increasing FPD severity in those that received carprofen (Figure 5). This model accounted for 30.3% of variation in the LTL dataset.

Butorphanol

Butorphanol treatment had no significant effect on latency to lie, either 1 or 2 hours after injection.

LTL results (s), mean ± SD, were as follows (raw data): lame + saline = 327.2 ± 287.0 (hr 0), 330.5 ± 336.9 (hr 1), 309.5 ± 285.9 (hr 2); lame + butorphanol = 338.9 ± 297.9 (hr 0), 311.9 ± 289.7 (hr 1), 364.5 ± 345.0 (hr 2); non-lame + saline = 867.2 ± 114.9 (hr 0), 833.7 ± 191.7 (hr 1) , 830.7 ± 190.3 (hr 2); non-lame + butorphanol = 900.0 ± 0.0 (hr 0), 900.0 ± 0.0 (hr 1), 843.6 ± 172.5 (hr 2).

The latencies to lie of saline- and drug-treated birds did not differ at Hour 0 (pre-treatment) and no significant main effect of *treatment*, nor any interaction with any other predictor, was found either 1 or 2 h following administration. The data for all 3 time points were, therefore, combined and modelled with test nested within bird. This resulted in model containing the predictors *lameness* (P <0.001) which increased LTL by 573s, *pathology* ($P = 0.003$) which increased it by 181s and *FPD* (P <0.001), where a one-unit increase in FPD score was predicted to decrease LTL by 108s (Table 3). This model explained 63.9% of the variance in latency.

Although *FPD* was a significant negative predictor of LTL, there was no evidence of any interaction between *FPD* and *treatment* within this data set.

Discussion

As described in the introduction, information about appropriate drug doses and pharmacokinetic parameters was fairly limited. This, and the knowledge that genetic factors can influence differential sensitivity to analgesia in the domestic fowl (Hughes, 1990), meant our experiments were necessarily exploratory. Nevertheless, the results revealed beneficial effects of carprofen and meloxicam on lame birds' latency to lie (LTL). At the doses used, these drugs did not appear to affect activity within the Obstacle test, and no beneficial effect of butorphanol was observed in either test. Our findings are consistent with our recent demonstration that carprofen and meloxicam obtunded elevated nociceptive threshold in a model of lameness (Caplen *et al*., 2013). As in the larger, pooled sample reported in Caplen *et al* (2014), lameness was again the most consistent predictor of performance in both the Obstacle and LTL tests: lameness (or Gait Score) was significant in every individual model except the measure of latency to cross the barrier, for carprofen only.

Within the Obstacle test, meloxicam and carprofen treatment were both ineffective at increasing the number of crossings, either overall or when we explored results within specific time intervals. The

lack of effect on latency was not surprising because bioavailability was anticipated to be highest several hours after dosing (Baert & DeBacker, 2002, 2003; Hothersall *et al.*, 2012). The LTL results were more promising, with standing times being longer in lame birds administered either NSAID 5h previously. Drug treatment was not expected to increase latency in non-lame broilers but, as most birds stood for the entire 15 minute test duration, an improvement in standing performance was impossible to rule out. In the meloxicam group, data from the drug-treated non-lame birds did not reveal a reduction in obstacle crossing or LTL. This was reassuring, as such a reduction might have indicated a soporific effect due to an overdose. These findings add to the existing literature from model lameness studies and pharmacokinetic data in parrots (Cole *et al.*, 2009; Molter *et al.*, 2013) and our nociceptive threshold data (Caplen *et al.*, 2013; Hothersall *et al.*, 2014) to suggest meloxicam and, to some extent, carprofen have some therapeutic effect in lame broilers.

It is worth noting that post-hoc analysis of obvious signs of pathology (hock only) revealed unexpectedly large differences in pathology type between some sub-groups. It was not a significant predictor in any of our models, but nevertheless, this variability could have confounded results if one type of pathology was more susceptible to treatment than the other types. In the meloxicam LTL test, for example, around one third of lame birds showed signs of pathology. *Of those*, 87.5% in the saline group were classified as showing signs of 'deformity' while 71.3% in the meloxicam group were scored positive for 'infection' (see Table 1). Lameness due to 'deformity' might be less amenable to improvement by anti-inflammatory drugs than lameness due to other causes, or might encompass more and less painful conditions. Reassuringly, over-representation of a pathology type could not explain the similar efficacy of carprofen in the LTL test, where distribution of pathologies was more consistent. There was no clear pattern overall and many birds exhibited signs of more than one pathology type. We also detected pathology in less than 50% of lame birds in any test group. Thus we believe that our classification of pathologies was of limited accuracy (rather than identifying a genuine imbalance in our groups) and requires further refinement. The data are included here for completeness and to inform future studies where lameness type might be of interest.

