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Biomechanics of landing in injured and uninjured chickens and the role of meloxicam

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ABSTRACT Birds use their legs and wings when transitioning from aerial to ground locomotion during landing. To improve our understanding of the effects of footpad dermatitis (**FPD**) and keel bone fracture (**KBF**) upon landing biomechanics in laying hens, we measured ground-reaction forces generated by hens (n = 37) as they landed on force plates (Bertec Corporation, Columbus, OH) from a 30 cm drop or 170 cm jump in a singleblinded placebo-controlled trial using a cross-over design where birds received an anti-inflammatory (meloxicam, 5 mg/kg body mass) or placebo treatment beforehand. We used generalized linear mixed models to test for effects of health status, treatment and their interaction on landing velocity (m/s), maximum resultant force (N), and impulse (force integrated with respect to time [N s]). Birds with FPD and KBF tended to show divergent alterations to their landing biomechanics when landing from a 30 cm drop, with a higher landing velocity and maximum force in KBF compared to FPD birds, potentially indicative of efforts to either reduce the use of their wings or impacts on inflamed footpads. In contrast, at 170 cm jumps fewer differences between birds of different health statuses were observed likely due to laying hens being poor flyers already at their maximum power output. Our results indicate that orthopedic injuries, apart from being welfare issues on their own, may have subtle influences on bird mobility through altered landing biomechanics that should be considered.

Key words: footpad dermatitis, keel bone fracture, landing velocity, ground-reaction force, NSAID

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INTRODUCTION

Flighted birds are bipedal animals that rely on their legs (hind limbs) for ground locomotion and wings (fore limbs) for aerial locomotion (Gatesy and Dial, 1996). Both hind limbs and fore limbs are active during take-off and landing (i.e., transition ground-aerial locomotion) (Gatesy and Dial, 1996); however, chickens are mainly ground-dwelling species spending between 48% and 70% of their time foraging on the ground (Savory et al., 1978; Dawkins, 1989). Aerial locomotion is mainly reserved for bursts of flight to escape predators (Tobalske and Dial, 2000) or to gain access to an elevated roost in most ground-dwelling birds. Despite this, housing systems for chickens require them to manoeuver

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increasingly complex spatial environments to access valuable resources such as feeders, nest boxes, or roosting spaces (Karcher and Mench, 2018). These systems are increasingly used as countries commit to phasing out more behaviorally restrictive cage housing (Egg Farmers of Canada (EFC), 2016; European Commission, 2021). In complex housing systems, elevated slatted or wire tiers are stacked in vertical space with ramps, perches, or platforms allowing hens to manoeuver vertically between tiers (Karcher and Mench, 2018). Birds' ability to navigate these structures and access resources depends on configuration such as height and angles (Scott et al., 1997; LeBlanc et al., 2018a; Rufener et al., 2020) but also factors such as strain (Kozak et al., 2016; LeBlanc et al., 2018a), environmental challenges and physical health challenges (Nasr et al., 2012a; LeBlanc et al., 2016; Campbell et al., 2016; Garant et al., 2022).

Physical health challenges occur in all housing systems but a few orthopedic injuries are of particular concern in chickens kept for egg-laying in complex housing systems (EFSA, 2005; Karcher and Mench, 2018; Mench and Rodenburg, 2018). These include conditions such as

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footpad dermatitis (FPD) or keel bone fracture (KBF) which are highly prevalent welfare issues (Lay Jr. et al., 2011). FPD is an infection of the plantar region of the foot leading to discoloration, erosion, and ulceration at the site of infection (Shepherd and Fairchild, 2010) which can develop into a painful affliction described as bumble foot as indicated by severe swelling and inflammation (Tauson and Abrahamsson, 1994). It is commonly promoted by prolonged contact with wet and dirty litter or poor perch design (Tauson and Abrahamsson, 1994; Wang et al., 1998). Bone fractures to the keel of the sternum from which the main flight muscles originate are a potentially painful condition that is reported in up to 97% of laying hens (Nasr et al., 2012b, 2013; Rufener and Makagon, 2020). The etiology of KBF is not fully understood (Harlander-Matauschek et al., 2015; Stratmann et al., 2016) but due to its higher prevalence in complex systems, KBF was often suggested to be due to collisions (Sandilands et al., 2009; Riber et al., 2018). More recent research indicates that most KBF are due to internal forces and not external trauma caused by high-energy collisions (Thøfner et al., 2020). However, KBF is known to reduce the strength of wingbeats and increase a hen's reluctance to use her wings (Sandilands et al., 2009; Wei et al., 2020a). Considering that domestic chickens are generally poor flyers that are already at the limit of their ability to control flight trajectories (Kozak et al., 2016; León et al., 2021), KBF may exacerbate this and be a risk factor for collisions that may worsen the occurrence of some KBF. Rufener et al. (2020) found that chickens landing from a 100 cm downward transition experienced a greater peak force on the keel than at 50 cm of descent. Finally, the relationships between different injuries should also be considered (Leishman et al., 2022). Hens with KBF spend increased time on the ground (Sandilands et al., 2009; Nasr et al., 2012a; Wei et al., 2020b) and this could potentially exacerbate the issue of FPD in poor litter conditions if no ramps are provided (Heerkens et al., 2016).

While the limited research on physical health conditions has mainly focused on how chickens maneuver to access resources and navigate tiers, less is known about the biomechanics involved in landing. Banerjee et al. (2014) observed landing forces of approx. 81 N and 107 N (5.2-6.9 times when as expressed multiples ofbody weight (\mathbf{BW}) assuming an average 1.58 kg) in laying hens jumping from 41 and 61 cm heights, respectively. Landing is reliant on both the fore limbs and hind limbs; hens must transition the downward force from their wings to their legs (Provini et al., 2014; Campbell et al., 2016). In the final wingbeat, a hen's wings act as a brake and can output 3 to 10 times the energy than the legs must absorb at touchdown (Moinard et al., 2004; Provini et al., 2014). Considering that FPD and KBF likely show some cardinal signs of inflammation (i.e., redness, swelling, heat, pain, loss of function) leading to a reluctance to bear weight on their feet (LeBlanc et al., 2016; Mikoni et al., 2022) or use their wings (Sandilands et al., 2009; Wei et al., 2020a), this could have implications for landing biomechanics. Several studies have investigated the use of analgesia and its impact on

locomotion in poultry including forces during walking (FPD) or latency to landing (KBF) with inconclusive results (Nasr et al., 2012b, 2015; Weber Wyneken et al., 2015; Rentsch et al., 2019). Furthermore, to the best of the authors' knowledge, these studies did not evaluate ground-reaction forces experienced during landing in birds with FPD or KBF; nor the influence of meloxicam, a cyclooxygenase-2 preferential nonsteroidal antiinflammatory drug (**NSAID**), which is the most commonly used pain and inflammation reliever in avian medicine (Summa et al., 2017).

