Genetic improvement of skeletal architecture and locomotion in domestic poultry

Brendan Michael Duggan

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Contents

Abstract

Breeding success in the broiler chicken has been accompanied by gait problems which are detrimental to productivity and welfare. Although these gait issues have not been reported to the same extent in Pekin ducks, there is concern that such problems will manifest if the duck continues on its current selection trajectory.

In order to understand how changes in morphology due to selection have affected gait in both species, divergent lines were objectively assessed for gait using a pressure platform (12 birds per line at three, five and seven weeks of age). The broiler chicken was compared to the slower growing layer chicken and the Pekin duck to its slower growing ancestor, the mallard. Two breeding lines of Pekin duck were also assessed. After gait assessment, the leg bones (femur and tibiotarsus) were scanned by computed tomography to measure morphological changes which have occurred due to selection for high growth and meat yield. Results were analysed by ANOVA, accounting for age and sex.

During walking, heavy lines walked at a slower velocity, displayed a wider stance and spent more time supporting their mass on both feet than their lighter conspecifics, strategies which are likely to improve balance. The foot angle while walking differed between lines; all duck lines rotated their feet internally whereas the layer chickens' feet were aligned with the direction of travel. Conversely the broiler chicken rotated its feet externally by seven weeks of age.

Morphologically, the main differences were between species. Duck lines reached adult leg size earlier than chickens, which may be a response to differing adaptive environments prior to domestication. This early cessation of bone growth in ducks may provide more opportunity for the bones to remodel to handle the loads imposed

on them. Lower levels of porosity and a unique cortical architecture observed in ducks endow relatively greater bone strength. Bone curvature also differed between species; the tibiotarsus curved more laterally in ducks than in chickens and may be a swimming adaptation that hinders locomotion on land in the modern production bird.

In order to improve the objectivity of selection for better gait in poultry, the genetic parameters of gait components selected on the basis of results in this thesis were estimated using a linear mixed model in a population of Pekin ducks of known pedigree. As they are a simpler measure, similar or improved heritability estimates were estimated for these gait components when compared with the standard commercial gait score which is based on a subjective view of walking ability.

Intense selection for economic traits has altered gait in similar ways in both species. To improve gait in poultry, greater breeding success may be achieved by focussing on those components of gait which have changed through selection, rather than using a subjective overall visual gait score. Furthermore, in both species, adaptations for pre-domesticated life may have affected the ability with which the selected lines have accommodated their gait to other morphological changes associated with increasing body mass.

Lay Summary

Poultry raised for meat production have been intensely selected for increased growth rates and meat yields over the last six decades. This selection has resulted in modern birds with greatly altered morphologies which have been associated with welfare problems such as sub-optimal walking ability. While these gait problems have not been reported to the same extent in the Pekin duck as in the chicken, concern exists that gait problems will manifest in Pekin ducks in the future if they continue along their current selection trajectory. The aim of this PhD is to understand the changes in morphology and gait which have occurred in chickens and ducks as a result of intense selection and to work with the breeding industry to develop a more objective method of selecting for better gait which can be used to improve the welfare of Pekin ducks worldwide.

When heavily-selected meat-producing ducks and chickens were compared to smaller 'unselected' ducks and chickens respectively, the meat producing birds had altered their gait in very similar ways, adopting a slower velocity, a wider stance and spending less time supporting themselves on a single leg. These are likely strategies to compensate for a lack of balance brought about by their large breast muscle mass. The architecture of the leg bones differed between ducks and chickens. Ducks have curved leg bones, which seem to be an adaptation to a swimming lifestyle. This adaptation may hinder walking ability on land and since the modern Pekin duck does not normally have access to water, this warrants attention from the breeding companies.

By using the results of gait analysis studies, a more objective method of selecting for better gait was developed by focusing on particular simple gait components rather than the current system which uses a subjective overall impression of walking ability. This simpler and more objective scoring system allows for more progress to be made when selecting for better gait in ducks.

The results of this thesis demonstrate that intense selection for greater meat production in birds has altered their leg architecture and their gait. The use of a simpler and more objective scoring system which focusses on components of gait rather than on an overall impression of walking ability can bring about greater improvements in the gait and welfare of meat-producing birds.

Introduction

Gait problems in poultry

The process of domestication through artificial selection changes an animal's form and function. For example the broiler chicken, often cited as an illustration of the successes of intense genetic selection, has increased its growth rate by 300% in the last 50 years (Knowles et al. 2008). Some of these changes brought about by intensive selection can be detrimental to welfare. Abnormality of gait is one such detrimental effect, and has been observed in dairy cattle, pigs, turkeys, broiler chickens and ducks (Mercer and Hill 1984, Martrenchar 1999, Jones and Dawkins 2010, Chapinal et al. 2012, de Koning et al. 2012). 'Gait' refers to the manner in which an animal walks or runs, the pattern of movements of the limbs that translates into overall movement of the body in a certain direction. It is a combination of the animal's conformation, stride pattern, balance and velocity. The gait of any particular animal will alter depending on the terrain over which the animal is moving, the velocity that the animal is trying to achieve, the functional capacity of its limbs to transport the mass of the body and the ability of its nervous system to process and respond to specific terrains, velocities and body capabilities. Each species generally has a range of gaits it can employ. Sub-optimal gait can be defined as a gait that is outside of the normal range of gaits observed in a particular species. An example of sub-optimal gait in humans is visible lameness, due to injury of the leg, or a shuffling gait, often seen in the elderly due to an inability to control lateral centre of mass movements.

Of the various welfare issues that affect the global livestock industry, poor gait in poultry (mainly broiler chickens) tends to be a prominent problem. Poultry are by far the most numerous animals raised for meat worldwide (FAO 2014); therefore, welfare problems within the poultry industry affect a vast number of individual animals. Each year, over 60 billion chickens and over 2 billion ducks are killed for meat globally. A survey of 2,800 birds in 28 broiler chicken flocks in Denmark found that 30% of them suffered from poor gait (Sanotra et al. 2001). A similar study using over 50,000 birds sampled from 176 flocks reported poor locomotion in 27% of the birds (Knowles et al. 2008) with 3% being unable to walk. Kestin *et al* (Kestin et al. 1992) also reported a similar prevalence of sub-optimal gait in chickens two decades ago. Prevalence rates of gait problems in the domestic duck are not as widely reported as in chickens but as these animals are undergoing a similar selection process to the broiler, it is reasonable to expect the incidence of sub-optimal gait in ducks to increase with selection for faster growth rates. In 1999 the standing committee of the European Convention for the Protection of Animals kept for Farming Purposes reported that "*the heavier domestic birds (ducks), in particular those selected for meat production, may be unable to fly, have difficulty in walking and be subject to leg disorders*" (Council of Europe 1999). The scale of gait problems in commercial duck populations is also poorly defined. The only study to date reporting the prevalence of gait abnormalities in commercial ducks estimated that 14% of 23 day old and 21% of 41 day old Pekin ducks show signs of moderate to significant walking impairment (when assessed using a three point scale of walking ability) (Jones and Dawkins 2010). Furthermore the ability of ducks to walk at any age seems to decrease as their weight increases (Robison et al. 2015), which implies that gait problems are (directly or indirectly) related to the selection for better production characteristics.

Causes of gait problems in poultry

Sub-optimal gait cannot be attributed to one particular cause. Both infectious and non-infectious leg problems can occur which affect gait. For example environmental factors such as litter quality can lead to conditions such as foot pad dermatitis (Mayne et al. 2006); feed quality or metabolic disorders can lead to rickets, and femoral head necrosis can be caused by infections (Thorp 1994). However, in meat type birds, sub-optimal gait is generally of non-infectious origin, often attributed to skeletal, conformational or morphological changes (Mench 2004). The heavily selected meat-type bird has undergone immense changes in leg morphology (Paxton et al. 2010, Duggan et al. 2015) and centre of mass location (Corr et al. 2003, Paxton et al. 2013). An increase in breast muscle mass has led to a cranial shift in the body's centre of mass, affecting balance and exerting increased strain on the hindlimbs which they are not evolved to deal with (Paxton et al. 2010). Hindlimb musculature has increased through selection (Paxton et al. 2014, Duggan et al. 2015) and, to deal with such large changes in body form, birds have adapted their gait by taking shorter strides and widening their stance (Corr et al. 2003, Paxton et al. 2013). This adaptation of gait to suit body morphology also occurs in wild avian species; slimmer king penguins reduce lateral movements during walking when compared to fat penguins (Willener et al. 2016).

Skeletal defects in the hindlimb can affect gait by altering the forces applied during walking. Common defects reported in meat-type poultry are long bone deformities of the hindlimb, such as valgus and varus bending and bone torsion (Mench 2004, Toscano et al. 2013). These are usually described affecting the tibiotarsus and tarsometatarsus but can also be found in the femur (Thorp 1994). The degree of bending seems to be associated with deterioration in gait, with severe bending associated with complete lameness (Leterrier and Nys 1992). These angular bone deformities may be caused by rapid growth which leaves insufficient time for the leg bones to remodel or by dietary deficiencies which can disrupt growth plate activity (Julian 1998). Some angular deformities, such as the lateral curvature observed in duck tibiotarsi (Duggan et al. 2015), may be swimming adaptations which have been retained from a previous adaptive environment and thus, while these are not deformities as such (as they occur naturally in most birds of the mallard species) they may contribute towards sub-optimal gait on land. Long bone torsion can affect gait by altering muscle attachment sites in the leg and thus affecting the forces they produce and by changing the orientation of the foot during the stance phase of the gait cycle, which can affect the direction in which forces are acting. The causes of abnormal bone torsion are unclear; altered load bearing may play a role in broiler chickens, bone torsion seems to be associated with increased body mass (Duff and Thorp 1985).

The link between tibial dyschondroplasia (TD) and poor gait is unclear. While birds exhibiting TD have been recorded as having poor gait, the issue is confounded by other factors associated with TD, such as long bone deformities or increased body mass, which are also associated with poor gait. The pathology has been reported in chickens, turkeys and ducks (Wise and Nott 1975, Hester and Ferket 1998, Hocking et al. 2009). TD results from a disruption of the chondrocyte maturation process during bone development at the growth plate and leads to the retention of cartilage at this site (Farquharson and Jefferies 2000). However the exact cause of the cellular disruption is unknown and its incidence is associated with many factors (Thorp 1994) although there seems to be a genetic component (Kapell et al. 2012). There is some evidence that TD is associated with tibiotarsal deformity in broiler chickens (Lynch et al. 1992).

Gait and Leg Health

While gait can be suboptimal in birds with perfect leg development, skeletal and non-skeletal leg health issues are responsible for some incidences of poor gait and genetic improvement of these general leg health issues has likely alleviated some of the sources of poor gait. Traits such as crooked toes, valgus and varus long bone deformities, hock burn and foot pad dermatitis have all be subject to long term selection within breeding companies, with some progress achieved (Kapell *et al* 2012,(Kapell et al. 2017)). Contact dermatitis, or hock burn, is a trait that manifests when birds spend long periods sitting on hocks in litter that contains ammonia (Bradshaw et al. 2002). This is usually due to an inability to stand caused by leg weakness. The leg weakness can be skeletal in origin or soft-tissue related (such as a slipped tendon). Foot pad dermatitis can be a painful condition that affects gait, caused by inflammation of the dermis layer of the foot in wet litter conditions (Bradshaw et al. 2002). There is a genetic component to foot pad dermatitis (Kapell et al. 2017) and populations can be selected either for an ability to tolerate wet litter or for a tendency to produce drier litter (by selecting for water intake). Progress made in selecting against these leg health traits will go some way to improving gait in a population. However the complete eradication of these negative traits will not solve the problem of poor gait in poultry, as birds with perfectly healthy legs can also experience sub-optimal gait, for example if a certain body morphology alters the position of the body's centre of mass and thus hinders balance when walking.

Gait, pain and economics

The issue of whether sub-optimal gait is painful or not remains unclear. When offered a choice between two types of feed, one containing analgesic and the other without, birds with poor gait have been shown to self-medicate, consuming proportionally more analgesic depending on the severity of their gait problems (Danbury et al. 2000). Caplen *et al*. (2013) observed that birds exhibiting severe gait problems improved their gait after treatment with analgesics. However, a study by Corr *et al.* (2007) found that analgesics had no effect on the birds' velocity or the peak vertical forces they exert while walking, suggesting that birds with poor gait were not in pain. Indeed, some altered gait may simply be a response to an altered morphology, rather than a response to pain (Corr et al. 2007, Paxton et al. 2013).

Regardless of the issue of pain, gait problems can still lead to compromised welfare; for example, poor gait can cause a failure to reach food and water or an inability to perform natural behaviours.

Sub-optimal gait also has economic consequences. McNamee *et al.* (1998) reported 10% of the flocks they observed were culled due to severely compromised gait. At the slaughterhouse, some carcasses may be downgraded due to leg problems (Kestin et al. 1999). In addition, the general negative publicity of lameness in poultry may cause economic losses for the poultry industry. The energy efficiency of poor gait may also have economic consequences on the industry. However, these issues have not been investigated in depth. On the one hand, birds with poor gait tend to be less active (Aydin et al. 2010)**,** therefore one would expect these individuals to expend less energy and perhaps have a lower feed conversion ratio (FCR). However, if gait problems are severe enough to affect a bird's ability to access food or water, FCR may worsen. Finally, a perfect gait is assumed to be the most energy efficient method of transporting the bird's mass. Intuitively, a sub-optimal gait is expected to be less energy efficient (in a hypothetical population in which all birds' feed intake and genotypes are the same, FCR would be higher in those individuals with poor gait) (Waters and Mulroy 1999). This hypothesis has yet to be tested in birds.

Gait Assessment

Gait is usually assessed on farm using a visual gait score, based on the Bristol Gait Score system developed by Kestin *et al.* (1992). A scorer visually assesses walking birds over a period of a few seconds and each bird is scored on a six-point scale from zero to five, with zero being perfect gait and five being fully lame. Birds are also scored *ad hoc* using a binary scoring system as staff walk through the farm, with fully lame birds being culled immediately. The visual gait scoring system is non-invasive, requires no equipment, set-up or data manipulation and minimal training for scorers. It enables high-throughput collection of data for thousands of birds with minimal cost or time investment and therefore it has become the method of choice for both on-farm welfare assessments and phenotypic measurement within breeding programmes. However, as the score is highly subjective, concerns have been raised as to whether it is an appropriate tool to accurately measure gait, particularly in breeding programmes where accurate measures for each individual are required (during welfare assessments, flock level data may suffice). It has long been suggested that the Bristol gait scoring system was too subjective and that a more objective system of measuring gait in poultry was needed (Anon 2000). Efforts were made to refine the Bristol gait score to improve its objectivity and reliability (Garner et al. 2002) (their intra-observer reliability for Kestin's original gait score was 0.90 compared to 0.95 for their improved version) but the authors of this work still acknowledge that the modified gait scoring system was too subjective (Mench 2004). A separate study found only moderate (0.63 - 0.78) inter-observer reliability for Kestin's gait score (Webster et al. 2008). A reduced, 3-point score, has recorded better reliabilities (Webster et al. 2008, Makagon et al. 2015), but it is difficult to foresee a use for this type of system, given the limited number of categories if offers. Visual gait scores cannot differentiate poultry with and without limb pathologies, and it has been concluded that "if gait scores were to continue to be used as a proxy measure of leg health, then there is a need to better understand the factors that contribute to gait scores, and how these factors affect leg health and, more generally, bird health and welfare" (Sandilands et al. 2011). Future gait selection may be informed by modelling morphological changes and their effect on gait and by focussing on key gait components, rather than the overall gait of each individual.

In a research setting, gait can be measured objectively using various techniques. There are two main categories: kinematic (measurement of spatial and temporal aspects of how the leg moves) and kinetic (measurement of the forces applied through the leg). Force plates have been used to investigate how birds convert potential energy into kinetic energy during the stance phase of the gait cycle (Muir et al. 1996, Usherwood et al. 2008) and to measure ground reaction forces in chickens as they walk (Corr et al. 2007). The force plate consists of a platform that sits atop a set of strain gauges. The gauges measure any forces applied to the platform as the bird walks over it; output from the gauges is integrated in computer software to calculate the overall force applied by the bird for each step. This force is measured in the vertical direction and also in the fore-aft and medio-lateral directions. However, the force plate cannot measure which parts of the foot are applying pressure to the platform or distinguish between two feet applying a force at the same time.

Pressure-based systems can record forces applied by individual feet simultaneously. A pedobarograph (Fig. 1) has been used to measure pressure distribution under the foot in chickens (Corr et al. 1998). Birds were walked over a plastic interface (unexposed, processed photographic paper) on a glass plate. The emulsion layer of the plastic interface scatters light as it passes through the glass plate, to different degrees depending on the pressure it is experiencing, and whether that pressure is applied statically or dynamically. The intensity of diffraction is proportional to the amount of pressure applied; thus, software can convert diffraction intensities (recorded by video camera) into pressure measurements. However, the system is unsuitable for analysis of a sequence of steps due to its size. An advantage of the pedobarograph system is that it can record kinetic measures of gait (pressures and, indirectly, forces applied through the foot) and also spatio-temporal aspects of each walk, although the latter must be calculated manually and is not suitable for high-throughput data collection.

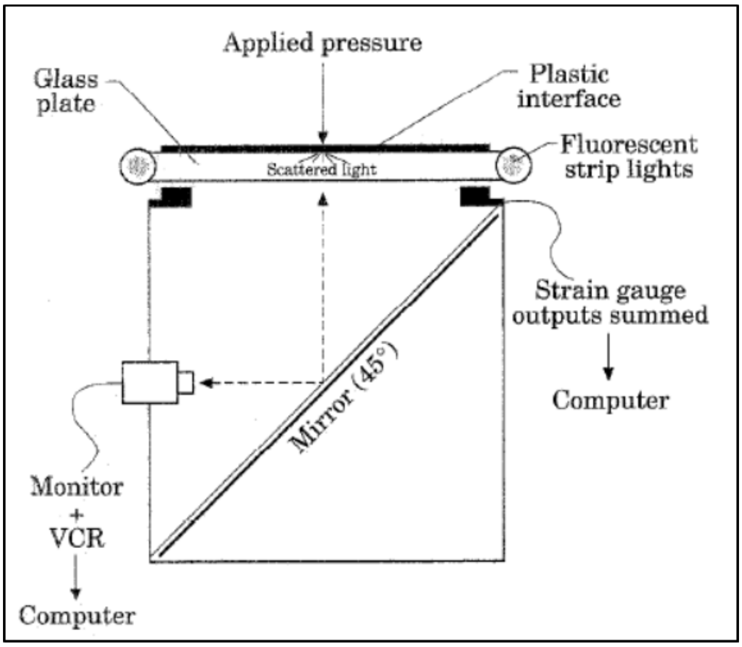

Fig. 1. Diagrammatic representation of a pedobarograph (from Corr *et al.*, 1998).

The pressure walkway (Fig. 2) employs a similar principal to the pedobaragraph but in digital form. The walkway consists of a series of mats each containing a circuit board embedded with an array of piezoelectric cells, which produce a varying voltage output depending on the pressure applied. As a bird walks over the mat, the system records the pressure applied across each foot (using a known calibration to convert voltage into pressure) as well as spatial and temporal information from each piezoelectric cell. The cells embedded in the mat are robust enough to record pressures of any animal’s gait and mats can be joined together in sequence to create a long pressure walkway capable of recording data over a series of steps. The pressure walkway is the most comprehensive of the kinematic gait measurement systems developed to date, as it can record kinetic, temporal and spatial information for each foot placement. Pressure data from each stance can be converted into forces (Fig. 3) and while force measurements obtained using the pressure walkway system are not considered as accurate as force plate measurements, the values are reliable to use for comparison within and between individual animals (Lascelles et al. 2006).

Fig. 2. A Pekin duck ready to walk over a pressure walkway (which is hidden under latex sheeting to provide the bird with grip). Data is recorded on the laptop in the background.

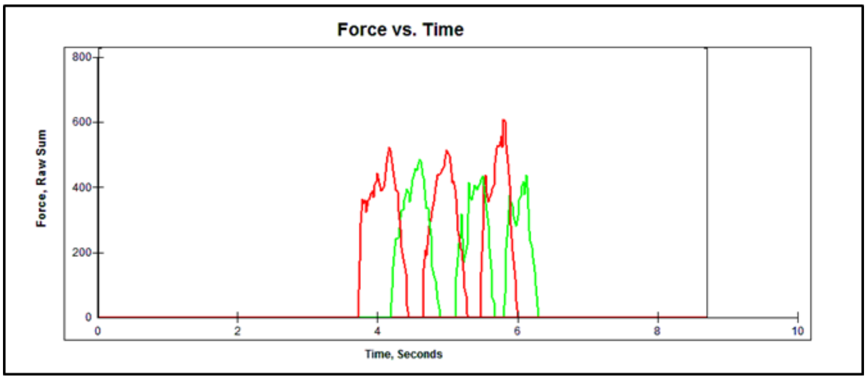

Fig. 3. Forces calculated using a pressure walkway from a Pekin duck while walking. The right foot is in red and the left foot is in green.