There was no indication from either test that 4mg/kg butorphanol had any beneficial analgesic effect on lameness. In fact, it was associated with a decrease in activity in non-lame birds within the first two hours of the obstacle test, and an increase in latency (of over 15 minutes) to first cross the obstacle. Previous data on effective analgesic doses for broilers were lacking, and suggestions that similar or lower doses would be effective in broilers (Singh *et al*., 2011) and parrots (Guzman *et al*., 2011) for around two hours were based on extrapolation from behavioural data in other studies or even species. A 2mg/kg sub-cutaneous dose improved mobility in laying hens with healed keel fractures (Nasr *et al.,* 2012) but pilot data at this dose had not appeared effective in our broilers. The lack of significant effect of butorphanol on total obstacle crossings across the 5 h data set as a whole provides some basic information about the duration of effect: it suggests that non-lame birds recovered normal behaviour and even showed some rebound effect during the latter stages of the test.

The positive effects of the NSAIDs on the LTL test - which does not require movement - combined with the effects of NSAIDs on broiler nociceptive threshold, may indicate that lameness is causing some degree of pain or discomfort that is (partially) alleviated by NSAID administration. However, the absence of any beneficial effect of butorphanol in either test makes it difficult to unequivocally separate pain from mobility. Intriguingly, our drug treatment findings are the opposite of those obtained by Nasr and colleagues in laying hens with healed keel bone fractures. In their studies, 2mg/kg butorphanol reduced latency to fly down from a perch to access a food reward (Nasr *et al*., 2012), whereas neither 5mg/kg meloxicam or 25mg/kg carprofen (all subcutaneously) were effective in the same test (Nasr *et al*., 2014). Previous studies have shown great variability in bioavailability and clearance rates of drugs in different avian species (eg Baert & deBacker 2003). Given the intense selection for growth rate and comparative immaturity at slaughter in broilers, differences between laying hens and broiler strains may also be considerable.

Effects of Foot Pad Dermatitis (FPD) on LTL

We previously found that broilers with worse FPD sat significantly quicker in water in the LTL (Caplen *et al*., 2014). This finding prompted us to conduct separate analyses to explore the relationship between analgesia and FPD in the LTL tests. In all three groups in this study, LTL again decreased with increasing severity of FPD. This appeared to be ameliorated by both meloxicam and carprofen, though not by butorphanol. However, FPD was only significant in the final (most parsimonious) model of LTL for butorphanol. FPD scores were highly variable and were not totally balanced across groups so this finding should be interpreted with caution, but may merit further systematic evaluation.

Utility of tests

As per Caplen *et al* (2014), for each group, the LTL test again explained the highest percentage of variance (range 31.9-69.6%), followed by Obstacle crossings (30.4-54.0%), with the lowest percentage of variance explained by Latency (3.7-35.7%). This suggests that the factors we modelled were able to explain more of the variation in LTL than of the birds' activity in the Obstacle test. An additional advantage of the LTL test is that it is quick and easy to set up. The obstacle test had the advantage of being non-invasive but while it was able to detect the relatively large effects of lameness or increasing Gait Score on activity, it did not appear to detect any putative influence of drug treatment. In this test, the greatest explanatory power was found for the butorphanol group, which was probably due in part to the rapid decrease in activity (and thus overall variability) caused by the drug. It seems likely that the LTL was more sensitive because the response was less 'voluntary', in that birds stood to avoid a mildly aversive stimulus. Consistent with this, butorphanol did not reduce latency to lie despite a strong sedative effect in the obstacle test. Weeks *et al* (2000) found that lame birds adapted their behaviour in a way that minimised movement while maintaining overall food intake; the single session of the Obstacle test may not have been long enough for lame birds to overcome habitual sedentary behaviour, though we expected them to be very motivated to reach the food.