The aim of this study was to test for differences in landing biomechanics (maximum force, impulse, and velocity) in birds that had FPD or KBF or were considered healthy. Secondly, we evaluated if an oral meloxicam treatment influenced these landing biomechanics. We predicted that birds with KBF would be reluctant to use their wings to brake before landing therefore experiencing greater forces, whereas birds with FPD would try to cushion their landing on the infected footpads. The provision of the meloxicam was expected to restore the landing biomechanics to similar values as observed in healthy birds. As redness and swelling are difficult to quantify in animals, heat can be quantified by thermal imaging (Yahav and Giloh, 2012). Therefore, thermal imaging was used as a noninvasive tool to provide a measure of the effectiveness of meloxicam in reducing inflammation, given the limited information on the pharmacodynamics of meloxicam in laying hens.

MATERIALS AND METHODS

Animals and Housing

This study was approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol # 2501) before being conducted.

Prior to the experiment, birds were group housed in a commercial style aviary containing adult, white-feathered laying hens (Lohmann LSL lite) at the Ontario Poultry Research Station (Guelph, Ontario, Canada). All birds were beak trimmed. Birds were screened by physical examination for injury status and uninjured birds (healthy), birds with FPD, and birds with KBF were selected. Injury status was based on visual inspection of the footpads and palpation of the keel bone. A bird was considered to have FPD when an inflammatory/degenerative condition of the avian foot (Grades III and IV) (Degernes, 1994) was present on the metatarsal pad and the affected footpad was recorded. For KBF, palpation was used to select hens with clear signs of fractures including ridges/bumps, callus(es), and deviations on the caudal end of the keel as extreme cases of KBF (Riber et al., 2018; Thøfner et al., 2021) (Figure 1).

Selected birds were housed in individual floor pens (183 L × 244 W × 290 H cm) bedded with wood shavings for the duration of the experiment. Pens were equipped with a round feeder in the middle of the pen and a nest box against the rear wall. Two platforms (122 L × 31 W, 70 cm above the ground) were placed on



Figure 1. Photographs of (A) footpad dermatitis present on the metatarsal pad and (B) fracture of the keel bone of the bird.

either side of the pen. The pens were also equipped with a perch toward the pen's rear, spanning the pen's width. Automatic nipple drinkers were placed beneath one of the platforms. The room that housed the hens averaged 21°C and had a 14:10 h light:dark light cycle with a 30min dawn and dusk period, following industry management guidelines. Due to restricted number of floor pens available, inclusion of birds was staggered across time with a similar proportion of birds from each injury status within each cohort. Birds were kept in the floor pens for approx. 2 wk before being returned to the commercial-style aviary. The experiment occurred between January and March 2017. A total of 37 birds were selected including 10 uninjured birds, 17 birds with FPD, and 10 birds with KBF. The number of birds with FPD was higher to compensate for data loss in this group due to technical issues (see further).

Force Plate and Test Apparatus

The testing apparatus to evaluate the biomechanics of landing was established in a dedicated test arena (366) $L \times 244 \text{ W} \times 290 \text{ H cm}$) in a separate room. Two 35 cm $\log \times 17$ cm wide $\times 3$ mm thick aluminum plates covered with a sanded, non-slip roofing membrane (RESISTO Self-Adhesive Roofing Underlay) were mounted to 2 identical $15 \text{ cm} \times 15 \text{ cm}$ custom-made force plates (Bertec Corporation, Columbus, OH) (LeBlanc et al., 2018b; Ross et al., 2019) using Scotch double-sided carpet tape, allowing for measurement of ground reaction forces from any location on the aluminum plates. The aluminum plates were positioned adjacent to each other along their long side, with a 3 mm gap between them (together these plates covered an area of approx. 35 $cm \times 35 cm$). The aluminum plates were centered inside a plywood frame measuring $60 \text{ cm} \times 60 \text{ cm}$ (with a 35.5

 $cm \times 35.5 cm$ cut-out to allow clearance around the aluminum plates), mounted so that the aluminum plates were flush with the top of the plywood frame. From each force plate, data were transmitted to a computer using a Bertec AM6500 digital preamplifier, and recorded using Bertec Digital Acquire software (version 4.0.11) at a frequency of 200 Hz with an initial low-pass filter at 1000Hz. The floors of the testing arena were covered with foam interlocking mats (Interlocking play foam floor tiles, Canadian Tire, Guelph, ON). A container of sweet corn kernels and dried mealworms was placed directly in front of the force plates to encourage the birds to land on the force plates. A Phantom Miro Ex4 high-speed camera (Vision Research, Wayne, NJ) was positioned 1 m to the left of and level with the force plate assembly for a pure sagittal view. The landing area was lit with a bright fullspectrum light to allow high-speed recording.

Birds were made to land on the force plates from a 30 cm manual drop or a 170 cm jump (see further). The jump tower apparatus used was the same as described in León et al. (2021). In brief, a wooden jump tower was placed to create a 60-degree angle between the floor and the hypotenuse that connected the force plate to the jump tower platform. The height of the startbox and platform assembly could be adapted from 10 to 170 cm in 20 cm increments. The startbox (30 W × 30 L × 45 H cm) had a plastic sliding door to allow birds to exit onto the platform (20 L × 30 W cm) from which they jumped down from jump tower onto the force plate apparatus.