Whereas kinetic gait analysis techniques measure the forces applied through the ground during the gait cycle, kinematic techniques record the movement of the limbs and body during the gait cycle. The majority of kinematic studies employ the use of reflective markers attached to key positions on the limbs and torso of the subject and custom software that uses high-speed cameras to track the position of each marker throughout the gait cycle and thus record the movement of the limbs and other body parts central to gait. Reflective markers attached directly on the bodies of broiler and layer chickens have been used with motion capture technology to identify differences in the kinematics of gait in birds displaying normal and severely sub-optimal gait (Reiter and Bessei 1997). Another method using markers attached to a wearable saddle (Fig. 4) has also been used to compare gait in broilers with optimal and sub-optimal gait (Caplen et al. 2012). The saddle method was used partly to circumvent the issue that feathers can obscure attached markers from view, especially those at the hip and knee joints. However, the authors suggest that the wearing of the saddle can affect the gait pattern. Provini *et al* (2012) circumvented this problem by using radio-opaque markers (using surgical attachment at one site) and X-ray video to measure hindlimb movement in teal during walking and swimming. Again, it could be argued that the attachment of the markers may have altered gait in these birds, and the invasive nature precludes its use on non-experimental birds, or the study of large populations. Dynamic X-rays have also been used to record kinematic parameters of leg movement in lapwing (Fig. 5) walking at varying speeds on a treadmill (Nyakatura et al. 2012). The use of a treadmill simplifies kinematic analysis somewhat in that the cameras are not required to follow the bird as it moves (Reiter and Bessei 1997, Nyakatura et al. 2012). However, due to the lateral position of the eyes, birds will correct their gait by 'head-bobbing' to retain visual focus during displacement. The area lateral to the path of movement will appear blurred due to the relative displacement of the eye. Head bobbing allows the bird to hold its head stationary (and thus maintain focus) for as long as possible when walking. As relative displacement does not occur on the treadmill, the bird may not employ this corrective movement and so birds walking on a treadmill may not exhibit natural gait. Kinematic gait analysis systems tend to be more expensive to implement than kinetic systems mentioned above and require more equipment. Also, as cameras are typically required to constantly track the movement of small markers reflecting light, these systems may be hindered by dusty and dirty environments on farm, or limited in their ability to track movement of the upper leg which is covered by the wing.

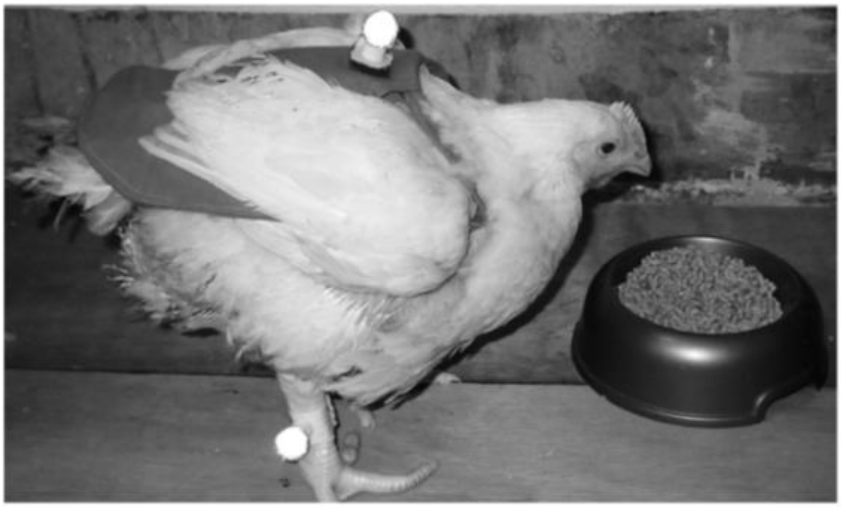

Fig. 4. Saddle with reflective markers (from Caplen *et al,* 2012).

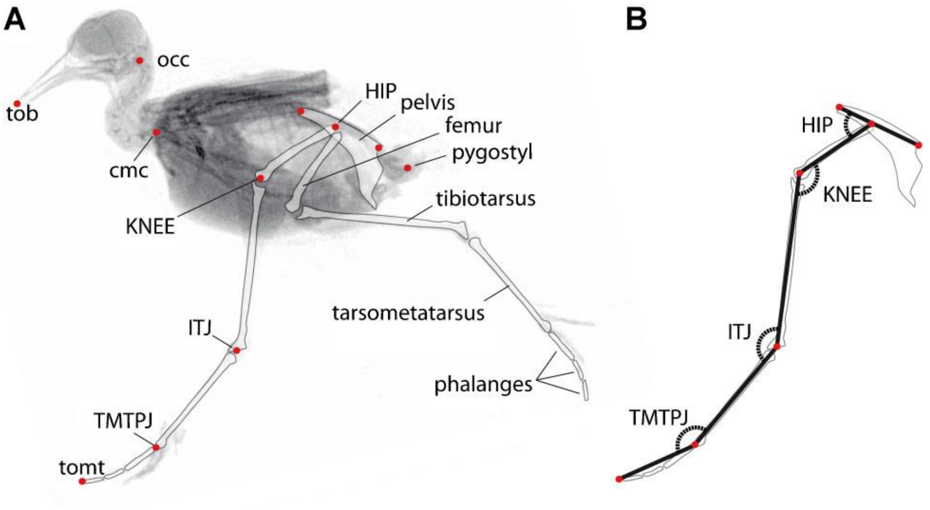

Fig 5. Landmarks used for the attachment of radio-dense markers (red dots) to obtain kinematic data using X-ray video (from Nyakatura *et al*, 2012)

Bird-mounted loggers (such as accelerometers) can record the body movement of a free-living individual. The gait of the bird can then be deduced from the patterns of body movement it exhibits (Willener et al. 2015). A benefit of this system is that it can record free movement of birds on farm, without restricting them to walkways (as is required with the methods detailed above) and accelerometry can be used to calculate the energy expenditure relating to locomotion (Green et al. 2009). The technique has even been used on species in their natural habitat (Wilson et al. 2006, Halsey et al. 2009). However, the amount of information that accelerometers record on gait dynamics is relatively limited and the technology may thus be more appropriately used as an addition to other gait measurement techniques.

Bone quality

Leg bone fractures can obviously hinder gait. Bone quality is poorer in fast growing meat lines compared with traditional lines (Hocking et al. 2009) and the incidence of bone fractures on farm, although poorly reported, is likely to be significant (Anon 2000). A lack of mineralisation has been observed in the long bones of chickens, ducks and turkeys (Williams et al. 2000, Williams et al. 2004, Charuta and Cooper 2012, Charuta et al. 2012, Van Wyhe et al. 2012). While poor mineralisation can be alleviated by dietary changes in certain cases, some bone quality issues may be due to underlying genetic causes related to accelerated growth. The leg bones of fast growing birds may not have the opportunity to remodel to handle the unnaturally large loads imposed on them. The strength of a bone can be due to many aspects of its morphology and sub-structural composition, such as overall geometry, cortical and trabecular proportions, porosity, and chemical properties of the bone itself (for example calcium to phosphorous ratios (Ammann and Rizzoli 2003). Bone strength is generally measured destructively, by dissecting out and breaking the leg bones in a three- or four-point-bending test, where a bone is fixed at both ends and a known force is applied in a given direction until failure occurs (Utz et al. 2009) (Fig. 6). Some non-invasive methods of bone quality assessment exist, such as the use of X-ray; however, this technology is more commonly used to assess the occurrence of TD rather than bone mineralisation/quality. However, computed tomography has been used to estimate bone strength (Jämsä et al. 1998, MacNeil and Boyd 2008). When reporting bone strength, various measures can be used. The maximum load that the bone can take before breaking can be measured. This can either be applied as a compressive load (parallel to the direction of the bone axis) or as a shear load (perpendicular to the direction of the bone axis) (Ammann and Rizzoli 2003). Loads can be applied as a singular force or within a repeated cycle of loading and unloading to calculate the fatigue point of a loads capability to maintain strength

(Kim et al. 2007). These measures of fatigue strength may lack some biological relevance, since in living bone, remodelling will constantly occur during long term loading to maintain the structural integrity of the bone. Stiffness refers to the ratio of stress to strain in a bone under loading and can be approximated by the ratio of load to deformation (for example the amount of force required to bend the centre point of a long bone by a given distance during loading when both ends of the long bone are fixed in place). Young's modulus of elasticity measures the ability of bone to undergo changes in length during compression while still maintaining functional integrity. The bending moment of bone refers to how much the midpoint of a long bone can deviate during shear loading (when bone ends are fixed) while still maintaining functional integrity (Utz et al. 2009).

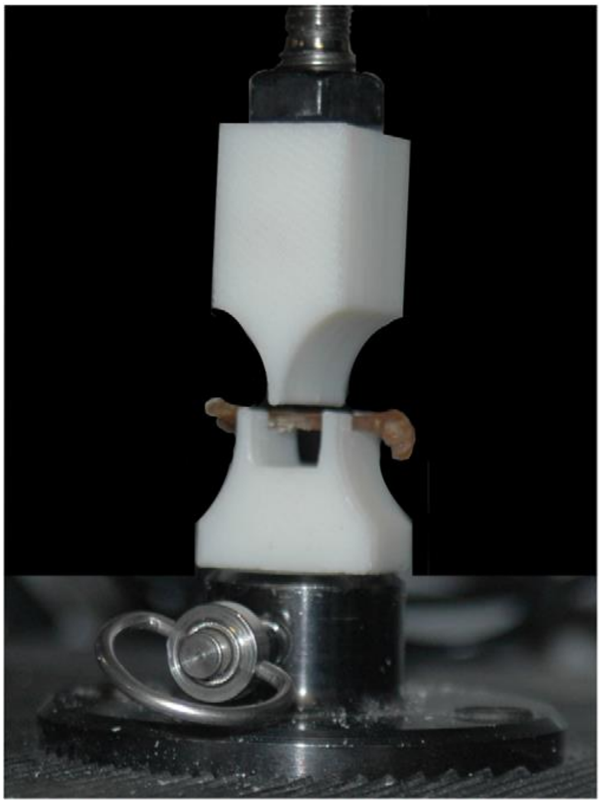

Fig. 6. Three-point bending (from Utz *et al, 2009*)

Breeding for better gait

To alter a trait such as gait in the general population, breeding companies must include this objective in their breeding goals. The breeding goals of a company are the traits upon which a high selection pressure is placed during selection of the next generation. The poultry industry lends itself well to making rapid and far-reaching changes to the global poultry population. For example, the vast majority of individual birds produced for meat globally are derived from only a handful of breeding companies. The breeding structure for each company is similar; selection is carried out on small nucleus flocks that are kept in bio-secure facilities. From these flocks, the birds with the most desirable traits are selected to become the parents that will contribute to the next generation. This group of selected birds are the great-grandparents of the production birds which eventually be reared for the table. As each great-grandparent female can produce over 200 offspring a year, and each of those offspring (the grandparents) can produce the same number of progeny, a vast number of parent stock can be produced to supply the chicks that will be reared as production birds from relatively few great-grandparents. This system, referred to as a ‘breeding pyramid’ (Fig. 7), ensures that trait changes brought about through selection in the small nucleus flocks are expressed in the global population of production birds a few generations later.

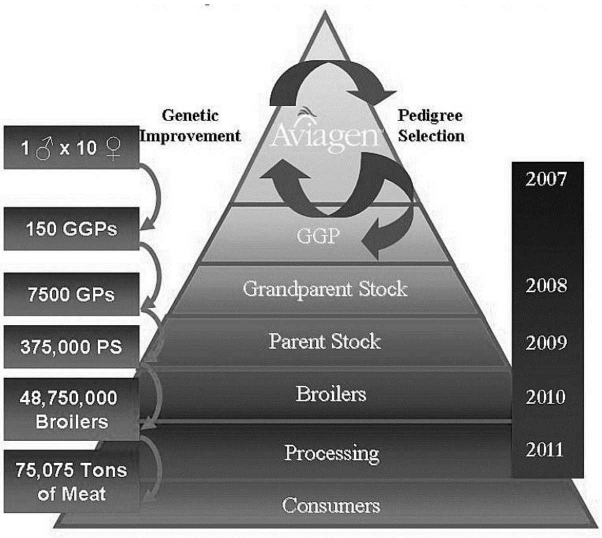

Fig. 7. An example of a poultry breeding pyramid (GGP = great grandparent stock). ©Aviagen

Trait selection is traditionally achieved by phenotypic measurement of individuals. The nucleus flocks are divided into particular lines and generally, a different selection pressure is applied to each line. These lines are crossed to produce the production birds. For example, lines that are chosen to be the paternal parents or grandparents of the production birds may be heavily selected for growth rate or breast muscle mass whereas selection in lines that occupy the maternal side of the cross may focus more on fertility. Certain traits are selected for in all lines, such as resistance to disease and other health traits. The methodology of phenotypic measurement varies for each trait. Certain traits, such as feed conversion ratio (FCR) are measured over a period of weeks using data recorded daily on feed intake and body mass. Other traits, such as breast muscle mass or gait, may only be assessed at a single time point in the birds' life. Although the breeding birds in the nucleus flock will survive beyond sexual maturity (in order to reproduce), measurement and selection of traits which are desirable in the production birds will occur in the first five to seven weeks of life (after which point, production birds are generally slaughtered). Certain trait measurements (such as assessment of bone strength) are destructive and so birds cannot contribute their genes to the next generation after phenotypic measurement for such traits. In these cases, sib-selection is carried out, where the close relatives of an individual displaying a desirable trait are selected. These siblings are likely to carry some of the genes that have led to the expression of the desirable trait; therefore, by selecting the individual's siblings, the genes for the desirable trait can be passed on to the next generation.

As each bird is simultaneously under selection for a variety of traits, a selection index is used to weight each trait depending on its economic importance (as well as their heritabilities and genetic correlations) (Hocking 2014). The weighting that each trait receives when calculating the index depends on current (and predicted future) market trends. For example, if particular traits such as breast size or leg health are expected to influence consumer choice in a few generations time, then these traits will receive a greater weighting when forming the index upon which individuals within the nucleus flock are selected to produce the next generation.

A final consideration when forming a selection index is the heritability of each trait and the genetic correlation between traits. Heritability is the proportion of the phenotypic variation which can be accounted for by additive genetic variation (or simply, the extent to which phenotypes are determined by the genes transmitted from the parents) (Falconer and Mackay 1996). The more heritable a trait is, the faster a response is observed when selecting for or against that trait. Genetic correlations are important when selecting for multiple traits simultaneously. A favourable correlation exists when two desirable traits are correlated and can be selected together without selection for one of the traits negatively impacting on the genetic progress of the other. Unfavourable genetic correlations generally slow genetic progress. For example, in chickens, a high body mass and low gait score are both desirable traits; however, body mass and gait score are positively correlated (Kestin et al. 1999), meaning the correlation is unfavourable. Therefore, if selection is heavily weighted towards a higher body mass, it will be difficult to make any progress selecting for better gait.

A key factor in the success of this system is the accurate measurement of phenotypic traits. Most traits (especially those that are economically important and thus receive a strong weighting in the selection index) are measured to a high degree of accuracy using bespoke equipment. For example, body mass is assessed using digital weighing scales; breast muscle size is assessed using ultrasound; FCR is measured in feeding stations which quantify feed intake for each bird using digital weighing scales and transponder tagging. However, some traits, such as gait, are still assessed subjectively; generally, these traits are not central to consumer choice and thus are not weighted strongly in selection indexes.

Traditionally, traits such as breast muscle mass, body mass, FCR were most important economically and so breeding companies have placed a large emphasis on these traits when performing selection (Hocking 2014). As animal welfare is a growing concern for consumers, breeding companies are now placing more emphasis on welfare traits such as gait. In addition to the subjective nature of the current best practice (the visual gait score), another problem faced by breeding companies in selecting for better gait is that, as suggested by Karcher *et al* (2013), little consensus exists on what the ideal gait should be, thus making it difficult to breed towards a certain goal.

Aims of the thesis

The main aims of this thesis are firstly, to understand how leg morphology has changed in the chicken and duck during the process of intense selection for growth rate and body mass; secondly, to understand how gait has changed during this process; and finally, to develop a more objective method of assessing gait in Pekin ducks which can bring about greater success in breeding towards an ‘ideal gait’.

The challenge of describing the ideal gait of either species is outside the scope of this thesis as there still exists a lack of complete understanding as to what should constitute the ideal gait for both broiler ducks and chickens.

It was hypothesised that the process of selection for larger body mass and growth rate has affected both leg morphology and the leg bone quality. The different adapted histories of chickens and ducks prior to domestication will also have affected their hindlimb morphology in different ways. These differences in leg morphology may affect gait.

References

Ammann, P., and R. Rizzoli. 2003. Bone strength and its determinants. Osteoporos Int **14**:Suppl 3: S13-18.

Anon. 2000. The welfare of chickens kept for meat production (broilers). European Commission, Brussels, Belgium.

Aydin, A., O. Cangar, S. E. Ozcan, C. Bahr, and D. Berckmans. 2010. Application of a fully automatic analysis tool to assess the activity of broiler chickens with different gait scores. Computers and Electronics in Agriculture **73**:194-199.

Bradshaw, R. H., R. D. Kirkden, and D. M. Broom. 2002. A review of the aetiology and pathology of leg weakness in broilers in relation to welfare. Avian and Poultry Biology Reviews **13**:45-103.

Caplen, G., G. R. Colborne, B. Hothersall, C. J. Nicol, A. E. Waterman-Pearson, C. A. Weeks, and J. C. Murrell. 2013. Lame broiler chickens respond to non-steroidal anti-inflammatory drugs with objective changes in gait function: A controlled clinical trial. The Veterinary Journal **196**:477-482.

Caplen, G., B. Hothersall, J. C. Murrell, C. J. Nicol, A. E. Waterman-Pearson, C. A. Weeks, and G. R. Colborne. 2012. Kinematic Analysis Quantifies Gait Abnormalities Associated with Lameness in Broiler Chickens and Identifies Evolutionary Gait Differences. Plos One **7**.

Chapinal, N., A. Sewalem, and F. Miglior. 2012. Short communication: Estimation of genetic parameters for gait in Canadian Holstein cows. Journal of Dairy Science **95**:7372-7376.

Charuta, A., and R. G. Cooper. 2012. Computed tomographic and densitometric analysis of tibiotarsal bone mineral density and content in postnatal Peking ducks (Anas platyrhynchos var. domestica) as influenced by age and sex. Polish Journal of Veterinary Sciences **15**:537-545.

Charuta, A., R. G. Cooper, M. Pierzchala, and J. O. Horbanczuk. 2012. Computed tomographic analysis of tibiotarsal bone mineral density and content in turkeys as influenced by age and sex. Czech Journal of Animal Science **57**:573-580.

Corr, S. A., M. J. Gentle, C. C. McCorquodale, and D. Bennett. 2003. The effect of morphology on walking ability in the modern broiler: A gait analysis study. Animal Welfare **12**:159-171.

Corr, S. A., C. McCorquodale, J. McDonald, M. Gentle, and R. McGovern. 2007. A force plate study of avian gait. Journal of Biomechanics **40**:2037-2043.

Corr, S. A., C. C. McCorquodale, and M. J. Gentle. 1998. Gait analysis of poultry. Research in Veterinary Science **65**:233-238.

Council of Europe. 1999. Standing committee of the European convention for the protection of animals kept for farming purposes - Recommendation concerning domestic ducks (*Anas platyrhynchos*).

Danbury, T. C., C. A. Weeks, A. E. Waterman-Pearson, S. C. Kestin, and J. P. Chambers. 2000. Self-selection of the analgesic drug carprofen by lame broiler chickens. Veterinary Record **146**:307-311.

de Koning, D. B., E. M. van Grevenhof, B. F. A. Laurenssen, B. J. Ducro, H. C. M. Heuven, P. N. de Groot, W. Hazeleger, and B. Kemp. 2012. Associations between osteochondrosis and conformation and locomotive characteristics in pigs. Journal of Animal Science **90**:4752-4763.

Duff, S., and B. Thorp. 1985. Patterns of physiological bone torsion in the appendicular skeletons of domestic fowl. Research in Veterinary Science **39**:307-312.

Duggan, B. M., P. M. Hocking, T. Schwarz, and D. N. Clements. 2015. Differences in hindlimb morphology of ducks and chickens: effects of domestication and selection. Genetics, Selection, Evolution : GSE **47**:88.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. 4th Edition edition. Pearson Education, UK.