The use of commercial, spontaneously lame birds was an important element of this study because they are representative of the populations and rearing conditions in which lameness occurs. However, it did inevitably compromise experimental control to some degree. We administered a single drug dose, whereas extended use of NSAIDS over several days might have increased any physiological or mechanical improvement to lame birds. However, repeated testing would have been subject to error from changes in Gait Score, including loss of birds that deteriorated over the testing period. The instability of Gait Score – even within our short testing period – is very likely to have reduced our ability to detect treatment effects. The aetiology of lameness in our birds could also not be fully known, and there was large variability in some parameters. We attempted to record (very obvious) signs of pathology or illness; these had little predictive power and were almost all found in the lame birds, but a small number of non-lame birds showed signs of pathology or did not, for example, make *any* obstacle crossings. Sandilands *et al* (2011) previously found that links between gait score and pathology in broilers were weak, and it is possible that at least some birds reaching our sample age group without becoming lame had some pathologies that were not detected in this study. We included measures of pathology opportunistically because we were unable to avoid some heterogeneity in our birds, focussing on the hock because early indications suggested this was the most common site of pathology in our samples. Our assessment appears to have been too blunt to accurately reflect lameness aetiology. If possible, application of the behavioural tests to groups of birds with known and consistent lameness aetiology would be informative.

Conclusions.

The Obstacle and LTL tests are minimally invasive behavioural assays that discriminate well between lame and non-lame birds. This study found promising evidence that either 5mg/kg of meloxicam or 35 mg/kg carprofen was able to partially ameliorate reduced latency to lie in spontaneously lame commercial broiler chickens. No evidence of a beneficial effect of 4 mg/kg butorphanol was found, and it was unclear how effectively we were able to identify heterogeneous causes of lameness that could have influenced the efficacy of drug treatments. As a result we were unable to unequivocally exclude a simply anti-inflammatory action by the NSAIDS. In conjunction with findings that these

NSAID drugs alter nociceptive threshold, the results suggest that meloxicam and carprofen may have an analgesic effect on lame broilers, but further testing of opioid analgesics is needed to differentiate between the influences of mobility and pain on LTL. Ideally, the tests outlined here should also be replicated in birds of homogeneous lameness type and severity, though this may be difficult to achieve within a commercial sample.

Animal welfare implications

Altered mobility (latency to lie) in commercially reared lame birds was attenuated by NSAID analgesics, adding to a growing body of evidence that such lameness involves an element of pain or discomfort.

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Tables

Table 1: Summary of the characteristics, including post-mortem pathology, of broilers used for the Obstacle and LTL tests: (a) Meloxicam; (b) Carprofen; (c) Butorphanol

(a)

Test	Characteristic	Saline		Meloxicam	
		Lame	Non-lame	Lame	Non-lame
Obstacle	Test population (n)	18	16	17	15
	Mass[1], kg	1.74±0.26 (1.27-2.27)	1.34±0.23 (1.01-1.72)	1.76±0.26 (1.12-2.19)	1.36±0.24 (0.83-1.715)
	Sex	M: n = 11 F: n = 7	M: n = 5 F: n = 11	M: n = 13 F: n = 4	M: n = 6 F: n = 9
	Hock Burn[1,2]	0.15±0.23 (0-0.75)	0.05±0.14 (0-0.5)	0.85±1.05 (0-3.0)	0.13±0.28 (0-1.0)
	Foot Pad Dermatitis[1,2]	1.47±0.90 (0-2.5)	0.91±0.76 (0-2.0)	1.32±0.91 (0-3.0)	0.60±0.86 (0-2.25)
	Pathology (%)	33.33	6.25	47.06	0
	Type[4] (%):				
	Infection	83.33	100.00	50.00	0.0
	Deformity	33.33	00.00	62.50	0.0
	Injury	00.00	00.00	00.00	0.0
LTL	Test population (n)	22	22	19	16
	Mass[1], kg	1.86±0.33 (1.01-2.39)	1.51±0.28 (0.99-2.11)	1.89±0.31 (1.28-2.39)	1.40±0.26 (1.02-1.95)
	Sex	M: n = 17 F: n = 5	M: n = 8 F: n = 14	M: n = 10 F: n = 9	M: n = 8 F: n = 8
	Hock Burn[1,2]	0.49±0.61	0.06±0.13	0.65±1.02	0.14±0.24

		(0-1.75)	(0-0.5)	(0-3.0)	(0-0.75)
	Foot Pad Dermatitis[1,2]	1.35±0.91 (0-2.5)	0.43±0.60 (0-1.75)	1.38±0.90 (0-3.0)	1.20±0.90 (0-2.25)
	Pathology (%)	36.36	0.00	36.84	0.00
	Type[4] (%):				
	Infection	37.50	0.00	71.43	0.00
	Deformity	87.50	0.00	42.86	0.00
	Injury	0.00	0.00	0.00	0.00

(b)

