Development of Domestic Fowl Locomotion over Inclined Surfaces and Use of Anticipation Strategies

by

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ABSTRACT

DEVELOPMENT OF DOMESTIC FOWL LOCOMOTION OVER INCLINED SURFACES AND USE OF ANTICIPATION STRATEGIES

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The aim of this thesis was to determine the safest method for vertical ascent in domestic fowl to prevent injuries and falls. The first objective was to determine locomotor style and climbing capacity (0-70°) in relation to age and the surface substrate (sandpaper or wire grid). The second was to measure modulation of hindlimb (step velocity, foot contact time and variation in center-of-pressure (COP)) and peak ground-reaction-force (GRF) in anticipating ramps (0, 40, 70°). Chicks and adults performed walking to climb 40° inclines and did not differ in the GRFs compared to 0°. They performed wing-assisted incline running or aerial ascent on steeper inclines (70°) and generated higher GRFs with longer foot contact times. Wire grid surfaces improved contact on steep inclines. Age did not have a significant effect upon GRFs relative to body weight, COPx, COPy, and velocity (x and y). Therefore we recommend incline angles of $\leq$ 40°.

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LIST OF SYMBOLS AND ABBREVIATIONS

h = thickness of material that covered the force plate = 0.006 m

t1 = time of initial step contact with force plate

t2 = time of final step contact with force plate

Fx: anterior-posterior force

Fy: medio-lateral force

Fz: vertical force

Mx: moment about the anterior-posterior axis

My: moment about the medio-lateral axis

Mz: moment about the vertical axis

Vx: velocity along anterior-posterior axis

Vy: velocity along medio-lateral axis

COPx: center of pressure along anterior-posterior axis

COPy: center of pressure along medio-lateral axis

GRF: vertical ground-reaction force

CHAPTER 1: GENERAL INTRODUCTION

1.1 The problem

One of the biggest welfare issues in the laying hen industry today is keel bone (bone extending from the sternum) damage (KBD) and fractures (FAWC, 2010; 2013). Birds housed in different systems (free-range, free-run, organic and furnished cage) (Wilkins et al., 2011; Petrik et al., 2015; Bestman and Wagenaar, 2014) and across different genetic lines will acquire KBD to some degree (Kappeli et al., 2011). KBD is a major welfare concern because of the likely pain associated with it (Nasr et al., 2012a; 2012c; 2015). Non-cage systems allow for more freedom of space and increased natural locomotion (Lay et al., 2011), however approximately 80% of birds will have KBD (Wilkins et al., 2004; 2011). Birds housed in conventional and furnished caged systems will have similar or less KBD (83% and 36% of birds, respectively) (Hester et al., 2013; Wilkins et al., 2011) which is assumed to be due to less collisions with the system (Harlander-Matauschek et al., 2015). With different frequencies of KBD in different housing systems, housing design and/or management plays an important role in the cause of these injuries (Rodenburg et al., 2008; Wilkins et al., 2011). There may be other contributing factors to KBD such as, genetics, nutrition (calcium uptake) or other environmental factors, but their specific importance and interactions are largely unknown (Whitehead, 2004a).

1.2 The housing system

With the ban of conventional cage systems in Europe in 2012 and the recent decision to phase out conventionally caged systems in Canada by 2026, the push towards adopting free-run and aviary systems is greater than ever. Currently in Canada, 90% of laying hen housing systems are conventional cage systems (Egg Farmers of Canada, 2016). However, large retailers in North America, such as McDonalds, are phasing out cage systems all together (conventional and furnished (addition of nest areas, more space, perches and scratch mats)) in order to improve laying hen welfare by allowing the birds to perform natural behaviour and locomotion. In addition, building multiple tier levels within systems is becoming attractive for producers since it allows for increased stocking density with three-dimensional space. The birds are required to reach different levels within the system to obtain essential resources such as food and water. However, *Gallus gallus domesticus* are poor fliers; they are ground birds with limited flight abilities (Dial, 2003). Therefore, an increase in collisions with the system or other birds in aviary systems is assumed to be due to imprecise movements (Gregory and Wilkins, 1996; Moinard et al., 2004a) or during "escape" behaviour and panic from a perceived life-threatening event (Richards et al., 2012). During the high-energy movements, the keel bone is at greater risk of becoming damaged or fractured since it protrudes out from the body and is generally the first body part to hit an object such as perches (Scott et al., 1997; Moinard et al., 2004a; Sandilands et al., 2009; Wilkins et al., 2011). The design of proper aviary systems for laying hens from the birds' point of view and to prevent injuries or falls is yet to be determined.

1.3 Locomotion

Understanding how these birds navigate between levels is essential to fully understand the appropriate design of housing systems to improve their welfare. Wild jungle fowl spend 70% of their daily time budget foraging by walking on the ground (Collias et al., 1966) and they seek elevated refuges only when threatened or roosting (Jones and Goth, 2008). Birds will fly to the lowest branch and move upward, branch by branch, to move to the highest branch for roosting. They return to the ground by flying directly onto the ground (Savory, personal communication; see Moinard et al., 2004a). In a commercial aviary system, birds are motivated to reach elevated surfaces where food, water and perches are located, or to escape a perceived threat or dominant conspecific. However, these domestic birds may have different challenges which could impair their movement such as overcrowding, foot-pad lesions or foot pad dermatitis (Wilkins et al., 2011), feather loss (Heers et al., 2011), and KBD (Nasr et al., 2012a). In a commercial aviary system, laying hens had a prevalence of 9-21% of failed landings after flight, with the majority of collisions being with other birds (Campbell et al., 2016). Therefore providing an inclined surface to connect tier levels may be the best solution to prevent injuries and falls. Stratmann and colleagues (2015a) demonstrated that the addition of short inclined platforms (ramps) and horizontal platforms within a commercial aviary system (for adult birds) to connect the tier levels reduced falls by 55%, collisions by 41%, and increased transitions between tiers by 44%. However, this did not result in lower keel-bone fracture rates (verified via dissection) (Stratmann et al., 2015a).

There has been very little focus on laying hen locomotion, however learning their basic locomotor abilities can provide insight to the cause of KBD and how they move within aviary systems. Comparable studies have examined climbing locomotion in wild-type birds including: Brush-turkeys (*Alectura lathami*) (Dial and Jackson, 2011), Black–billed magpies (*Pica hudsonia*) (Dial et al., 1997), Chukar partridges (*Alectoris chukar*) (Bundle and Dial, 2003; Dial et al., 2008; Jackson et al., 2009; Tobalske and Dial, 2007) and Rock doves (*Columba livia*) (Dial, 1992; Jackson et al., 2011). These studies explored age-dependent locomotor abilities and flapping behaviour, but this has never been done in the domestic chicken. Wild Galliform birds are fully capable of flight but they prefer to perform wing-assisted incline running (WAIR) to ascend inclines at all ages (Dial et al., 2008). WAIR is described as running with hindlimbs in combination with wing-flapping of the forelimbs. WAIR provides an energy saving strategy for birds compared to flight since it requires less aerodynamic energy output (Tobalske and Dial, 2000; 2007). Exploring these processes in domestic fowl on inclined surfaces throughout development can provide insight to the appropriate degree of incline to connect tier levels within housing systems that will improve locomotion. Birds use their breast muscles (pectoralis major), which originate from the keel-bone, to drive the wing and generate aerodynamic force during climbing (Tobalske and Dial, 2007; Jackson et al., 2011). Therefore, when domestic fowl perform vigorous flapping behaviour it may detrimental to the health of the keel-bone due to tensile force on an already weakened keel. Understanding the locomotor patterns and capabilities in domestic fowl will not only provide insight to the appropriate design of aviary systems from the birds' point of view, but will also allow us to reduce injuries or falls within these systems.

1.4 Anticipating inclines

As birds approach inclined surfaces, it is important to understand whether they can anticipate the steepness of the incline and how they appropriately modulate their hindlimbs. If birds cannot predict how they should effectively prepare to climb a ramp, it can lead to many falls and injuries. Anticipation has recently been defined as an actively organized system within the central nervous system which has, at some point, been outlined by potential acts or environmental features that can be connected to result in a specific behaviour (Jarvilehto *et al.,* 2013). This behaviour is triggered by a stimulus from the environment, which allows the body to retrieve the information from the organized system (Jarvilehto *et al.,* 2013). Once a sensory input (i.e. sight, touch, temperature) is conveyed to the brain, a response will be carried out by the motor system or musculo-tendon system to react appropriately (Borghuis *et al.,* 2008). For example, when driving a car, we must anticipate that the traffic light will turn red to press the brakes in a timely manner to prevent car accidents.

Research has shown that experience or training aids in anticipation by allowing the subject to build an organized framework by exploring an environment and identifying action alternatives for different circumstances (Jarvilehto *et al.,* 2013). This priming technique is what allows for fast action and skilled responses (i.e. driving in humans, Jarvilehto *et al.,* 2013). It is often used for research measuring motor behaviour (Rosenbaum, 1985). Although, if there is an impairment in a sensory input (Lord et al., 2006) or motor response (Sturnieks et al., 2008; Brauer et al., 2001; Szulc et al., 2005) such as inadequate lighting, obstructed walkways (Carter et al., 1997), or uneven surfaces, the body's ability to maintain balance is challenged and there is a great risk of falling incidences.

When an animal wants or anticipates a goal, it may adjust its behavioural patterns, which can be locomotive or investigatory, before retrieving a reward (Spruijt et al., 2001). The behaviour of "anticipation" when expecting a reward has been described in laying hens as showing attentive movements and stretching their necks (Moe et al., 2009). Zimmerman et al. (2011) also found that when birds expect a positive event they display comfort behaviour (preening or wing flapping) and stand alert more often, however regardless of whether the anticipated event was positive or negative, laying hens increase the frequency and intensity of head movements. These behavioural adjustments are related to different decision-making processes. A more difficult decision (such as, a steeper incline) involves finely balanced options, for example, a bird may be highly motivated to reach a reward but the cost of falling or experiencing injuries is high due to a steep surface. A bird must decide if the risk of falling is worth it based on priority and/or the cost-benefit analysis. It has been shown that significantly more cognitive effort is required for a finely balanced decision (Moffatt, 2005), which demonstrates the importance of investigating anticipatory behaviour leading up to a decision. Whether birds can anticipate various inclines effectively is crucial for the proper design of aviary systems to provide safe transitions between tier levels.

Humans can anticipate steeper inclines by generating greater hindlimb ground reaction forces (GRFs) (Sheehan and Gottschall, 2012b) in the last steps before making the transition to climb. In addition, during stair and slope walking, humans are less stable in the anterior-posterior and medial-lateral directions compared to walking on level ground (Sheehan and Gottschall, 2012a). Transitions to inclined surfaces appear to be more challenging compared to transitions from a slope to level ground (Sheehan and Gottschall, 2012a). On average, transitions to sloped surfaces lead to a 40% increase in fall risk due to less stability in the transitional steps (Sheehan and Gottschall, 2012a). Lastly, over 50% of all the reported fall injuries have occurred during slope or stair walking (CDC, 2007). Based on these findings, we can infer that bipedal birds could respond similarly to inclined surfaces.

1.5 Aims of thesis:

The purpose of this thesis was to investigate the development of locomotion skills in chicks, pullets and laying hens during ramp ascent for the design of appropriate aviary systems to improve their welfare. Each paper had specific aims.

Manuscript 1: How does the locomotor style and climbing capacity differ between different strains (Lohmann Brown, Hyline Brown, Dekalb White, Lohmann LSL lite) of domestic fowl throughout development during ramp ascent of various inclines to reach elevated tier levels? Differences in conformation across strains could affect their abilities to perform WAIR and the three-dimensional space utilized. Locomotor style may depend on the age of the birds as it has been shown that wild ground birds perform asymmetric wing flapping as fledglings then transition to WAIR, and preferentially perform WAIR as adults.

Manuscript 2: Examines the anticipation of different inclines in adult domestic fowl (Lohmann Brown, Hyline Brown, Dekalb White, Lohmann LSL lite). It was investigated whether birds can anticipate steeper inclines by adjusting their hindlimbs accordingly. The ability to anticipate inclines can reduce the incidences of falls and injuries in commercial aviary systems.

CHAPTER 2: Development of locomotion over inclined surfaces in domestic fowl

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2.1 ABSTRACT

During post-fledging development, precocial birds are hypothesized to routinely rely on the integrated use of their hindlimbs and forelimbs to navigate in three-dimensional environments and to reach elevated surfaces. Previous studies have suggested fledgling chukar partridges will use wing-assisted incline running (WAIR) before they are able to fly, but the generality of this behaviour to other species remains largely unknown. The goal of the present study was to evaluate locomotor strategies during development in domestic chickens (*Gallus gallus domesticus*); we were motivated, in part, by current efforts to improve the design of housing systems in agriculture. Using four different commercial strains, we tested for locomotor style and climbing capacity (0-70°) in relation to the degree of incline, the age of the birds and the surface substrate (sandpaper or wire grid). Chicks and adult fowl performed walking behaviour in order to climb 40° inclines, and performed WAIR or aerial ascent on steeper inclines. Wire grid surfaces were important for improved hindlimb contact on steep inclines and reduced the amount of wing use. When birds increased in age/ experience this resulted in increased WAIR or aerial behaviour. Consistent with a fundamental wing-stroke hypothesis, wing kinematics of chicks were of a similar pattern to that of mature birds, as indicated by a conserved wing angular velocity. White feathered strains performed more wing-associated locomotor behaviour compared to brown feathered strains. The implication of the results for the design of three-dimensional housing systems involves providing 40° inclines which are easily negotiated (without wing use) by chicks and adult fowl.

2.2 KEYWORDS: incline, avian locomotion, performance, WAIR, chicks

2.3 INTRODUCTION

For most animals, locomotion is fundamental for survival: to escape predators, to search for essential food resources, to mate and to thermoregulate (Drake et al., 2001; Walker et al., 2005; Dial and Jackson, 2011). Animals usually rely on several types of locomotion to varying degrees depending on factors including their morphology and features of their habitat (Sapir and Dudley, 2012; Bueno and Motta-Junior, 2015; Enriquez-Urzelai et al., 2015).

Although locomotion in the field can occur on horizontal and planar ground, the real world is often neither planar nor simple in structure. For example, ascending to a tree branch will usually require motion in the vertical direction. The nature and orientation of the branch influences whether and how animals move onto it (Higham and Biewener, 2008). Moving along an incline in comparison to a level surface involves much greater effort and may be challenging to control, especially for 'ground-dwelling' birds (Dial and Jackson, 2011; Jeffery et al., 2013). Climbing requires additional effort to move because gravity acts against the motion depending on the angle of inclination (Birn-Jeffery and Higham, 2014). In order to avoid injury during climbing, birds prevent uncontrolled movement using their feet as the first and last contact with the surface (Pike and Maitland, 2004) and maintain balance with their wings (Necker, 2006). There are also some birds that use their tails to balance themselves (e.g. woodpeckers; Short, 1970). Mammals grasp substrates using their extremities and the frictional properties of the surfaces are essential for securely holding and climbing on inclined planes (Adolph et al., 1993; Schmitt, 2010). Studies examining bird locomotion on inclines have mainly focused on the mechanical demands of hindlimbs (e.g., Daley and Biewner, 2003; Gabaldon et al., 2004), with less focus on the forelimbs or wing mechanics (Birn-Jeffery and Higham, 2014). Wing and forelimb functions are important factors that should be considered when investigating inclined surface locomotion (Autumn et al., 2006; Lammers et al., 2006; Lee, 2011). It is known that several wild birds such as the Brush-turkey (*Alectura lathami*), Black–billed magpie (*Pica hudsonia*), Chukar partridge (*Alectoris chukar*) and the Rock dove (*Columba livia*) employ age-dependent modes of locomotion during walkway ascent: asymmetric flapping immediately after fledging transitions to wing-assisted incline running (WAIR), in which the wings generate forces that supplement the work of the hindlimbs by accelerating the animal upward and toward the substrate (Dial et al., 2008; Jackson et al., 2009; Tobalske and Dial, 2007). Later, even when the birds are fully capable of flying upward, in some circumstances they still preferentially perform WAIR.

As natural environments are spatially complex, precocial birds, including ducks (*Anseriformes*) and pheasants and their allies (*Galliformes*), are likely compelled to move through this complexity during their development and before they are fully capable of flight. Recent research has demonstrated that they vary the relative contributions of their legs and wings as a function of their developmental state (Dial and Carrier, 2012). In a laboratory setting Muir et al., (1996) and Muir (2000) showed that domestic fowl chicks are able to locomote using only their legs within 6-8 hours after hatching and that young chicks prefer to be in contact with the horizontal ground supported by two legs. However, when escaping on level ground young chicks prefer to walk or run with the additional assistance of their wings (Collias and Collias, 1967). Under natural conditions, adult domestic fowl spend 70% of their active time foraging while walking on the ground (Collias et al., 1966). When threatened, Galliformes can outrun predators and use their wings for brief escape flights (Dial and Jackson, 2011). Domestic hens seek elevated refuges only when they are threatened or roosting (Jones and Göth, 2008). When roosting, they fly up to the lowest branch of a tree and from there make their way further up by moving from branch by branch. They return by flying directly to the ground (Savory, personal communication; see Moinard et al., 2004).