Experimental Design and Testing Procedures

A single-blinded placebo-controlled trial using a crossover design was performed where birds were medicated with meloxicam or placebo before jump landings onto the force plate apparatus. Birds spent approx. 12 d being acclimatized to the testing environment and procedures, including the voluntary consumption of capsules containing crushed meloxicam tablets or placebo (feed), thermal imaging and jumping off a platform and landing on the force plates. During this time, birds were trained to voluntary consume 2.5 cm long white capsules filled with crushed feed and meloxicam (0.5 mg per gelatine)capsule) or capsules filled with feed (placebo). A maximum safe dosage was set at 5 mg/kg BW based on previous research (Hothersall et al., 2016; Summa et al., 2017) and a typical dosage of 1 to 1.5 mg/kg q12h. The number of capsules offered therefore varied according to the birds' BW, but typically 10 capsules were offered. The voluntary consumption of capsules was used to evaluate whether birds with injuries would self-medicate depending on the severity of the injuries. The experimenters were blinded to the capsule medication treatments.

The final 2 d were considered the true testing days which occurred when birds were approx. 70 wk of age. Prior to the capsule medication, birds were weighed (Ohaus Ranger, Fisher Scientific, 0.1 g readability) and thermal images were taken of the feet and keel area. Images were taken using an infrared camera (320×240) pixels, FLIR T430sc) set to emissivity of 0.95 and distance of 1 m as per Wilcox et al. (2009). The image of the keel area was taken while separating the feathers to expose the underlying skin of the breast area. The underside of the footpads was imaged and any dirt was gently brushed of the footpads beforehand. The capsule medication treatment always occurred in the same location as the force plate and jump tower apparatus in a 0.5 m^3 training box for 5 min, after which the number of consumed capsules was recorded. The order of the medication treatment was randomized so that 20 birds received the placebo on d 1 and 17 birds received the meloxicam treatment on d 1. This ensured a 24 h washout period between medication treatments. After each medication treatment, birds were returned to their individual pen. The medication was considered to have reached its maximum effect 2 h after being consumed based on previous literature (Molter et al., 2013; Hothersall et al., 2016) and pilot testing.

At this point, the hen was removed from the individual pen and carried to the separate room where thermal images were re-taken as described above. The hen then had to complete the 30 cm drop and 170 jump landings onto the force plate (Figure 2). A feed bowl containing their preferred treat (e.g., sweet corn kernels, hardboiled egg) was placed directly in front of the force plate, and hens were allowed to eat for approx. 3 s once they landed successfully on the force plate. The researcher conducting the force plate tests was blinded to the birds' medication status. Hens were trained to land on the force plates by dropping them straight down onto the plates from a height of 30 cm. Drop height was kept consistent between hens by lining their feet up to a line that was marked on the wall adjacent to the force plate. A trial was deemed successful when the hen landed with their left and right feet fully contacting the left and right

force plates respectively (with no more than one digit of the opposite foot crossing over onto the plate) and remaining in this position for at least 1 s. The inter-trial interval (time between drops) ranged between approx. 15 and 60 s. Five successful drops were aimed for, but the hen was only dropped a maximum of 10 times to minimize fatigue and pain.

Immediately after completing the 30 cm drops, birds were placed in the startbox of the jump tower apparatus to conduct the 170 cm jumps (Figure 2). During the previous 12 d birds had been trained to gradually increase their jump height from 30 cm by increasing the platform height with 20 cm after 3 successful jumps. After approx. 10 s, the sliding door was opened and the bird was allowed to exit and voluntary jump from the platform. Birds that did not jump within 60 s were gently encouraged to jump and were positioned straight on the platform for subsequent jumps. Similar to the 30 cm drops, birds had to land successfully in order to be allowed to consume their feed reward. The intertrial interval (time between jumps) ranged between approx. 20 and 60 s. Due to difficulty in seeing if feet crossed during the experiment, jumps where birds landed with both feet on the force plate were considered successful in the case of the 170 cm jumps. Jumps were repeated with the aim to achieve 2 to 5 successful landings to avoid fatigue and pain. In the end, variation between birds meant that the total number of jumps from 170 cm ranged between 3 and 11 jumps.

DATA EXTRACTION AND PREPARATION

Thermal images of each bird were analyzed using ResearchIR 4 (Teledyne FLIR LLC, Wilsonville, Oregon) to determine the impact of the medication treatment on skin surface temperature (°C) along the keel bone and footpads. The maximum temperature along the keel bone area was determined. Following Wilcox et al. (2009), the difference between the maximum and minimum temperature on the line from the middle digit across the metatarsal pad for foot most affected by FPD was calculated. For birds without FPD, the right footpad was considered.

Force plate data were manually synchronized between the 2 plates to measure timing of landing events and to be able to calculate a total resultant landing force. When the hen made contact with the force plate it recorded force (Newton, N) in 3 directions: front and back/fore-aft (x-axis), left and right/lateral (y-axis), and downward (z-axis). Individual files were inputted into the analysis software IgorPro 6 (Wavemetrics, Lake Oswego, OR) to observe the 3D kinematics of landing. Custom macros were used to extract the relevant data. Data were smoothed using a low pass filter (End of Pass Band 0.02, Start of Reject Band 0.05; Number of Coefficients 101, Window Hanning). The resultant force (N) was calculated by taking the square root of the squared x, y, and z forces of both force plates. Body mass was determined from the mean force applied when the resultant force was stabilized and only the hen's weight was



Figure 2. Photographs of the testing procedure including (A) a bird jumping from the 170 cm jump tower platform toward the force plate and the feed reward, (B) a bird approaching the force plate during the 30 cm drop, and (C) a bird approaching the force plate from the 170 cm jump.

recorded. This force was converted to kilograms by dividing the force by the acceleration of gravity (9.805 m s^{-2}) . If body mass was not able to be calculated using the previously mentioned method, the BW recorded on a balance at the start of the test day was used (see earlier). The hen's mass was then subtracted from the resultant landing force to isolate the forces involved in landing. Landing was considered to start when the first positive force was applied to the force plates (toe-down) and landing was considered finished when the first negative occurred (touchdown) after landing had started. After isolating the resultant landing force, the macro extracted details on the landing biomechanics including the impulse (integrated force with respect to time). Landing velocity (m/s) was calculated by dividing the impulse by body mass. The maximum resultant force was expressed as a multiple of body mass by dividing the force by the bird's body mass (kg) multiplied by the 9.805 m s^{-2} conversion factor. Due to the variability in the number of 30 cm drops and 170 cm jumps between birds, we chose the first drop or jump for which we could successfully extract the force plate data.