Test	Characteristic	Saline		Carprofen	
		Lame	Non-lame	Lame	Non-lame
Obstacle	Test population (n)	19	n/a	16	n/a
	Mass[1], kg	2.10±0.37 (1.46-2.76)	n/a	2.04±0.48 (1.33-3.04)	n/a
	Sex	M: n = 11 F: n = 8	n/a	M: n = 13 F: n = 3	n/a
	Hock Burn[1,2]	0.11±0.20 (0-0.5)	n/a	0.12±0.21 (0-1.0)	n/a
	Foot Pad Dermatitis[1,2]	0.94±0.93 (0-2.5)	n/a	1.21±0.92 (0-3.0)	n/a
	Pathology (%) Type[4] (%):	5.26	n/a	37.50	n/a
	Infection*	100.00		50.0	
	Deformity	0.00		33.33	
	Injury	0.00		33.33	
LTL	Test population (n)	16	n/a	16	n/a

Characteristic				
Mass[1], kg	1.74±0.42 (0.97-2.57)	n/a	1.73±0.34 (1.18-2.39)	n/a
Sex	M: n = 11 F: n = 5	n/a	M: n = 9 F: n = 7	n/a
Hock Burn[1,2]	0.19±0.19 (0-0.5)	n/a	0.20±0.37 (0-1.0)	n/a
Foot Pad Dermatitis[1,2]	0.69±0.54 (0-1.5)	n/a	0.50±0.66 (0-2.0)	n/a
Pathology (%)	43.75	n/a	43.75	n/a
Type[4] (%): Infection*	57.14		57.14	
Deformity	42.86		42.86	
Injury	28.57		14.29	

(c)

Test	Characteristic	Saline		Butorphanol	
		Lame	Non-lame	Lame	Non-lame
Obstacle	Test population (n)	24	25	18	15
	Mass[1], kg	1.92±0.31 (1.40-2.40)	1.62±0.21 (1.28-2.20)	2.06±0.29 (1.50-2.59)	1.68±0.17 (1.47-2.12)
	Sex	M: n = 21 F: n = 3	M: n = 5 F: n = 20	M: n = 18 F: n = 2	M: n = 2 F: n = 13
	Hock Burn[1,2]	0.19±0.28 (0-0.75)	0.05±0.18 (0-0.75)	0.11±0.20 (0-0.75)	0.08±0.18 (0-0.5)
	Foot Pad Dermatitis[1,2]	0.80±1.08 (0-3.0)	0.64±0.71 (0-3.0)	0.83±0.82 (0-2.5)	0.37±0.60 (0-2.0)
	Pathology (%) Type[4] (%):	37.50	4.00	38.89	0.00

	Infection*	55.56	100.00	57.14	0.00
	Deformity	77.78	0.0	57.14	0.00
	Injury	22.22	0.0	0.00	0.00
LTL	Test population (n)	21	24	17	15
	Mass[1], kg	1.98±0.25 (1.44-2.40)	1.61±0.23 (1.12-2.19)	2.03±0.27 (1.54-2.40)	1.74±0.20 (1.34-2.20)
	Sex	M: n = 18 F: n = 3	M: n = 5 F: n = 19	M: n = 15 F: n = 2	M: n = 2 F: n = 13
	Hock Burn[1,2]	0.4±0.89 (0-3.0)	0.25±0.73 (0-3.0)	0.19±0.27 (0-0.75)	0.17±0.26 (0-0.75)
	Foot Pad Dermatitis[1,2]	0.87±1.08 (0-3.0)	0.64±0.73 (0-3.0)	0.60±0.87 (0-2.5)	0.35±0.43 (0-1.0)
	Pathology (%)	28.57	4.17	47.06	0.00
	Type[4] (%): Infection	33.33	100.00	50.00	0.00
	Deformity	100.00	0.0	62.50	0.00
	Injury	16.67	0.0	12.50	0.00

[1] Mean ± SD (range)

[2] 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 the test population.

[4] Of those individuals with an identified pathology the prevalence of each pathological 'type' was also calculated. The different types were tallied separately; an individual could display more than one so percentages may total >100%.

* Either localised hock joint infection or systemic infection.

Table 2: Final models of significant predictors for broiler performance in the obstacle test.