FAO. 2014. FAOSTAT. Live Animals, National Production. FAO, Rome, Italy.

Farquharson, C., and D. Jefferies. 2000. Chondrocytes and longitudinal bone growth: The development of tibial dyschondroplasia. Poultry Science **79**:994-1004.

Garner, J. P., C. Falcone, P. Wakenell, M. Martin, and J. A. Mench. 2002. Reliability and validity of a modified gait scoring system and its use in assessing tibial dyschondroplasia in broilers. British Poultry Science **43**:355-363.

Green, J. A., L. G. Halsey, R. P. Wilson, and P. B. Frappell. 2009. Estimating energy expenditure of animals using the accelerometry technique: activity, inactivity and comparison with the heart-rate technique. Journal of Experimental Biology **212**:471-482.

Halsey, L. G., S. J. Portugal, J. A. Smith, C. P. Murn, and R. P. Wilson. 2009. Recording raptor behavior on the wing via accelerometry. Journal of Field Ornithology **80**:171-177.

Hester, P. Y., and P. R. Ferket. 1998. Relationship between long bone distortion and tibial dyschondroplasia in male turkeys. Poultry Science **77**:1300-1302.

Hocking, P. M. 2014. Unexpected consequences of genetic selection in broilers and turkeys: problems and solutions. British Poultry Science **55**:1-12.

Hocking, P. M., D. A. Sandercock, S. Wilson, and R. H. Fleming. 2009. Quantifying genetic (co)variation and effects of genetic selection on tibial bone morphology and quality in 37 lines of broiler, layer and traditional chickens. British Poultry Science **50**:443-450.

Jämsä, T., P. Jalovaara, Z. Peng, H. K. Väänänen, and J. Tuukkanen. 1998. Comparison of three-point bending test and peripheral quantitative computed tomography analysis in the evaluation of the strength of mouse femur and tibia. Bone **23**:155-161.

Jones, T. A., and M. S. Dawkins. 2010. Environment and management factors affecting Pekin duck production and welfare on commercial farms in the UK. British Poultry Science **51**:12-21.

Julian, R. 1998. Rapid growth problems: ascites and skeletal deformities in broilers. Poultry Science **77**:1773-1780.

Kapell, D. N. R. G., W. G. Hill, A. M. Neeteson, J. McAdam, A. N. M. Koerhuis, and S. Avendano. 2012. Twenty-five years of selection for improved leg health in purebred broiler lines and underlying genetic parameters. Poultry Science **91**:3032-3043.

Kapell, D. N. R. G., P. M. Hocking, P. K. Glover, V. D. Kremer, and S. Avendano. 2017. Genetic basis of leg health and its relationship with body weight in purebred turkey lines. Poultry Science **96**:1553-1562.

Karcher, D. M., M. M. Makagon, G. S. Fraley, S. M. Fraley, and M. S. Lilburn. 2013. Influence of raised plastic floors compared with pine shaving litter on environment and Pekin duck condition. Poultry Science **92**:583-590.

Kestin, S., T. Knowles, A. Tinch, and N. Gregory. 1992. Prevalence of leg weakness in broiler chickens and its relationship with genotype. Veterinary Record **131**:190-194.

Kestin, S. C., G. Su, and P. Sorensen. 1999. Different commercial broiler crosses have different susceptibilities to leg weakness. Poultry Science **78**:1085-1090.

Kim, J. H., M. Niinomi, T. Akahori, and H. Toda. 2007. Fatigue properties of bovine compact bones that have different microstructures. International Journal of Fatigue **29**:1039-1050.

Knowles, T. G., S. C. Kestin, S. M. Haslam, S. N. Brown, L. E. Green, A. Butterworth, S. J. Pope, D. Pfeiffer, and C. J. Nicol. 2008. Leg disorders in broiler chickens: prevalence, risk factors and prevention. Plos One **3**:e1545.

Lascelles, B. D. X., S. C. Roe, E. Smith, L. Reynolds, J. Markham, D. Marcellin-Little, M. S. Bergh, and S. C. Budsberg. 2006. Evaluation of a pressure walkway system for measurement of vertical limb forces in clinically normal dogs. American Journal of Veterinary Research **67**:277-282.

Leterrier, C., and Y. Nys. 1992. Clinical and anatomical differences in varus and valgus deformities of chick limbs suggest different aetio-pathogenesis. Avian Pathology **21**:429-442.

Lynch, M., B. H. Thorp, and C. C. Whitehead. 1992. Avian tibial dyschondroplasia as a cause of bone deformity. Avian Pathology **21**:275-285.

MacNeil, J. A., and S. K. Boyd. 2008. Bone strength at the distal radius can be estimated from high-resolution peripheral quantitative computed tomography and the finite element method. Bone **42**:1203-1213.

Makagon, M. M., R. Woolley, and D. M. Karcher. 2015. Assessing the waddle: An evaluation of a 3-point gait score system for ducks. Poultry Science **94**:1729-1734.

Martrenchar, A. 1999. Animal welfare and intensive production of turkey broilers. Worlds Poultry Science Journal **55**:143-152.

Mayne, R. K., P. M. Hocking, and R. W. Else. 2006. Foot pad dermatitis develops at an early age in commercial turkeys. British Poultry Science **47**:36-42.

McNamee, P. T., J. J. McCullagh, B. H. Thorp, H. J. Ball, D. Graham, S. J. McCullough, D. McConaghy, and J. A. Smyth. 1998. Study of leg weakness in two commercial broiler flocks. Veterinary Record **143**:131-135.

Mench, J. 2004. Lameness.*in* C. A. Weeks and A. Butterworth, editors. Measuring and auditing broiler welfare. CABI Publishing.

Mercer, J. T., and W. G. Hill. 1984. Estimation of genetic parameters for skeletal defects in broiler chickens. Heredity **53**:193-203.

Muir, G. D., J. M. Gosline, and J. D. Steeves. 1996. Ontogeny of bipedal locomotion: Walking and running in the chick. Journal of Physiology-London **493**:589-601.

Nyakatura, J. A., E. Andrada, N. Grimm, H. Weise, and M. S. Fischer. 2012. Kinematics and Center of Mass Mechanics During Terrestrial Locomotion in Northern Lapwings (*Vanellus vanellus*, Charadriiformes). Journal of Experimental Zoology Part A: Ecological Genetics and Physiology:000-000.

Paxton, H., N. B. Anthony, S. A. Corr, and J. R. Hutchinson. 2010. The effects of selective breeding on the architectural properties of the pelvic limb in broiler chickens: a comparative study across modern and ancestral populations. Journal of Anatomy **217**:153-166.

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

Paxton, H., P. G. Tickle, J. W. Rankin, J. R. Codd, and J. R. Hutchinson. 2014. Anatomical and biomechanical traits of broiler chickens across ontogeny. Part II. Body segment inertial properties and muscle architecture of the pelvic limb. Peerj **2**.

Provini, P., P. Goupil, V. Hugel, and A. Abourachid. 2012. Walking, Paddling, Waddling: 3D Kinematics Anatidae Locomotion (*Callonetta leucophrys*). Journal of Experimental Zoology Part a-Ecological Genetics and Physiology **317A**:275-282.

Reiter, K., and W. Bessei. 1997. Gait analysis in laying hens and broilers with and without leg disorders. Equine veterinary journal. Supplement:110-112.

Robison, C. I., M. Rice, M. M. Makagon, and D. M. Karcher. 2015. Duck gait: Relationship to hip angle, bone ash, bone density, and morphology. Poultry Science.

Sandilands, V., S. Brocklehurst, N. Sparks, L. Baker, R. McGovern, B. Thorp, and D. Pearson. 2011. Assessing leg health in chickens using a force plate and gait scoring: how many birds is enough? The Veterinary record **168**:77.

Sanotra, G. S., J. D. Lund, A. K. Ersboll, J. S. Petersen, and K. S. Vestergaard. 2001. Monitoring leg problems in broilers: a survey of commercial broiler production in Denmark. Worlds Poultry Science Journal **57**:55-69.

Thorp, B. H. 1994. Skeletal disorders in the fowl - a review. Avian Pathology **23**:203-236.

Toscano, M. J., M. A. F. Nasr, and B. Hothersall. 2013. Correlation between broiler lameness and anatomical measurements of bone using radiographical projections with assessments of consistency across and within radiographs. Poultry Science **92**:2251-2258.

Usherwood, J. R., K. L. Szymanek, and M. A. Daley. 2008. Compass gait mechanics account for top walking speeds in ducks and humans. Journal of Experimental Biology **211**:3744-3749.

Utz, J. C., S. Nelson, B. J. O'Toole, and F. van Breukelen. 2009. Bone strength is maintained after 8 months of inactivity in hibernating golden-mantled ground squirrels, Spermophilus lateralis. Journal of Experimental Biology **212**:2746-2752.

Van Wyhe, R. C., T. J. Applegate, M. S. Lilburn, and D. M. Karcher. 2012. A comparison of long bone development in historical and contemporary ducks. Poultry Science **91**:2858-2865.

Waters, R. L., and S. Mulroy. 1999. The energy expenditure of normal and pathologic gait. Gait & Posture **9**:207-231.

Webster, A. B., B. D. Fairchild, T. S. Cummings, and P. A. Stayer. 2008. Validation of a Three-Point Gait-Scoring System for Field Assessment of Walking Ability of Commercial Broilers. The Journal of Applied Poultry Research **17**:529-539.

Willener, A. S. T., Y. Handrich, L. G. Halsey, and S. Strike. 2015. Effect of walking speed on the gait of king penguins: An accelerometric approach. Journal of Theoretical Biology **387**:166-173.

Willener, A. S. T., Y. Handrich, L. G. Halsey, and S. Strike. 2016. Fat King Penguins Are Less Steady on Their Feet. Plos One **11**.

Williams, B., S. Solomon, D. Waddington, B. Thorp, and C. Farquharson. 2000. Skeletal development in the meat-type chicken. British Poultry Science **41**:141-149.

Williams, B., D. Waddington, D. H. Murray, and C. Farquharson. 2004. Bone strength during growth: Influence of growth rate on cortical porosity and mineralization. Calcified Tissue International **74**:236-245.

Wilson, R. P., C. R. White, F. Quintana, L. G. Halsey, N. Liebsch, G. R. Martin, and P. J. Butler. 2006. Moving towards acceleration for estimates of activity-specific metabolic rate in free-living animals: the case of the cormorant. Journal of Animal Ecology **75**:1081-1090.

Wise, D. R., and H. Nott. 1975. Studies on tibial dyschondroplasia in ducks. Research in Veterinary Science **18**:193-197.

Chapter One – Bookending section

Context and rational behind the study

In order to approach the problem of improving gait in the domestic duck, it was first necessary to understand how both gait and morphology have changed throughout the period of intense selection for higher meat yield in Pekin ducks. It is important to consider both morphology and gait, as the two are closely linked. As gait problems were already well documented in broiler chickens, chickens were included in the study as a useful benchmark with which to compare with the Pekin duck. In addition, the broiler chicken and Pekin duck grow at very similar rates and reach similar body weights in their first 7 weeks. An experiment was planned to compare the heavy, fast growing Pekin duck and broiler chicken with their ancestral phenotypes. The ancestral phenotype of the Pekin is the mallard and differences between the two can demonstrate the effect that intense artificial selection has had on morphology and gait. The ancestral phenotype of the broiler chicken is the red jungle fowl. However, as these birds are expensive and hard to source, the layer chicken was used instead, as an example of a lighter, slower growing chicken. Like the mallard, this bird has not been subject to same intensity of selection for growth rate and body mass as the broiler chicken or Pekin duck. However, a possible limitation to using the layer chicken is that some selection for skeletal integrity has occurred along with selection for egg production traits. This should be kept in mind when interpreting data on bone strength in the layer chicken.

As the work was funded by a duck breeding company, two breeding lines of Pekin duck were also included in this study. Ideally, the six lines would have been raised in the same research shed together at the same time. This was not possible due to space availability and the study was split into two experiments; in the first experiment the broiler chicken, layer chicken and Pekin duck were raised to seven weeks in a converted pig unit and in the second experiment the two breeding lines of Pekin duck and the mallard were raised to the same age in the same unit. The first experiment ran from early February to the end of March and the second ran from early May to the end of June. While the environment was controlled to industry standards as much as possible, some minor differences may have existed in light and temperature profiles between experiments. In order to allow for mortalities, 2 extra birds were stocked in each pen. Within each pen, 8 birds were stocked – 2 were removed for measurement of both gait and morphology at 3 weeks, 2 at 5 weeks and 2 at 7 weeks and 2 remained as spare birds. In each pen therefore, 8 birds were stocked and data was collected on 6 of these birds. Because birds were removed from each pen during the experiment and not replaced, stocking density reduced as time passed, which would not happen in a commercial setting. After culling, a brief post mortem was carried out on each bird during their dissection and prior to recording morphology. No pathologies or defects were observed in the birds used for data collection.

Corrections and Clarifications

For each of the 6 lines studied in this chapter, morphology was recorded on 36 birds: 12 at 3 weeks, 12 at 5 weeks and 12 at 7 weeks of age. At each time point 6 males and 6 females were assessed. A further 12 birds from each line were reared as spare birds but no data were collected from these spare birds.

In the final paragraph of the Introduction, it is suggested that “selection has led to a cranial shift in the body’s centre of mass, thus altering the loading forces which act on the legs of both lines”. While the principle is likely to also apply to the Pekin duck, the citation given in support of this statement only refers to broiler chickens.

Birds were weighed (data in Figure 1) on days when staff were available to do so. Lines from Experiment 1 (layer chicken, broiler chicken and Pekin hybrid) were weighed on days 1, 15, 25, 36 and 50. Lines from Experiment 2 (Pekin male line, Pekin female line and mallard) were weighed on days 7, 14, 21, 28, 35, 42 and 49.

A more detailed description of methods described in Chapter One

Bone morphology measurement by computed tomography

All bones were evaluated with a computed tomography (CT) scan using a ‘Somatom Volume Zoom’ helical 4-slice CT unit (Siemens, Germany). A helical scan of the intact femur and tibiotarsus was performed with the following settings: 120kV, 130 mAs, 1.5s tube rotation time, 1mm slice width, collimator pitch of 0.75, high resolution image reconstruction kernel (proprietary name U90u).

Bone morphology was assessed using a dedicated DICOM viewing software (Osirix, Geneva, Switzerland, version 5.8.5-32bit) on a computer workstation (Apple IMac 27 inch, Apple, USA) with a calibrated monitor. The multiplanar reconstruction application of the software was used for angular and other measurements. Morphological measurements taken for both femur and tibiotarsus included functional length, diameter and cortical cross-sectional area at the mid-diaphysis, curvature in both frontal and sagittal planes and torsion.

Length was measured viewing the medio-lateral plane by drawing a straight line between the midpoint of the distal end of the bone to the midpoint at the proximal end of the bone.

The mid-diaphysis was located by halving the length measure above. As the cross section (in the transverse plane) of the bone is approximately elliptical in shape, the lengths of both the major and minor axes were recorded. Area of the whole cross-section of bone at the mid-diaphysis was measured using the ‘area’ tool within the software by highlighting the whole cross-sectional area. Internal area was measured by highlighting the non-cortical area of the cross-section. This was subtracted from the total area to obtain the cortical area of the bone at the mid-diaphysis.

To measure cranio-caudal curvature, the midpoint of the bone was located by halving the distance between the lateral periosteal surfaces of the mid-diaphysis when viewing the medio-lateral plane. Two lines were drawn from this midpoint of the mid-diaphysis – one to the midpoint of the distal end and another to the midpoint of the proximal end. The caudal angle made by these two lines was recorded as the cranio-caudal curvature of the bone. The same process was used to measure the mediaolateral curvature when viewing the craniocaudal plane, with the medial angle measured in this plane.

Bone torsion was measured by creating a separate DICOM file in the transverse plane of the bone, covering its whole length. A slice from the distal end was then overlaid on a slice from the proximal end of the bone. A line was drawn between selected landmarks chosen for each site. The angle between these two lines (one line drawn on the distal slice and one line on the proximal slice) was recorded as the relative torsion of the whole bone. Details of which slices were compared and which landmarks were used to form each line are given in Table S1. An example of how these lines were used to calculate relative torsion is shown in Figure S5.

Bone	**Figure**	**Position along proximal-distal axis**	**Landmarks**
Proximal femur	S1	The slice at which the diameter of the femoral head is greatest.	A line joining the centre of the femoral head to the most cranial point of the *trochanter femoris*.
Distal femur	S2	The slice at which both the *condylus lateralis* and *condylus medialis* are at their most pronounced.	A line joining the most caudal points of the *condylus lateralis* and *condylus medialis*
Proximal tibiotarsus	S3	Approaching from the proximal end, the slice at which the first sign of mineralisation appears in the *facies articularis femoralis*.	A line joining the most caudal point of the *crista cnemialis cranialis* with the most caudal point of the *facies articularis lateralis*.
Distal tibiotarsus	S4	The slice at which both the *condylus lateralis* and *condylus medialis* are at their most pronounced.	A line joining the most caudal points of the *condylus lateralis* and *condylus medialis*

Table S1. Anatomical landmarks used to calculate bone torsion

Details of anatomical terms used are from The Anatomical Atlas of *Gallus* [1].

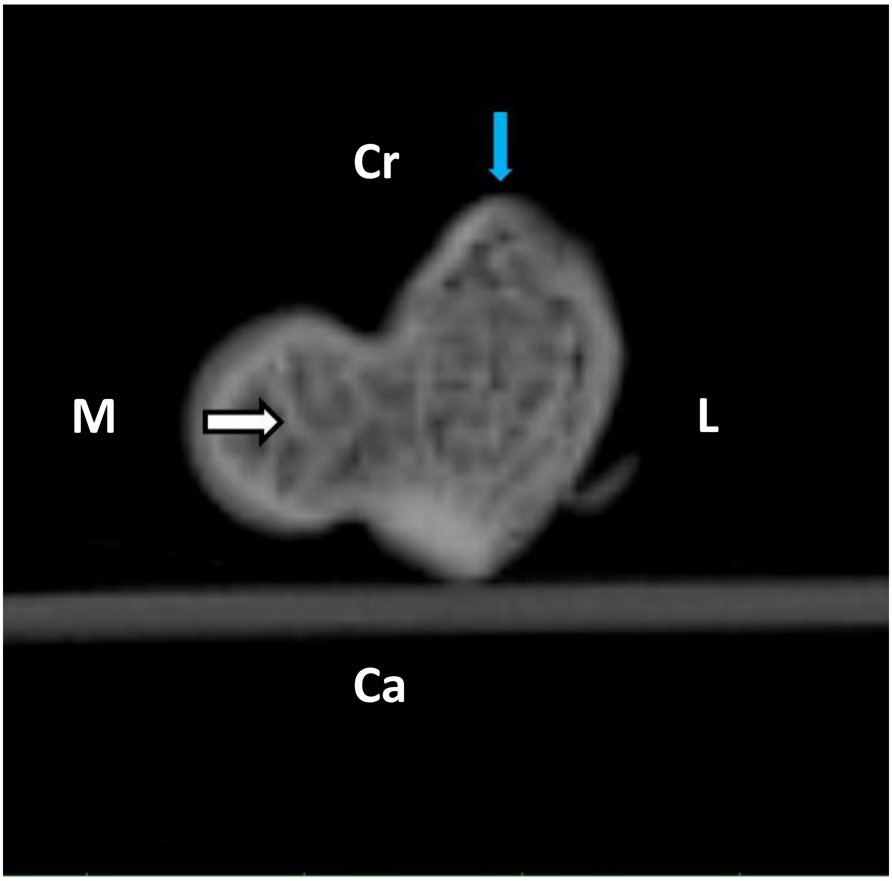

Fig S1. Proximal femur in cross-section, with the centre of the femoral head (white arrow) and *trochanter femoris* (blue arrow) highlighted.