In complex non-cage housing systems for commercial egg production, domestic fowl are at a high risk of bone damage from collisions with the environment (Harlander-Matauschek et al., 2015) due to increased locomotion (up to 80% in adult birds; Wilkins et al., 2004). Although these systems vary in design, they generally consist of multiple stacked tiers (as high as 4 m (C. LeBlanc, unpublished observation)) in a shelf-like manner allowing for increased space as well as providing essential resources on the different tiers (food, water, nests, or perches) (Aerni et al., 2005). On average, the heights of the 1st two tiers are 70 cm and 160 cm in commercial settings. Domestic fowl must be able to reach these tier heights in order to gain access to resources but are often not provided with a climbing aid to do so. Providing a continuous path or walkway may be important for birds to make safe transitions from the ground to the multiple tier levels. A primary motivation for our present study was to improve understanding of mechanisms developing chickens use for navigating complex 3D environments; as such information may be useful for improving animal welfare in agricultural systems.

In the current study, we investigated age-dependent responses of domestic fowl of four commercial strains (Lohmann LSL lite, Dekalb White, Lohmann Brown, and Hyline Brown) to various inclined surfaces in order to reach an elevated tier in a test arena. To determine potential differences in locomotor performance on various inclines, we assessed the following: (1) Do domestic fowl successfully climb various inclined surfaces for two different tier heights? (2) How do birds differ in their mode of locomotion (for example: walking, WAIR or aerial ascent) as inclines become steeper? (3) Are the success rates and modes of locomotion dependent on (a) experience, (b) strain or (c) surface material? (4) Do bird morphometrics or kinematics contribute to locomotor performance?

First, we hypothesized that domestic fowl would require different strategies for successfully managing the two different tier heights. Based on prior research using wild-type birds (Dial, 2003; Jackson et al., 2009), we predicted that domestic chickens would show an increase in the use of wing-assisted locomotion when ascending steeper inclines and that the performance on steeper inclines should vary with experience (age), wing loading (weight per unit wing area), wing kinematics and surface substrates with different friction or grip.

2.4 MATERIALS AND METHODS

2.4.1 Ethical statement

This study was approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol Number 2501) prior to testing.

2.4.2 Animals and housing

A total of 40 chicks (Lohmann Brown, Lohmann LSL lite, Dekalb White and Hyline Brown) were used in this experiment. There were approximately 10 birds in each home pen (strains were separated by pen), and 20 test birds were chosen randomly (5 birds of each strain) at the start of the experiment (1^{st} week of life) and individually tagged. The home pens (182 x 243 x 280 cm) contained wood shavings on the floor, a total of four platforms at two platform heights mirrored within each pen (70 cm and 160 cm), and a wooden ramp (width = 15 cm) and ladder (width = 15 cm) at 45° to vertically connect them. The birds were provided with a dark brooder box with a heat pad until they were 8 weeks of age, and nest boxes were put in each pen at 16 weeks of age. Birds were provided with nipple drinkers and commercial feed in round feeders ad libitum. Also, the birds were supplemented with hay and straw. Standard commercial management by the University of Guelph Arkell Poultry Research Station staff was provided.

2.4.3 Experimental design

Starting in their second week of life, the 20 domestic fowl comprising four different strains (5 birds per strain) (Lohmann Brown, Lohmann LSL lite, Dekalb White and Hyline Brown) were tested on seven walkway inclines (0 (horizontal control), 20, 30, 40, 50, 60, 70º), with two different walkway (15 cm wide) surface materials (60-grit Mastercraft sandpaper and 2.5 cm^2 wire mesh grid), at two constant tier heights (70 cm and 160 cm). Horizontal starting (37 cm length on the ground) and landing (41 cm length) platforms flanked the ramp sections. Chicks were trained to move up the walkway in the first week of life. The inclines tested for the higher tier (160 cm) did not include 0º and 20º due to space limitations within the test arena.

Each test consisted of an individually neck-tagged (Ketchum, Canada) subject beginning in the start box until the start door was lifted after a 3 second pause by the experimenter. The subject then had one minute (determined from pilot study) to climb up to the tier height being tested. Chicks were provided with motivation to climb using 5 same-age and same-strain chicks (pen mates) in a crate on each tier in addition to providing a tablespoon of their commercial feed along with 5 raisins for each tier. Experimenters were blinded to the strains as much as possible during the entire testing period. All subjects were presented with inclines in a systematic variation of increments/decrements (weeks 2, 3, 4, 5, 6, 7, 8, 9, 11, 13, 15, 17, 19, 21, 26, 31, and 36). In each testing week, all birds were tested once on both surface materials for every ramp incline for each tier height. Once the birds started to lay eggs at 17 weeks of age, testing began in the late morning to allow for eggs to be laid before testing. Experimenters observed the behaviour of each subject for each incline tested (See Ethogram, Figure 2.7.1) from outside the test arena via a Japan's Victor Company (JVC) application on an ipad® mini (see *video*, below) in order to avoid visual distractions for the birds. When birds were successful (reached the tier height and did not reach the one minute maximum time to initiate ascent), the mode/modes of locomotion were observed and recorded for each test.

2.4.4 Video

Each individual test was video recorded using a JVC GC-PX100BU HD Everio camcorder (high speed video capture, 600 Hz) that was mounted within the test arena. The videos served several functions in addition to capturing the behavioural data of each bird. From these videos, we calculated wing beat frequency (Tobalske and Dial, 1996), wingbeat amplitude (distance from wingtip at peak upstroke and downstroke) (Tobalske and Dial, 2000), and change in angle of wingbeat (degrees) to calculate angular velocity (radians/second) (Dial et al., 2008).

2.4.5 Morphometrics and kinematics

Each subject was weighed and photographed with their wings spread against a solid background in order to obtain wing area (analysed in ImageJ®) to calculate wing-loading (body mass divided by surface area of both wings) (Dial and Jackson, 2011). These variables were measured on a weekly basis for weeks 2-9 of testing, biweekly for weeks 11-21, and every 5 weeks from weeks 26-36. Low wing-loading (large wings relative to body size) represents the ability to produce high aerodynamic forces relative to body weight.

In weeks 2-9 of life, video records from the individual birds that performed WAIR on the 50° incline (majority of WAIR behaviour was performed on the 50° incline) with the coarse sandpaper (60-grit Mastercraft) surface substrate were digitized frame by frame, in order to calculate wingbeat frequency, wingbeat amplitude and wing angular velocity. All values for body mass, wingbeat frequency, wingbeat amplitude and angular velocity were log transformed to derive linear scaling relationships. Angular velocity of the wing was calculated as total angular excursion of the wing divided by wingbeat duration (radians per second).

2.4.6 Statistical analyses - behaviour

Data were analyzed in 3 groups: weeks 2-4, 5-7, and 8-36. The purpose of the analysis of weeks 2-4 was to compare how chicks managed the inclines for only the 1st tier height (70 cm) and using only the sandpaper covered ramps. Testing on the wire mesh grid began when birds were 5 weeks of age when their feet were physically large enough to grip the grid and not fall through. Analysis of weeks 5-7 compared the behaviour of the birds on both (sandpaper and wire mesh grid) surface materials for the 1st tier height only (70 cm). Birds were only tested on the 2nd tier height (160 cm) when they were observed to reach the same height in their home pens (8 weeks of age). Finally, analysis of weeks 8-36 compared the behaviour of the birds on both surface materials (sandpaper and raised wire mesh grid) for both tier heights (70 and 160 cm).

Statistical analyses were performed using the generalized linear mixed model procedure (PROC GLIMMIX) in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA, 2012). Plots of studentized residuals were used to assess normality and confirm the best fitting distribution. Full models with all fixed and random effects were then fit to the appropriate distribution and model fitting criteria (AIC) were used to select the best fitting model.

The proportion of success, walking, WAIR, and aerial ascent for each walkway test was calculated (N= 5 for each strain, total N= 20 birds) based on a binary distribution. For weeks 2-4 analysis, the strain, week (age), incline and interactions (strain*week, strain*incline and week*incline) were included as fixed effects. For weeks 5-7 analysis, the strain, week (age), incline, material, and interactions (strain*week, strain*incline, strain*material, material*week, week*incline, and incline*material) were included as fixed effects. For weeks 8-36, the strain, week (age), incline nested within tier height, material, and interactions (strain*week, strain*incline, material*week, week*incline, incline*material, tier*material, tier*week, tier*strain, incline (tier), material*incline (tier), strain*incline (tier), and week*incline (tier)) were included as fixed effects. The incline was nested within tier height because there were different inclines tested for each tier height (0° and 20° were not tested for the higher (160 cm) tier height). All 3 sets of analysis (weeks 2-4, weeks 5-7 and weeks 8-36) included the co-variable of week*wingloading. Parameters were estimated using LAPLACE integral approximation (METHOD=LAPLACE). A compound symmetry structure (TYPE=CS) was fit with bird nested within strain as the repeated measure subject. All dependent variables (count outcomes) were fit with complementary log-log link (LINK= CCLL). Denominator degrees of freedom were determined using the Containment method in the GLIMMIX procedure (Littell et al., 2006). Results are presented as mean estimates ± standard error of the mean in the results, and raw data are presented as percentage of birds ± standard error in the graphs. Statistical comparisons performed on the model scale were considered significant at $P < 0.05$, and displayed in the graphs if $P<0.05$, $P<0.01$ and $P<0.001$.

2.4.7 Statistical analyses – morphometrics and kinematics

The same statistics program and model were used for the morphological and kinematic data except for some minor differences. The Gaussian distribution was fit for all the following variables: wingloading, log body mass, log wingbeat frequency, log wingbeat amplitude and log angular velocity. A first order autoregressive covariance structure (TYPE=AR (1)) was fit as the repeated measure subject for the wingloading, log body mass, log wingbeat frequency and log angular velocity data. A heterogeneous AR (1) covariance structure (TYPE= ARH (1)) was fit as the repeated measure subject for the log wingbeat amplitude variable. The Kenward-Roger method (DDFM= KR) was used to determine denominator degrees of freedom for wingloading and log body mass data. The Containment method (DDFM= CONTAIN) was used to determine denominator degrees of freedom for log wingbeat amplitude, log wingbeat frequency and log angular velocity.

Wingloading results are presented as an average for each strain across week ± standard error of the mean.

Results are presented as raw data for log wingbeat frequency, log wingbeat amplitude and log angular velocity plotted against log body mass ± standard error and all statistical differences were considered significant for $P < 0.05$.

2.5 RESULTS

2.5.1 Weeks 2-4 (1^{st} tier height only and sandpaper covered inclines)

All chicks were successful on 0°, 20°, 30°, and 40° inclines. When considering 50° and 60° ($F_{1, 129.5}$ =4.19, P=0.0427), the number of successful tests (per bird) decreased (93% and 62%) as the inclines became steeper ($t_{129.5}$ = 2.05, 2.79±1.36, P= 0.0427). All chicks performed walking behaviour on 0°, 20°, 30°, and 40°, and therefore did not employ WAIR.

When considering 50° and 60° inclines, chicks performed walking and WAIR behaviour on the 50° incline, but did not perform walking behaviour on the 60° incline. There was a significant ($F_{3, 128.9}$ = 3.24, P=0.0243) strain*incline effect for WAIR behaviour which can be attributed to the fact that Lohmann LSL lite chicks performed more WAIR on 60° (83% of birds) compared to 50° (42% of birds) ($t_{128.9}$=-2.23, -1.52±0.70, P= 0.0275). All other strains performed more WAIR on 50° (71% of birds) compared to 60° (53% of birds).

2.5.2 Weeks 5-7 (1st tier height and both surface materials on the inclines)

All birds were successful on 0°, 20°, 30°, and 40° inclines for both materials. When considering 50°, 60° and 70° for both materials, there was a significant ($F_{4,279.3}$=2.86, P=0.0237) week*incline effect. This could be attributed to an increase in success rate across weeks (weeks 5, 6 and 7) for the 50° incline, but not for the 60° and 70° inclines (Fig. 2.7.2A). In week 6, there was a lower number of successful tests compared to weeks 5 and 7 on the 60° and 70° inclines (Fig. 2.7.2A).

There were also significant material*incline ($F_{2, 279.3}$=4.75, P=0.0093) and material*week ($F_{2, 279.3}$=4.73, P=0.0096) effects for successful tests. For both materials, there was no difference in the number of successful tests on the 50° incline; however as inclines became steeper (60° and 70°), birds were more successful on the wire grid material (60°: $t_{279.3}$= - 3.70, - 1.29±0.35, P= 0.0003; 70°: $t_{279.3}$= - 4.89, - 2.27±0.46, P< 0.0001) compared to the sandpaper material. In addition, as the birds aged, they were more successful on the wire grid material. However, on sandpaper covered inclines, birds were more successful in weeks 5 and 7 compared to week 6 (Fig. 2.7.2B).

All birds performed walking behaviour on 0°, 20°, and 30° inclines, and did not perform WAIR behaviour. There was a significant ($F_{1, 58}$= 7.35, P= 0.0074) material*incline effect when considering walking behaviour on 40° and 50° inclines. As inclines became steeper (50°) there was much less walking behaviour performed on the sandpaper material (40°: 85% of birds; 50°: 17% of birds) compared to wire grid material (40°: 95% of birds; 50°: 78% of birds) (t_{158}= -5.16, -3.14±0.61, P< 0.0001). Birds did not perform any walking behaviour on 60° and 70° inclines for both surface materials.

Similar to weeks 2-4, birds did not perform WAIR behaviour until ascending 50°, 60° and 70° inclines. There was a significant ($F_{4,265}$=3.50, P= 0.0083) week*incline effect which may be due to the fact that in weeks 6 and 7 birds performed WAIR behaviour less often as the inclines became steeper, but birds in their 5th week of life performed more WAIR on 60° (45% of birds) compared to 50° (34% of birds) (t_{265} = -2.82, -1.49±0.53, P= 0.0051). There was also a significant ($F_{2,265}$ = 18.94, P< 0.0001) incline*material effect for WAIR behaviour. Birds performed more WAIR behaviour on the sandpaper material for the 50° incline (t_{265} = 5.24, 2.25±0.43, P< 0.0001), but the opposite occurred for the 70° incline due to birds performing more WAIR on wire grid material (Fig. 2.7.3A). There was also a significant ($F_{2,265}$ = 5.83, P= 0.0033) week*material effect, which can be attributed to the fact that birds performed more WAIR on the sandpaper material in week 5, but as the birds increased in age (weeks 6 and 7), they performed more WAIR on the wire grid material (Fig. 2.7.3B).

Aerial ascent did not begin until the birds were in their 4th week of life. However, during weeks 5-7 when considering 40°, 50°, 60° and 70°, there were significant ($F_{3,372}$ =7.42, P< 0.0001) incline and ($F_{1,372}$ =15.10, P= 0.0001) material main effects. Birds performed significantly more aerial ascent on the 60° (25% of birds) and 70° (29% of birds) inclines compared to 40° (6% of birds) and 50° (7% of birds) (60° vs. 40°: t_{372} = -3.27, -1.68±0.51, P= 0.0012; 60° vs. 50°: t_{372} = -3.33, -2.04±0.61, P= 0.0010; 70° vs. 40°: t_{372} = -3.39, -1.75±0.52, P= 0.0008; 70° vs. 50°: t_{372} = -3.43, -2.11±0.62, P= 0.0007). In addition, birds performed more aerial ascent on inclines with wire grid surface material (24% of birds) compared to sandpaper surface material (9% of birds) (t_{372} = -3.89, -1.60±0.41, P= 0.0001).

2.5.3 Weeks 8-36 (1st and 2nd tier heights with both surface materials for each)

During this stage of development, birds were successful at mastering 20°, 30°, and 40° inclines. When considering 50°, 60° and 70° there was a significant ($F_{2,2330}$ = 5.74, P= 0.0033) material*incline(tier) effect (Fig. 2.7.4A). Interestingly, when birds were tested on the higher tier (160 cm), the wire grid material was more important for success, which was reflected by this interaction. For sandpaper covered inclines, there was a significant decrease in success rate for the 2nd tier height (39%, 17%, 5%), but not for the 1st tier height (77%, 67%, 66%) (Fig. 2.7.4A).