Group	Measure	Predictor	Coef	SE $_{(Coef)}$	χ^2	df	z	p
Meloxicam	*Total crossings*[1]	*Constant*	4.37	0.23			18.77	<0.001
		Lameness	1.91	0.23			8.20	<0.001
		Sex	0.80	0.23			3.46	<0.001
	Latency[2]	*Constant*	6.70	0.55			12.20	<0.001
		Lameness	0.92	0.41			2.27	0.023
Carprof en	*Total crossings*[2]	*Constant*	1.44	0.27			5.26	<0.001
		Gait Score	-0.29	0.09			-3.15	0.002
Butorphanol	*Total crossings*[1]	*Constant*	2.38	0.09			27.96	<0.001
		Lameness	-1.06	0.12			-8.91	<0.001
	0-2h crossings[1]	*Constant*	2.48	0.08			32.16	<0.001
		Lameness	-0.9	0.11			-8.07	<0.001
		Treatment	-0.42	0.09			-4.85	<0.001
		Lameness*Treatment			65.72	1		<0.001
	Latency[1]	*Constant*	25.28	6.61			3.82	<0.001
		Lameness	53.83	9.47			5.68	<0.001
		Mass	-37.48	16.54			-2.27	0.023
		Hock burn	-38.11	17.56			-2.29	0.022
		Treatment	14.63	7.30			2.00	0.046

[1]square-root transformed

[2]natural log transformed

Table 3: Final models of significant predictors for broiler latency to lie.

Treatment Group	Predictor	Coefficient	SE (Coefficient)	χ^2	df	z	p
Meloxicam	*Constant*	844.05	45.27			18.65	<0.001
	Lameness	-733.66	64.78			-11.33	<0.001
	Treatment	-17.55	69.77			-0.25	ns
	Lameness*Treatment			5.63	1		0.017
Carprofen[1]	*Constant*	7.24	1.43			5.08	<0.001
	Treatment	4.56	2.02			2.26	0.024
	GS	-7.83	2.39			-3.28	0.001
Butorphanol	*Constant*	910.13	32.75			27.78	<0.001
	Lameness	-573.12	47.07			-12.17	<0.001
	FPD	-108.46	26.28			-4.13	<0.001
	Pathology	180.57	60.56			2.98	0.003