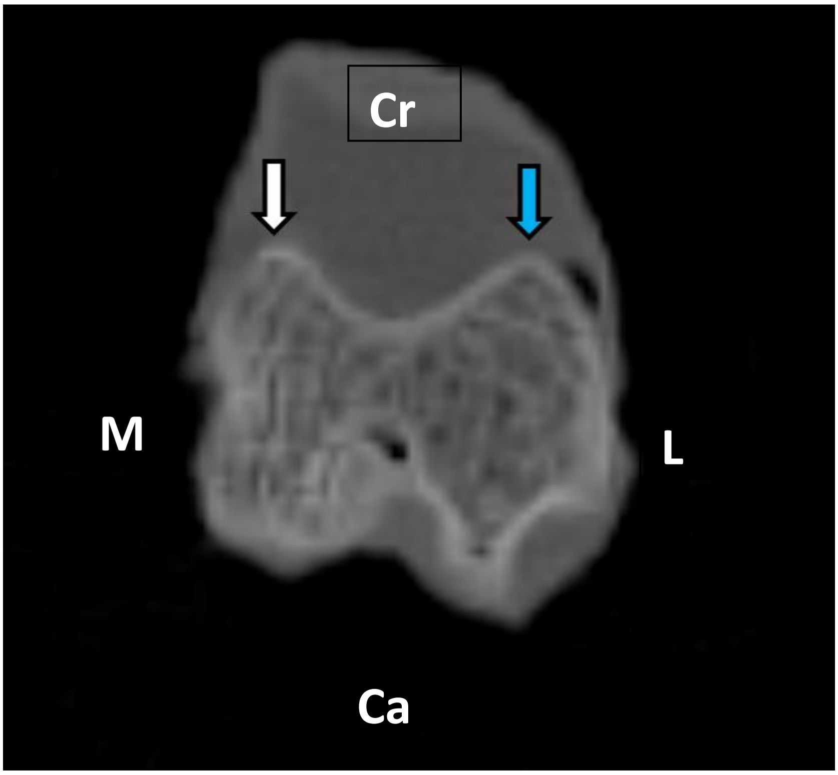

Fig S2. Distal femur in cross-section, with the *condylus medialis* (white arrow) and *condyles lateralis* (blue arrow) highlighted.

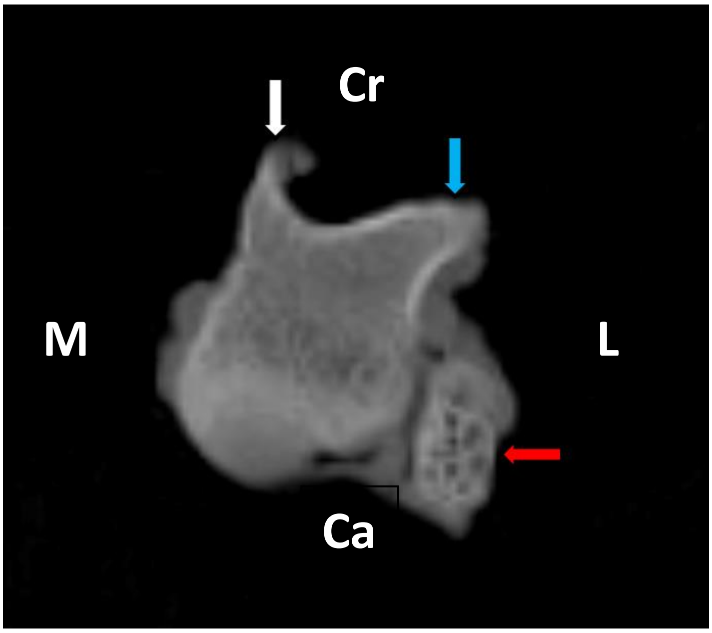

Fig S3. Proximal tibiotarsus in cross-section, with the *crista cnemialis cranialis* (white arrow), the *facies articularis lateralis* (blue arrow) and the mineralised *facies articularis femoralis* (red arrow) highlighted.

Fig S4. Distal tibiotarsus in cross-section, with the *condylus medialis* (white arrow) and *condylus lateralis* (blue arrow) highlighted.

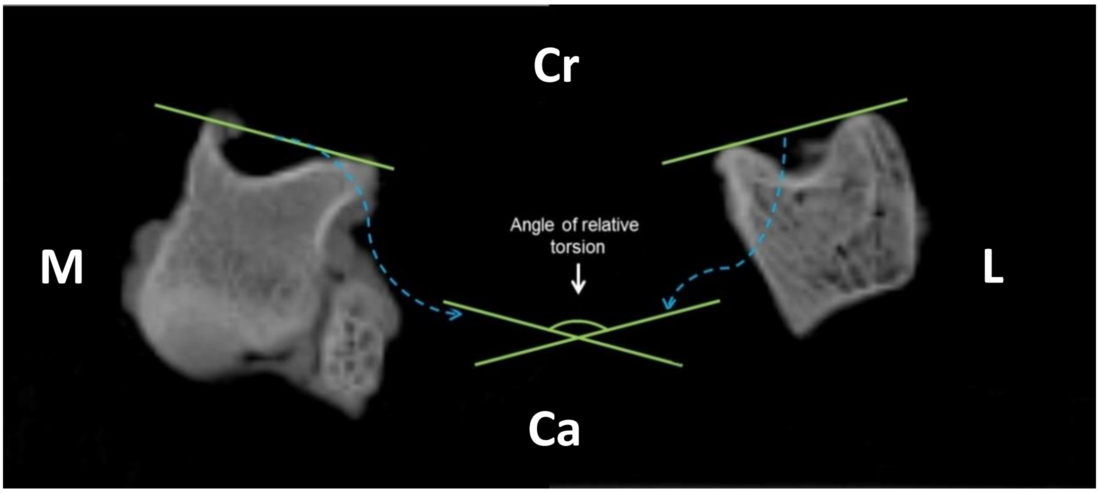

Fig S5. An example of how the angle of relative bone torsion was calculated in the tibiotarsus.

Bending stress calculations

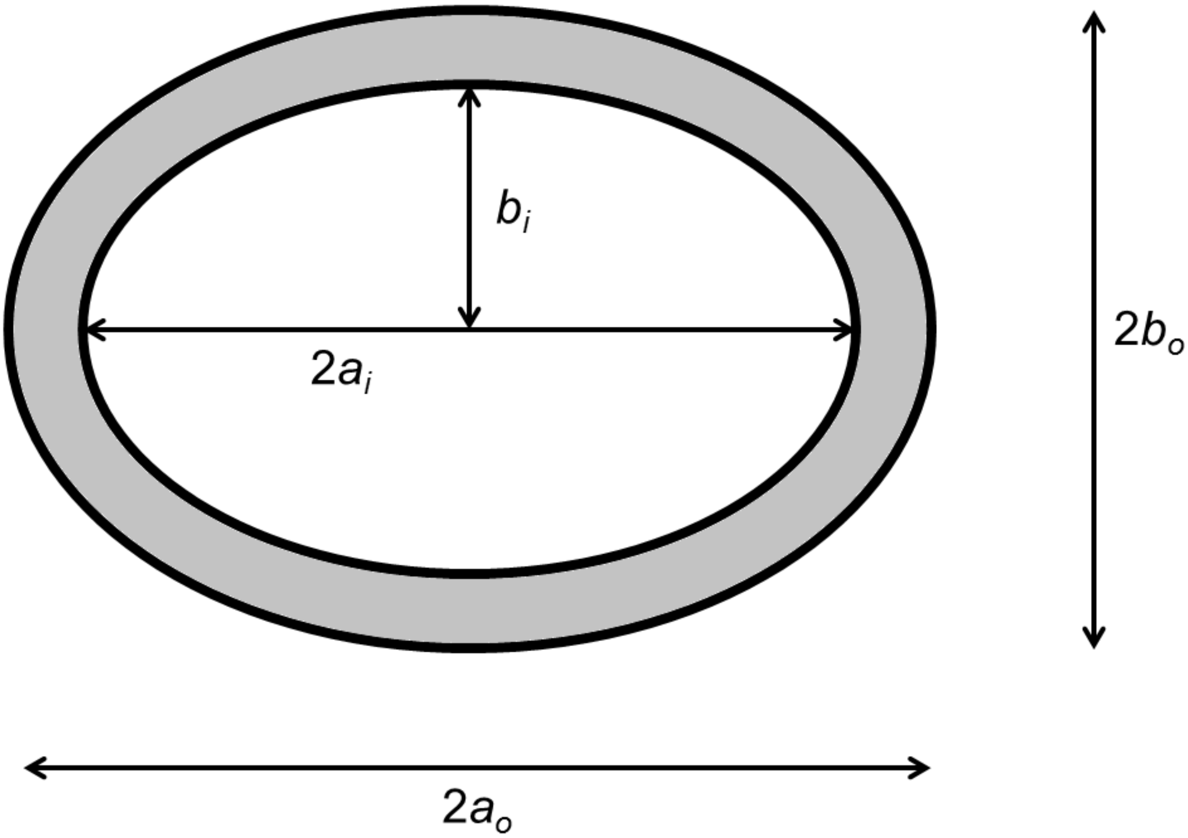

Fig. S6. Schematic of mid-diaphyseal tibiotarsal cross-section showing measurements taken or deduced as below.

The second area moment of inertia (I) was calculated by assuming the bone to be a perfect hollow elliptical cylinder of constant wall thickness and outer semi-major axis a_o, inner semi-major axis a_i, outer semi-minor axis b_o and inner semi-minor axis b_i as:

$$I = \frac{\pi}{4}\left(a_o {b_o}^3 - a_i {b_i}^3\right) \quad \dots \; a > b \quad [2]$$

This formula to calculate the second area moment of inertia (I) requires the assumption of a constant wall thickness. Therefore, rather than using measurements of a_i and b_i from CT analysis, it is assumed that $a_o : b_o = a_i : b_i$

Assume $a_o : b_o = x$

So if $a_o = xb_o$ then $a_i = xb_i$

$$Area_i = \pi a_i b_i$$

$$A_i = \pi x b_i b_i$$

$$\frac{A_i}{\pi x} = {b_i}^2$$

$$b_i = \sqrt{\frac{A_i}{\pi x}}$$

$$a_i = xb_i$$

Both a_o and b_o were taken from CT measurements.

The second area moment of inertia (*I*) is then used to calculate the bending stress (*B*) experienced by the bone, using the formula given in the main text.

Histology protocol for porosity measurement

Staining and mounting was carried out by the Veterinary Services department at the Royal (Dick) School of Veterinary Studies, UK.

To assess the porosity of each bone, 1 cm samples of the mid-diaphysis of the left tibiotarsus from each bird were cut using a bench based bone saw. The tissues were placed into 10% buffered formalin for about a week followed by decalcification in Decal I solution from Leica until soft enough to cut through easily. They were checked every 3 days until soft. The tissue was then washed in running water to remove the decal fluid for 1 hour, then processed over 2 days through graded ethanol xylenes and fixed into wax. Sections were cooled on ice and cut at 3 microns. Sections were picked up onto electrostatically charged microscope slides. The sections were dried overnight at 37°C then heated to 60°C for 30 minutes before staining with haematoxylin and eosin.

Porosity was measured on a Leica DMRB fluorescent microscope using a x5 objective lens. Photographs were taken of north, south, east and west sections of cortical bone mounted on slides. Porosity was then calculated for each photograph by converting the image to a greyscale 32 bit image and counting the percentage of pixels in each image which were black (the percentage porosity) using the 'analyse particles' function within ImageJ, a public domain image analysis software (NIH, USA, version 1.6.0_20, 64-bit). Porosity results of the north, south, east and west sections of each bird were averaged to obtain an overall cortical porosity for each bird.

Mineral content analysis protocol

Bone mineral analysis was carried out by DM Scientific (Thirsk, UK).

To measure the mineral content of each bone, 2 cm samples of the mid-diaphysis of the right tibiotarsus were cut using a bench based bone saw. Bone samples were stored at -20°C and defrosted prior to being sent for analysis.

From this 2cm cut, a 1 cm portion was taken and weighed on a 4 place balance. This 1 cm portion was dried in an oven at 103°C, the bone was then cooled and weighed to calculate the dry matter. To defat, the sample was wrapped in a filter paper and placed in a soxhlet extraction apparatus and extracted for four hours under reflux with petroleum ether. The sample was then air dried overnight and placed in an oven at 103°C for four hours to remove any residual solvent.

Ash content was calculated by placing the sample into a pre-weighed ash crucible and heating overnight in a furnace at 550°C. Once removed and cooled the sample and crucible were weighed to obtain the ash percentage.

The resulting ash sample was then used for the calcium and phosphorus analysis.

Sample was placed in a beaker and heated with 50% hydrochloric acid; once cooled the solution was placed in a 250 ml volumetric flask and diluted with deionised water.

The sample solution was diluted in a strontium chloride solution and the calcium concentration was determined on an atomic absorption spectrometer using a standard calibration curve.

The sample solution was diluted in an ammonium molybdovanadate solution and phosphorous content was determined on a visible spectrometer at 430nm using a standard calibration curve.

References

1. Yasuda M. The Anatomical Atlas of Gallus. Tokyo: Unviversity of Tokyo Press; 2002.

2. Utz JC, Nelson S, O'Toole BJ, van Breukelen F. Bone strength is maintained after 8 months of inactivity in hibernating golden-mantled ground squirrels, *Spermophilus lateralis*. J Exp Biol. 2009;212(17):2746-52. doi:10.1242/jeb.032854.

Supplementary data and enlarged figures are included below.

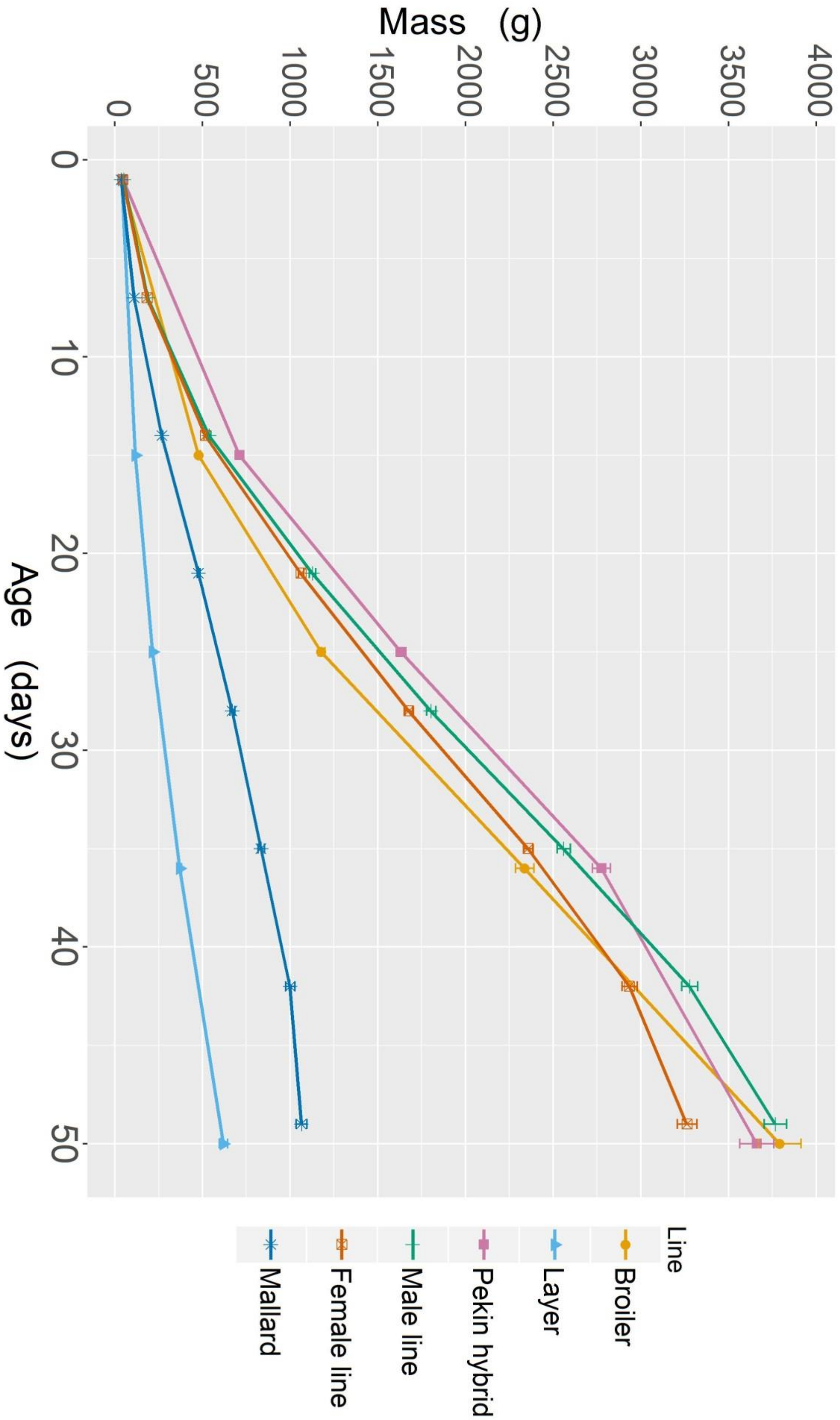

Figure 1. Body mass measurements (means and standard errors) from hatch to seven weeks of age.

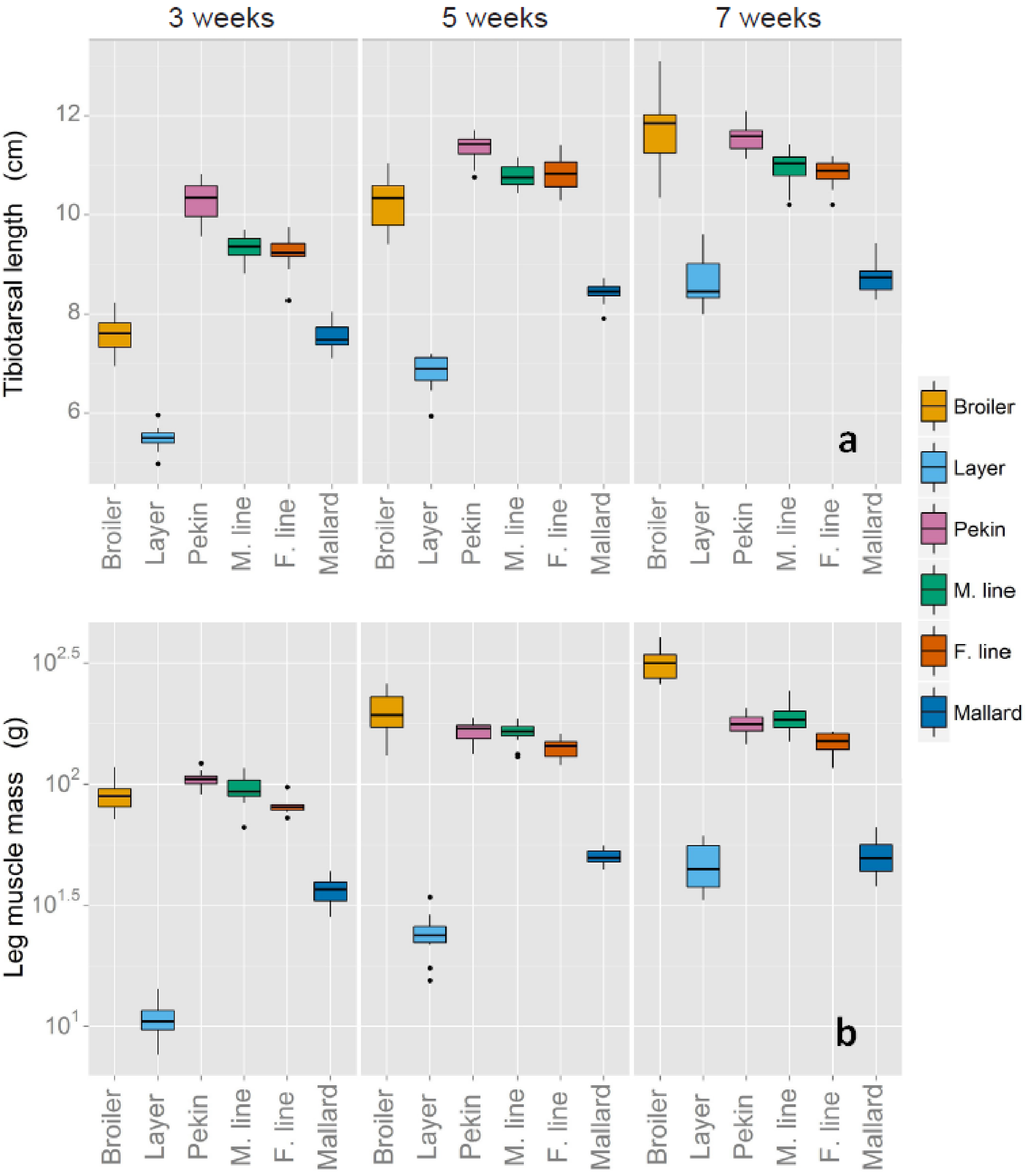

Figure 2. Tibiotarsal length (a) and leg muscle mass (b) from three to seven weeks of age.