There was also a significant ($F_{6,2330}$ =2.47, P= 0.0218) strain*incline (tier) interaction for bird success (Fig. 2.7.4B, 2.7.5). On the 1st tier height, birds were always more successful at 50° while there was no difference in success at 60° and 70° (Fig. 2.7.4B). However, on the 2nd tier, as inclines became steeper, Lohmann LSL lite, Lohmann Brown and Dekalb White birds experienced a significant decrease in their success rates, but Hyline Brown birds did not have a significant decrease in success from 60° to 70° (Fig. 2.7.5).

All birds performed walking behaviour on 0°, 20° and 30° inclines, and did not perform WAIR. When considering a comparison between 40° and 50° there was a significant ($F_{3,\ 1481}$ = 5.31, P= 0.0012) strain*incline interaction. For sandpaper material covered inclines, the Lohmann Brown strain was the only strain to perform walking behaviour on the 50° incline for both tier heights (Fig. 2.7.6A). For the wire grid material, the Hyline Brown, Lohmann Brown and Dekalb White birds demonstrated a decrease in walking behaviour as the inclines became steeper (50°), but the Lohmann LSL lite strain demonstrated the same amount of walking behaviour for both inclines (Fig. 2.7.6B). Birds did not perform any walking behaviour on 60° and 70° inclines for both surface materials.

There were also significant ($F_{3,\ 1481}$ = 3.12, P= 0.0252) strain*material and tier*strain ($F_{3,\ 1481}$ = 4.19, P= 0.0058) interactions. All strains performed more walking behaviour on the wire grid material compared to the sandpaper material however; the difference was much greater for Hyline Brown birds compared to the other strains. This was demonstrated by the fact that Lohmann Brown birds performed significantly more walking behaviour on the sandpaper material compared to Hyline Brown (t_{1481} = -2.61, -1.11±0.43, P= 0.0092), and conversely, the Hyline Brown birds performed significantly more walking behaviour on the wire grid material compared to the Dekalb White birds (t_{1481} = 1.96, 1.04±0.53, P= 0.0497). Lohmann LSL lite and Lohmann Brown demonstrated no differences in the amount of walking behaviour when comparing both tier heights. Hyline Brown birds (t_{1481} = 2.28, 1.23±0.54, P= 0.0229) and Lohmann Brown birds (t_{1481} = 2.87, 1.43±0.50, P= 0.0042) performed significantly more walking behaviour compared to Dekalb White birds on the 1^{st} tier height.

Birds started to perform WAIR at 40° and continued to do so at 50°, 60° and 70°. There was a significant ($F_{30,\ 3180}$= 1.62, P= 0.0179) week*incline (tier) interaction. When considering the 1^{st} tier height, birds in weeks 8 (t_{3180} = -3.41, -1.91±0.56, P= 0.0007), 9 (t_{3180} = -3.68, -2.46±0.67, P= 0.0002) and 11 (t_{3180} = -3.66, -2.45±0.67, P= 0.0003) performed more WAIR behaviour on steeper inclines (60°) compared to 40°. As the birds grew older in week 13 (t_{3180} = 3.11, 2.63±0.85, P= 0.0019), 17(t_{3180} = 1.98, 1.62±0.82, P= 0.0476), 19 (t_{3180} = 3.87, 4.56±1.18, P= 0.0001), 21 (t_{3180} = 2.77, 1.98±0.71, P= 0.0057), 26 (t_{3180} = 5.08, 4.10±0.82, P<0.0001), 31

(t_{3180} = 2.99, 3.64±1.22, P= 0.0028) and 36 (t_{3180} = 4.15, 3.65±0.88, P< 0.0001) they performed more WAIR on shallower inclines (40° and 50°) compared to the steeper inclines (60° and 70°). For the 2nd tier height, when the birds were 8 weeks of age (t_{318} = -3.71, -3.99±1.06, P= 0.0002) they performed more WAIR on 70° incline compared to 40°, and as they matured (weeks 13-36) they demonstrated no differences in WAIR behaviour for the inclines.

Birds started to perform aerial ascent on the 40° incline and continued for 50°, 60° and 70° for this age class. There were significant ($F_{3,\ 3183}$ = P< 0.0001) material*incline and ($F_{10,\ 3183}$ = 2.51, P= 0.0084) week*material interactions. Birds performed significantly more aerial ascent on the sandpaper material at 50° (t_{3183} = 6.13, 2.30±0.40, P< 0.0001) and 60° (t_{3183} = 4.13, 0.93±0.22, P= <0.0001), but this was the opposite for 70° (t_{3183} = -2.85, -0.68±0.24, P= 0.0044), where birds performed more aerial ascent on the wire grid material. As birds developed, there was an increase in the number of birds performing aerial ascent on both materials, however there was more of a dramatic effect for the sandpaper material; the difference in the amount of aerial ascents performed for the materials was much greater. Birds at 31 weeks of age (t_{3183} = -2.55, -2.55±1.00, P= 0.0108) and 36 weeks of age (t_{3183} = -2.42, -3.32±1.37, P= 0.0157) performed significantly more aerial ascents compared to birds at 15 weeks of age on the sandpaper material.

2.5.4 Morphometrics and kinematics (weeks 2-9)

The body mass of the birds had a significant strain*week interaction ($F_{21,54.44}$=4.21, P<0.0001) which could be attributed to Hyline Brown birds and Lohmann Brown birds having the lowest body masses in week 2 of life, but then increased to the highest in week 9. Conversely, the Lohmann LSL lite birds and Dekalb White birds demonstrated the highest body mass in week 2 but then decreased to lowest in week 9. There was an obvious increasing trend in wing-loading throughout development, with brown strains having higher wingloading compared to white strains (Fig. 2.7.7). However, there was no effect of week*wingloading as a co-variable across all age groups.

The wingbeat frequency and wingbeat amplitude of the birds changed as the bird's increased in body mass (wingbeat frequency: $F_{1,\ 35.9}$ = 9.05, P= 0.0048; wingbeat amplitude: $F_{1,\ 9.89}$ = 45.27, P< 0.0001) (Fig. 2.7.8A and 2.7.8B). The wingbeat frequency gradually decreased as body mass increased while the wingbeat amplitude gradually increased. There was no effect of strain for wingbeat frequency and wingbeat amplitude (wingbeat frequency: $F_{3, 26.8}$ =1.70, wingbeat amplitude: P= 0.1916; $F_{3, 8.88}$ =1.28, P= 0.3393). The angular velocity did not change as the bird's body mass increased ($F_{1, 35.9}$ =0.02, P= 0.8904) and there was also no effect of strain ($F_{3, 32.3}$ =1.15, P= 0.3442) (Fig. 2.7.8C).

2.6 DISCUSSION

In this study we tested four strains of domestic fowl throughout development in their ability to ascend inclined ramps of varying heights, angles and surfaces. The main results indicate that chicks and mature birds were able to master inclines of up to 40° with only walking behaviour, and performed WAIR to ascend steeper ramps. In addition, wire grid surfaces provided grip to help birds climb – especially steeper inclines. We observed that chicks were more successful in performing WAIR than older birds, and particular strains were more successful than others.

All birds from two weeks old through maturity were successful in ascending ramps at 20°- 40° angles at 70 cm and 160 cm heights without the use of their wings. These results do not reflect the expectation that motor development would first require birds to undergo significant morphological changes (Dial and Carrier, 2012). These include body size, tail muscle function, feather development, and claw length and curvature. Chicks' heads and torsos are relatively large compared to their legs and wings, and vice versa for adult domestic birds. It has been observed that tail and feather function contributes to lift, while claw function allows for more controlled motion up the ramp (Gatesy and Dial, 1996; Tobalske and Dial, 2007). More developed claws are able to interact with the grooves on the ramp and provide an upward force (Bock and Miller, 1959). Since no differences were observed within the 20°- 40° range in the present study, these morphological changes did not alter the birds' abilities to walk on these inclines.

Chicks and mature birds had different amounts and different sources of motivation to ascend the inclines (Ryan and Deci, 2000). The birds might have found the task itself rewarding, associating a positive experience with exercising and extending their willingness to climb. Incentives included food rewards, seeing or hearing a conspecific, and desiring to reach the top with intrinsic motivation. Without providing social attraction or food rewards, birds would not be motivated to use elevated surfaces, especially at less than 9 weeks of age (Kozak et al., 2015 submitted). In this study, reaching conspecifics as chicks seemed to be the most important and this desire decreased over time. It has been demonstrated chicks become distressed when they are alone or away from their group, and this behaviour stops once they are reunited with their conspecifics (Collias and Collias, 1967). Date from the current study indicates that as birds matured they ignored their conspecifics and food rewards or intrinsic motivation took precedence. Other studies have also found that after 2 weeks of age, chicks begin to increase their distance from their conspecifics and explore their environments (Muir et al., 1996; Wood-Gush, 1971). This distance continues to increase as the chick's mature as they develop positive associations with other resources such as raisin food rewards.

When ramps were configured at angles greater than 40° for both tier heights (70 cm and 160 cm), there was an increased use of WAIR. The birds used their wings to create an upward force or to maintain balance and control while ascending the ramp (Heers et al., 2014; Dial, 1992; Dial, 2003; LeBlanc et al., 2015 submitted). This type of motion, WAIR, requires much more energy compared to walking (Crabtree and Newshome, 1972). The birds make use of their breast muscles (pectoralis major) to generate enough force to assist with climbing (Tobalske and Dial, 2007; Jackson et al., 2011). In the case of inclined motion, gravity acts down the ramp and opposes the motion. The larger the angle, the more force required by the bird to move. In this study, the amount of WAIR performed on steep inclines (60° and 70°) was dependent on age and strain. Young birds demonstrated successful WAIR more frequently than older birds, especially for the Lohmann LSL lite strain. They were able to do this by making use of their fluffy, feathered wings to climb the steep slopes. In general, young birds were found to have lower wing-loading (ratio of weight to surface area of the wings) which requires less propulsion to move. Birds of the white strain (Lohmann LSL lite and Dekalb White) had the lowest wing-loading when compared to the brown strains (Lohmann Brown and Hyline Brown). An interesting finding was that morphological differences at a young age did not affect the angular velocity of the wings during WAIR, which means that young birds performed it with similar kinematics to mature birds. This is consistent with a "fundamental wing stroke hypothesis" that juveniles perform a similar wing pattern until adulthood, which may be intrinsic to the motor program of developing birds (Dial et al., 2008).

It is important to animal welfare that domestic fowl develop these appropriate techniques at a young age (Harlander-Matauschek et al., 2015), which we observed in this study. The force that propels a bird (air resistance on the wings) depends mostly on the surface area and angular velocity of its wings. As a result, younger birds are more capable of ascending steep ramps using WAIR despite not having undergone many morphological developments.

The capacity to use WAIR and, in general, the ability to ascend ramps depended on the amount of friction and grip provided by the ramp. Domestic birds may act on similar information to that of children to assess an incline, who make use of exploratory looking and touching movements (Adolph, 1995). Children do this by stretching the skin of their digits/toes to touch the inclined surface and adjust their movement to avoid a backward or sideways slip. Similar patterns are exhibited by wild-type chukars (*Alectoris chukar*), where birds make little to no forward progress on non-textured inclines due to slippage, even though the birds used their fully functional wings to assist them (Dial, 2003). In our trials, all birds were more successful in reaching the tier levels on wire grid surfaces than sandpaper ramps. Hyline Brown birds demonstrated increasingly more walking behaviour on the wire grid surfaces as they increased in age (weeks 8-36). This could be because white strains have retained more instinctive tendencies to perform wing-associated behaviours associated with predator-escape throughout selection in comparison to brown strains (Hakansson and Jensen, 2005).

As birds mature, they develop morphological features that can have variable effects upon ground and aerial movement. Tail muscle function assists locomotion by providing lift, movement control and balance (Gatesy and Dial, 1996). This has been observed in peacocks (Askew, 2014) and barn swallows (Balmford et al., 1993). Also, tail feather coverage may decreases the amount of lift required from the wings during ascent. Alternatively, the additional surface area from the feathers results in more drag acting against the motion (Veit and Jones, 2003). Whole body feather coverage has been shown to affect balance in domestic fowl, with poor feather coverage demonstrating a decrease in rotational and linear balance on an elevated moving perch (LeBlanc et al., 2015 submitted). In this study the birds had intact feather cover throughout the entire testing period, which may have contributed to the high success rates. Another feature affecting locomotion is claw length and curvature. In woodpeckers, claw length and curvature have been shown to be important for assisting in climbing, especially in nature where it is essential for bird's claws to dig into the bark of a tree while climbing it (Bock and Miller, 1959). This is applicable to the wire grid surfaces, where developed claws would be able to grasp the wire more effectively.

On inclines greater than 40°, birds performed aerial ascent behaviour more frequently as they increased in age (weeks 8 -36). Mature birds had larger wing-loading and had to produce larger aerodynamic forces to overcome gravity. Why is it that birds perform more aerial behaviour when they need to produce larger aerodynamic forces? Perhaps the morphological developments mentioned previously contributed to the success by providing more control, balance and lift (Gatesy and Dial, 1996; LeBlanc et al., 2015 submitted). Increased development of the mass of flight muscles was probably important (Dial and Carrier, 2012). Other features may have increased the bird's coordination and confidence over time. Interestingly, birds performed more successful aerial behaviour when tested on the wire grid surface compared to the sandpaper surface. They began by grasping the wire grid before flight to provide a take-off force (C. LeBlanc, unpublished observation). When a bird lands, the momentum from their motion tends to cause difficulties and instability. Therefore, grip on the landing surface was also essential. It is possible that other features of the surfaces contributed to the amount of aerial ascent behaviour performed such as ramp width (15 cm), but this was not tested in the current study.

Keel bone damage can have an effect on wing-associated behaviours, since the wing muscles are attached to the keel bone and pain may inhibit use of the muscles (Proctor and Lynch, 1993). In this study, there were no keel bone injuries or fractures found in the birds at the end of the study, which was determined via palpation. This may be because they were trained and tested on inclines starting from their first week of life until they were fully developed. During this time the birds may have developed sufficient muscle and bone strength to protect their keels from fractures or damage.

This research provides new insight on how domestic chicks and mature fowl can walk up ramp inclines up to 40° without the assistance of their wings, but must use their wings on steeper inclines. A wire grip surface enabled more effective ascent than sandpaper on all inclines and also facilitated takeoff and landing. Lastly, presumably because of muscle development, mature birds were more willing to perform aerial behaviour compared to young chicks regardless of increased wing-loading. Providing inclined walkways within rearing environments in agricultural settings will likely provide chicks with locomotor experience that should improve their capacity to safely negotiate complex rearing and laying environments (LeBlanc et al., 2015 submitted; Harlander-Matauschek et al., 2015).

2.7 TABLES AND FIGURES

Success: bird reaches the tier height being tested, with both feet on the tier

Fail: after 1 minute, the bird does not move up the ramp OR the bird moves away from the test arena

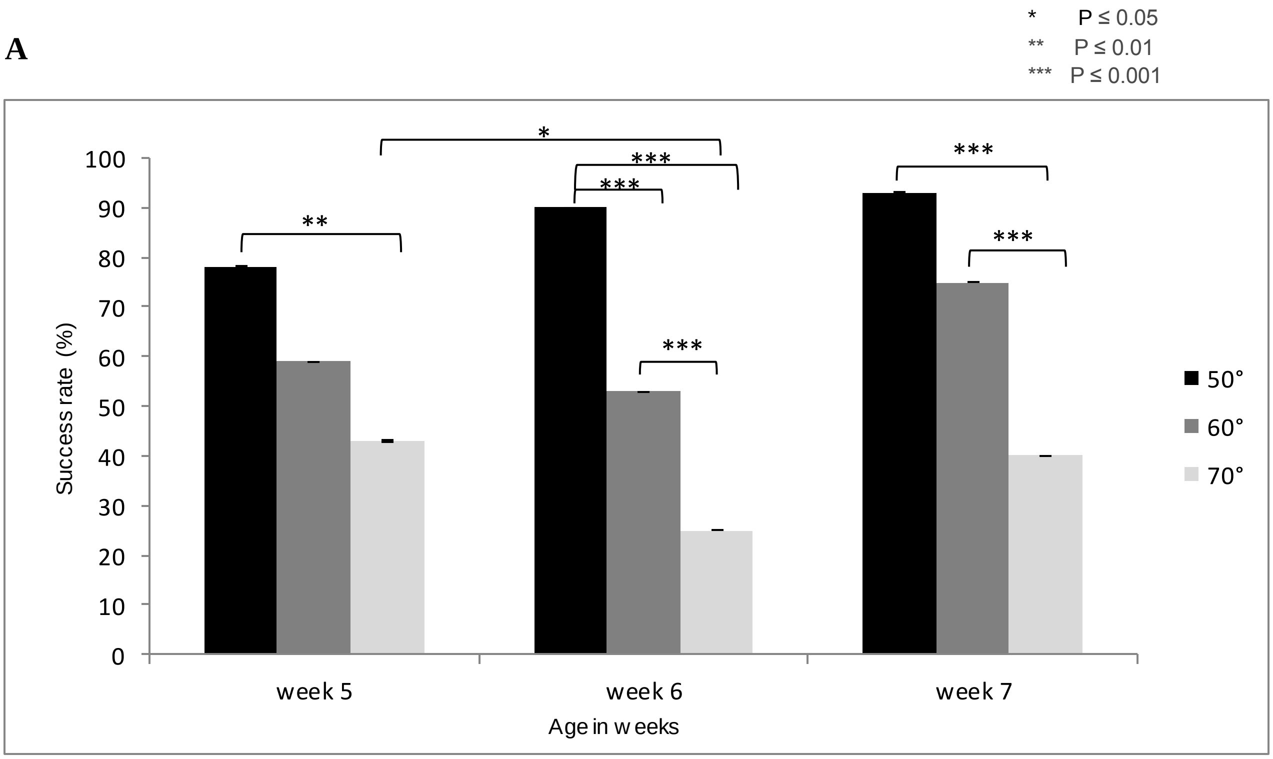
Walk: putting one foot in front of another at any pace (bipedal), without any assistance from the wings (no wing movement)

Wing-assisted incline running (WAIR): a combination of bipedal walking and wing flapping, with no aerial time

Aerial ascent: use of hindlimb jumping in addition to aerial wing flapping.