STATISTICAL ANALYSIS

A total of 37 birds were used in the experiment. Five birds did not consume any meloxicam on their assigned treatment days and were omitted from the analysis. This left a final 32 birds from which thermal images of the footpad and keel were collected and analyzed. Due technical issues with the force plate and bird behavior (e.g., crossing of feet) we ended with a total 22 birds that had correct landings on the force plate during both days on the 30 cm drop and 15 birds for the 170 cm jump.

To evaluate whether the meloxicam had an impact, we determined if the temperature measurement from the thermal images differed using a generalized linear mixed model with health status (healthy, FPD, KBF), treatment (placebo, meloxicam), time (1: before and 2: after treatment) and their interactions using a repeated statement for bird within treatment. The simple effect of time within the 3-way interaction was used to evaluate mean comparisons within each treatment-health status combination.

Generalized linear mixed models were used to evaluate the effect of health status (healthy, FPD, KBF), treatment (placebo, meloxicam) and their interaction on outcomes of bird landing biomechanics. Separate models were used for the 30 cm drop and 170 cm jump. Outcomes that were analyzed included landing velocity, maximum resultant force expressed as multiples of BW, and impulse. A random effect of bird and day was used to account for the cross-over design. Simple effects of the interaction were used to specifically examine the effect of health status or treatment, respectively. All statistical procedures were conducted using SAS V9.4 (SAS Inst. Inc., Cary, NC). Assumptions of normally distributed residuals and homogeneity of variance were examined graphically and data were transformed where necessary. A Holm–Tukey adjustment was used to account for multiple comparisons. Results are presented as LS means \pm SE, unless stated otherwise. Statistical significance was considered at P < 0.05 and tendencies are reported at $0.05 \le P \le 0.1$.

RESULTS

Five birds refused to consume the meloxicam and were excluded from the analysis as previously mentioned. The average BW of the birds was 1.78 ± 0.03 kg (mean \pm SD, range: 1.38-2.23 kg) and they consumed on average 4.5 ± 1.22 mg/kg BW of the meloxicam. Birds that were healthy consumed on average 4.5 ± 1.01 mg/kg BW (95% CL of the mean 3.81-5.26), birds with FPD consumed 4.1 ± 0.23 mg/kg BW (95% CL of the mean 4.92 - 5.21), and birds with KBF consumed 3.9 ± 1.81 mg/kg BW (95% CL of the mean 2.61-5.19).

Effect of Meloxicam on Thermal Measurements of FPD and KBF

Birds with FPD had a larger temperature difference $(F_{2,29} = 12.00, P = 0.0002)$ as calculated by the subtraction of the maximum and minimum temperature measured on the line from the middle digit across the metatarsal pad (as per Wilcox et al., 2009). Overall, the average temperature difference was $7.9 \pm 0.60^{\circ}$ C in hens with FPD compared to 5.5 ± 0.63 °C in healthy birds $(t_{29} = -3.64, P = 0.0029)$ and 4.8 ± 0.63 °C birds with KBF ($t_{29} = 4.57, P = 0.0002$), whereas healthy and KBF birds had the same temperature difference $(t_{29} = 0.89, P = 0.6496)$. Moreover, we observed that the temperature difference decreased 2h after receiving the meloxicam (time 2) in hens with FPD (-17%), $t_{58} = 2.59, P = 0.0120$, whereas no such reduction was observed in birds with FPD that received the placebo $(-4\%, t_{58} = 0.51, P = 0.6136)$ (Figure 3A). This suggests that the meloxicam treatment was effective at decreasing foot temperature associated with inflammation, thereby reducing the temperature difference in the birds' feet, however, it should be noted that differences were small. Birds that did not have FPD (either healthy or with KBF) showed no decrease in temperature difference of the feet whether they were on the placebo (all P >(0.05) or had received the meloxicam (all P > 0.05) (Figure 3A). Keel temperature increased with time in birds with FPD (+1.0%, $t_{58} = -2.48$, P = 0.0160) and with KBF (+1.7%, $t_{58} = -3.00$, P = 0.0040) when on the placebo treatment (Figure 3B); an increase that was not observed in healthy birds. However, when birds received the meloxicam this increase was also no longer observed when birds had FPD ($t_{58} = -0.50, P = 0.6156$) and only

remained a tendency when birds had KBF (+1.0%, $t_{58} = -1.84$, P = 0.0704).

Biomechanics During Landing

The results of each outcome for both the 30 cm drop and 170 jump are presented by treatment, health status, and their interaction in Table 1.

Effect of Health Status and Meloxicam on Biomechanics of Landing From a 30 cm Drop

The average landing velocity of hens during the 30 cm drop was 2.1 ± 0.34 m/s (mean \pm SD, range: 1.5-3.0 m/s). The variance in landing velocity was not explained by the interaction between the health status and the treatment ($F_{2,19} = 0.64, P = 0.5405$) or treatment itself $(F_{2,19} = 0.13, P = 0.7238)$. However, health status tended to change landing velocity $(F_{2,19} = 3.34,$ P = 0.0571) with KBF birds tending to have a 17% higher landing velocity than FPD birds $(2.3 \pm 0.10 \text{ m/s})$ vs 2.0 ± 0.10 m/s). When examining the birds' health status within the placebo treatment, birds with KBF had a higher landing velocity than birds with FPD $(+21\%, t_{-28.94} = -2.57, P = 0.0471)$ but similar landing velocity as healthy birds $(+18\%, t_{-28.94} = -2.11,$ P = 0.1152). Birds with FPD and no injuries showed a similar landing velocity ($t_{-28.94} = 0.27, P = 0.9597$) (Figure 4). Once birds were on the meloxicam treatment, the same pattern was observed with highest landing velocity in birds with KBF, followed by healthy, and finally birds with FPD. However, no differences in landing velocity were found between birds with the different health conditions under meloxicam treatment (Figure 4, all P > 0.05).

While birds with different health statuses did not differ in their landing velocity when on the meloxicam treatment, further investigation showed that this cannot be attributed to the meloxicam treatment per se. Birds with a certain health status had the same landing velocity whether they received the placebo or meloxicam treatment (all P > 0.05).