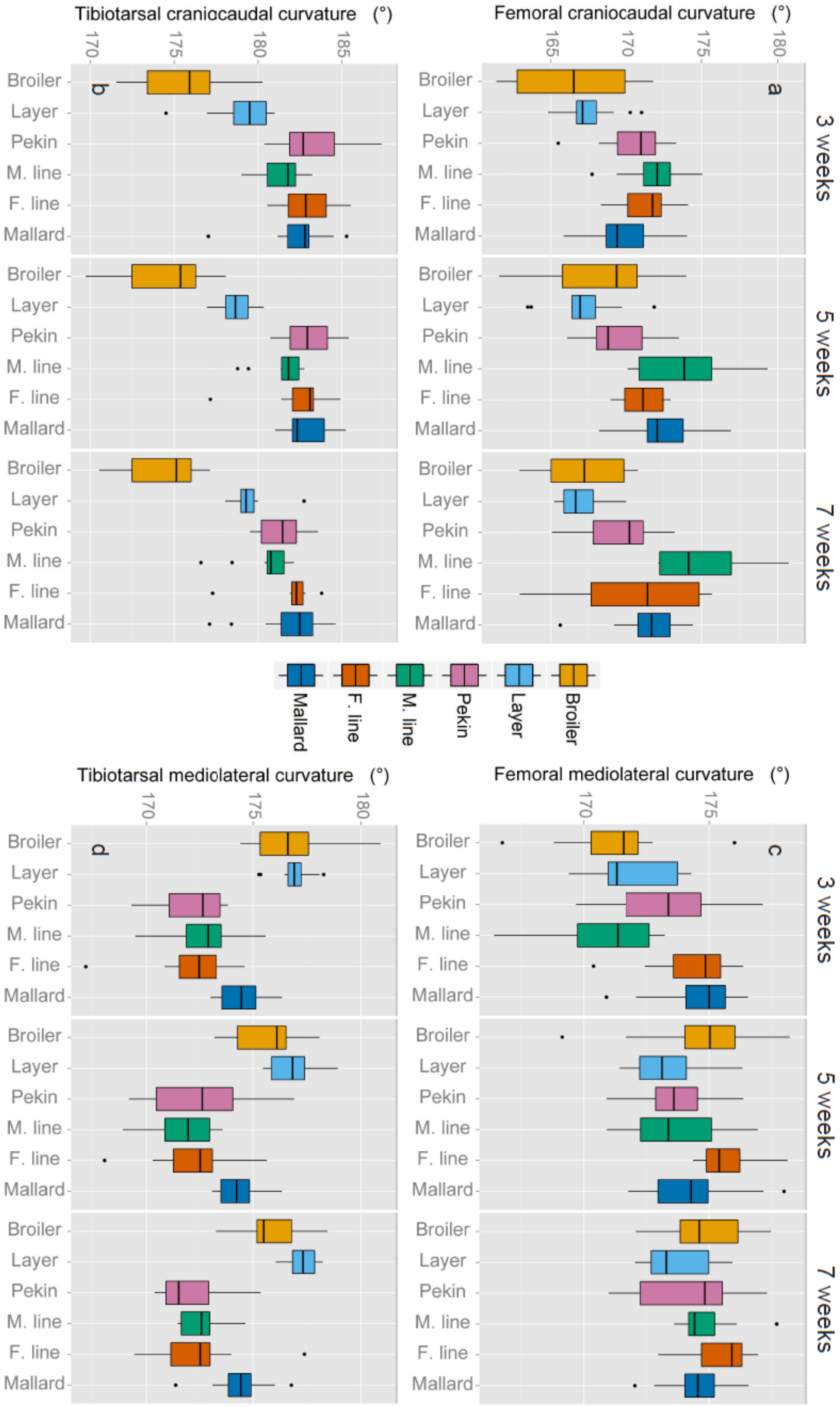

Figure 3. Cranio-caudal and medio-lateral curvature of the femur and tibiotarsus (in degrees).

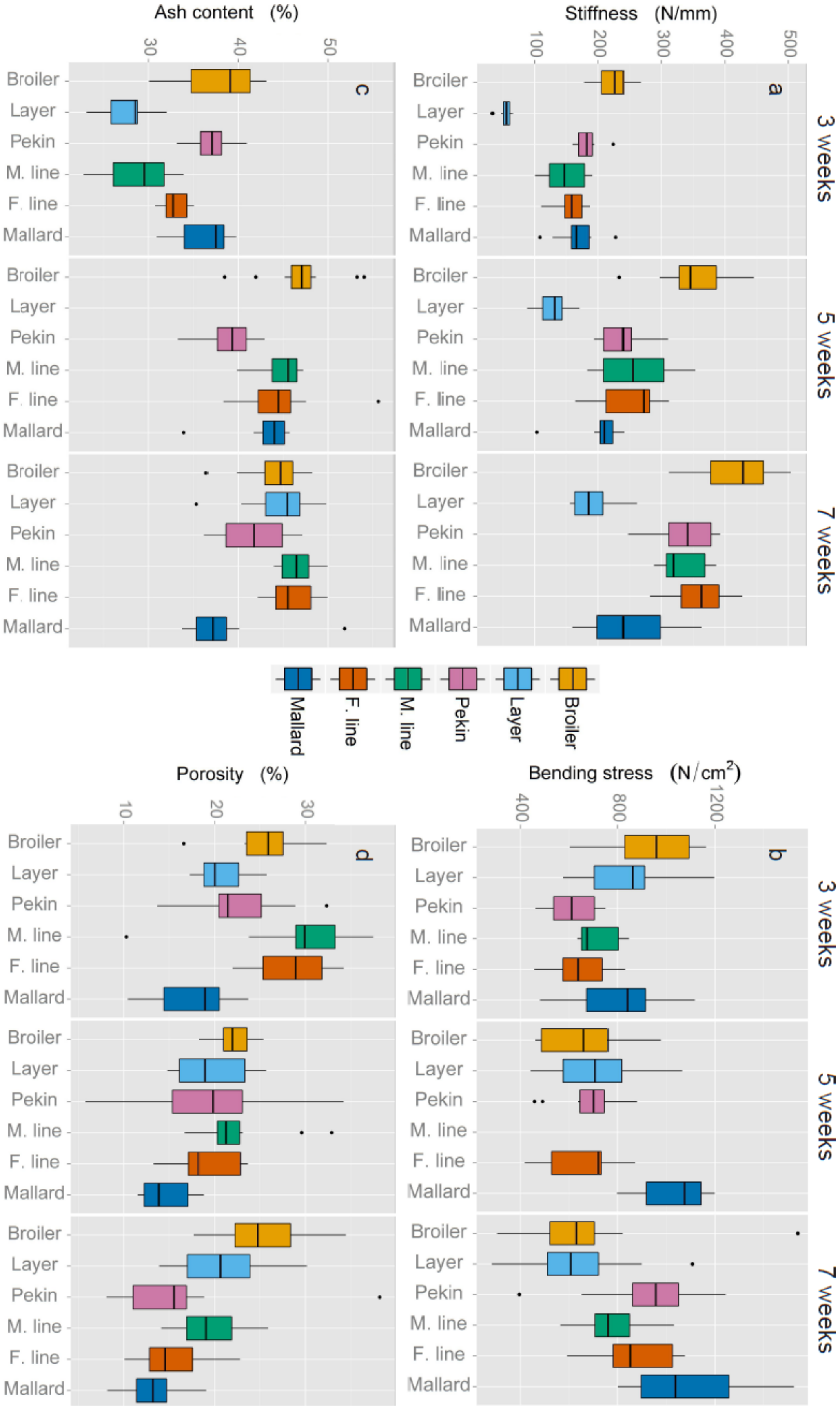

Figure 6. Tibiotarsal bone quality measurements.

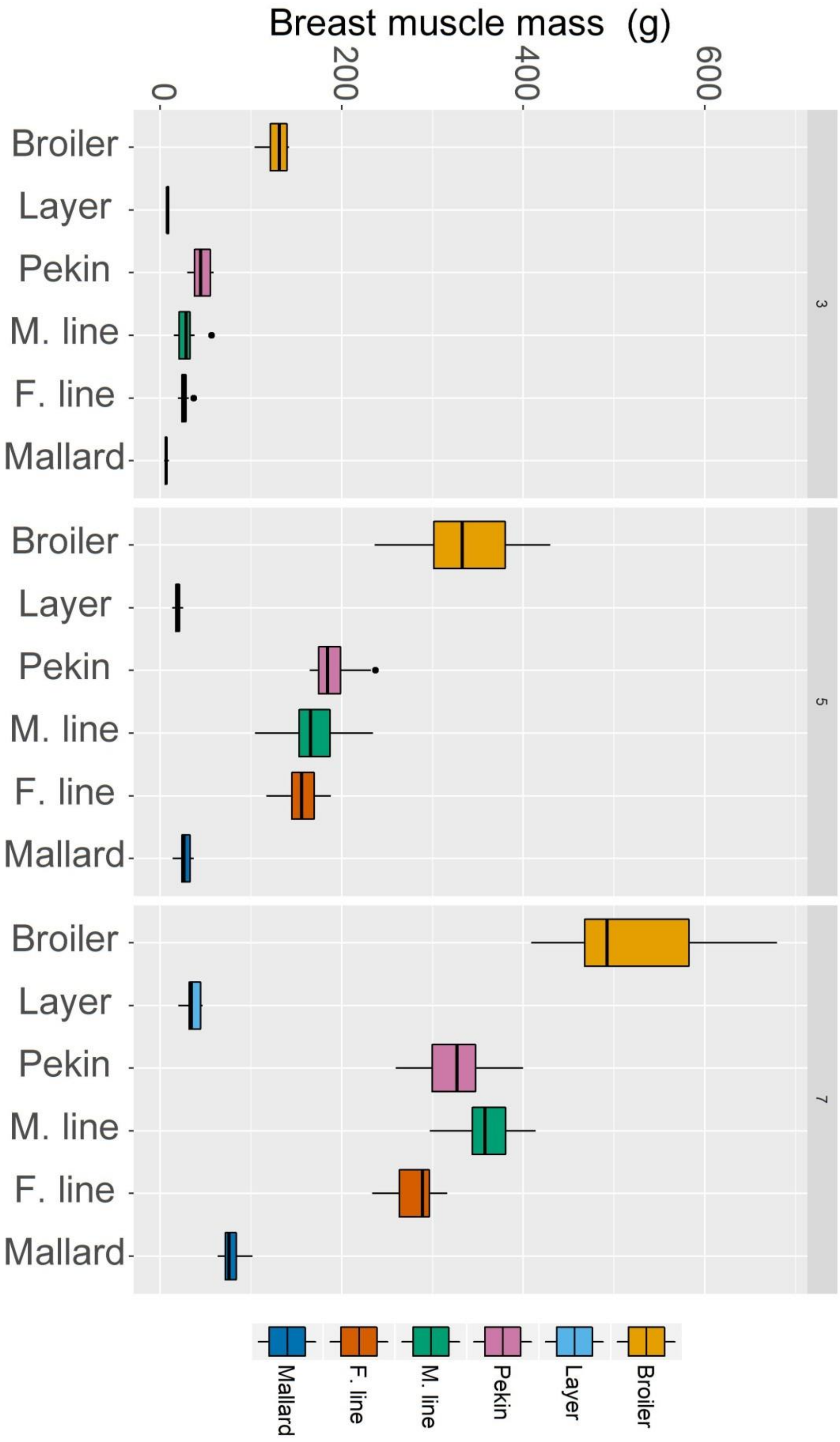

Figure B1. Left pectoral muscle mass in each line at 3 weeks, 5 weeks and 7 weeks of age.

Line	**Length (cm)**			**Cranio-caudal curve (°)**			**Medio-lateral curve (°)**			**Torsion (°)**			**Cortical Area (cm^2)**		
Experiment 1	*3 wks*	*5 wks*	*7 wks*	*3 wks*	*5 wks*	*7 wks*	*3 wks*	*5 wks*	*7 wks*	*3 wks*	*5 wks*	*7 wks*	*3 wks*	*5 wks*	*7 wks*
Broiler chicken	7.59 (0.27)	10.24 (0.37)	11.72 (0.53)	175.5 (2.03)	174.6 (1.75)	174.4 (1.64)	176.7 (1.24)	175.5 (1.09)	175.9 (1.15)	174.2 (2.97)	167.8 (5.20)	170.1 (3.71)	0.18 (0.022)	0.35 (0.065)	0.37 (0.058)
Layer chicken	5.49 (0.17)	6.82 (0.26)	8.69 (0.40)	179.1 (1.31)	178.7 (0.75)	179.5 (0.86)	176.9 (0.65)	176.8 (0.76)	177.2 (0.51)	173.6 (3.50)	174.2 (1.78)	174.6 (2.53)	0.06 (0.005)	0.10 (0.012)	0.13 (0.018)
Pekin hybrid	10.25 (0.29)	11.35 (0.20)	11.57 (0.22)	183.2 (1.46)	183.0 (1.11)	181.4 (0.96)	172.2 (1.14)	172.7 (1.83)	172.1 (1.09)	166.3 (4.99)	153.5 (7.70)	153.9 (8.04)	0.15 (0.014)	0.21 (0.019)	0.23 (0.023)
SED	0.16 P<0.001 df=4 [line, age, sex, age*sex]			0.74 P=0.151 df=4 [line, line*sex]			0.66 P=0.285 df=4 [line]			3.08 P=0.005 df=4 line, age]			0.02 P<0.001 df=4 [line, age, sex, line*sex]		
Experiment 2	*3 wks*	*5 wks*	*7wks*	*3 wks*	*5 wks*	*7wks*	*3 wks*	*5 wks*	*7wks*	*3 wks*	*5 wks*	*7wks*	*3 wks*	*5 wks*	*7 wks*
Pekin male line	9.35 (0.19)	10.79 (0.18)	10.93 (0.28)	181.3 (0.98)	181.6 (0.89)	180.6 (1.11)	172.7 (1.16)	171.7 (1.05)	172.5 (0.70)	168.2 (4.20)	167.1 (6.35)	151.3 (4.68)	0.14 (0.013)	0.18 (0.010)	0.20 (0.014)
Pekin female line	9.22 (0.27)	10.81 (0.25)	10.84 (0.20)	183.0 (1.19)	182.5 (1.35)	182.0 (1.12)	172.2 (1.37)	172.3 (1.41)	172.5 (1.44)	175.0 (2.33)	169.6 (4.66)	157.5 (4.97)	0.12 (0.009)	0.15 (0.006)	0.17 (0.009)
Mallard	7.54 (0.21)	8.43 (0.15)	8.73 (0.24)	182.4 (1.45)	182.9 (0.98)	181.9 (1.60)	174.6 (0.78)	174.3 (0.71)	174.4 (0.97)	171.9 (3.39)	165.4 (5.34)	154.2 (7.27)	0.08 (0.005)	0.09 (0.006)	0.10 (0.009)
SED	0.11 P=0.001 df=4 [line, age, sex]			0.71 P=0.887 df=4			0.65 P=0.756 df=4 [line]			2.86 P=0.712 df=4 [age]			0.02 P=0.001 df=4 [line, age, sex]		

Table S2: Tibiotarsal morphology - Least squares means (and standard errors) in all lines at each age. SED = Standard Errors of Difference. P values refer to line*age interactions. Other significant effects are presented in square brackets for each trait.

Line	**Stiffness (N/mm)**			**Maximum load (N)**			**Bending stress (N/cm^2)**			**BMD (%)**			**Porosity (%)**		
Experiment 1	*3 wks*	*5 wks*	*7wks*	*3 wks*	*5 wks*	*7wks*	*3 wks*	*5 wks*	*7wks*	*3 wks*	*5 wks*	*7wks*	*3 wks*	*5 wks*	*7wks*
Broiler chicken	224 (18.8)	349 (39.5)	415 (44.6)	284 (33.8)	483 (59.6)	627 (76.1)	951 (125)	652 (117)	664 (219)	38.0 (3.01)	47.0 (2.97)	44.1 (2.32)	25.8 (2.94)	22.2 (1.51)	25.6 (3.63)
Layer chicken	53 (7.5)	130 (18.1)	189 (24.0)	60 (10.4)	121 (14.7)	177 (34.6)	821 (125)	717 (125)	615 (160)	27.6 (1.96)	38.9 (NA)	44.3 (2.82)	20.8 (1.89)	19.6 (2.80)	20.7 (3.43)
Pekin hybrid	183 (12.3)	241 (26.5)	336 (34.9)	193 (18.6)	290 (19.9)	435 (46.8)	614 (72)	684 (85)	926 (166)	37.2 (1.53)	39.2 (1.93)	41.6 (2.56)	22.7 (3.53)	19.7 (5.46)	16.1 (5.46)
SED	14.6 P=0.002 df=4 [line, age, sex]			19.9 P<0.001 df=4 [line, age, sex, line*sex]			75.4 P<0.001 df=4 [line*sex]			1.4 P<0.001 df=3 [line, age]			2.1 P=0.067 df=4 [line]		
Experiment 2	*3 wks*	*5 wks*	*7wks*	*3 wks*	*5 wks*	*7wks*	*3 wks*	*5 wks*	*7wks*	*3 wks*	*5 wks*	*7wks*	*3 wks*	*5 wks*	*7wks*
Pekin male line	148 (22.2)	257 (62.8)	333 (24.5)	201 (27.6)	314 (52.0)	410 (31.5)	721 (57)	883 (367)	781 (85)	28.8 (3.72)	45.0 (1.59)	46.5 (1.37)	29.0 (7.18)	22.9 (3.32)	19.2 (2.55)
Pekin female line	158 (14.8)	248 (35.0)	360 (33.1)	171 (15.7)	274 (30.2)	382 (33.0)	645 (79)	660 (109)	867 (117)	32.9 (1.06)	44.5 (3.26)	45.9 (1.72)	28.6 (2.96)	19.0 (2.55)	15.1 (2.53)
Mallard	168 (21.7)	207 (25.3)	248 (47.0)	108 (7.16)	173 (12.3)	206 (30.8)	827 (131)	1020 (99)	1103 (165)	36.5 (1.99)	43.1 (3.38)	38.1 (3.36)	17.9 (2.79)	14.5 (1.86)	13.1 (2.18)
SED	16.2 P<0.001 df=4 [line, age]			12.1 P<0.001 df=4 [line, age, sex, age*sex]			80.1 P=0.019 df=3 [line, age]			1.4 P<0.001 df=4 [age]			1.7 P=0.012 df=4 [line, age]		

Table S3: Tibiotarsal bone quality - Least squares means (and standard errors) in all lines at each age. SED = Standard Errors of Difference. P values refer to line*age interactions. Other significant effects are presented in square brackets for each trait.

Line	**Length (cm)**			**Cranio-caudal curve (°)**			**Medio-lateral curve (°)**			**Torsion (°)**			**Cortical Area (cm^2)**		
Experiment 1	*3 wks*	*5 wks*	*7 wks*	*3 wks*	*5 wks*	*7 wks*	*3 wks*	*5 wks*	*7 wks*	*3 wks*	*5 wks*	*7 wks*	*3 wks*	*5 wks*	*7 wks*
Broiler chicken	5.70 (0.11)	7.21 (0.24)	8.17 (0.28)	166.6 (2.81)	168.5 (2.54)	167.2 (1.90)	171.3 (1.60)	174.8 (1.81)	174.8 (1.25)	126.2 (3.21)	125.9 (3.98)	127.7 (4.85)	0.23 (0.028)	0.40 (0.059)	0.42 (0.065)
Layer chicken	4.01 (0.11)	5.01 (0.17)	6.21 (0.22)	167.6 (1.24)	167.2 (1.61)	166.9 (1.13)	172.0 (1.28)	173.4 (1.07)	173.8 (0.95)	134.9 (4.28)	135.7 (2.34)	133.6 (1.92)	0.07 (0.006)	0.12 (0.015)	0.16 (0.015)
Pekin hybrid	6.27 (0.17)	7.18 (0.18)	7.38 (0.16)	170.5 (1.61)	169.3 (1.63)	169.4 (1.75)	173.3 (1.50)	173.7 (1.14)	174.2 (1.53)	138.2 (2.87)	128.9 (3.60)	123.9 (4.39)	0.19 (0.021)	0.24 (0.017)	0.22 (0.029)
SED	0.10 P<0.001 df=4 [line, age, sex]			1.06 P=0.428 df=4 [line]			0.80 P=0.094 df=4 [age]			1.86 P<0.001 df=4 [line, age]			0.02 P<0.001 df=4 [line, age, sex, line*sex]		
Experiment 2	*3 wks*	*5 wks*	*7wks*	*3 wks*	*5 wks*	*7wks*	*3 wks*	*5 wks*	*7wks*	*3 wks*	*5 wks*	*7wks*	*3 wks*	*5 wks*	*7wks*
Pekin male line	5.65 (0.16)	6.89 (0.16)	7.14 (0.11)	171.9 (1.42)	173.7 (2.15)	174.9 (1.99)	170.8 (1.65)	173.6 (1.35)	174.8 (0.80)	137.9 (2.06)	130.3 (4.50)	124.4 (4.02)	0.22 (0.018)	0.22 (0.010)	0.22 (0.019)
Pekin female line	5.54 (0.09)	6.81 (0.15)	6.76 (0.14)	171.5 (1.20)	171.1 (1.04)	170.7 (3.11)	174.4 (1.22)	175.7 (0.80)	175.5 (0.91)	136.2 (3.23)	130.4 (2.89)	125.5 (3.57)	0.17 (0.014)	0.17 (0.013)	0.18 (0.011)
Mallard	4.41 (0.12)	5.03 (0.10)	5.24 (0.13)	169.9 (1.51)	172.5 (1.66)	171.4 (1.65)	174.6 (1.22)	174.3 (1.40)	174.5 (0.85)	139.8 (3.39)	135.8 (3.10)	133.2 (3.44)	0.09 (0.005)	0.10 (0.007)	0.10 (0.008)
SED	0.07 P<0.001 df=4 [line, age, sex]			1.07 P=0.1 df=4 [line]			0.71 P<0.001 df=4 [line, age]			1.9 P=0.084 df=4 [line, age]			0.01 P=0.589 df=4		

Table S4: Femoral morphology - Least squares means (and standard errors) in all lines at each age. SED = Standard Errors of Difference. P values refer to line*age interactions. Other significant effects are presented in square brackets for each trait.