Figure 2.7.1 Ethogram describing the behaviour of birds observed on the walkway inclines.

A



B

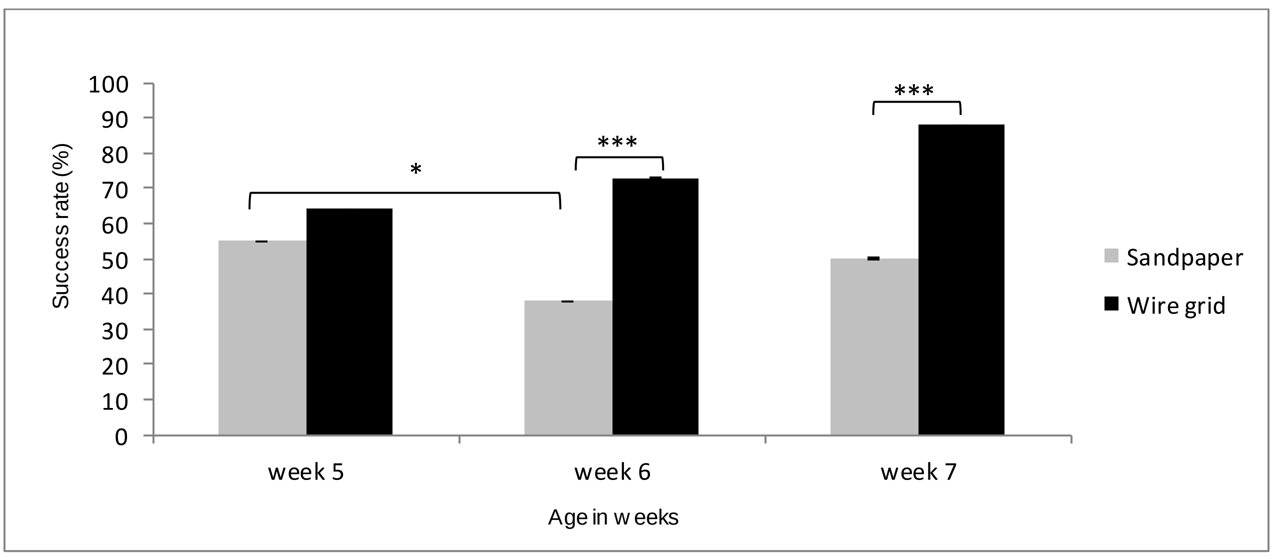
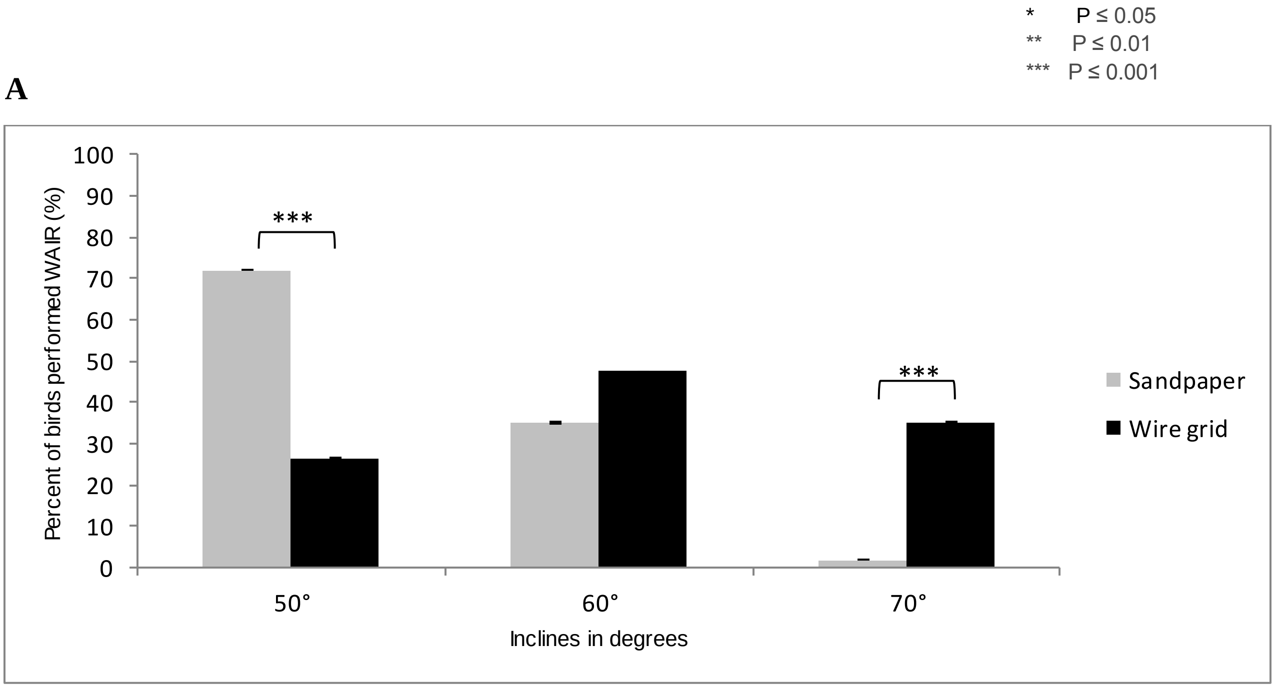


Figure 2.7.2 Success rate to reach the 1st tier height on 50°, 60° and 70° inclines for four strains (Hyline Brown, Lohmann Brown, LSL lite and Dekalb White) (*N* = 5 for each strain, displayed together, total = 20) of birds in weeks 5, 6, and 7 of life (± s.e.m.).

(A) each incline across weeks.

(B) each material across weeks.



A

B

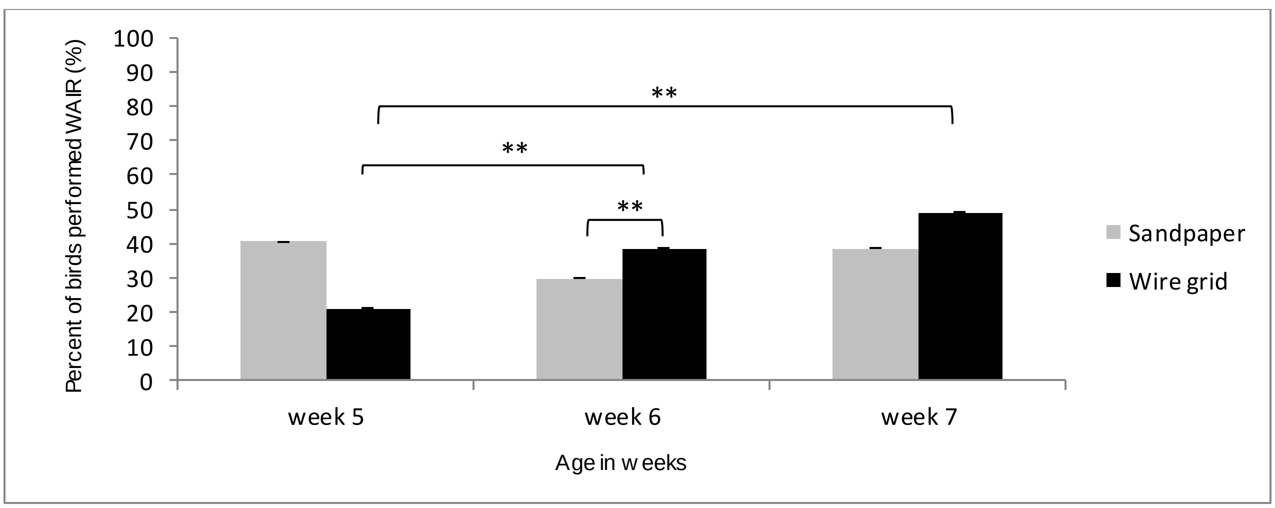
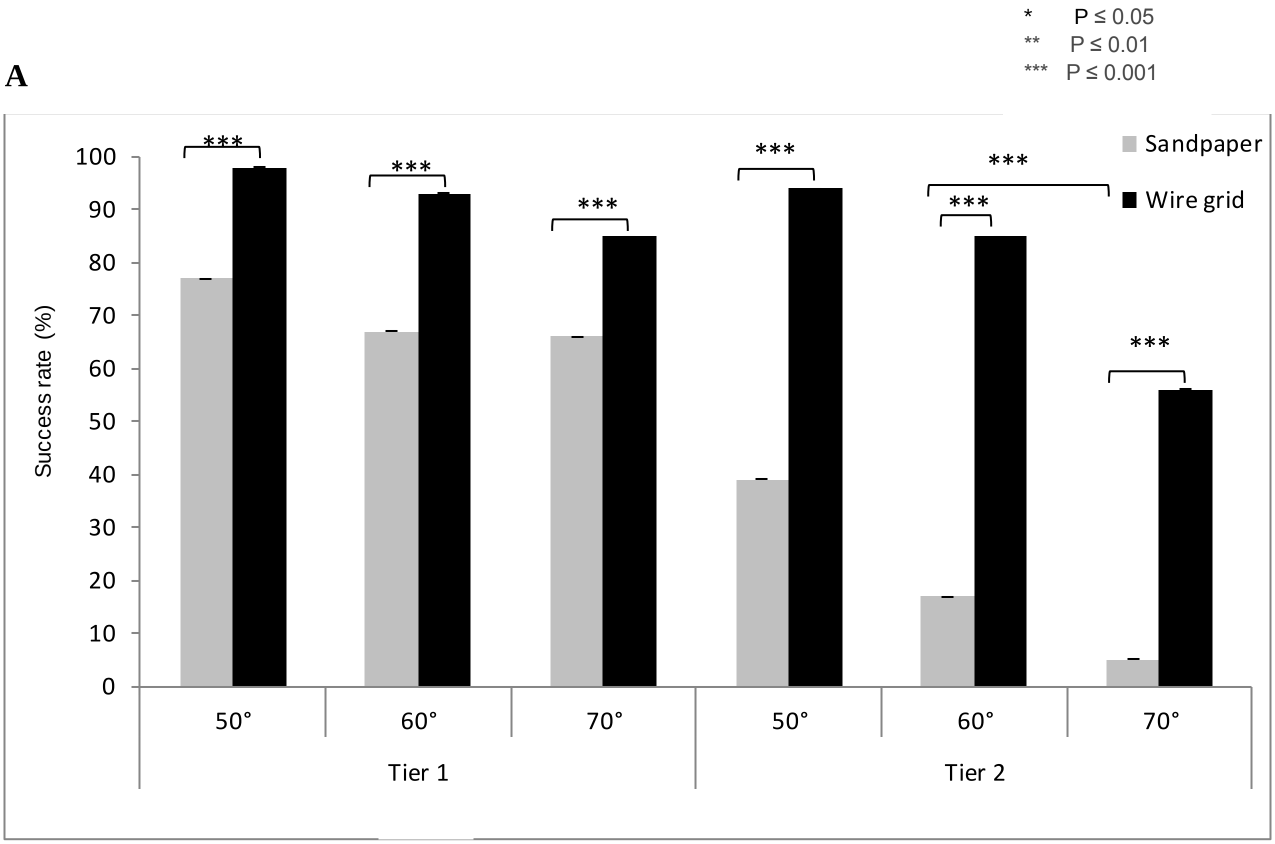


Figure 2.7.3 WAIR behaviour performed to reach the 1st tier height on 50°, 60° and 70° inclines for four strains (Hyline Brown, Lohmann Brown, LSL lite and Dekalb White) (*N* = 5 for each strain, displayed together) of birds in weeks 5, 6, and 7 of life (± s.e.m.).

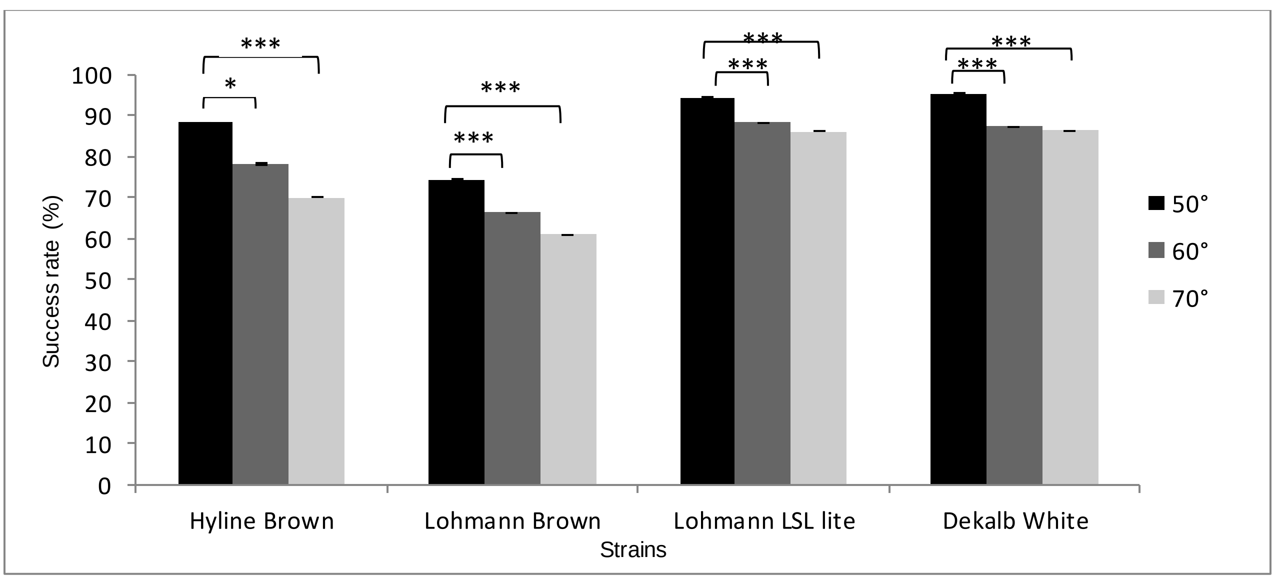
(A) each material on the inclines.

(B) each material across weeks.

A



B



2.7.4 Success rate to reach the tier on 50°, 60° and 70° inclines for four strains (Hyline Brown, Lohmann Brown, LSL lite and Dekalb White) (*N* = 5 for each strain) of birds in weeks 8-36 of life (± s.e.m.).

(A) each incline for both tier heights comparing the materials.

(B) each incline for the 1st tier height comparing the different strains.