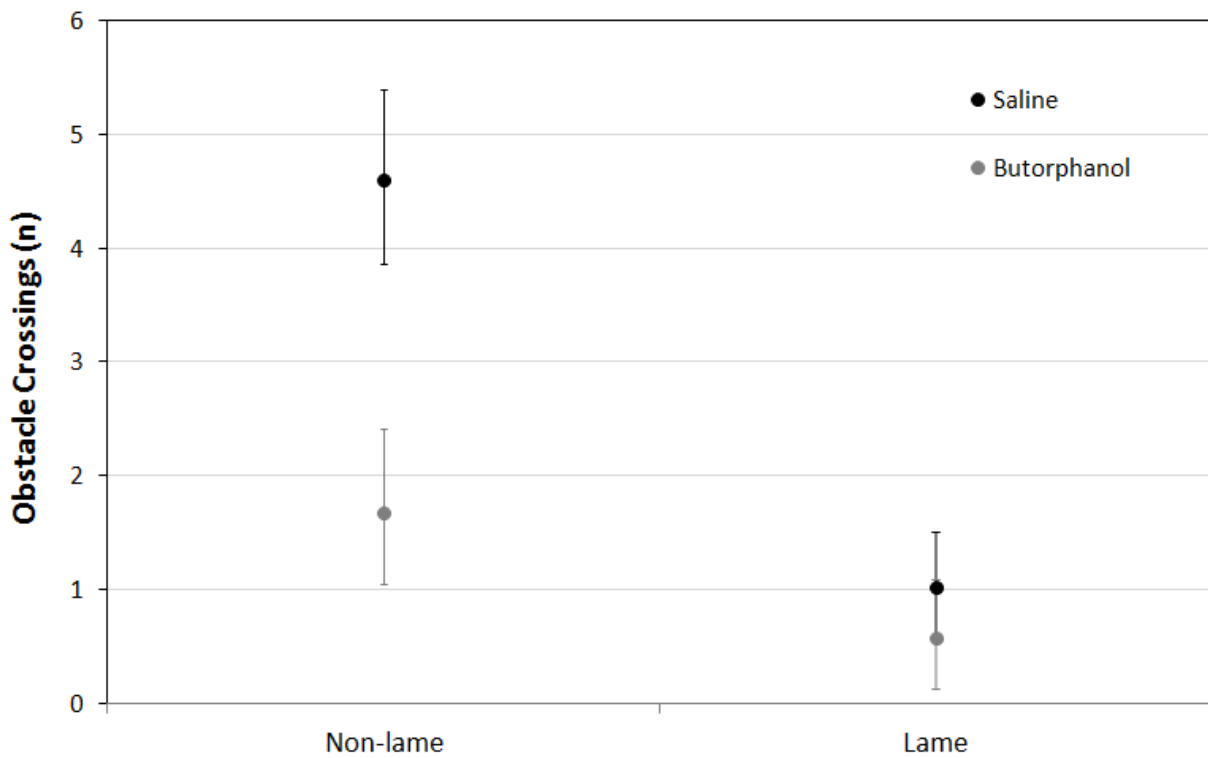[1]square-root transformed

Figures

Figure 1: Effect of butorphanol on obstacle crossings. Back-transformed model estimates of the number of obstacle crossings (mean ± 95% CI) by lame and non-lame broilers in the two hours after treatment (saline or 4mg/kg butorphanol).
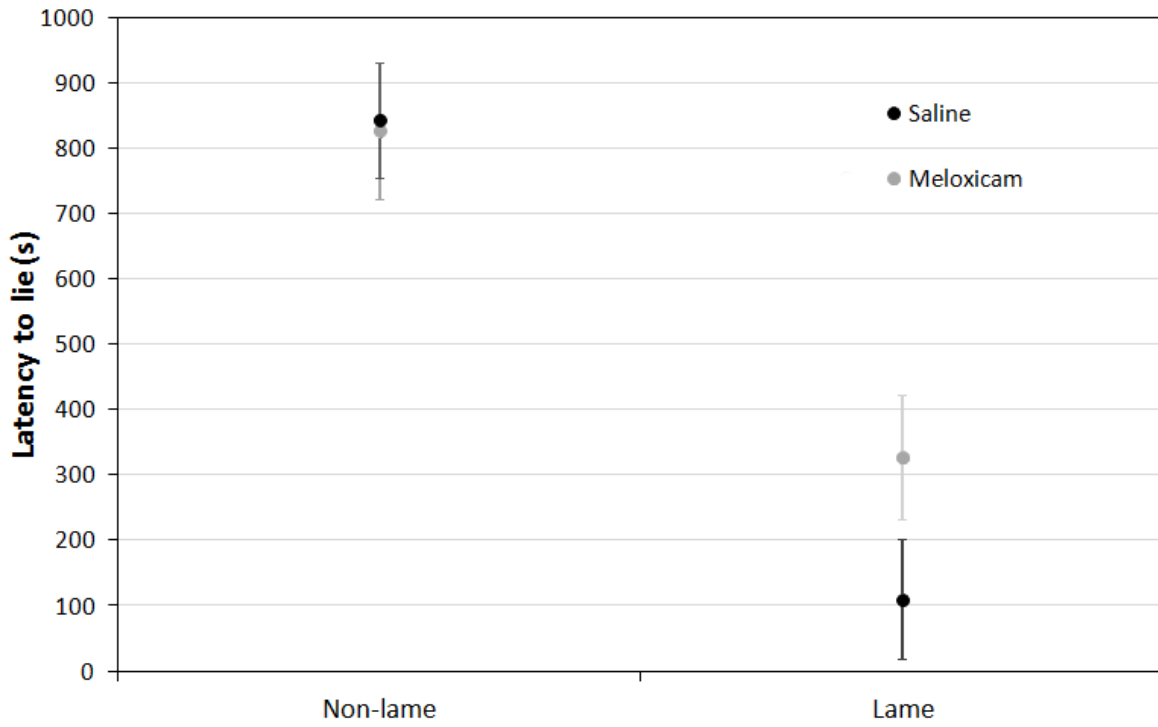
Figure 2: Effect of meloxicam on latency to lie (LTL). Model estimates for LTL (mean ± 95% CI) in