The average maximum force as measured during 30 cm drops of laying hens onto the force plate apparatus was 56.5 ± 11.84 N (mean \pm SD, range: 29.3-78.2 N). This maximum resultant force translates to a force 3.3 ± 0.69 times the birds' BW (mean \pm SD, range: 1.4 -4.9) and tended to differ between birds of a different health status ($F_{2,19} = 3.06$, P = 0.0704). This tended to be 24% higher in KBF birds compared to FPD birds in general ($t_{19} = -2.37$, P = 0.0699). Mostly, this tendency held true under the placebo treatment ($t_{25.44} = -2.24$ P = 0.0903) and approached tendency on the meloxicam treatment ($t_{25,44} = -2.14$, P = 0.1087) (Figure 5). No differences were observed between healthy and FPD birds (all P > 0.05) or healthy and KBF birds (all P >(0.05) on either treatment (Figure 5). In general, there was no effect of treatment ($F_{1,19} = 1.63, P = 0.2165$) or treatment by health status interaction ($F_{2,19} = 0.46$,

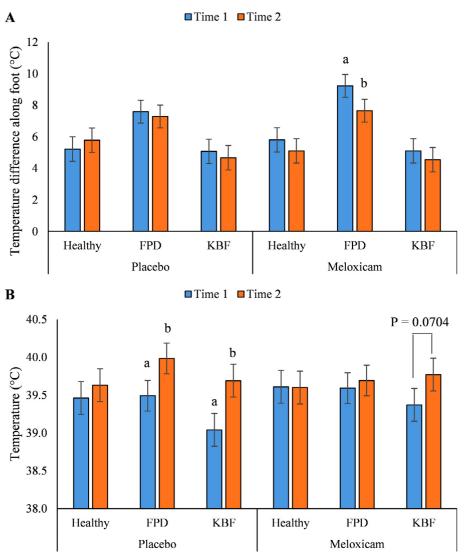


Figure 3. Thermal measurements of (A) the difference (°C) between the maximum and minimum temperature measured along the middle digit and metatarsal footpad, or (B) maximum temperature along the keel of laying hens. Birds were healthy (n = 10), had footpad dermatitis (FPD, n = 12), or had keel bone fracture (KBF, n = 10) and received a placebo or a meloxicam treatment in a cross-over design. Birds were measured before (time 1) or 2 h after (time 2) receiving a placebo or a meloxicam treatment. In birds with FPD (n = 12) the affected foot was measured, whereas in birds without FPD (n = 20), the right foot was measured. Results are presented as LSM and SE. Significance of the simple effect of time within treatment and health status is indicated; different lower case superscript letters indicate statistically significant differences in means before and after consumption of capsules within treatment and health status categories.

P = 0.6389) on the force expressed as multiples of BW. Providing meloxicam additionally did not alter the force expressed as multiples of BW in healthy (t₁₉ = -0.07, P = 0.9463), FPD (t₁₉ = 1.12, P = 0.2787), or KBF (t₁₉ = 1.3, P = 0.2099) birds.

Similar results were observed for the impulse of the 30 cm drop (health status $F_{2,19} = 1.38$, P = 0.2767, treatment $F_{1,19} = 0.92$, P = 0.3507, interaction $F_{2,19} = 0.86$, P = 0.4385) which averaged at 3.8 ± 0.59 N s (mean \pm SD, range: 2.6–5.5). The impulse did not differ between healthy, FPD, or KBF birds whether on placebo or meloxicam treatment (Table 1), however the impulse was numerically highest for KBF birds and lowest for FPD birds. Providing meloxicam additionally did not alter the impulse in healthy ($t_{19} = -0.52$, P = 0.6108), FPD ($t_{19} = 1.09$, P = 0.2898), or KBF ($t_{19} = 1.26$, P = 0.2246) birds.

Effect of Health Status and Meloxicam on Biomechanics of Landing from a 170 cm Jump

Birds (n = 15) had an average landing velocity of 3.9 ± 0.57 m/s (3.0–5.8 m/s). Neither health status (F_{2,12} = 0.93, P = 0.4220), or the interaction between health status and treatment (F_{2,12} = 0.62 P = 0.5557) significantly explained the variance in landing velocity (Table 1). However, at 170 cm treatment birds on the meloxicam treatment tended to show a 7% increase in landing velocity compared to the placebo treatment (F_{1,12} = 3.76 P = 0.0765). However, the meloxicam treatment did not appear to affect individual health conditions (all P > 0.05). Furthermore, there were no differences in birds that were healthy or had FPD or KBF within the placebo (all P > 0.05) or meloxicam treatment (all P > 0.05).

Table 1. Landing velocity (velocity, m/s), maximum resultant force (Max $F_{res}[N]$), maximum resultant force expressed as multiples of body weight (Max F_{res} times BW), and impulse (area under the force-time curve) of laying hens following a drop from 30 cm or jump from 170 cm height