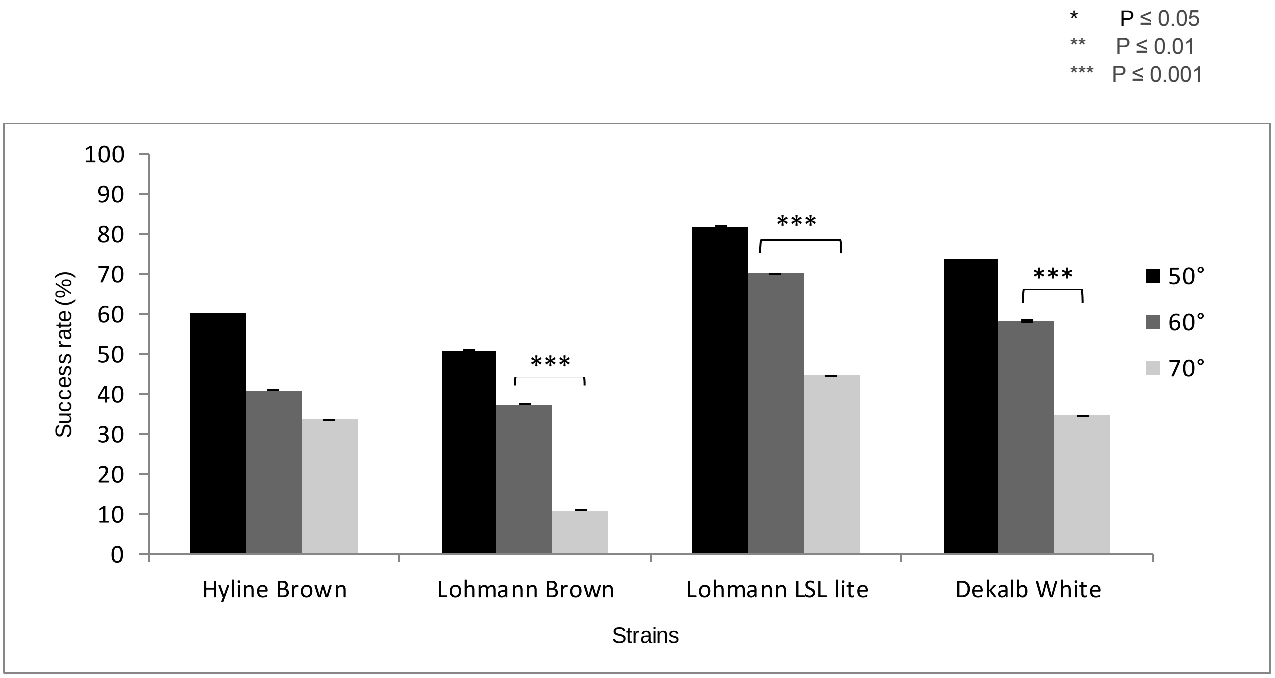
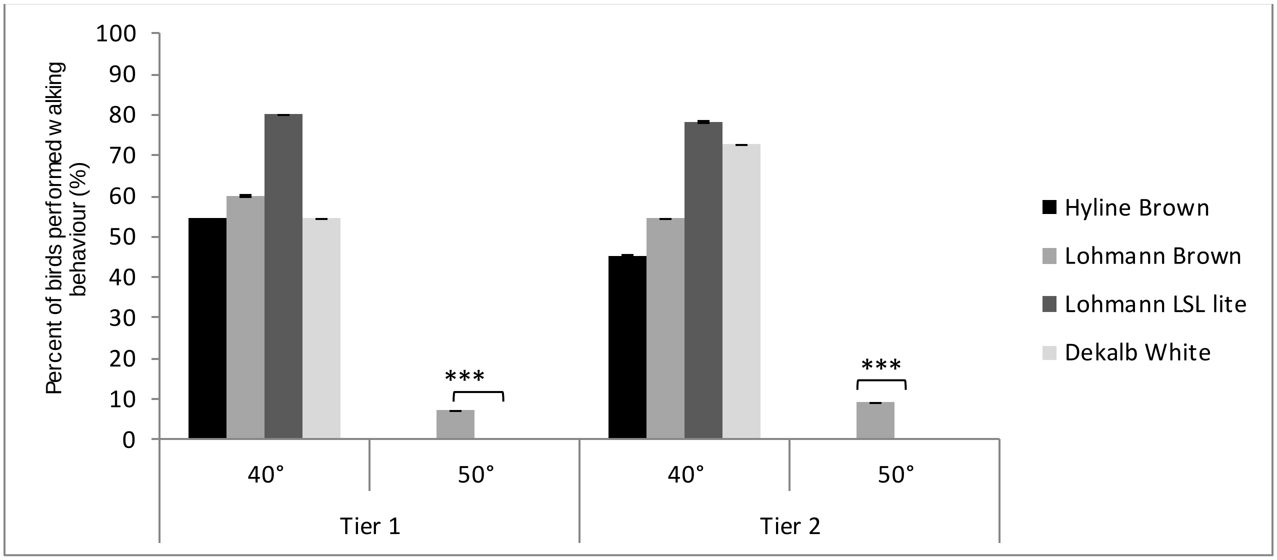


Figure 2.7.5 Success rate to reach the 2nd tier height on 50°, 60° and 70° inclines for four strains (Hyline Brown, Lohmann Brown, LSL lite and Dekalb White) (*N* = 5 for each strain) of birds in weeks 8-36 of life (± s.e.m.).

* P ≤ 0.05
** P ≤ 0.01
*** P ≤ 0.001

A



B

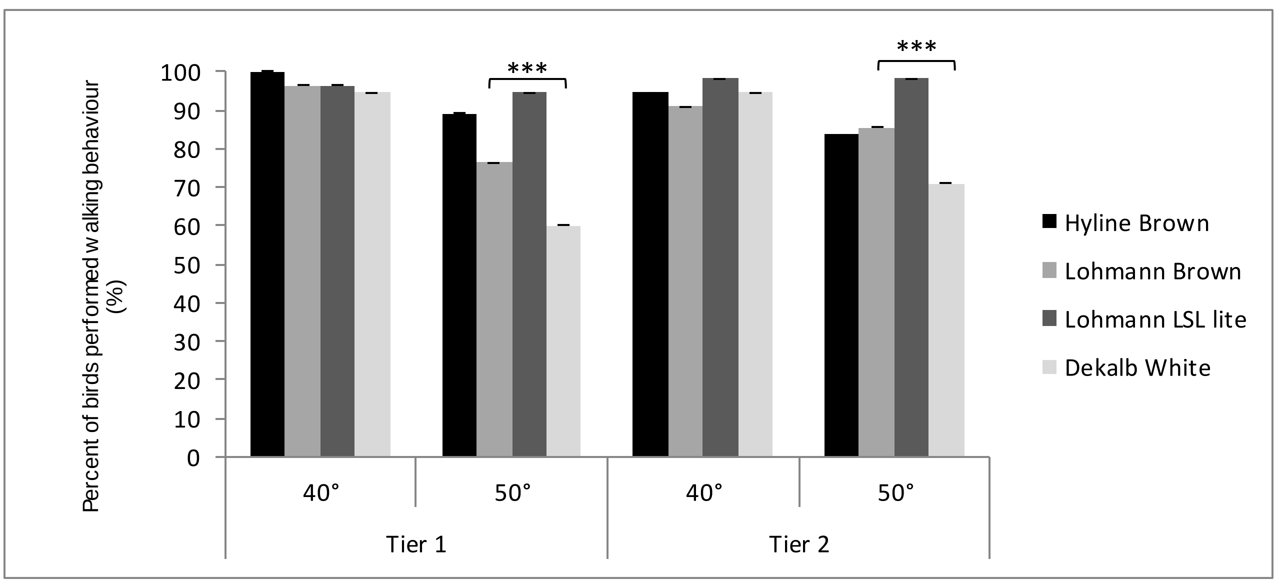


Figure 2.7.6 Walking behaviour performed to reach the 1st and 2nd tier heights on 40° and 50° inclines for four strains (Hyline Brown, Lohmann Brown, LSL lite and Dekalb White) (*N* = 5 for each strain) of birds in weeks 8-36 of life (± s.e.m.).

(A) sandpaper covered inclines for both tier heights within each strain.

(B) wire grid covered inclines for both tier heights within each strain.

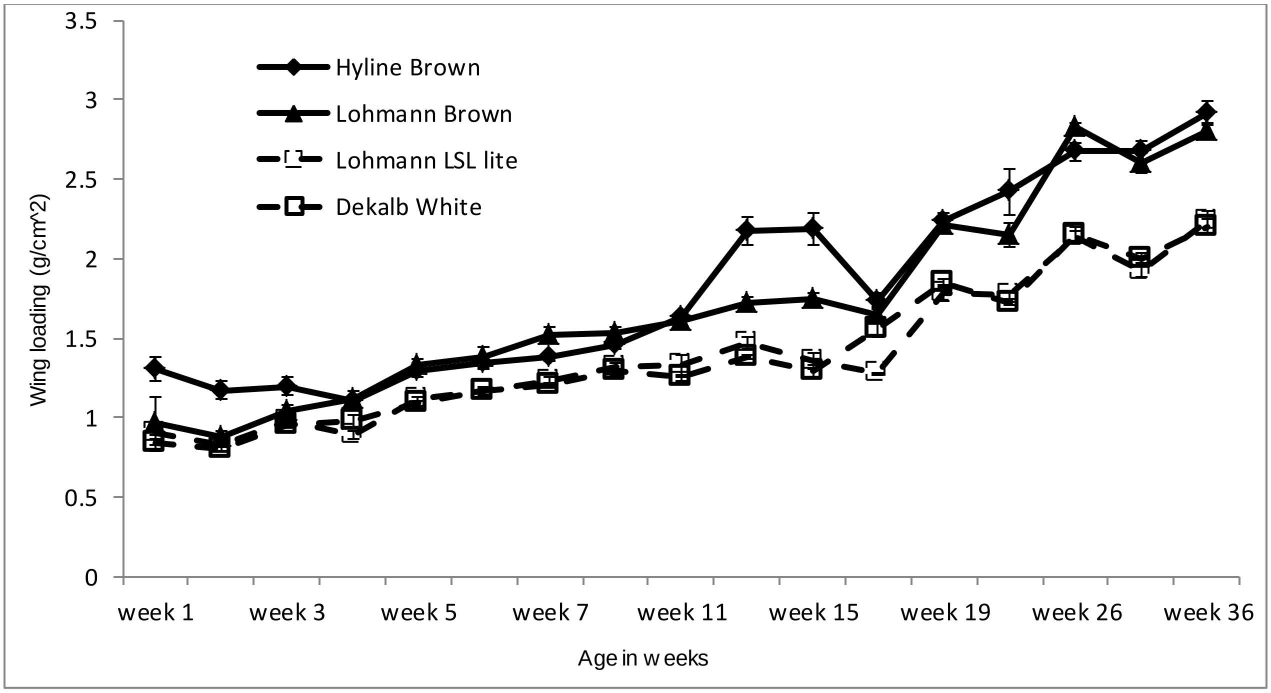
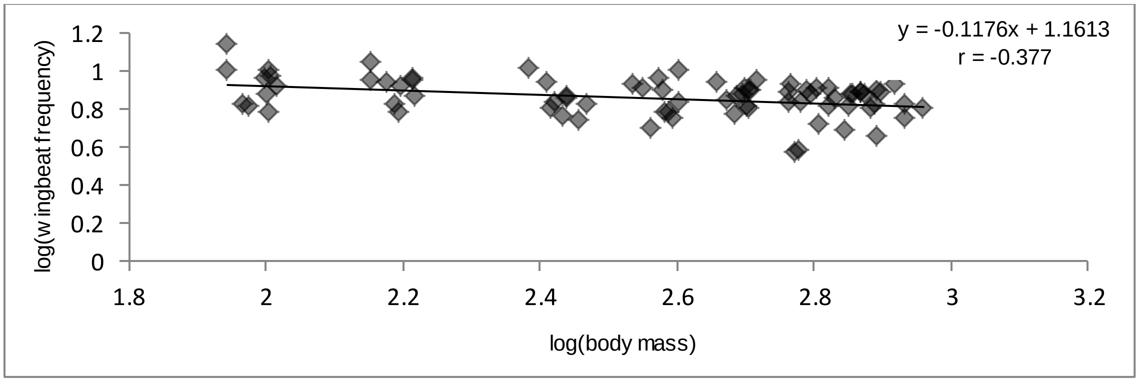
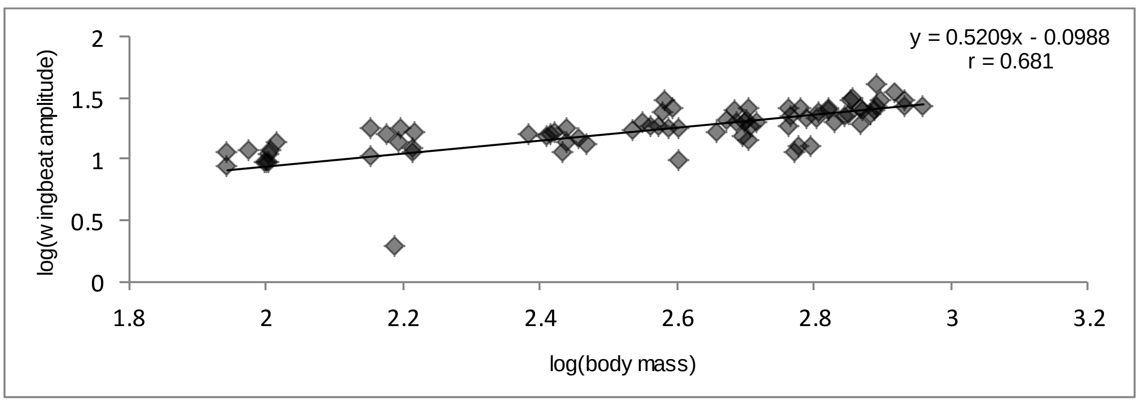


Figure 2.7.7 Average wing-loading (± s.e.m.) represented in a graph within four strains of birds (Hyline Brown, Lohmann Brown, LSL lite and Dekalb White) (*N* = 5 for each strain) throughout development.

A



B



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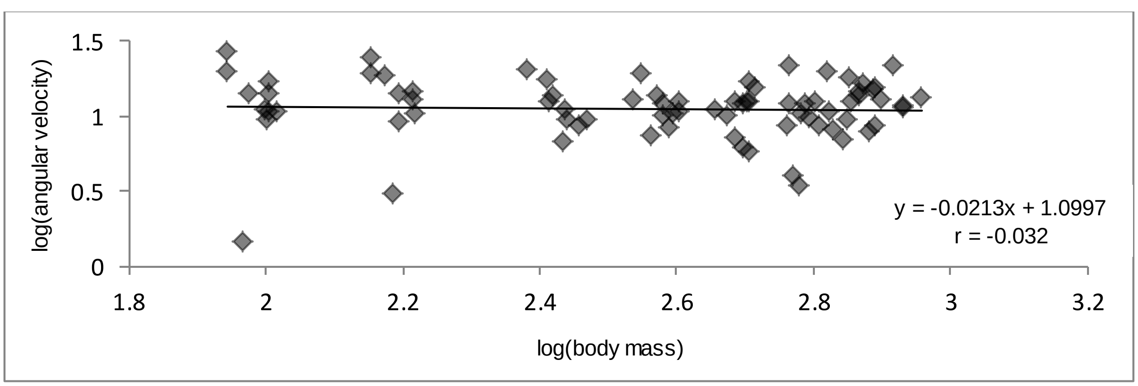

Figure 2.7.8 Log body mass relative to (A) log wingbeat frequency (B) log wingbeat amplitude and (C) log wing angular velocity of four strains of birds (Hyline Brown, Lohmann Brown, LSL lite and Dekalb White) (*N* = 5 for each strain, displayed together) in weeks 2-9 of life tested on the sandpaper covered 50° incline.

CHAPTER 3: Anticipation of inclines during terrestrial locomotion in domestic fowl

Manuscript format ready for submission.

3.1 ABSTRACT

Control of movement is important during daily life to prevent falls and injuries, especially when encountering uneven surfaces. The present investigation measured modulation of hindlimb movements and ground-reaction forces in domestic fowl (*Gallus gallus domesticus*, n = 20 birds) anticipating the navigation of inclined surfaces. Determining the degree of incline that domestic fowl anticipate appropriately is essential for providing the safest method to ascend vertically in commercial housing systems. The effects of incline angle (0, 40 and 70 deg) and age (17 – 36 weeks) upon characteristics for the last foot contact before climbing were examined including: vertical ground reaction force (GRF), step velocity, foot contact time and variation in center of pressure (COP). Birds generated higher peak GRFs and had the longest foot contact time prior to the steepest incline (70 degrees). There were no significant differences in peak GRFs between 0 and 40 degrees, but the birds had longer foot contact times before a 40-degree incline. Age did not have a significant effect upon peak GRFs relative to body weight. Age and incline did not have a significant effect upon COPx, COPy, and velocity (x and y). Overall, these data reveal that even when birds are experienced at climbing inclines, the time they require for sensory processing or decision making varies directly with the degree of the challenge before them and that modulation of GRF is only required for the steepest inclines. Thus to minimize stress on domestic fowl in aviaries, we recommend incline angles of $\leq$ 40 degrees.