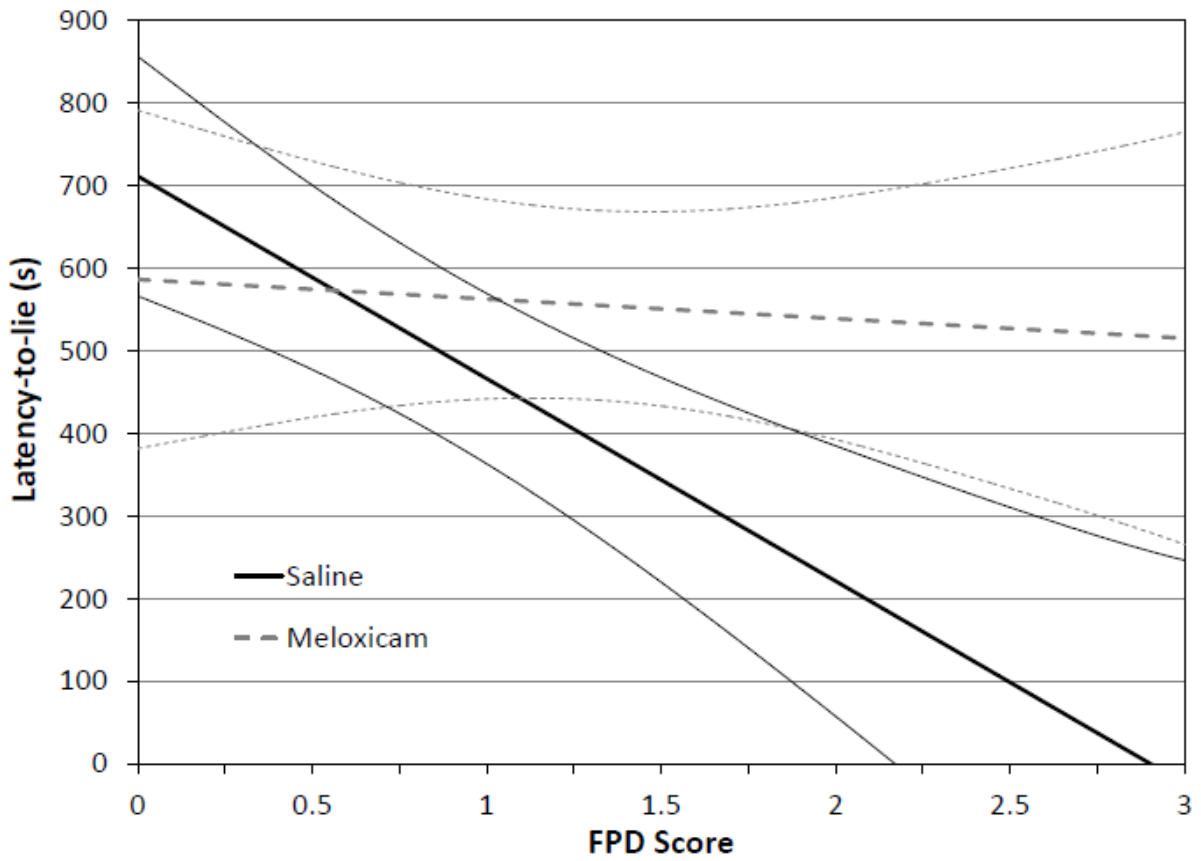lame and non-lame broilers tested 5 hours following treatment (saline or 5mg/kg meloxicam).