	$30 \operatorname{cm} \operatorname{drop} (n = 22)$				$170~{ m cm~jump}~(n=15)$			
	Velocity (m/s)	$MaxF_{\mathrm{res}}\left(N\right)$	${\rm Max}\ F_{\rm res}\ {\rm times}\ {\rm BW}$	Impulse $(N s)$	Velocity (m/s)	$MaxF_{\mathrm{res}}\left(N\right)$	${\rm Max} {\rm F}_{\rm res} {\rm times} {\rm BW}$	$Impulse (N \; s)$
Treatment								
Placebo	2.1 ± 0.1	58.0 ± 2.5	3.3 ± 0.1	3.8 ± 0.1	3.8 ± 0.2	109.4 ± 5.2	6.6 ± 0.3	6.5 ± 0.2
Meloxicam	2.1 ± 0.1	55.0 ± 2.5	3.2 ± 0.1	3.7 ± 0.1	4.1 ± 0.2	113.2 ± 5.2	7.1 ± 0.3	6.7 ± 0.2
Health status								
Healthy	2.1 ± 0.1	56.6 ± 4.2	3.1 ± 0.2	3.8 ± 0.2	4.2 ± 0.3	113.2 ± 8.9	6.6 ± 0.6	7.4 ± 0.4
FPD	2.0 ± 0.1	51.2 ± 3.7	3.0 ± 0.2	3.5 ± 0.2	3.9 ± 0.2	106.3 ± 8.0	6.9 ± 0.6	6.1 ± 0.4
KBF	2.3 ± 0.1	61.6 ± 3.7	3.7 ± 0.2	4.0 ± 0.2	3.8 ± 0.2	114.4 ± 7.3	7.1 ± 0.5	6.3 ± 0.3
Interaction								
Placebo								
Healthy	2.0 ± 0.1	56.8 ± 4.7	3.1 ± 0.3	3.7 ± 0.2	4.0 ± 0.3	113.4 ± 9.9	6.3 ± 0.7	7.4 ± 0.4
FPD	2.0 ± 0.1	54.0 ± 4.1	3.1 ± 0.2	3.6 ± 0.2	3.7 ± 0.3	101.1 ± 8.9	6.4 ± 0.6	6.0 ± 0.4
KBF	2.4 ± 0.1	63.2 ± 4.1	3.8 ± 0.2	4.1 ± 0.2	3.8 ± 0.2	113.8 ± 8.1	7.1 ± 0.5	6.2 ± 0.4
Meloxicam								
Healthy	2.1 ± 0.1	56.5 ± 4.7	3.1 ± 0.3	3.8 ± 0.2	4.5 ± 0.3	113.1 ± 9.9	6.8 ± 0.7	7.4 ± 0.4
FPD	2.0 ± 0.1	48.4 ± 4.1	2.9 ± 0.2	3.4 ± 0.2	4.0 ± 0.3	111.5 ± 8.9	7.4 ± 0.6	6.3 ± 0.4
KBF	2.3 ± 0.1	60.1 ± 4.1	3.5 ± 0.2	3.9 ± 0.2	3.9 ± 0.2	114.9 ± 8.1	7.0 ± 0.5	6.5 ± 0.4
Treatment	P = 0.7238	P = 0.1731	P = 0.2165	P = 0.3507	P = 0.0765	P = 0.4308	P = 0.0609	P = 0.4458
Health status	P = 0.0571	P = 0.1596	P = 0.0704	P = 0.2767	P = 0.4220	P = 0.7373	P = 0.8328	P = 0.0818
Interaction	P = 0.5405	P = 0.6270	P = 0.6389	P = 0.4385	P = 0.5557	P = 0.6008	P = 0.1692	P = 0.8679

Results are presented as LSM and SE. Birds were either healthy (30 cm n = 6; 170 cm n = 4), had footpad dermatitis (FPD, 30 cm n = 8; 170 cm n = 5), or had keel bone fracture (KBF, 30 cm n = 8; 170 cm n = 6) and received a placebo or a meloxicam treatment in a cross-over design.

The average maximum force as measured during jumps from 170 cm height onto the force plate apparatus was 11.4 \pm 18.67 N (mean \pm SD, range: 83.9–154.6 N). The high maximum resultant force values meant that birds landed with a force 6.9 \pm 1.27 times their BW (mean \pm SD, range: 5.1–9.6). In line with the landing velocity, this outcome tended to be higher in birds treated with meloxicam than when birds consumed the placebo (F_{1,12} = 4.28, P = 0.0609, Table 1). This was driven by birds with FPD who landed with a 16% higher force as expressed as multiples of BW when on the meloxicam compared to the placebo treatment (t₁₂ = -2.51, P = 0.0275). However, birds with FPD did not show a different pattern from healthy or KBF birds on either the placebo (all P > 0.05) or the meloxicam (all P > 0.05) treatment. Finally, the variance in force expressed as multiples of BW was not explained by health status ($F_{2,12} = 0.19$, P = 0.8328) or the interaction between health status and treatment overall ($F_{2,12} = 2.07$, P = 0.1692).

Although treatment ($F_{1,12} = 0.62$, P = 0.4458) and the interaction between health status and treatment ($F_{2,12} = 0.14$, P = 0.8679) were not significantly related to differences in impulse of the 170 cm jump, there was a tendency for birds with different health status to show a different impulse ($F_{2,12} = 3.11$, P = 0.0818). Birds with FPD tended to have a lower impulse than healthy birds ($t_{12} = 2.35$, P = 0.0868). This result was mainly observed within the placebo treatment with impulse of FPD birds being 18% lower than in healthy birds ($t_{17.21} = 2.33$, P = 0.089) (Figure 6).

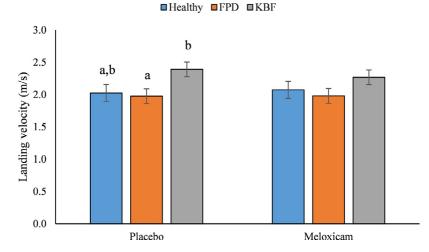


Figure 4. Effect of health status on the landing velocity (m/s) of laying hens treated with a placebo or meloxicam treatment during a 30 cm drop onto force plates. Birds were either healthy (n = 6), had footpad dermatitis (FPD, n = 8), or had keel bone fracture (KBF, n = 8) and received a placebo or a meloxicam treatment in a cross-over design. Results are presented as LSM and SE. Significance of the simple effect of health status within treatment is indicated; different lower case superscript letters indicate significant differences between health status within the placebo treatment.

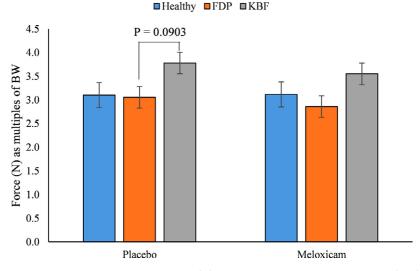


Figure 5. Effect of health status on the maximum resultant force (N) expressed as multiple of body weight (BW) of laying hens treated with a placebo or meloxicam treatment during a 30 cm drop onto force plates. Birds were either healthy (n = 6), had footpad dermatitis (FPD, n = 8), or had keel bone fracture (KBF, n = 8) and received a placebo or a meloxicam treatment in a cross-over design. Results are presented as LSM and SE. Tendency of the simple effect of health status within treatment is indicated.