3.2 KEYWORDS: domestic fowl, inclines, locomotion, decision making, anticipation

3.3 INTRODUCTION

Locomotion can be categorized into three basic components: production of locomotor movement, maintaining stability, and making adjustments to environmental cues or desired goals (Forssberg et al., 1980). Control of movement is important during daily life, especially when faced with uneven surfaces. When anticipating what forces are required to overcome an inclined surface, animals must first predict or be aware of what is coming and take the appropriate action. The ability to perceive the consequences of a given choice provides important information during cost-benefit analysis. When a task becomes difficult and the energetic cost is greater (for example, walking over a steep incline), significantly more cognitive effort is required to make a decision for climbing (Moffat, 2005).

Anticipatory behaviour is defined as goal-directed activity which occurs in the appetitive or wanting phase just prior to receiving or obtaining a reward when the reward is not visible (Craig, 1918). The behaviour can be locomotive or investigatory (Spruijt et al., 2001) and we can quantify it in this case by measuring hindlimb motor responses (such as GRFs) (Sheehan and Gottschall, 2012b). In order to maintain stability during motion, a sensory input such as vision or touch conveys a message to the brain and a response is carried out by the motor system (Borghuis et al., 2008). Failure to anticipate a surface or maintain balance effectively can lead to falling or injuries (Sheehan and Gottschall, 2012a).

Animal locomotion on inclined surfaces is important in nature for escaping predators and searching for food. Previous wild-bird locomotion studies have focused on age-dependent incline maneuverability and wing-associated behaviour, such as wing-assisted incline running (WAIR) (Dial et al., 2008; Jackson et al., 2009; Tobalske and Dial, 2007). WAIR involves the wings producing forces that assist the work of the hindlimbs by accelerating the animal upward and towards the substrate. Even when the birds are capable of flight, they may still choose to perform WAIR. In a recent study, domestic fowl were tested on similar inclines to the wild-bird study and investigated how chicks and adult fowl traversed up inclines of varying degrees (LeBlanc et al., 2016 submitted). The results indicated that chicks and adult fowl can ascend 40-degree inclines without the use of their wings. As the birds increased in age/experience they performed more WAIR or aerial behaviour. Understanding which inclines domestic fowl can manage and the mode of locomotion they use to navigate them can provide insight for the safe and effective use of continuous vertical paths within commercial systems. However, it is also important to consider what the domestic fowl visually perceive when approaching an incline that they are motivated to climb.

Measuring motor responses from limbs provides information about how the motor system reacts to sensory stimuli such as inclined surfaces. These responses can be measured through their interaction with force plates on the ground. Ground reaction forces (GRFs) can provide information on how different inclines are anticipated. Also, force plates can gather data pertaining to the stability of the transition step which are important for the prevention of falls. This includes monitoring the centre of pressure and how it changes throughout a step. Stability will depend on the speed and direction of this change (Kendell et al., 2010; Lemaire et al., 2006; Schmid et al., 2005). To my knowledge, there have only been studies involving humans to examine how sloped surfaces have an effect on the transitioning steps and the anticipation of inclines. It was found that humans adapt to inclined surfaces with a unique anticipatory step producing higher vertical ground reaction forces (GRFs) as inclines became steeper (Prentice et al., 2004; Sheehan and Gottschall, 2011; Sheehan and Gottschall, 2012b).

The purpose of this study was to test for effects of inclination and age upon hindlimb movement and ground-reaction forces during anticipation of climbing in domestic fowl during their last foot-contact before transitioning. I measured locomotor variables including ground reaction forces (GRF), step velocity, foot contact time and variation in center of pressure (COP).

Based on previous studies I hypothesized that domestic fowl will exert higher peak GRFs (Sheehan and Gottshall, 2012a; Minetti et al., 1999), decreased velocity (Huey and Hertz 1984; Farley 1997; Zaaf et al., 2001; Claussen et al., 2002; Pinch and Claussen 2003; Lammers et al., 2006; Russell and Higham 2009; Prenter et al. ,2012), increased foot contact time and more variation in COP (Chou et al., 2003) in the last step before transitioning as inclines become steeper. These factors will also depend on experience/age as it has been shown to affect gait variability in humans (Hausdorff et al., 2011).

3.4 MATERIALS AND METHODS

3.4.1 Ethical statement

This study was approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol Number 2501) prior to testing.

3.4.2 Animals and housing

Refer to Chapter 2 section 2.4.2.

3.4.3 Experimental design

The present study provides quantified measurements of the 20 domestic fowl comprising four strains (Lohmann Brown, Lohmann LSL lite, Dekalb White and Hyline Brown) aged from 17-36 weeks of age (see Table 1 for body measurements) and how they anticipate three incline treatments: 0, 40 and 70 degrees. The inclined surfaces (15 cm wide) were covered in a wire mesh grid surface (2.5 cm^2) and reached a constant tier height (160 cm). Only adult hens, after they began laying, were used for this study to maximize external validity with relation to commercial housing systems. By this age, birds are housed in a more complex environment with multiple tier heights and various ramp inclines. Refer to Chapter 2 for details on a broader range of ages and treatments.

Similar to Chapter 2 section 2.4.3, each test began with the individual bird standing in a closed box for 3 seconds until the start door was lifted by the experimenter. The subject then had one minute (determined from pilot study) to step on the force plate (one or two feet) and climb up to the tier height. Each bird was given a maximum of 2 trials if they failed to ascend the incline or if they failed to step on the force plate (only successful trials where the bird placed their entire foot/feet on the force plate were used in this study). The force plate and the 15 cm in front of the start box were covered with sanded roofing material, and the birds had experience with this material from their 1st week of life. A fail was defined as refusing to move up the surface, moving away from the test arena or reaching the one minute maximum. Birds were provided with motivation to climb using 3 same-age and same-strain birds (pen mates) in a crate on the tier to indicate a refuge, with the additional lure of a tablespoon of their commercial feed along with 5 raisins. All subjects were presented with inclines in random systematic order of increments/decrements weekly (weeks 17, 21, 26, 31, and 36).

3.4.4 Limb measurements

Each bird was photographed on a weekly basis while being held on their left side against a gridded background. The photos were used to measure tibiotarsus and metatarsus length of the right leg (analyzed using ImageJ).

3.4.5 Video

To avoid visual distractions for the birds, the experimenters observed each test from outside the test arena using a high-speed video camera (JVC GC-PX100BU HD Everio; 720p/1080p *resolution;* high speed video capture, 600 Hz) remotely connected via wifi streaming to an iPad mini (Apple, Inc.) The videos also served to confirm placement of one or two feet on the force plate.

3.4.6 Force plate

The force plate (Bertec Corporation, Colombus, Ohio, United States) used was custom made (15 cm X 15 cm) and had an internal digital preamplifier with a 16-bit digital signal output via RS-485 format. The output was amplified using BERTEC AM6500, a digital signal converter, which connected directly to a computer. Bertec Digital Acquire software was used to obtain the force plate data recorded at a frequency of 1000 Hz. It recorded 6 channels of internally-calibrated data including three axes of force (N) and three axes of moment (N*m): vertical force (Fz), anterior-posterior force (Fx), medial-lateral force (Fy), and moments around Mx, My, and Mz about the respective axes. Raw data was filtered using Loess (low-pass) via R programming (Cleveland et al., 1992). Our variables for subsequent analysis included onset and offset time of ground-reaction force (t_1 and t_2; s), peak Fz, variance in center of pressure about the x and y axes (meters; COPx and COPy), and velocity about the x and y axes ($m\ s^{-1}$; Vx and Vy) computed using change in COPx and COPy as a function of time (i.e., $t_2 - t_1$).

3.4.7 Statistical analyses

Data files were categorized according to the number of feet (one or two) on the force plate in the bird's transitions to climb. Only the tests where the birds placed one foot on the force plate were analyzed.

Statistical analyses were conducted using the generalized linear mixed model procedure (PROC GLIMMIX) in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA, 2012). Plots of studentized residuals were created to evaluate normality and determine the best fitting distribution. Full models with all fixed and random effects were then fit to the appropriate distribution and model fitting criteria (AIC) were used to select the best fitting model.

A repeated measures variance analysis for each variable (peak Fz relative to body weight, variance of center of pressure (COPx and COPy), foot contact time and velocity (Vx and Vy) was conducted. The week (age), incline and interaction (week*incline) were included as fixed effects. Strain of the birds was removed from analysis due to no significant strain effects. A first order autoregressive covariance structure (TYPE=AR (1)) was fit with bird as the repeated measure subject. Due to heterogeneity of error, separate error variances were fit at each incline. All dependent variables were fit with identity link (LINK= ID) and denominator degrees of freedom were calculated using the Kenward-Roger method (DDFM=KR) in the GLIMMIX procedure (Littell et al., 2006). Planned comparisons of interaction means were conducted by comparing incline least square means within each week.

Analysis of variance for the tibiotarsus, metatarsus and total limb length measurements was performed. The week (age) was included as the fixed effect. Due to heterogeneity of error, separate error variances were fit. The dependent variable was fit with identity link (LINK= ID) and denominator degrees of freedom were calculated using the Kenward-Roger method (DDFM=KR) in the GLIMMIX procedure (Littell et al., 2006).

Results are presented as mean estimates ± standard error of the mean. Statistical comparisons performed on the model scale were considered significant at $P < 0.05$.

3.5 RESULTS

There was an effect of incline upon capacity to perform the test. All 20 birds were successful in completing the task and stepped one foot on the force plate for the control (0 degrees) at each of the five weeks of testing; however this was not the case for 40 and 70 degree inclines (Table 3.7.1). There were some birds that used two feet on the force plate to prepare to ascend the 70 degree incline (week 17: N= 3, 15% ; week 21: N= 5, 25%; week 26: N= 9, 45%; week 31: N= 6, 30%; and week 36: N= 4, 20%). Reasons for using two feet to ascend steep inclines (70 degrees) include using jumping or flight to initiate climbing. For subsequent force analysis, we only present data from one-foot contacts with the force plate to maintain consistency.

The hindlimb ground reaction forces (peak Fz relative to bodyweight) during the transitions were dependent on the angle of inclination ($F_{2, 37.55}$= 28.88, P<0.0001). Tests on the 70 degree incline experienced higher peak GRFs compared to the 40 degree inclines (-1.31±0.18, $t_{17.49}$ = - 7.32, P<0.0001) and the control (0 degrees) (-1.34±0.18, $t_{17.08}$= - 7.58, P<0.0001) (Table 3.7.2). There were no significant differences when comparing 40 degrees to the control (Table 3.7.2).

Domestic fowl did not demonstrate a significant difference in the Vx and Vy when comparing the 3 different inclines across ages.

The force plate contact time of the domestic fowl depended on the angle of the incline ($F_{2, 41.28}$ = 10.73, P= 0.0002). There were longer foot contact times when transitioning to the 70 degree incline compared to the 40 degree incline (-11.27± 4.95, $t_{26.36}$ = -2.27, P= 0.0313) and the control (-17.12± 4.60, $t_{19.81}$ = -3.72, P= 0.0014) (Table 3.7.2). There were also longer foot contact times when transitioning to the 40 degree incline versus the control (-5.85±1.99, $t_{78.28}$ = - 2.95, P= 0.0042) (Table 3.7.2). In addition, there were no significant differences in variance of COPx and COPy for all inclines tested across ages.

Tibiotarsus length of domestic fowl depended on their age ($F_{4, 58}$ = 3.41, P= 0.0143). At 36 weeks of age, birds had a significantly longer tibiotarsus length compared to week 17 (-0.65±0.19, t_{58} = -3.52, P= 0.0009) and week 21 (-0.52±0.24, t_{58} = -2.19, P= 0.0324) (Table 3.7.1). There was no age effect for metatarsus length.

3.6 DISCUSSION

This study investigated how domestic fowl of different ages anticipate inclined surfaces by generating greater F_z GRFs, decreasing their step velocity, increasing foot contact time or by altering their COP. All birds generated higher peak GRFs and had the longest foot contact time for the steepest incline (70 degrees). There were no significant differences in the peak GRFs when comparing 0 degrees (control) to 40 degrees, but the birds had longer foot contact times on the 40 degree incline. The results indicate that domestic fowl exhibited the capacity to modulate their movement to successfully prepare for different inclines.

Consistent with the hypothesis that domestic fowl would generate higher peak GRFs for the 70 degree incline, the birds adjusted their motor responses for these transitions. They perceived the need to increase hind limb vertical forces prior to ascending the ramps in order to be successful in the climb. Similar results were found in a study involving humans as they adjusted their walking strategy prior to moving up a hill by increasing their GRFs (Sheehan and Gottschall, 2012b). Anticipation of inclined surfaces had not been examined in other animal species. However, species such as lizards (*Sceloporus malachiticus*) (Kramer and McLaughlin, 2001; Kohlsdorf and Biewener, 2006) and guinea fowl (*Numida meleagris L.,*) (Daley et al., 2006) have shown to modify their locomotion based on environmental cues. These guinea fowl were able to anticipate changes in their walking surfaces by adjusting their hind limb mechanics based on the visual information they were provided (Daley et al., 2006). With the results of this study, we have shown that birds are not only visually aware of their surroundings but also adjust their neuromuscular responses appropriately.

In general, inclined surfaces require more locomotive adjustments within the musculoskeletal system when compared to flat surfaces (Biewener and Daley, 2007). Contrary to the hypothesis, this study showed no significant differences in the peak F_z GRFs when comparing the flat surface control to the 40 degree incline. The hypothesis was based on a human study where they produced significantly higher vertical GRFs in their transition onto a 7 degree incline compared to level walking (Sheehan and Gottschall, 2012). The ground reaction forces for the control and 40 degree incline are comparable to a previous study conducted on domestic broiler chickens. Paxton and colleagues (2013) found that broiler chickens walking on a flat surface tended to produce forces equal to or slightly more than their body weight during a step, with peak GRFs not exceeding 1.4 times their body weight. The laying hens in the current study had forces that did not exceed 1.34 times their body weight. By producing similar GRFs for 0 and 40 degree inclines, most birds did not anticipate the extra force required and likely had to adapt their gait once on the inclined surface. These results are supported in that birds do not change their mode of locomotion when transitioning to 40 degree inclines compared to flat surfaces (LeBlanc et al., 2016 submitted).

There were no age based differences in the peak GRFs relative to body weight across the inclines in this study (birds from 17- 36 weeks of age did not differ in the peak GRFs relative to their body weight). However, the birds had significantly longer tibiotarsus length at 36 weeks of age compared to younger birds (17 and 21 weeks of age). This might have gave the birds decreased torque about the ankle joint, which results in more effort required to produce a GRF. Therefore, the 36 week old birds may have required more muscular effort than the others to provide the necessary GRF (per their weight) to ascend the inclines.

In order to ascend different inclines, birds had to consider both the strength and direction of the force generated by their hind limbs. To increase their vertical speed, they had to either increase GRF strength or the time the force is applied for. Both of these quantities are proportional to the momentum change of an object in the direction of that force. The birds spent a significantly longer period of time in contact with the force plate for steeper inclines, which could be attributed to generating larger forces and needing more time to make a decision. Gatesy and Biewener (1991) suggest that birds may keep their feet in contact with the ground for longer time periods in order to gain better stability during locomotion. The birds of this study took 2.7 times longer to complete the step for the 40 degree incline and 5.8 times longer for the 70 degree incline compared to a flat surface. Humans also tend to hesitate more when anticipating inclined surfaces in order to avoid falls or injuries (Sheehan and Gottschall, 2012a).

Average COPx, COPy, Vx and Vy did not have any significant differences when comparing across different inclines and ages of birds. We predicted that as inclines became steeper birds would demonstrate an increase in variance of COP which would suggest that the birds were not prepared to make the climb. With differences among means being insignificant, it suggests the birds were prepared to make the transitions by consistently stepping in the same spot to push off for climbing. This can be explained as birds were trained on these ramps from their 1st week of life and they were provided with one minute to make their decision. Another important observation is that the Vx values were similar in all cases despite there being less forward and more vertical momentum required to ascend steeper inclines.

Anticipating steep inclines effectively through cost-benefit analysis and understanding how birds process information about their environment is important to consider for the design of non-cage commercial systems and the prevention of falls and injuries. This research demonstrates that domestic fowl anticipate steeper inclines (70 degrees) differently by producing greater hind limb forces allowing them to safely transition onto the inclined surfaces. In the case of the 40 degree incline, birds did not show a difference in GRFs compared to flat surfaces which suggests birds had to adapt to the incline post transition or that hindlimb modulation was not necessary. Longer force plate contact times for steeper inclines emphasizes that even when the birds are trained on inclines, they still require more time for sensory processing or decision making, generating additional force, or to gain stability for a more challenging task. The lack of significance for COPx, COPy, Vx and Vy may indicate the birds were in control for each transition, probably as a function of their training. Furthermore, this may indicate that birds have the ability to use adaptive decision-making (Weller et al., 2010) by using their prior experiences to properly evaluate potential gains and losses to make a choice in a given circumstance.