Figure 3: Model estimates for the relationship between latency to lie (± 95% CI) and foot pad dermatitis (FPD) severity in broilers treated with saline or 5 mg/kg meloxicam.
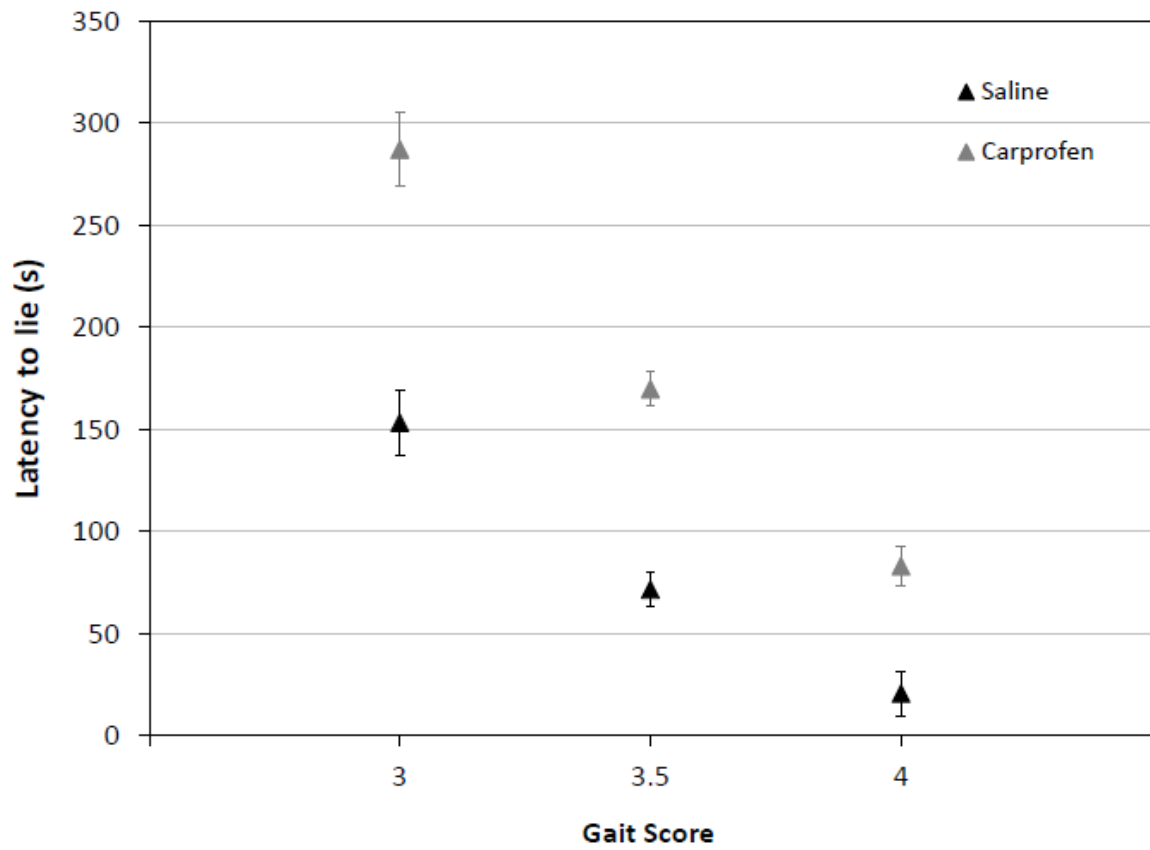
Figure 4: Effect of carprofen on latency to lie (LTL). Back-transformed model estimates (means ± 95% CI) of the effect of treatment (saline versus 35 mg/kg carprofen) on LTL in lame broilers differing in gait score.
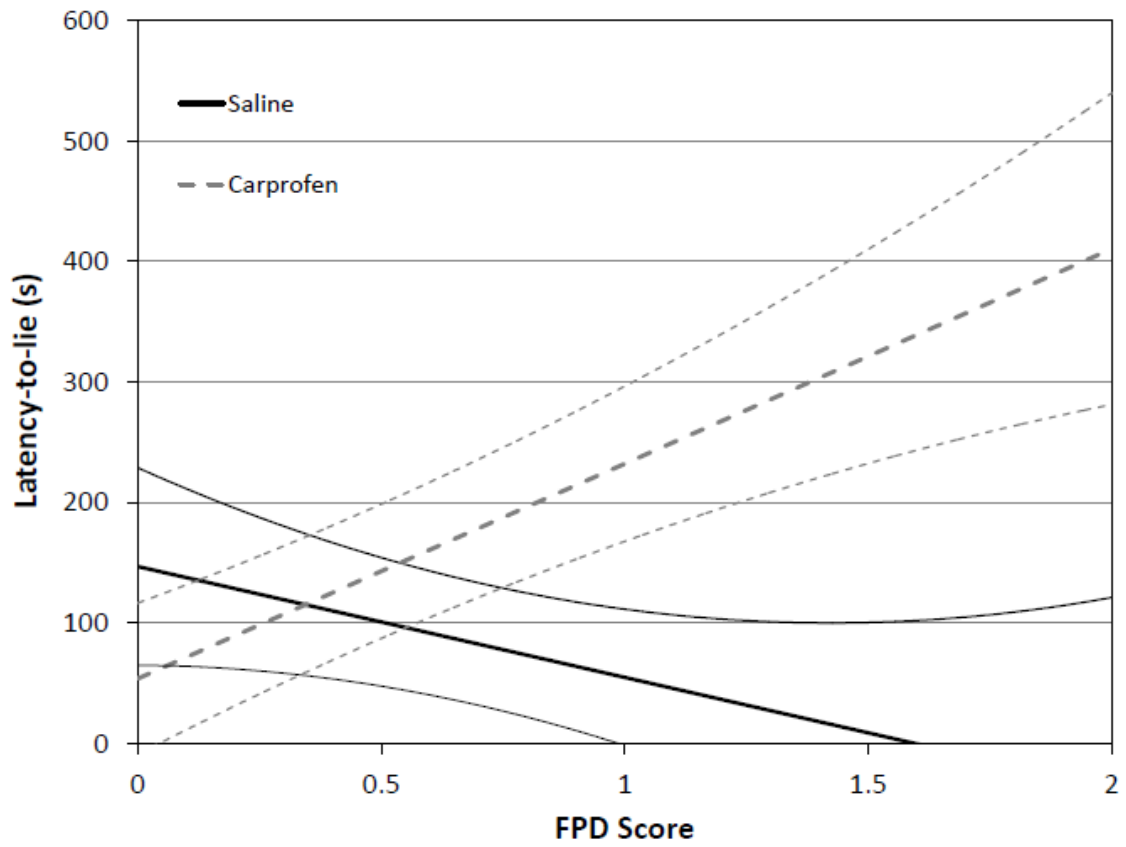
Figure 5: Model estimates for the relationship between latency to lie (± 95% CI) and foot pad dermatitis (FPD) severity in broilers treated with saline or 35 mg/kg carprofen.