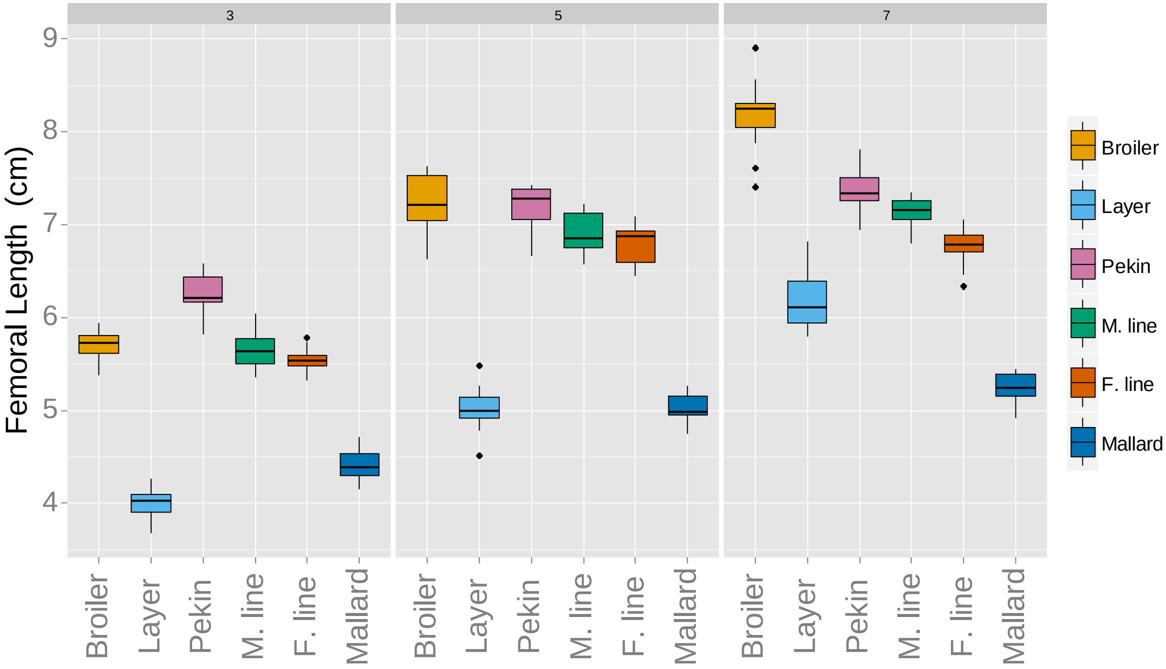

Fig. S7: Changes in the length of the left femur with age from Experiment 1 (broiler chicken, layer chicken, Pekin duck commercial hybrid) and Experiment 2 (male line Pekin, female line Pekin and mallard). The upper and lower boxplot whiskers extend to within 1.5 times above and below the interquartile range respectively.

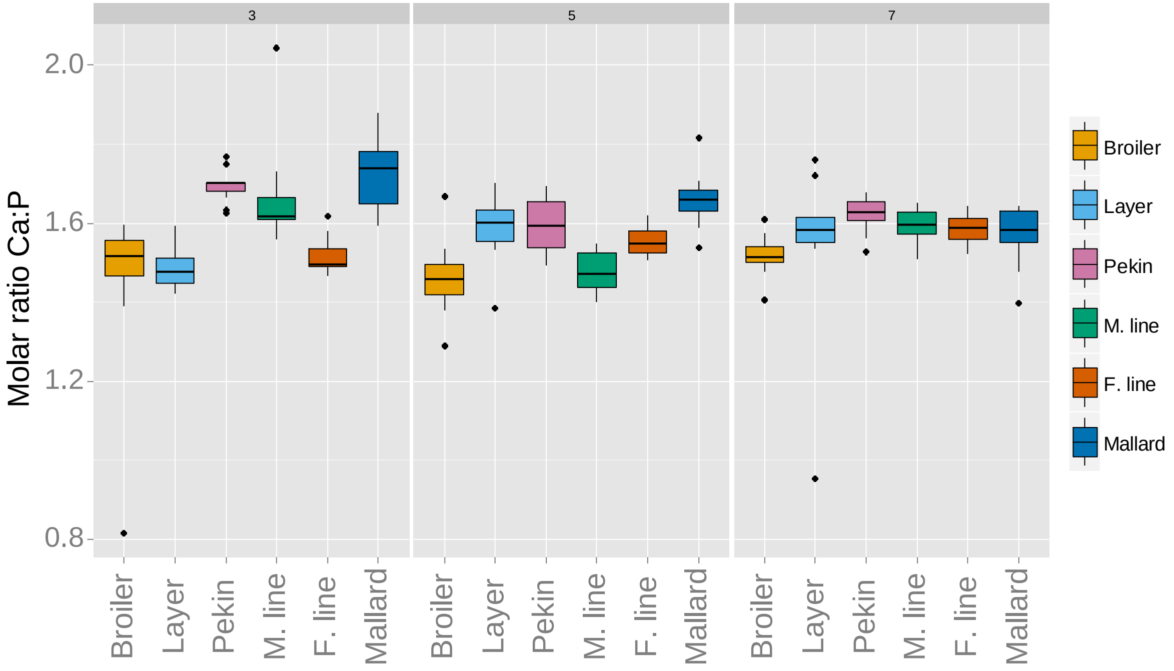

Fig. S8: Molar ratio of Ca:P in mid-diaphyseal cortical bone of the tibiotarsus in each line at each age. The upper and lower boxplot whiskers extend to within 1.5 times above and below the interquartile range respectively.

Chapter One

Differences in hindlimb morphology of ducks and chickens - effects of domestication and selection

This chapter was published in Genetics Selection Evolution on November 17th, 2015:

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Author contributions:

Brendan Duggan contributed to all aspects of this work. Paul Hocking and Dylan Clements provided supervision support. Tobias Schwarz provided advice on recording bone morphology measurements through the use of computed tomography.

Email addresses:

BMD: Brendan.Duggan@roslin.ed.ac.uk

PMH: Paul.Hocking@roslin.ed.ac.uk

TS: Tobias.Schwarz@ed.ac.uk

DNC: Dylan.Clements@ed.ac.uk

Abstract

Background

Poultry account for the most numerous species farmed for meat and have been subject to intense selection over approximately 60 generations. To assess morphological changes which have occurred in the avian leg due to selection for rapid growth and high meat yields, divergent lines of chicken (*Gallus gallus*) and duck (*Anas platyrhynchos*) were studied between three and seven weeks of age. For each line, femoral and tibiotarsal morphology was recorded using computed tomography scanning and tibiotarsal bone quality measures (stiffness, bending stress and porosity) were assessed.

Results

In chicken and duck, divergence in hindlimb morphology has occurred in the commercial meat lines compared to their lighter conspecifics. As expected, the differences were largest between species. Leg development nears completion much earlier in ducks than in chickens. Duck tibiotarsi showed a large degree of lateral curvature, which is expected to affect foot position during swimming and walking, and thus to influence gait. All lines have adapted their tibiotarsal morphology to suit the loading forces they experience; however bone quality was found to be poorer in chickens.

Conclusions

We demonstrate that intensive selection for growth rate in both chickens and ducks has resulted in leg morphology changes, which are likely to influence gait. Ducks represent an interesting compromise of adaptation for efficient locomotion in two media – on land and in water. Some aspects of bone morphology in the duck, such as lateral curvature of the tibiotarsus, may result from adaptation to swimming, which potentially imposes limitations on terrestrial locomotion.

Introduction

Poultry are the most numerous animals farmed for meat. The Food and Agriculture Organisation of the United Nations reported that over 60 billion chickens and 2 billion ducks are produced worldwide each year, with this figure increasing annually [1]. During approximately 60 generations of selection, the meat type (broiler) bird has undergone intensive selection for rapid growth and increased pectoral muscle mass [2, 3]. For example, the broiler chicken has experienced a 300% increase in body mass over this period [4]. One unwanted side effect of this genetic gain has been an increased incidence of locomotion problems (termed “leg weakness”) [5].

Various studies have reported figures for the prevalence of leg weakness in broiler chickens that range from 15 to 30% [6, 4, 7, 8]. Accurate figures are difficult to obtain due to variation between studies in the genotypes and gait scoring systems used, the age at which birds are assessed and management factors [9]. While recent reliable information on the prevalence of leg weakness in poultry is not available, it is likely that this issue causes economic losses to the producer [10]. Leg weakness in livestock is also a welfare issue; since it has been associated with pain [11-13] and modified behaviour [14, 15]. However, selection strategies over the last 25 years have addressed some leg weakness issues [16]. Data on the prevalence of welfare issues in domestic ducks is scarce [17, 18] but given that, in duck and chicken breeding, selection intensities and achieved growth rates and carcass weights are similar, it is likely that locomotion problems also exist to some extent in the domestic duck.

The causes of poor gait are varied. In broiler chickens, an increase in pectoral muscle mass has shifted the body’s centre of mass cranially which is associated with relatively poor stability [19, 20]. Bone deformities may also play a role: valgus, varus and torsional deformities are generally seen in the tibiotarsus and have previously been associated with gait abnormalities [21]. Some gait problems can be due to bone fractures, which either occur due to trauma or are secondary to other bone pathologies [21]. Fracture risk is often linked to bone quality; cortical bone has been shown to be less well mineralised and more porous in broiler chickens that are selected for rapid growth than in slower growing lines [22, 23]. In Pekin ducks, tibiotarsal bone mineral density seems to have remained within a similar range during the last two decades, although tibiotarsal length and body mass have increased [24].

The aim of this study was to assess skeletal changes which have occurred in the Pekin duck during its selection for rapid growth and to compare these with different lines of chickens. Since such a vast number of fast-growing ducks and chickens are reared for meat each year, a better understanding of these birds' gait may lead to welfare improvements on a large scale. The duck leg represents an interesting compromise of adaptation for efficient locomotion in two media, i.e. on land and in water; it is expected that adaptations which are beneficial to swimming will create a leg morphology which differs from that of a strictly cursorial species such as the chicken. To represent divergent lines of chicken, broiler chickens were used as an example of a line selected for rapid growth, and layers were used to represent a growth rate more similar to their ancestral phenotype, the red jungle fowl (*Gallus gallus*). For Pekin ducks, a commercial hybrid and two breeding lines were used as examples of high growth rate birds; these were compared to their ancestral phenotype, the mallard (*Anas platyrhyncos*). Selection for high feed efficiency and breast muscle yield in both species was anticipated to have led the heavier meat lines to diverge from their lighter conspecifics in their skeletal morphology. Both the Pekin duck and the broiler chicken have undergone intense selection for breast muscle mass over many decades. This selection has led to a cranial shift in the body's centre of mass, thus altering the loading forces which act on the legs of both lines [2]. As well as recording data on leg morphology, allometric scaling patterns of various traits were also compared; normally, aspects of hindlimb morphology would be expected to scale isometrically (that is, with geometric similarity) to body mass. However, due to differences in the natural habitats and locomotor modes of the ancestral phenotypes of chickens and ducks, some deviations from isometry were expected.

Methods

Animals and husbandry

A total of 216 birds of different lines were culled at three ages in two separate experiments. During the first experiment, 36 broiler chickens (Ross 308s), 36 layer chickens (Lohman Brown) and 36 Pekin ducks (Cherry Valley commercial hybrid) were raised in walled research pens. The second experiment used the same pens to house 36 Pekin ducks of a male line, 36 Pekin ducks of a female line (both Cherry Valley breeding stock) and 36 mallards (Hy-Fly Game Hatcheries, Poulton-le-Fylde, UK). The male Pekin line, which are the eventual male grandparents of the commercial hybrid line, are selected primarily for growth and feed efficiency while the female Pekin line, which are the female grandparents of the commercial hybrid line, were selected for fertility, as well. Both breeding lines contained equal numbers of both males and females.

Birds were raised following industry guidelines as much as possible. All birds were initially housed from day of hatch under brooder lamps in a single pen per line to regulate temperature. At seven days, birds were randomly allocated in a randomised block design to two blocks of nine pens, separated by a 3 m passage. Each pen (2.16 m^2) contained four males and four females housed in an area of 0.27 m^2 per bird, increasing to 0.36 m^2 per bird from 21 days and 0.54 m^2 per bird from 35 days onward as birds were removed for measurement. The lighting regime was 23 hours light: one hour dark at hatch, reducing by one hour light per day for the first seven days and remaining at 16 hours light: 8 hours dark thereafter. The mean light intensity in each pen was 120 lux. Barn temperature was 25°C at two weeks, reducing to 24°C at three weeks, 22°C at four weeks and remained at 20°C from five weeks until termination, as per industry guidelines. Experiment 1 used wood shavings as a substrate since this is the industry norm for chickens. Experiment 2 used straw as a substrate, as is the case on most UK duck farms. All birds were provided with food and water *ad libitum*. Broilers were given a commercial starter feed for the first 10 days, grower feed from days 11 to 35 and finisher feed from day 36 onwards. Layers were fed on a commercial starter feed for the first 35 days before transferring to grower feed from day 36 onwards. All duck lines in both experiments were fed on a starter feed until day 10 and then a grower feed thereafter; both duck diets were supplied by the breeding company.

The use of animals for this study was approved by the University of Edinburgh Ethics Committee.

Data collection

At three ages (21, 35 and 49 days), two randomly selected birds (one male and one female) from each pen (six males and six females per line) were euthanized by intravenous sodium pentobarbital injection (Euthatal, Merial, Toulouse, France) and immediately dissected. These ages were chosen to cover the typical lifespan of a production bird of both species. Body mass of each bird was recorded two days prior to euthanasia. The left pectoral muscle and thigh and shank muscle groups of the femur and tibiotarsus were dissected out and weighed. Both tibiotarsi and femora were left intact at the stifle joint and stored at -20°C for future measurement. At a later date, the bones were thawed and the left tibiotarsus and femur were evaluated with a computed tomography scan (CT). A helical 4-slice CT unit (Somatom Volume Zoom, Siemens, Germany) was used. For each scan, six legs were laid parallel to each other in supine position (cranial aspect facing upward), and scanned along their full length using a 1 mm slice width. The tibiotarsus was parallel to the table while the femur, still attached to the tibiotarsus, was at an approximately 10 degree angle (with the proximal end of the femur closer to the table than the distal end). Bone morphology was assessed using a 3D multi-planar reconstruction in dedicated DICOM viewing software (OsiriX, Geneva, Switzerland, version 5.8.5 – 32bit). Morphological measurements for both femur and tibiotarsus include functional length, diameter and cortical cross-sectional area at the mid-diaphysis, curvature in both frontal and sagittal planes and torsion. Detailed methods are in Appendix II.

Bone breaking tests were performed using an LRX materials tester running ‘Nexygen 2.2’ software (Lloyds Instruments, Bognor Regis, UK) to assess stiffness and ultimate breaking strength. Stiffness is a measure of the force required to displace the mid-diaphysis of a bone by a known distance when the ends are fixed. Ultimate breaking strength is the maximum load the bone can withstand before breaking. Compress to rupture tests were carried out on the right tibiotarsus using a three-point-bending jig – each bone was balanced in supine position on two curved rests 10 mm in diameter and 30 mm apart with a downward force (also curved, 10 mm diameter) centrally applied at the mid-diaphysis at a rate of 30 mm/min until rupture.

A 1.5 cm portion of the mid-diaphysis was cut from the broken (right) tibiotarsus using a circular bone saw and sent for mineral content analysis (DM Scientific, Thirsk, UK) to determine bone mineral density. A 1 cm section was also cut from the mid-diaphysis of the left tibiotarsus for porosity assessment by histology [See Appendix II].

Data analysis

Bending stress (*B*) is a measure of the maximum force experienced by the tibiotarsi before breaking, corrected for the anatomical shape of the bone. Bending stress was calculated using the formula:

$$B = \frac{My}{I},$$

where M is the bending moment (the maximum load applied to the bone multiplied by the distance over which it is applied), y is the distance from the cross-sectional centre of mass in the direction of loading (in this case, the outer semi-minor axis as the cross-section is a hollow ellipse) and I is the second area moment of inertia.

Full details of the calculation are in Appendix II.

A split-plot statistical model was analysed in Genstat statistical software (version 16.1.0.10916 (64-bit), VSN International, Ltd.) using ANOVA, with effects for pen nested within block and treatment effects of genetic line, age and sex. Because some conditions differed between experiments, the six lines were not analysed together; separate ANOVA were performed to compare the lines from Experiment 1 (broiler chicken, layer chicken and Pekin commercial hybrid) and the lines from Experiment 2 (male Pekin line, female Pekin line and mallard).

Scaling relationships through ontogeny were analysed by regressing the log of each trait against the log of body mass. Since both body mass and bone/muscle measurements were expected to contain some error, reduced major axis (also called Model II) regression was performed. The slope (scaling exponent) of the resulting regression equation for each trait was compared to the expected scaling exponent for that trait. Assuming that traits scale isometrically (that is, they grow with geometric similarity to body mass) and considering that the predictive trait (body mass) is volumetric, lengths were expected to scale to body mass$^{0.33}$, measurements of area were expected to body mass$^{0.67}$ and mass measurements were expected to scale to body mass^{1}. Non-dimensional measurements (such as bone torsion angles) were expected to scale to body mass^{0}; in other words, they were not expected to change as body mass increased. See Allen *et al.* [25] for a detailed description of this analysis.

Results

Least squares means and standard errors of the differences for each trait in all lines at all ages are in Appendix III.

Fig. 1 shows changes in body mass for each line over seven weeks. There was a significant difference in body mass between lines in both experiments (*P* < 0.001). The broiler chicken and all three Pekin duck lines grew at a faster rate than both the layer chicken and the mallard (*P* < 0.001).

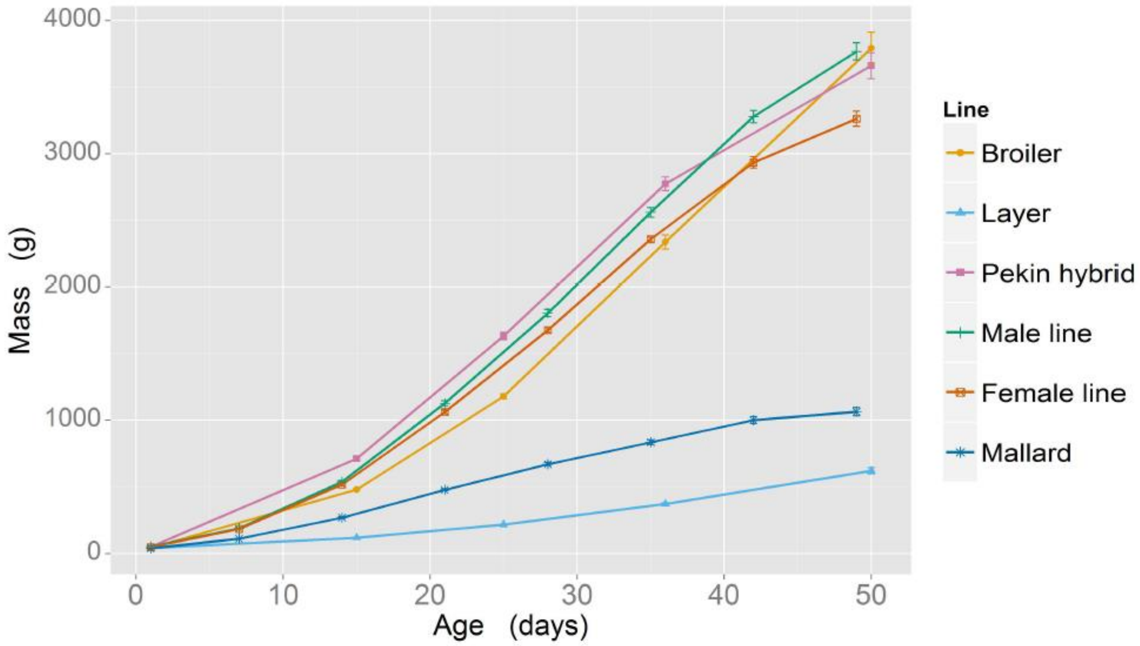


Figure 1. Body mass measurements (means and standard errors) from hatch to seven weeks of age.