DISCUSSION

We investigated differences in landing biomechanics (velocity, maximum force, and impulse, and) in birds that were healthy or had injuries that may impact the use of their fore and hind limbs. Health status was associated with different landing biomechanics depending on the type of injury (KBF or FPD) and the height from which birds landed.

Effect of Health Status on Bird Landing

KBF birds landed with a greater velocity and greater force compared to FPD birds at 30 cm. This finding, combined with the lack of difference in the impulse between KBF and FPD birds could suggest that birds with FPD may be trying to mitigate and cushion their landing on their feet, whereas birds with KBF perform less of a "brake" with their wings. Restrictions of movement or loading are beneficial behavioral responses to inflammation (Ackermann, 2012). Several studies have shown that birds with footpad inflammation alter their behavior (Weber Wyneken et al., 2015; LeBlanc et al., 2016; Mikoni et al., 2022). For example, chickens spent less time perching on inflamed footpads (LeBlanc et al., 2016) and turkeys with FPD showed lower walking speeds and stride lengths (Weber Wyneken et al., 2015). Thus, FPD birds may have adapted to spread the force with which they land more evenly. At the same time, birds rely on wing muscles to perform controlled landings to avoid injuries (Dial, 1992; Warrick et al., 2002) and need to be able to process distances and rates of deceleration (Lee et al., 1993). While not officially measured in the current study, most birds showed one (partial) wingbeat when dropped from 30 cm. In previous work, we observed that chickens performed on average 3 wingbeats from a 150 cm jump (León et al., 2021). Because the 30 drops were involuntary, birds were likely

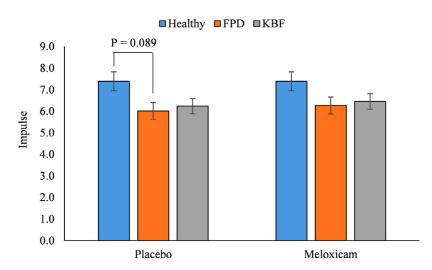


Figure 6. Impulse (area under the force-time curve) of landing of laying hens after a 170 cm jump onto force plates. Birds were either healthy (n = 4), had footpad dermatitis (FPD, n = 5), or had keel bone fracture (KBF, n = 6) and received a placebo or a meloxicam treatment in a cross-over design. Results are presented as LSM and SE. Tendency of simple effect of health status within treatment is indicated.

unable to fully decelerate by using their wings before touch-down, but potentially those birds with FPD were quicker to stretch and use their wings in any capacity than the KBF birds. However, the short time to react during the 30 cm drop may explain why no differences were observed with healthy birds.

According to the Canadian Code of Practice, the minimum height that must be provided to laying hens between all tiers including the floor level is 45 cm (National Farm Animal Care Council NFACC, 2017). At a drop from 30 cm, the birds already land with a velocity of 2 m/s and a force that is approx. 3 times theirown BW (50-65 N). However, the highest tiers or perches can be at much greater heights between 135 and 170 cm (National Farm Animal Care Council NFACC, 2017; Ali et al., 2019). At 170 cm, chickens experienced forces 6 to 7 times their BW (100–115 N) while landing with a 4 m/s velocity. This was similar to descent velocities measured in chickens jumping from 150 cm with intact or clipped wing feathers (León et al., 2021). Similarly, Banerjee et al (2014) observed landing forces of approx. 5 and 7 times their BW (81 N and 107 N, assuming 1.58 kg BW) in laying hens jumping from 41 and 61 cm heights, respectively. In contrast, birds which have not been exposed to selection pressures against flight, such as zebra finches and diamond doves, previously were recorded with landing velocities of approx. 1 m/s and forces of about 3.5 or 2.5 times their BW over a 1 m flight path distance at a 45 degree angle, respectively (Provini et al., 2014). Chickens kept for egg-laying as used in the current study are known to be poor flyers that are at the limit of their ability to control flight trajectories (Tobalske and Dial, 2000; Kozak et al., 2016; León et al., 2021), and so the higher landing velocities and force with which they landed are not surprising.

Potentially, their poor flight ability could explain why less differences between healthy, FPD and KBF birds were observed at 170 cm. Previous research had suggested laying hens with KBF had prioritized flight paths within an aviary though cause-and-effect could not be determined (Rufener et al., 2019). The need for a controlled landing where birds only had one specific flight path as in the current study might have forced all birds to use their wings to decelerate the same way, or they were all already at the maximal power output (León et al., 2021) and might not have more capacity to buffer their landing for injuries such as FPD or KBF. Thus, at 170 cm the differences between FPD and KBF birds may have become diluted. A tendency was observed where FPD birds had a smaller impulse while landing from 170 cm compared to healthy birds while landing with a similar velocity and force. As impulse and velocity are linear conversions via body mass, we would have expected differences in either force or velocity as well. While this was not statistically the case, it is likely that the small numerical differences observed (e.g., lower velocity and lower maximum force in FPD birds) contributed to this observed tendency. Alternatively, FPD birds may have shortened the duration of landing while not increasing maximum force by converting more of their kinetic energy at landing into work used to increase angular momentum. However, we did not evaluate these aspects in the current study. Finally, we should acknowledge that we worked with a limited sample size due to the nature of the research and some technical issues. As such, the power of the analysis to detect differences between birds of different health status ranged between 0.5 and 0.7 depending on the outcome, jump height and comparison. Future studies should aim to increase the sample size when exploring biomechanics in laying hens.

Effect of Meloxicam Treatment on Bird Landing

We predicted meloxicam would restore the landing biomechanics to similar values as observed in healthy birds, yet the meloxicam treatment did not have statistically significant effect on any landing measurements during the 30 cm drops. However, at 170 cm jumps the meloxicam treatment was associated with a tendency for a higher velocity and higher force while landing in all birds. This was driven by FPD birds on meloxicam which showed a greater force expressed as multiples of bird BWs compared to FPD birds on the placebo. Potentially, the FPD birds benefitted the most from the anti-inflammatory effects of the meloxicam (see next section). This then allowed these birds to land with a 16%higher force expressed in multiples of BW when on meloxicam compared to the placebo. Meloxicam was previously shown to increase thermal nociceptive thresholds and being antihyperalgesic (Caplen et al., 2013a; Hothersall et al., 2014, 2016) and lame birds provided with meloxicam showed an increase in latency to lie (Hothersall et al., 2016), walking velocity, and improved stability at slow speeds but imbalance at fast speeds (Caplen et al., 2013b). Possibly, a similar effect is observed in the current study where the pain threshold due to FPD is increased in birds receiving the meloxicam which translated to birds landing with greater force. However, the impulse remained the same in FPD birds on meloxicam or the placebo suggesting that FPD birds are still not fully able to cushion the higher landing force at touch-down, potentially increasing the risk of further injuries.