Overall, these data reveal that even when birds are experienced at climbing inclines, the time they require for generating force varies directly with the degree of the challenge before them and that modulation of GRF is only required for the steepest inclines. As a recommendation to minimize stress on domestic fowl in commercial egg-production aviaries, we recommend incline angles of $\leq$ 40 degrees.

3.7 TABLES AND FIGURES

Table 3.7.1 Average raw data (mean ± s.d.) body mass, tibiotarsus length, tarsometarsus length and total right limb length (N values are presented for each measurement).

Age (weeks)	40 degrees		Age (weeks)	70 degrees		Right limb measurements		
	N values	Body mass (kg)		N values	Body mass (kg)	Tibiotarsus Length (cm) N= 20	Tarsometatarsus Length (cm) N= 20	Total (cm) N= 20
17	16	1.34 ± 0.08	17	6	1.31 ± 0.06	9.4 ± 0.6	7.2 ± 0.6	16.6 ± 0.9
21	14	1.72 ± 0.14	21	2	1.65 ± 0.01	9.5 ± 0.3	7.2 ± 0.2	16.8 ± 0.5
26	19	1.78 ± 0.17	26	4	1.86 ± 0.21	9.7 ± 0.1	7.3 ± 0.7	17 ± 0.3
31	18	1.95 ± 0.17	31	4	1.89 ± 0.15	9.9 ± 0.4	7.3 ± 0.4	17.2 ± 0.7
36	13	1.97 ± 0.18	36	6	1.92 ± 0.17	10.0 ± 0.8	7.4 ± 0.6	17.4 ± 1.1

Table 3.7.2 Average raw data values of the transition step for all conditions in domestic fowl (See Table 3.7.1 for N values). Bold numbers indicate significant from level walking. Bold numbers with asterisk indicate significant from level walking and 40 degrees.

17 weeks of age:	**0 degrees (control)**	**40 degrees**	**70 degrees**
Max. peak Fz (Newtons)	14.57	14.23	**24.53***
Max. peak Fz/Body weight	1.09	1.09	**1.91***
Velocity x-direction (metres/second)	-0.0242	-0.0158	-0.0286
Velocity y-direction (metres/second)	-0.0439	-0.0201	-0.0229
Contact time (seconds)	3.79	**12.09**	**33.56***
Variance of COPx (metres)	0.0007	0.0007	0.0018
Variance of COPy (metres)	0.0002	0.0003	0.0011
21 weeks of age:	**0 degrees (control)**	**40 degrees**	**70 degrees**
Max. peak Fz (Newtons)	17.52	16.76	**61.65***
Max. peak Fz/Body weight	1.04	0.99	**3.81***
Velocity x-direction (metres/second)	0.3687	0.0367	0.0125
Velocity y-direction (metres/second)	0.2365	-0.008	-0.0075
Contact time (seconds)	2.86	**7.49**	**4.07***
Variance of COPx (metres)	0.0221	0.246	0.0001
Variance of COPy (metres)	0.0136	0.1458	0.0001
26 weeks of age:	**0 degrees (control)**	**40 degrees**	**70 degrees**
Max. peak Fz (Newtons)	20.01	21.7	**44.80***
Max. peak Fz/Body weight	1.09	1.17	**2.5***
Velocity x-direction (metres/second)	0.047	0.0968	-0.0032
Velocity y-direction (metres/second)	-0.001	0.0061	-0.004
Contact time (seconds)	3.3	**6.72**	**23.19***
Variance of COPx (metres)	0.0011	0.1541	0.0015
Variance of COPy (metres)	0.0003	0.0914	0.0005
31 weeks of age:	**0 degrees (control)**	**40 degrees**	**70 degrees**
Max. peak Fz (Newtons)	20.93	20.31	**34.70***
Max. peak Fz/Body weight	1.1	1.06	**1.83***
Velocity x-direction (metres/second)	-0.0515	0.4367	-0.1725
Velocity y-direction (metres/second)	-0.1219	0.243	-0.1673
Contact time (seconds)	5.16	**6.5**	**6.2***
Variance of COPx (metres)	0.0617	0.0749	0.0239
Variance of COPy (metres)	0.0519	0.0264	0.017
36 weeks of age:	**0 degrees (control)**	**40 degrees**	**70 degrees**
Max. peak Fz (Newtons)	22.25	21.14	**37.76***
Max. peak Fz/Body weight	1.14	1.09	**2.05***
Velocity x-direction (metres/second)	0.1584	-0.0078	0.0039
Velocity y-direction (metres/second)	0.0585	-0.034	-0.0013
Contact time (seconds)	1.3	**10.72**	**28.47***

Variance of COPx (metres)	0.0078	0.0048	0.0009
Variance of COPy (metres)	0.004	0.0021	0.0003

CHAPTER 4: CONCLUSION

The purpose of this thesis was to investigate domestic laying hen locomotion skills and abilities throughout development to facilitate the design of proper housing systems. Determining the most appropriate ramp incline for vertical movement within aviary systems is essential for the improvement of domestic hen welfare. In order to provide an answer to this question, we must understand the locomotive style and capabilities of laying hens across development. In addition, determining whether laying hens can anticipate steeper inclines and how they modulate their hindlimb forces in preparation for climbing can provide information on how they perceive different stimuli. It is important to investigate the most appropriate ramp incline domestic laying hens can negotiate safely for the reduction or prevention of unnecessary falls or injuries within aviary housing systems. With 80% of domestic hens acquiring KBD within non-cage housing systems, which is assumed to be due to collisions and falls (Wilkins et al., 2004a, 2011); it is crucial to improve their movement in the third dimension.

In chapter two, locomotor capacity and strategies on inclined surfaces were evaluated during development in domestic chickens (*Gallus gallus domesticus*) of four different strains (Lohmann LSL lite, Dekalb White, Lohmann Brown, Hyline Brown). Starting from their 2nd week of life to 36 weeks of age, birds were tested on various inclines (0-70 degrees) to determine if they can successfully reach two elevated tier heights and what style of locomotion they used to do so. It was found that chicks and adult fowl performed only walking behaviour in order to climb 40 degree inclines, and performed WAIR or aerial ascent on steeper inclines. At all ages, the birds did not require the use of their wings on the 40 degree incline to successfully reach the tiers. This result is very important for commercial aviary systems because vigorous wing-use can potentially cause keel-bone fractures due to unequal bone loading from a keel-bone deviation or from a collision with the system or flock mates. In addition, birds were more successful at negotiating the ramp surfaces covered in wire grid and reduced wing use on these ramps, which could be attributed to improved hindlimb contact. When birds increase in age/ experience we observed an increase in WAIR or aerial behavior. The conserved wing angular velocity with an increase in age demonstrated that the wing kinematics of chicks were of a similar pattern to that of mature birds. White feathered strains performed more wing-associated locomotor behaviour compared to brown feathered strains. This study revealed that a ramp incline of 40 degrees does not require the use of wings in domestic fowl across development and chicks climb with a locomotor style similar to that of more mature birds, revealing that chicks have the locomotor abilities to be housed in three-dimensional systems similar to mature birds.

In chapter three, we measured modulation of hindlimb responses in the last step prior to successfully climbing wire grid covered inclines (0, 40 and 70 degrees) in adult domestic chickens (*Gallus gallus domesticus*). These response variables included: vertical ground reaction force (GRF), step velocity, foot contact time and variation in center of pressure (COP). I found that birds generated significantly higher peak GRFs and had the longest foot contact time with the force plate prior to the 70 degree incline. There were no significant differences in peak GRFs between 0 and 40 degrees; however the birds had double the amount of foot contact time before a 40 degree incline. This could be due to the birds adjusting their hindlimbs post-transition or it could be that it was not necessary to modulate their hindlimb GRFs for 40 degrees. Age did not have a significant effect upon peak GRFs relative to body weight. In addition, age and incline did not have a significant effect upon average COPx, COPy, Vx and Vy. Therefore, this experiment revealed that when birds are trained on inclined surfaces, it presumably takes significantly longer for sensory processing or decision making depending on the degree of incline they are challenged with indicating that adult domestic hens can perceive the steepness of uneven terrain before making a decision to climb. Overall, chapter two and three demonstrated that domestic chickens easily negotiate inclines up to 40 degrees and 70 degree inclines pose a significant challenge for them.

4.1 RECOMMENDATIONS AND FUTURE RESEARCH

From the results of these studies, I can make three recommendations for the commercial poultry industry. First I recommend using a raised wire grid (2.5 cm^2) surface material at inclines $\leq$ 40 degrees for adult domestic fowl in commercial aviary systems. However, further research is required for chicks and pullets in order to determine the appropriate wire grid size to fit the size of their feet and leg length. Exposing chicks to the appropriate wire grid inclines as early as possible is important to train them and for them to gain the cognitive skills required. In chapter 2, it was revealed that even when the birds were trained on ramps in their 1st week of life, they still required increased time for sensory processing just prior to climbing 40 and 70 degree inclines. Therefore, the second recommendation is to allow the chicks to train themselves on a daily basis on the inclined surface as early as possible because it allows them to gain the cognitive skills for perceiving different surfaces (that may not have been present otherwise). However, it requires further research in order to compare trained birds to untrained birds on inclined surfaces.

The birds did not acquire severe KBD throughout the course of these experiments (data not present), which could also be attributed to the training the birds received which allowed them to gain the appropriate musculoskeletal development. Lastly, it was found that as chicks, the ramp width (15 cm) was not ideal for wing flapping on the sandpaper surface material. Therefore, for the third recommendation we need to consider the ramp width when installing ramp inclines in systems for housing of chicks and pullets to prevent unnecessary wing collisions with the ramp surface. For example, installing a narrower ramp width for chicks and pullets due to their small wing size and short wing span, but increasing the ramp width for adult hens would be a suitable solution.

To conclude, novel research reported herein on locomotion in domestic laying hens offers significant insight for researchers designing aviary systems to ameliorate risk of KBD and other accidental damage due to falls and collisions.

BIBLIOGRAPHY

Adolph, K.E. (1995). Psychophysical assessment of toddlers' ability to cope with slopes. *J. Exp. Psychol. Hum. Percept. Perform.* **21(4)**, 734-750.

Adolph, K.E., Eppler, M.A. and Gibson, E.J. (1993). Crawling versus walking infants' perception of affordances for locomotion over sloping surfaces. *Child. Dev.* **64(4)**, 1158-1174.

Aerni, V., Brinkhof, M.W.G., Wechsler, B., Oester, H and Frohlich, E. (2005). Productivity and mortality of laying hens in aviaries: a systematic review. *WPSA*. **61**, 130-142.

Askew, G.N. (2014). The elaborate plumage in peacocks is not such a drag. *J. Exp. Biol.* **217**, 3237-3241.

Autumn, K., Hsieh, S. T., Dudek, D.M., Chen, J., Chitaphan, C. and Full, R.J. (2006). Dynamics of geckos running vertically. *J. Exp. Biol.* **209**, 260-272.

Balmford, A., Thomas, A.L.R. and Jones, I.L. (1993). Aerodynamics and the evolution of long tails in birds. *Nature.* **361**, 628-630.

Bestman, M. and Wagenaar, J.P. (2014). Health and welfare in Dutch organic laying hens. *Animals.* **4**, 374-390.

Biewener, A.A. and Daley, M.A. (2007). Unsteady locomotion: integrating muscle function with whole body dynamics and neuromuscular control. *J. Exp. Biol.* **210**, 2949-2960.

Birn-Jeffery, A.V. and Higham, T.E. (2014). The scaling of uphill and downhill locomotion in legged animals. *Integr. Comp. Biol.* **54(6)**, 1159–1172.

Bock, W.J and Miller, W.D. (1959). The scansorial foot of the woodpeckers, with comments on the evolution of perching and climbing feet in birds. *American Museum Novitates.*1931.

Borghuis, J., Hof, A.L. and Lemmink, K.A.P.M. (2008). The importance of sensory-motor control in providing core stability. *Sports Med.* **38(11)**, 893-916.

Brauer, S.G., Woolacott, M. and Shumway-Cook, A. (2001).The interacting effects of cognitive demand and recovery of postural stability in balance-impaired elderly persons. *J. Gerontol. A Biol. Sci. Med. Sci.* **56A (8),** M489–M496.

Bueno, A.A. and Motta-Junior, J.C. (2015). Behavioural and morphological strategies by small savannah rodents to avoid predation. *Mamm. Biol.* **80**, 401–408.

Bundle, M.W. and Dial, K.P. (2003). Mechanics of wing-assisted incline running (WAIR). *J. Exp. Biol.* **206**, 4553-4564.

Campbell, D.L.M., Goodwin, S.L., Makagon, M.M., Swanson, J.C. and Siegford, J.M. (2016). Failed landings after laying hen flight in a commercial aviary over two flock cycles. *Poult. Sci.* **95**, 188–197.

Carter, S.E., Campbell, E.M., Sanson-Fisher, R.W., Redman, S. and Gillespie, W.J. (1997). Environmental hazards in the homes of older people. *Age. Ageing.* **26**, 195-202.

CDC. (2007) Web-based injury statistics query and reporting system (WISQARS) [Online]. Centers for Disease Control and Prevention, National Center for Injury Prevention Control.

Chou, L., Kaufman, K.R., Hahn, M.E. and Brey, R.H. (2003). Medio-lateral motion of the center of mass during obstacle crossing distinguishes elderly individuals with imbalance. *Gait Posture*. **18**, 125-133.

Claussen, D.L., Lim, R., Kurz, M. and Wren, K. (2002). Effects of slope, substrate, and temperature on the locomotion of the Ornate Box Turtle, *Terrapene ornate*. *Copeia.* **2**, 411-418.

Cleveland, W.S., Grosse, E. and Shyu, W.M. (1992). Local regression models. Chapter 8 of *Statistical Models in S* eds J.M. Chambers and T.J. Hastie, Wadsworth & Brooks/Cole.

Collias, N.E and Collias, E. C. (1967). A field study of the Red Jungle Fowl in North-Central India. *The Condor.* **69(4)**, 360-386.

Collias, N.E., Collias, E.C., Hunsaker, D. and Minning, L. (1966). Locality fixation, mobility and social organization within an unconfined population of red jungle fowl. *Anim. Behav.* **14**, 550-559.

Crabtree, B. and Newsholme, E.A. (1972). The activities of phosphorylase, hexokinase, phos-phofructokinase, lactate dehydrogenase and the glycerol 3-phosphate dehydrogenases in muscles from vertebrates and invertebrates. *Biochem. J.* **126**, 49–58.

Craig, W. (1918). Appetites and aversions as constituents of instincts. *Biological Bulletin.* **34**, 91–107.

Daley, M. A. and Biewener, A. A. (2003). Muscle force–length dynamics during level *versus* incline locomotion: a comparison of *in vivo* performance of two guinea fowl ankle extensors. *J. Exp. Biol.* **206**, 2941-2958.

Daley, M.A., Usherwood, J.R., Felix, G. and Biewener, A.A. (2006). Running over rough terrain: guinea fowl maintain dynamic stability despite a large unexpected change in substrate height. *J. Exp. Biol.* **209**, 171-187.

Dial, K.P. (1992). Avian forelimb muscles and nonsteady flight: can birds fly without using the muscles in their wings? *The Auk*. **109(4)**, 874-885.

Dial, K.P. (2003). Evolution of avian locomotion: correlates of flight style, locomotor modules, nesting biology, body size, development, and the origin of flapping flight. *The Auk*. **120(4)**, 941–952.

Dial, K.P., Biewener, A.A., Tobalske, B.W. and Warrick, D.R. (1997). Mechanical power output of bird flight. *Nature*. **390**, 67-70.

Dial, T. R. and Carrier, D. R. (2012). Precocial hindlimbs and altricial forelimbs: partitioning ontogentic strategies in mallards (*Anas platyrhynchos*). *J. Exp. Biol.* **215**, 3703-3710.

Dial, K. P. and Jackson, B. E. (2011). When hatchlings outperform adults: locomotor development in Australian brush turkeys (*Alectura lathami*, Galliformes). *Proc. R. Soc. B.* **278**, 1610–1616.

Dial, K. P., Jackson, B. E. and Segre, P. (2008). A fundamental avian wing-stroke provides a new perspective on the evolution of flight. *Nature*. **451**, 985-989.

Drake, K R., Thompson, J. E. and Drake, K. L. (2001). Movements, habitat use, and survival of nonbreeding piping plovers. *The Condor*. **103**, 259–267.

Enriquez-Urzelai, U., Montori, A., Llorente, G.A. and Kaliontzopoulou, A. (2015). Locomotor mode and the evolution of the hindlimb in Western Mediterranean Anurans. *Evol. Biol.* **42**, 199–209.

Farley, C.T. (1997). Maximum speed and mechanical power output in lizards. *J. Exp. Biol.* **200**, 2189-2195.