Data are combined from Experiment 1 (broiler chicken, layer chicken, Pekin duck commercial hybrid) and Experiment 2 (male Pekin line (M. line), female Pekin line (F. line) and mallard).

Differences in leg bone length mirrored those of body mass; the tibiotarsus (Fig. 2a) was significantly shorter in the layer chicken and mallard compared to the broiler chicken and Pekin lines ($P < 0.001$). There was an age effect ($P < 0.001$) i.e. duck lines in each experiment showed a decline in tibiotarsal growth from five weeks of age whereas chicken tibiotarsi continued to grow throughout the experiment. When analysed allometrically, chicken tibiotarsi grew with positive allometry and the tibiotarsi of all four duck lines grew with negative allometry (Table 1). A sex effect ($P < 0.001$ in Experiment 1 and $P = 0.002$ in Experiment 2) was also observed; males of all lines had longer tibiotarsi than females. In Experiment 1 an age by sex interaction was observed ($P < 0.001$) i.e. females (broiler chicken and Pekin hybrid, but not layer chicken) had longer tibiotarsi than males at three weeks but not at five and seven weeks of age ($P < 0.001$).

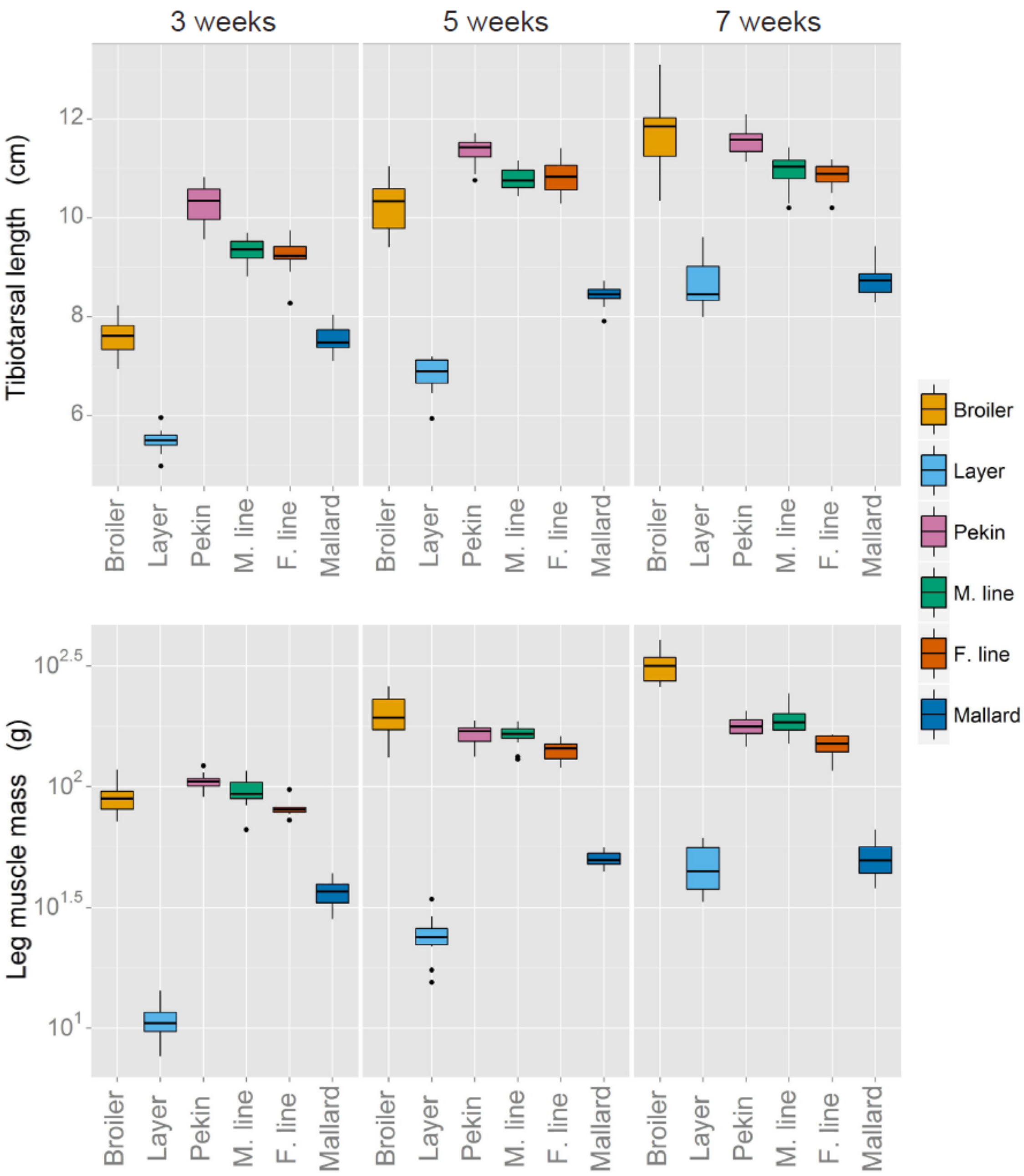


Figure 2. Tibiotarsal length and leg muscle mass from three to seven weeks of age.

Leg muscle mass (drumstick and thigh muscles) is presented using a log-scale for clarity. The upper and lower boxplot whiskers extend to within 1.5 times above and below the interquartile range, respectively. Dots outside this range are marked as outliers. Note the lack of growth in both bone length and muscle mass in all duck lines after five weeks of age.

In the cranio-caudal plane, the tibiotarsus of the broiler chicken was significantly more curved (cranially) than the tibiotarsus of the layer chicken, which was in turn more cranially curved than that of the Pekin hybrid ($P < 0.001$). In Experiment 2, the mallard tibiotarsus displayed significantly more caudal curvature than that of the male Pekin line ($P = 0.013$) but did not differ from that of the female line (Fig. 3b). Male birds in Experiment 1 exhibited greater cranio-caudal curvature of their tibiotarsi than females. Both species differed in the direction of tibiotarsal curvature in this plane, i.e. all four duck lines curved caudally whereas both chicken lines curved cranially. In the medio-lateral plane (Fig. 3d), the tibiotarsi of both the broiler chicken and Pekin duck displayed greater lateral curvature than their lighter conspecifics; however, this difference was statistically significant ($P < 0.001$) only between the mallard and Pekin breeding lines. In this plane, the duck tibiotarsi were more laterally curved than those of both chicken lines ($P < 0.001$).

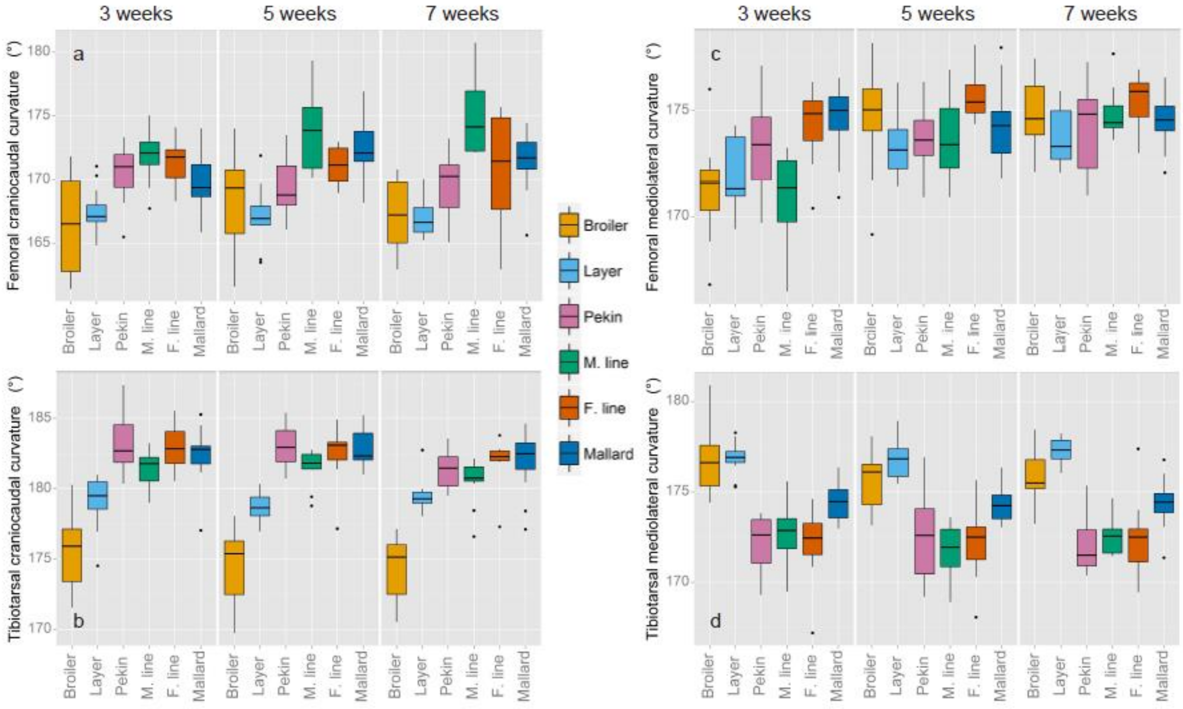


Figure 3. Cranio-caudal and medio-lateral curvature of the femur and tibiotarsus (in degrees).

180° represents a straight bone. A value below 180° represents cranial and lateral bending in the craniocaudal and mediolateral planes, respectively.

Tibiotarsal torsion occurred to a similar extent in both chicken lines. The Pekin hybrid differed significantly from the chicken lines (*P* < 0.001). There was a line by age interaction, i.e. at three weeks of age, the Pekin and chicken lines displayed a similar range of tibiotarsal torsion but by seven weeks of age the distal part of the tibiotarsus of the Pekin hybrid had rotated internally in relation to the proximal tibiotarsus (*P* = 0.005) (Fig. 5b). Internal rotation occurs when the cranial aspect of the distal part of the tibiotarsus turns to face medially. No difference in tibiotarsal torsion was observed between the mallards and Pekin breeding lines in Experiment 2; however, the distal tibiotarsi of the male Pekin line rotated internally to a greater extent than that of the female Pekin line (*P* = 0.024). There was an age interaction (*P* < 0.001) with tibiotarsi in all three duck lines of Experiment 2 rotating internally as they aged. A line by age interaction also occurred in Experiment 1, i.e. the distal tibiotarsi of the Pekin commercial hybrid rotated internally as the bird aged (*P* = 0.005) whereas the tibiotarsi of the chicken lines did not. The R^2 values from regressions of the log of bone torsion on the log of body mass were very low for both chicken lines (Table 1), suggesting no relationship. In the duck lines, tibiotarsal torsion deviated slightly from isometric growth.

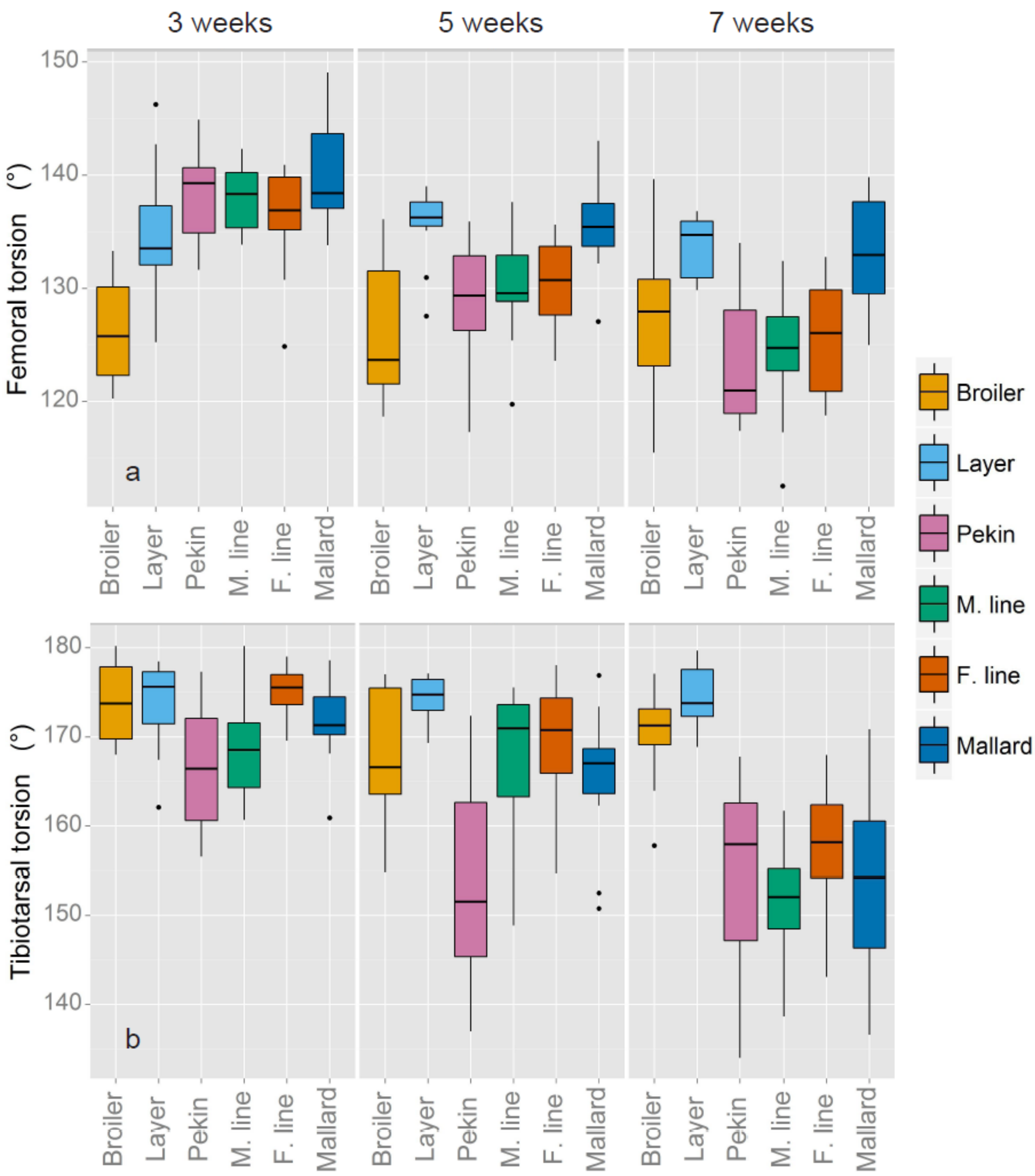


Figure 5. Rotation of the distal part of the femur and tibiotarsus in relation to the proximal part of the femur and tibiotarsus, respectively.

Angles at the starting point at three weeks of age are based on the relative position of bone landmarks and are not a measure of initial rotation. A decrease in angle after 3 weeks represents external femoral rotation and internal tibiotarsal rotation.

Tibiotarsal bone quality

Tibiotarsal stiffness differed significantly within both chickens and ducks ($P < 0.001$). Lines selected for rapid growth had stiffer tibiotarsi than lines with a slow growth (Fig 6a). There was an age effect in Experiment 2, i.e. the tibiotarsi of the fast-growing Pekin lines had the same stiffness as the mallard at three weeks of age but, by seven weeks, they were significantly stiffer than those of the mallard ($P < 0.001$). Tibiotarsal stiffness scaled isometrically in all lines except for the layer chicken, which displayed very positive allometry (Table 1). Tibiotarsal strength (maximum load to rupture) for all lines at all ages was significantly greater in fast-growing lines compared to their slow-growing conspecifics ($P < 0.001$). Tibiotarsal strength scaled with positive allometry for all lines except for the male and female Pekin breeding lines, which scaled with isometry (Table 1).

Bending stress (Fig 6b) did not differ significantly between the Pekin hybrid and both chicken lines at all ages. However, there was a line by age interaction ($P < 0.001$), i.e. Pekin hybrid tibiotarsi tolerated greater bending stresses as the animals grew older whereas the bending stresses tolerated by the tibiotarsi of both chicken lines decreased. The mallard tibiotarsi resisted significantly more bending stress than those of the heavier male Pekin line and female Pekin line ($P < 0.001$). There was also an age effect, i.e. the tibiotarsi of all three duck lines tolerated more bending stress as they aged ($P < 0.001$). Data for the male Pekin line at five weeks of age was not analysed since these bones moved during loading, causing error.

Data on tibiotarsal ash content for the layer chicken line at five weeks of age is not available due to measurement error. The ash content of the bone before drying (Fig. 6c) was significantly greater in the broiler compared with both the layer chicken and Pekin hybrid ($P < 0.001$). All lines had increased bone mineralisation as they aged ($P < 0.001$), although the broiler chicken reached its 7-week level of ash content earlier than the Pekin commercial hybrid ($P < 0.001$). In Experiment 2, all duck lines had increased bone mineralisation as they grew older (*P* < 0.001); however, for the mallard, bone mineralisation increased until five weeks of age and then decreased from seven weeks (*P* < 0.001). The molar Ca:P ratio across all lines over all ages ranged from 1.4 to 1.8 [See Appendix III].

Porosity differed significantly between lines in Experiment 1 (*P* = 0.003), i.e. the tibiotarsi of broiler chicken were more porous at the mid-diaphysis than those of both the layer chicken and Pekin hybrid (Fig. 6d). In Experiment 2, all three duck lines significantly differed in tibiotarsal porosity (*P* < 0.001) with the male line having the highest mid-diaphyseal porosity and the mallard having the lowest. An age interaction was also observed in the duck lines with the tibiotarsi becoming less porous as the birds aged (*P* < 0.001).

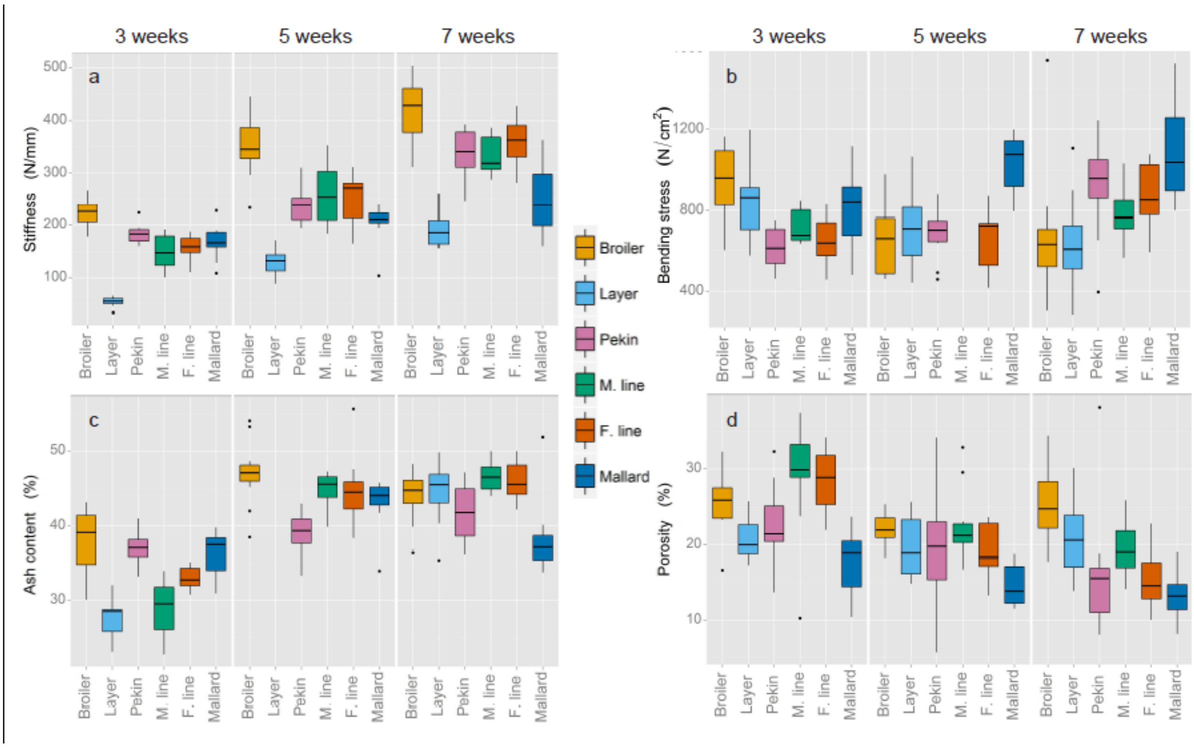


Figure 6. Tibiotarsal bone quality measurements.