We hypothesized that meloxicam would also function as an analgesic for KBF birds, however, birds with KBF did not alter their landing velocity, force or impulse following the meloxicam treatment. Despite being an important welfare issue in laying hens, less research has evaluated the use of analgesia in relation to KBF (Nasr et al., 2012b, 2015; Rentsch et al., 2019). Nasr et al. (2015) found no influence of meloxicam or carprofen (NSAID) on latency to fly down from perches in laying hens with KBF; however, latencies were decreased under butorphanol (opioid analgesic) treatment while mobility indices otherwise remained largely unchanged (Nasr et al., 2012b). Provision of paracetamol (also named acetaminophen) in drinking water also did not improve bird mobility or walking speed on ramps in birds with KBF (Rentsch et al., 2019). Rentsch et al. (2019) suggested that changes in mobility could also be independent of pain perception and that severe fractures may be needed for animals to experience pain. In humans, pain is experienced when the fracture breaks the periosteum or the outer layer (Mach et al., 2002). Potentially, the end-of-lay birds in the current study did not suffer from extreme enough KBF, or all KBF were healed at 70 wk of age, to pick up differences following meloxicam treatment. Edgar et al. (2023) reported no difference in hens with potentially healed or unhealed KBF at 68 wk of age in fear, anxiety or transitional movements. The KBF itself could have caused changes in bird landing biomechanics irrespective of pain or behavior due to biological limits. It would be interesting to examine whether more granular measurements of KBF through radiographs or necropsy would yield different results. The inconclusive results regarding the effect of analgesia on laying hen mobility require further investigation. Furthermore, it should be acknowledged that the study was likely underpowered to detect small differences in landing biomechanics following meloxicam treatment (upper range: 0.4 - 0.5).

Meloxicam as Anti-Inflammatory Treatment of Footpad and Keel Injuries

Meloxicam appeared effective as an anti-inflammatory by reducing the footpad temperature differences in FPD birds and somewhat preventing the increase in keel temperature in FPD and, to a lesser extent, KBF birds. It should be acknowledged that, as poultry has been selected for increasing metabolism, which came with the expense of thermoregulation, thermal imaging has been found to have a lower sensitivity for detecting inflammatory changes in poultry (Beaufrère and Mills, 2023). The observed differences were small (<2°C difference in temperature values), but for FPD birds the meloxicam led to a 17% reduction in temperature difference along the footpad. A reduction of 2°C along the middle digit (III) to the metatarsal footpad could indicate a change from clinical to mildly clinical or sound feet as per Wilcox et al. (2009). The observed anti-inflammatory effects may not have been sufficient to also influence the birds' pain perception per se. However, meloxicam previously increased weight-bearing, caused changes in gait, increased walking velocity, latency to lie, and lowered nociceptive thresholds in birds with lameness or arthritis (Cole et al., 2009; Caplen et al., 2013b, a; Hothersall et al., 2014, 2016). Similarly, birds that received meloxicam showed more weight-bearing and lower pain scores with a quicker return to behavior activity before induced femur fracture compared to birds that received saline (Desmarchelier et al., 2012).

Meloxicam also appeared to reduce the increase in temperature along the keel bone observed in FPD and KBF birds under placebo treatment. The feet are a source of heat dispensation as opposed to the general feathered and insulated body in different birds (Powers et al., 2015). In chickens with FPD, it is possible that they cannot sufficiently dissipate heat through their feet and therefore dissipate proportionally more through modulation of feather placement over sternal region which is featherless between the pterylae. Furthermore, an inflammatory response is suggested in laying hens with healing KBF (Wei et al., 2019) which could potentially explain the increase in keel temperature under placebo conditions which was not observed in healthy birds. In contrast, others found lower temperatures in the keel area of KBF birds via thermal imaging and suggested that healed KBF may no longer be associated with inflammation (Nasr et al., 2015). We could not determine the age or healing status of the KBF of the birds in the current study. Regardless, the analysic properties of meloxicam may still influence bird behavior through influencing nociceptive processing (Nasr et al., 2015).

The studies described above provided the meloxicam subcutaneous or intra-muscular in contrast to the voluntary consumption approach used in the current study. As such, birds in the current study consumed a variable dose close to the max. 5 mg/kg BW. We had hoped to use the voluntary consumption as a measure of self-medication depending on the severity of the injuries when designing the experiment; however, this was not explored further due to most birds consuming all capsules offered. On the other hand, not all birds consumed the meloxicam when needed, leading to 5 birds being excluded from the analysis. Additionally, most studies used different avian species (e.g., pigeons, parrots, broilers) or types of analgesic (e.g., meloxicam, carprofen, butorphanol, betamethasone, bupivacaine) (Cole et al., 2009; Desmarchelier et al., 2012; Caplen et al., 2013b, a; Hothersall et al., 2014, 2016), which could explain discrepancies among results considering differences in pharmacokinetic parameters such as volume of distribution and clearance rate among bird species (Baert and De Backer, 2003).

We described landing biomechanics in chickens that were injured or uninjured. In general, laying hens landed with high velocities and subsequently high forces that need to be absorbed at touchdown. Tendencies for relatively large changes in landing velocity (+17% in KBF)birds) and force (+24% in KBF birds) during 30 cm drops were found, possibly reflecting a reluctance of wing use in birds with KBF and an attempt to cushion landing on inflamed footpads in birds with FPD. At jumps from greater heights (170 cm) birds' limited flight abilities likely required maximum use of fore and hind limbs such that birds might not have capacity to buffer their landing for injuries such as FPD or KBF. Implications of injuries for bird welfare may be more far reaching and affect biomechanics during landing which should be considered in laying hen housing systems.

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DISCLOSURES

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