FAWC. (2010). Opinion on Osteoporosis and Bone Fractures in Laying Hens. Farm Animal Welfare Council, London.

FAWC. (2013). An open letter to Great Britain Governments: Keel bone fracture in laying hens.

Forssberg, H., Grillner, S. and Halbertsma, J. (1980). The locomotion of the low spinal cat

1. Coordination within a hindlimb. *Acta. Physiol. Scand.* **108**, 269-281.

Gabaldón, A. M., Nelson, F.E. and Roberts, T. J. (2004). Mechanical function of two ankle extensors in wild turkeys: shifts from energy production to energy absorption during incline *versus* decline running. *J. Exp. Biol.* **207**, 2277-2288.

Gatesy, S.M. and Biewener, A.A. (1991). Bipedal locomotion: effects of speed, size and limb posture in birds and humans. *J. Zool. Lond.* **224**, 127-147.

Gatesy, S.M. and Dial, K.P. (1996). Locomotor modules and the evolution of avian flight. *Evolution.* **50(1)**, 331-340.

Gregory, N. G. and L. J. Wilkins. (1996). Effect of age on bone strength and the prevalence of broken bones in perchery laying hens. *N. Z. Vet. J.* **44**, 31–32.

Hakansson, J. and Jensen, P. (2005). Behavioural and morphological variation between captive populations of red junglefowl (*Gallus gallus*) - Possible implications for conservation. *Biol. Cons.* **122**, 431-439.

Harlander-Matauschek, A., Rodenburg, T.B., Sandilands, V., Tobalske, B.W. and Toscano, M.J. (2015). Causes of keel bone damage and their solutions in laying hens. *WPSA*. **71**, 461-472.

Hausdorff, J.M., Rios, D.A. and Edelberg, H.K. (2001). Gait variability and fall risk in community-living older adults: A 1-year propspective study. *Arch. Phys. Med. Rehabil.* **82**, 1050-1056.

Heers, A.M., Dial, K.P. and Tobalske, B.W. (2014). From baby birds to feathered dinosaurs: incipient wings and the evolution of flight. *Paleobiology*. **40(3)**, 459–476.

Heers, A. M., Tobalske, B.W. and Dial, K.P. (2011). Ontogeny of lift and drag production in ground birds. *J. Exp. Biol.* **214**, 717–725.

Hester, P.Y., Enneking, S.A., Haley, B.K., Cheng, H.W., Einstein, M.E. and Rubin, D.A. (2013). The effect of perch availability during pullet rearing and egg laying on musculoskeletal health of caged White Leghorn hens. *Poult. Sci.* **92**, 1972-1980.

Higham, T. E. and Biewener, A. A. (2008). Integration within and between muscles during terrestrial locomotion: effects of incline and speed. *J. Exp. Biol.* **211**, 2303-2316.

Huey, R.B. and Hertz, P.E. (1984). Effects of body size and slope on acceleration of a lizard (*Stelljo Stellio*). *J. Exp. Biol.* **110**, 113-123.

Jackson, B. E., Segre, P. and Dial, K.P. (2009). Precocial development of locomotor performance in a ground-dwelling bird (*Alectoris chukar*): negotiating a three-dimensional terrestrial environment. *Proc. R. Soc. B*. **276**, 3457-3466.

Jackson, B.E., Tobalske, B.W. and Dial, K.P. (2011). The broad range of contractile behaviour of the avian pectoralis: functional and evolutionary implications. *J. Exp. Biol.* **214**, 2354-2361.

Jarvilehto, T., Nurkkala, V., Koskela, K. and Kalermo, J. (2013). Anticipation as a main principle of neural function and mastering of driving, in: *Driver Behaviour and Training, Volume 6*. Ashgate Publishing Limited.

Jeffery, K. J., Jovalekic, A., Verriotis, M. and Hayman, R. (2013). Navigating in a 3D world. *Behav. Brain. Sci.* 1-71.

Jones, D.N. and Goth, A. (2008). Mound-builders. Collingwood, Australia: CSIRO publishing.

Kappeli, S., Gebhardt-Henrich, S.G., Frohlich, E., Pfulg, A. and Stoffel, M.H. (2011). Prevalence of keel bone deformities in Swiss laying hens. *Br. Poult. Sci.* **52**, 531-536.

Kendell, C., Lemaire, E.D., Dudek, N.L. and Kofman, J. (2010). Indicators of dynamic stability in transtibial prosthesis users. *Gait. Posture.* **3**, 375–379.

Kohlsdorf, T. and Biewener, A.A. (2006).Negotiating obstacles: running kinematics of the lizard *Sceloporus malachiticus*. *J. Zool.* **270**, 359–371.

Kozak, M., Tobalske, B., Martins, C., Bowley, S., Wuerbel, H. and Harlander-Matauschek, A. Use of space by domestic chicks in complex aviaries. *Appl. Anim. Behav. Sci.* Submitted for publication.

Kramer, D.L. and Mclaughlin, R.L. (2001). The behavioral ecology of intermittent locomotion. *Amer. Zool.* **41**, 137-153.

Lammers, A. R., Earls, K. D. and Biknevicius, A. R. (2006). Locomotor kinetics and kinematics on inclines and declines in the gray shorttailed opossum *Monodelphis domestica*. *J. Exp. Biol.* **209**, 4154-4166.

Lay, D.C., Fulton, M., Hester, P.Y., Karcher, D.M., Kjaer, J.B., Mench, J.A., Mullens, B.A., Newberry, R.C., Nicol, C.J., O'Sullivan, N.P. and Porter, R.E. (2011). Hen welfare in different housing systems. *Poult. Sci.* **90**, 278–294.

LeBlanc, S., Springthorpe, D., Quinton, M., Szkotnicki, B., Tobalske, B., Wuerbel, H. and Harlander-Matauschek, A. (2015). Effects of physical health problems and environmental challenges on balance in laying hens. *PLOS ONE*. Submitted for publication.

LeBlanc, C., Tobalske, B., Bowley, S. and Harlander- Matauschek, A. (2016). Development of locomotion over inclined surfaces in domestic fowl. *PLOS ONE*. Submitted for publication.

Lee, D.V. (2011). Effects of grade and mass distribution on the mechanics of trotting in dogs. *J. Exp. Biol.* **214**, 402-411.

Lemaire, E.D., Biswas, A. and Kofman, J. (2006). Plantar pressure parameters for dynamic gait stability analysis. In: Conference Proceedings Annual International Conference of the IEEE Engineering in Medicine & Biology Society 1, pp. 4465-4468.

Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D. and Schabenberger, O. (2006). SAS for Mixed Models, 2^{nd} ed. Cary: SAS Institute Inc.

Lord, S. R., Menz, H.B. and Sherrinton, C. (2006). Home environment risk factors for falls in older people and the efficacy of home modifications. *Age. Ageing.* **35-S2**, ii55–ii59.

Minetti, A.E., Ardigo, L.P., Reinach, E. and Saibene, F. (1999). The relationship between mechanical work and energy expenditure of locomotion in horses. *J. Exp. Biol.* **202**, 2329-2338.

Moe, R.O., Nordgreen, J., Janczak, A.M., Spruijt, B.M., Zanella, A.J. and Bakken, M. (2009). Trace classical conditioning as an approach to the study of reward-related behaviour in laying hens: A methodological study. *Appl. Anim. Behav. Sci.* **121**, 171–178.

Moffat, P.G. (2005). Stochastic choice and the allocation of cognitive effort. *Exper. Econ.* **8**, 369–388.

Moinard, C., Statham, P., Haskell, M.J., McCorquodale, C., Jones, R.B. and Green, P.R. (2004a). Accuracy of laying hens in jumping upwards and downwards between perches in different light environments. *Appl. Anim. Behav. Sci.* **85**, 77–92.

Muir, G.D. (2000). Early ontogeny of locomotor behaviour: A comparison between altricial and precocial animals. *Brain. Res. Bull.* **53(5)**, 719–726.

Muir, G.D., Gosline, J.M. and Steeves, J.D. (1996). Ontogeny of bipedal locomotion: walking and running in the chick. *J. Physiol.* **493(2)**, 589-601.

Nasr, M.A.F., Murell, J., Wilkings, L.J. and Nicol, C.J. (2012a). The effect of two classes of opioid drug on the landing ability of laying hens with and without keel fractures, in:

UFAW Animal Welfare Conference: Recent Advances in Animal Welfare Science III, York, UK.

Nasr, M.A.F., Murell, J., Wilkings, L.J. and Nicol, C.J. (2012c). Do laying hens with keel bone fractures experience pain? *PLoS One.* **7**, e42420.

Nasr, M.A.F., Murell, J., Wilkings, L.J. and Nicol, C.J. (2015). The effects of two non-steroidal anti-inflammatory drugs on the mobility of laying hens with keel bone fractures. *Vet. Anaesth. Analg.* **42**, 197-204.

National News: Egg Farmers of Canada Announces Industry-Wide Transition Away from Conventional Housing. (2016, February). Retrieved from http://www.northumberlandview.ca/index.php?module=news&type=user&func=display&sid=39978.

Necker, R. (2006). Specializations in the lumbosacral vertebral canal and spinal cord of birds: evidence of a function as a sense organ which is involved in the control of walking. *J. Comp. Physiol. A.* **192**, 439–448.

Paxton, H., Daley, M.A., Corr, S.A. and Hutchinson, J.R. (2013). The gait dynamics of the modern broiler chicken: a cautionary tale of selective breeding. *J. Exp. Biol.* **216**, 3237-3248.

Petrik, M.T., Guerin, M.T., Widowski, T.M. (2015). On-farm comparison of keel fracture prevalence and other welfare indicators in conventional cage and floor-housed laying hens in Ontario, Canada. *Poult. Sci.* **94**, 579-585.

Pinch, F.C. and Claussen, D.L. (2003). Effects of temperature and slope on the sprint speed and stamina of the Eastern Fence Lizard, *Sceloporus undulates. J. Herpetol.* **37(4),** 671–679.

Pike, A. V. L. and Maitland, D. P. (2004). Scaling of bird claws. *J. Zool. Lond.* **262**, 73–81.

Prenter, J., Fanson, B.G. and Taylor, P.W. (2012). Whole-organism performance and repeatability of locomotion on inclines in spiders. *Anim. Behav*. **83**, 1195-1201.

Prentice, S.D., Hasler, E.N., Groves, J.J. and Frank, J.S. (2004). Locomotor adaptations for changes in the slope of the walking surface. *Gait Posture.* **20**, 255-265.

Proctor, N.S. and Lynch, P.J. (1993). Manual of ornithology: Avian structure & function. New Haven, CT, United States: Yale University.

Richards, G.J., Brown, S.N., Booth, F., Toscano, M.J. and Wilkins, L.J. (2012). Panic in free range laying hens. *Veterinary Record.* **170**, 519.

Rodenburg, T.B., Tuyttens, F.A.M., De Reu, K., Herman, L., Zoons, J. and Sonck, B. (2008). Welfare assessment of laying hens in furnished cages and non-cage systems: an on-farm comparison. *Anim. Welf.* **17**, 363-373.

Rosenbaum, D.A. (1985). Motor programming: a review and scheduling theory, in: *Motor Behavior*. Spring-Verlag Berlin Heidelberg.

Russell, A.P. and Higham, T.E. (2009). A new angle on clinging in geckos: incline, not substrate, triggers the deployment of the adhesive system. *Proc. R. Soc. B.* **276**, 3705–3709.

Ryan, R.M. and Deci, E.L. (2000). Intrinsic and extrinsic motivations: classic definitions and new directions. *Contemp. Edu. Psychol.* **25**, 54–67.

Sandilands, V., Moinard, C. and Spark, N.H.C. (2009). Providing laying hens with perches: fulfilling behavioural needs but causing injury? *Br. Poult. Sci.* **50**, 395-406.

Sapir, N. and Dudley, R. (2012). Backward flight in hummingbirds employs unique kinematic adjustments and entails low metabolic cost. *J. Exp. Biol.* **215**, 3603-3611.

Schmid, M., Beltrami, G., Zambarbieri, D. and Verni, G. (2005). Centre of pressure displacements in trans-femoral amputees during gait. *Gait. Posture*. **21**, 255–262.

Schmitt, D. (2010). Primate locomotor evolution: biomechanical studies of primate locomotion and their implications for understanding primate neuroethology, in: *Primate Neuroethology*. Oxford: Oxford University Press.

Scott, G., Lambe, N.R. and Hitchcock, D. (1997). Ability of laying hens to negotiate horizontal perches at different heights, separated by different angles. *Br. Poult. Sci.* **38**, 48-54.

Sheehan, R.C. and Gottschall, J.S. (2011). Stair walking transitions are an anticipation of the next stride. *J. Electromyogr. Kinesiol.* **21**, 533-541.

Sheehan, R.C. and Gottschall, J.S. (2012a). At similar angles, slope walking has a greater fall risk than stair walking. *Appl. Ergon.* **43**, 473-478.

Sheehan, R.C. and Gottschall, J.S. (2012b). Walking strategies change with distance from hill transition and scale with hill angle. *J. Appl. Biomech.* **28**, 738-745.

Short, L.L. (1970). Reversed sexual dimorphism in tail length and foraging differences in woodpeckers. *Bird Banding.* **41(2)**, 85-168.

Spruijt, B.M., van den Bos, R. and Pijlman, F.T.A. (2001). A concept of welfare based on reward evaluating mechanisms in the brain: anticipatory behaviour as an indicator for the state of reward systems. *Appl. Anim. Behav. Sci.* **72**, 145–171.

Stratmann, A., Frohlich, E.K.F., Gebhardt-Henrich, S., Harlander-Matauschek, A., Wurbel, H. and Toscano, M.J. (2015a). Modification of aviary design reduces incidence of falls, collisions and keel bone damage in laying hens. *Appl. Anim. Behav. Sci.* **165**, 112-123.

Sturnieks, D.L., George, R.S. and Lord, S.R. (2008). Balance disorders in the elderly. *Clin. Neurophysiol.* **38**, 467-478.

Szulc, P., Beck, T.J., Marchand, F. and Delmas, P.D. (2005). Low skeletal muscle mass is associated with poor structural parameters of bone and impaired balance in elderly men—The MINOS study. *J. Bone. Miner. Res.* **20**, 721-729.

Tobalske, B.W. and Dial, K.P. (2007). Aerodynamics of wing-assisted incline running in birds. *J. Exp. Biol.* **210**, 1742-1751.

Tobalske, B.W. and Dial, K.P. (1996). Flight kinematics of Black-billed magpies and pigeons over a wide range of speeds. *J. Exp. Biol.* **199**, 263–280.

Tobalske, B.W. and Dial, K.P. (2000). Effects of body size on take-off flight performance in the Phasianidae (aves). *J. Exp. Biol.* **203**, 3319–3332.

Veit, A.C. and Jones, I.L. (2003). Function of tail streamers of red-tailed tropicbirds (*Phaethon rubricauda*) as inferred from patterns of variation. *The Auk*. **120(4)**, 1033–1043.

Walker, J.A., Ghalambor, C.K., Griset, O.L., McKenney, D. and Reznick, D.N. (2005). Do faster starts increase the probability of evading predators? *Funct. Ecol.* **19**, 808–815.

Weller, J.A., Levin, I.P. and Bechara, A. (2010). Do individual differences in Iowa Gambling Task performance predict adaptive decision making for risky gains and losses? *J. Clin. Exp. Neuropsyc.* **32 (2)**, 141–150.

Whitehead, C.C. (2004a). Skeletal disorders in laying hens: the problem of osteoporosis and bone fractures, in: *Welfare of the Laying Hen*. Wallingford, CABI Publishing.

Wilkins, L. J., Brown, S.N., Zimmerman, P.H., Leeb, C. and Nicol, C.J. (2004). Investigation of palpation as a method for determining the prevalence of keel and furculum damage in laying hens. *Veterinary Record.* **155**, 547-549.

Wilkins, L.J., Mckinstry, J.L., Avery, N.C., Knowles, T.G., Brown, S.N., Tarlton, J. and Nicol, C.J. (2011). Influence of housing system and design on bone strength and keel bone fractures in laying hens. *Veteterinary Record.* **169**, 414.

Wood-Gush, D.G.M. (1971). The Behaviour of the Domestic Fowl. London: Heinemann Educational Books LTD.

Zaaf, A., Van Damme, R., Herrel, A. and Aerts, P. (2001). Spatio-temporal gait characteristics of level and vertical locomotion in a ground-dwelling and a climbing gecko. *J. Exp. Biol.* **204**, 1233-1246.

Zimmerman, P.H., Buijs, S.A.F., Bolhuis, J.E. and Keeling, L.J. (2011). Behaviour of domestic fowl in anticipation of positive and negative stimuli. *Anim. Behav.* **81**, 569-577.