(a) Stiffness and (b) bending stress were measured on the whole bone. (c) Ash content (wet bone) and (d) porosity were taken from a 1 cm section of the mid-diaphysis. Ash content data for layer chickens at five weeks of age and bending stress values for the male Pekin line at five weeks of age were excluded due to measurement error.

Femoral measurements

The length of the femur was significantly shorter in the layer chicken and the mallard than in the broiler chicken and Pekin male and female lines ($P < 0.001$). Age effects, sex effects and line-by-age interaction effects were observed in both experiments ($P < 0.001$). Femoral length scaled with negative allometry for all duck lines (Table 1). The femoral length of the broiler chicken increased isometrically with body mass and that of the layer chicken showed slightly positive allometric growth.

There was no difference in cranio-caudal curvature of the femur between the broiler and layer chicken in Experiment 1 (Fig. 3a). However, the femora of the chicken lines were more cranially curved than the Pekin hybrid in this plane ($P < 0.001$). In Experiment 2, the femora of the mallard and the female Pekin line displayed more cranial curvature than those of the male Pekin line ($P = 0.001$). In the medio-lateral plane, no significant differences in femoral curvature of the broiler chicken, layer chicken or Pekin duck hybrid were observed. However, there was an age effect ($P < 0.001$), i.e. lateral curvature of the femora of all three lines decreased as the birds aged. In Experiment 2, the femora of the male Pekin line were significantly more laterally curved than those of the female line and the mallard ($P < 0.001$). An age effect and a line-by-age interaction effect were observed in these lines ($P < 0.001$); the femora of the male line became less curved in this plane as the birds aged whereas the female line and mallard maintained the same curvature. By seven weeks of age, the femora of all lines were curved to a similar degree in the medio-lateral plane (Fig. 3c).

Femoral torsion differed significantly between chicken lines in Experiment 1 (Fig. 5a); the distal femur of the broiler was rotated more externally to the proximal end when compared to that of the layer at all ages (*P* < 0.001). There was also a line-by-age interaction (*P* < 0.001); at three weeks of age, the distal femur of the Pekin commercial hybrid was rotated internally compared to that of both chicken lines. However, as the Pekin individuals aged, the distal femur rotated externally, reaching a similar degree of femoral torsion as in the broiler chicken by seven weeks of age. A similar age interaction occurred in Experiment 2, with the distal femur of all duck lines rotating externally in relation to the proximal femur (*P* < 0.001). The mallard femur underwent less rotation as it aged (*P* = 0.002), reaching a degree or femoral torsion similar to that of the layer chicken by seven weeks of age.

Femoral cortical area grew isometrically in the broiler chicken and with positive allometry in the layer chicken. The R^2 value for this trait in duck lines was low, suggesting a weak relationship with body mass.

Tibiotarsal length	Expected slope	Slope	Lower CI	Upper CI	R^2
Broiler	0.33	0.40 (+)	0.37	0.44	0.93
Layer	0.33	0.43 (+)	0.41	0.46	0.98
Pekin hybrid	0.33	0.17 (-)	0.15	0.20	0.78
Pekin male line	0.33	0.15 (-)	0.13	0.18	0.84
Pekin female line	0.33	0.17 (-)	0.15	0.20	0.82
Mallard	0.33	0.21 (-)	0.18	0.23	0.88
Femoral length	Expected slope	Slope	Lower CI	Upper CI	R^2
Broiler	0.33	0.33 (=)	0.30	0.35	0.95
Layer	0.33	0.41 (+)	0.38	0.44	0.97

Pekin hybrid	0.33	0.23 (-)	0.20	0.26	0.85
Pekin male line	0.33	0.22 (-)	0.20	0.24	0.93
Pekin female line	0.33	0.21 (-)	0.18	0.23	0.88
Mallard	0.33	0.24 (-)	0.21	0.26	0.91
Leg muscle mass	Expected slope	Slope	Lower CI	Upper CI	R^2
Broiler	1	1.15 (+)	1.09	1.21	0.98
Layer	1	1.41 (+)	1.34	1.48	0.98
Pekin hybrid	1	0.70 (-)	0.62	0.80	0.86
Pekin male line	1	0.64 (-)	0.58	0.71	0.91
Pekin female line	1	0.59 (-)	0.53	0.66	0.90
Mallard	1	0.59 (-)	0.48	0.72	0.68
Tibiotarsal torsion	Expected slope	Slope	Lower CI	Upper CI	R^2
Broiler	0	-0.08	-0.11	-0.06	0.10 *
Layer	0	0.05	0.03	0.07	0.00 *
Pekin hybrid	0	-0.21	-0.28	-0.15	0.22
Pekin male line	0	-0.13	-0.17	-0.10	0.35
Pekin female line	0	-0.12	-0.15	-0.09	0.44
Mallard	0	-0.19	-0.25	-0.15	0.43
Femoral torsion	Expected slope	Slope	Lower CI	Upper CI	R^2
Broiler	0	0.09	0.07	0.13	0.01 *
Layer	0	-0.07	-0.10	-0.05	0.02 *
Pekin hybrid	0	-0.17	-0.22	-0.13	0.52
Pekin male line	0	-0.11	-0.14	-0.09	0.59

Pekin female line	0	-0.10	-0.13	-0.08	0.47
Mallard	0	-0.11	-0.15	-0.08	0.24
Tibiotarsal cortical area	Expected slope	Slope	Lower CI	Upper CI	R^2
Broiler	0.67	0.81 (+)	0.68	0.96	0.75
Layer	0.67	0.77 (+)	0.67	0.88	0.86
Pekin hybrid	0.67	0.60 (=)	0.49	0.74	0.66
Pekin male line	0.67	0.34 (-)	0.29	0.41	0.74
Pekin female line	0.67	0.31 (-)	0.26	0.37	0.86
Mallard	0.67	0.48 (-)	0.41	0.55	0.80
Femoral cortical area	Expected slope	Slope	Lower CI	Upper CI	R^2
Broiler	0.67	0.68 (=)	0.57	0.81	0.75
Layer	0.67	0.80 (+)	0.71	0.90	0.89
Pekin hybrid	0.67	0.47 (-)	0.35	0.64	0.24
Pekin male line	0.67	0.19 (-)	0.14	0.27	0.02 *
Pekin female line	0.67	0.21 (-)	0.15	0.29	0.07 *
Mallard	0.67	0.35 (-)	0.28	0.45	0.53
Tibiotarsal stiffness	Expected slope	Slope	Lower CI	Upper CI	R^2
Broiler	0.67	0.63 (=)	0.54	0.73	0.81
Layer	0.67	1.28 (+)	1.14	1.43	0.90
Pekin hybrid	0.67	0.79 (=)	0.66	0.95	0.73
Pekin male line	0.67	0.78 (=)	0.67	0.90	0.81
Pekin female line	0.67	0.77 (=)	0.66	0.90	0.81
Mallard	0.67	0.79 (=)	0.62	1.00	0.52

Tibiotarsal maximum load	Expected slope	Slope	Lower CI	Upper CI	R^2
Broiler	0.67	0.78 (+)	0.68	0.89	0.85
Layer	0.67	1.13 (+)	0.99	1.27	0.88
Pekin hybrid	0.67	1.01 (+)	0.89	1.15	0.86
Pekin male line	0.67	0.67 (=)	0.59	0.76	0.87
Pekin female line	0.67	0.74 (=)	0.65	0.84	0.88
Mallard	0.67	0.88 (+)	0.79	0.99	0.89

Table 1. Allometric analysis of reduced major axis regressions

Slopes and R^2 values for various bone traits are provided, along with their 95% confidence intervals. Regressions presented here were significant ($p < 0.01$) with the exception of those marked *. All length measurements that are regressed against body mass have an expected slope of 0.33 and measurements of areas have an expected slope of 0.67. Angular measurements such as torsion are expected to have a slope of 0. The symbols next to each slope indicate positive allometry (+), negative allometry (-) or isometry (=).

Discussion

Both the Pekin duck and broiler chicken have undergone major changes in body size and leg morphology since divergence from their ‘unselected’ conspecifics occurred through artificial selection. Other studies have reported that these changes affect gait [26, 5, 27, 28]. Body mass of both chicken and duck meat lines has also considerably increased since divergence from their unselected (or ancestral) phenotype. While the layer chicken cannot be regarded as the broiler’s ancestral phenotype, it has not been submitted to such intensive selection for increased body mass (selection has mainly focused on reproductive traits) and its growth rate is similar to that of the mallard. Therefore, it is a useful baseline for comparison with the broiler (Fig. 1).

The length of the tibiotarsus scales differently in both species (Table 1); the leg bones of all duck lines undergo a similar rapid early development which is in contrast to that of the chicken lines. The duck’s tibiotarsal and femoral growth begins to plateau at five weeks of age whereas the chicken’s leg bones continue to grow. A similar pattern of growth is seen in leg muscle mass (Fig. 2b). In other words, leg growth displays positive allometry in chickens and negative allometry in ducks (Table 1). These findings are consistent with a previous study of mallard ontogeny, which demonstrated that leg development plateaus to a level close to that of the adult at four weeks post-hatch whereas wing development does not really begin until three weeks post-hatch [29]. These alternate strategies of leg development may be due to differences in the behavioural ecology of the birds’ wild ancestors. Predation on chicks represents an intensive selective pressure. The standard predator escape mechanism for ducklings is to run to water and swim away from the bank [30], whereas for jungle fowl chicks, the predator escape mechanism involves periods of immobility and short bursts of flight [31], neither of which require intensive or prolonged use of the legs. Therefore, there may have been a higher selective pressure for well-developed legs early in life in the duck ancestor, which would explain the patterns of hindlimb growth observed in both the mallard and the Pekin lines.

Tibiotarsal morphology

Curvature of the tibiotarsus in the cranio-caudal plane differs between both species (Fig. 3c). In chickens, the birds selected for rapid growth rate are more cranially curved than their slow-growing conspecifics but this is not the case in ducks, for which all lines display a similar curvature. Whereas the increased curvature observed in the broiler chicken may be a side-effect of the rapid growth rate, it is not clear why a similar effect does not occur in the Pekin duck lines. The divergence of bone angulation in different directions from 180° (a straight bone) as observed in each species may represent an adaptation to specialised leg use in the ancestor such as paddling in ducks or cursorial or perching behaviour in chickens (Fig. 4).

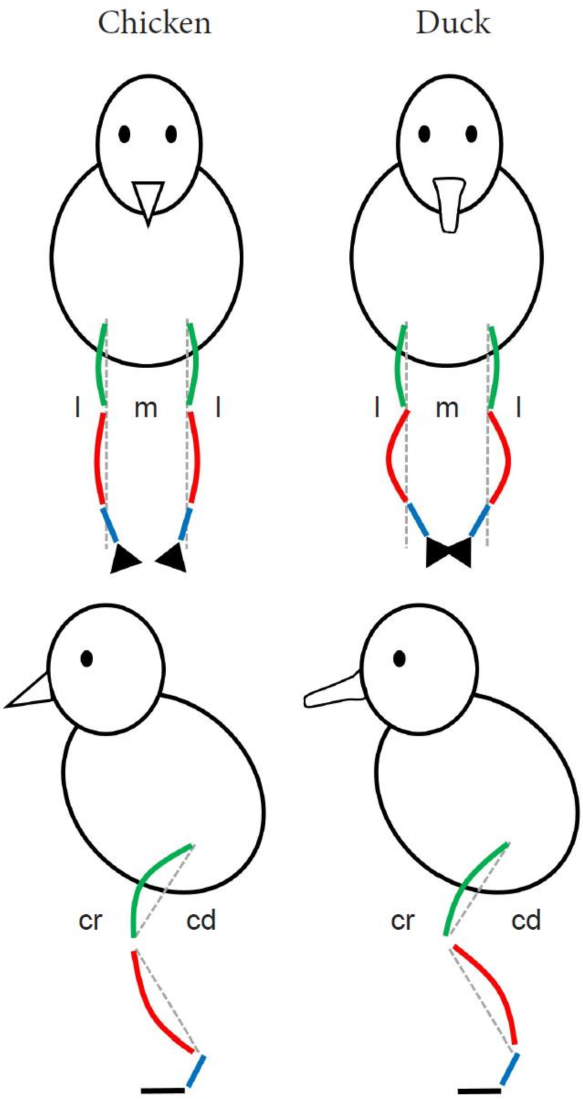


Figure 4. Curvature of the femur (green) and tibiotarsus (red) in the chicken and duck, shown in both frontal and lateral views.

The tarsometatarsus (blue) is represented by a straight line since curvatures were not recorded on this bone. Curvatures are exaggerated for clarity. Note the increased lateral curvature of the duck tibiotarsi (and subsequent foot placement) in the medio-lateral plane and the differing directions of tibiotarsal curvature in the cranio-caudal plane. l = lateral; m = medial; cr = cranial; cd = caudal.

In the medio-lateral plane of the tibiotarsus, ducks selected for rapid growth rate experience more lateral curvature than their ‘unselected’ conspecifics, the mallards (Fig. 3d). This may be a side-effect of rapid growth (although it was not observed in broiler chickens), or it may have developed as an adaptation to loading through the limb. Lateral bending of the tibiotarsus would increase the angle that the tarsometatarsus makes with the sagittal plane of the body, thereby moving the foot to a more medial position which would place the foot under the centre of mass during stance time and thus, increase stability. Divergence for this trait is also observed between species, with the duck lines displaying more curved tibiotarsi. This suggests that greater lateral curvature may be beneficial to the duck but not to the chicken. Simplistically, the varus deviation of tibiotarsi in ducks would permit the feet to be positioned in a more medially aligned position when they paddle, given that swimming birds typically paddle with their tibiotarsi positioned in a more abducted position than when they walk [32]. The angles of the distal tibiotarsal (intertarsal) joint plane have been reported to differ between the ringed teal (a semi-aquatic species) and the quail (a cursorial species), which supports our findings on tibiotarsal bending in the medio-lateral plane [33]. A lateral curvature of the distal tibiotarsus (Fig. 4) would lead to a change in the angle of the intertarsal joint plane and, thus, affect the position of the tarsometatarsus and move the foot to a more medial position. In guinea fowl, during walking the tibiotarsus and tarsometatarsus are adducted so that the foot remains underneath the centre of mass during stance [34]. Gatesy [35] suggested that the tibiotarsus moves laterally (abducts) to bring the protracting foot clear of the stance limb during its swing phase before adducting again for ground contact. The lateral curvature observed in the duck may be a swimming adaptation which hinders this process during walking. Previous work has demonstrated that unperturbed mallards swim at speeds which minimise the energetic cost of transport [36]. Mallard ducklings will swim in formation which reduces their energy expenditure and it has been suggested that while this is partly due to the drag wake of the leading ducklings, energy may also be ‘recycled’ from vortices shed during the power phase of the lead ducking’s paddling stroke [37]. The lateral curvature of the duck tibiotarsi may assist in harvesting the energy from these shedding vortices to reduce the energetic cost of swimming, but clearly more detailed investigation is required to confirm this. Bone curvature was not analysed allometrically since this trait does not change with increasing body size (Fig.3).

Figure 7. Curvature of the femur (above) and tibiotarsus (below) in the broiler chicken (Br), layer chicken (L), Pekin commercial hybrid (P) and the mallard (M), in both frontal (a) and lateral (b) views.

These bones were taken from birds aged 7 weeks. Note the lateral curvature of the both the Pekin and mallard tibiotarsi (a) and the extreme cranial curvature of the broiler tibiotarsus (b).

Rotation of the leg bones can have a major effect on the orientation of some more distal elements of the limb and, thus, greatly influence gait dynamics. The tibiotarsi of both species show a similar degree of torsion at three weeks of age (Fig. 5b). However, as ducks grow older, their tibiotarsi twist with the distal end rotating internally. Allometric analysis (Table 1) shows that the scaling exponent of the tibiotarsal torsion in duck lines deviates further from the expected value of zero than that in chickens (here, a negative scaling exponent indicates an increase in internal rotation, rather than negative allometry). The R^2 values of the regressions for these traits were very close to zero in both chicken lines, which suggests that bone torsion does not scale to body mass in this species. This rotation occurs earlier in the Pekin commercial hybrid than in the male and female Pekin lines and the mallard, a difference which is probably associated with early maturity (Fig. 5b). It is not known why the tibiotarsi of the duck lines rotate as the ducks develop, but one effect of this rotation would be to position the foot more medially during stance and during swimming. In effect, tibiotarsal rotation in this case is complementing the lateral curvature discussed previously. However, if this was a swimming adaptation, one would expect this rotation to occur earlier when a high selection pressure on swimming ability exists in ducklings due to predation (if the same morphological constraints on swimming ability exist across ages). Previous studies have reported a link between tibiotarsal torsion and rapid growth rate [27] but this explanation is contradicted by the observation that duck lines with rapid and slow growths display similar ranges of tibiotarsal rotation. Finally, it is also worth noting that these measures of torsion are calculated using bone landmarks; changes in the relative size/position of these landmarks throughout development may affect the amount of torsion recorded and also affect comparisons between lines.

Tibiotarsal bone quality

Selection for production traits was expected to affect aspects of the leg which would normally be subjected to a strong level of natural selection. The stiffness of the tibiotarsi in all lines scaled with isometry (geometrically similar to body mass) with the exception of the layer chicken, which scaled with very positive allometry (Table 1). This may be a strategy to counteract the comparatively small radius of the layer bones. The radius of a bone exponentially affects its strength, so relatively narrow bones (such as those of the layer) will be exponentially weaker. Surprisingly, the stiffness of the mallard bones does not scale with a similarly positive allometry. In chickens, the cortical area of the tibiotarsus increased at a faster rate than the rest of the body; in ducks the tibiotarsal cortical area displayed negative allometry (with the exception of the Pekin hybrid, which grew isometrically). This is another indication that legs reach adult size and slow down their growth earlier in the duck lines than in the chicken lines. The tibiotarsi of the broiler, layer, Pekin hybrid and mallard became relatively stronger as the bird grew; the maximum load tolerated by the tibiotarsi before breaking scaled positively in these lines (the male and female Pekin breeding lines scaled isometrically). The range of bending stresses measured in the tibiotarsi during breaking were similar for all lines, which suggests that the birds, regardless of their size, adapt their bone morphology in a similar way to suit the forces subjected on them (Fig. 6b). However, by seven weeks of age, the tibiotarsi of the duck lines tolerated more stress than those of the chicken lines, which indicates that either the selection pressure on chicken lines for production traits has occurred at the expense of the mechanical properties of their bones, or that the composition of bone rather than its gross morphology allowed the bones of the duck lines to tolerate relatively high forces. The mallard tibiotarsi tolerated slightly more bending stress than those of the selected duck lines, which would support this theory. Also, as the chicken tibiotarsi are still growing at this developmental stage, they may not be as mechanically robust as the duck tibiotarsi, which have slowed considerably in growth and thus have more opportunity to remodel to handle the loads imposed on them. Previous studies on broiler chickens suggested that lines selected for rapid growth, while having tibiotarsi of the correct dimensions for supporting greater loads, have greater porosity and lower levels of cortical bone mineralisation than slower growing lines [23, 22].

Differences in bone mineral content did not explain the mechanical changes; all lines showed an increase in mineralisation of the mid-diaphyseal tibiotarsus until five weeks of age, and thereafter the rate of mineralisation stabilised (Fig 6c). The differential mechanical properties of avian bone were consistent with the histological measures of porosity. The duck tibiotarsi became less porous (and thus stronger) as the birds aged, allowing them to tolerate greater bending stresses, whereas the chicken lines maintained the same levels of tibiotarsal porosity throughout the same growth period (Fig. 6d). Neither bending stress nor porosity scaled to body mass. Bending stress is a metric which has already been corrected for body mass in its calculation and it is thus expected to show no relationship with body mass. It is likely that porosity is mainly influenced by genotype and environmental factors (such as feed) rather than by the size of the bird (although loading forces acting on the bone due to body mass will affect porosity through bone remodelling).

These findings on the divergence of pelvic limb morphology within two species of poultry provide useful information, which can be used to lay the foundations for further investigations on the link between anatomy and gait in poultry.

Conclusions

It is clear that artificial selection for increased growth rate has resulted in diverging hindlimb architectures within species that have been domesticated. Natural selection that acted on these species prior to domestication, has also affected leg morphology. Since the terrestrial lifestyle of the domestic duck differs from that of its semi-aquatic ancestors, it is possible that some hindlimb adaptations for aquatic locomotion, such as lateral curvature of the tibiotarsus, may be a hindrance to effective terrestrial locomotion in the commercial Pekin duck. Indeed, it is interesting that the Pekin duck can ambulate with relative ease compared to the broiler chicken, which reaches a similar size in the same growth period. Future investigations on the differences in leg morphology between strictly cursorial species such as the chicken and swimmers such as ducks may shed some light on these adaptations and their possible effects on gait.

References

1. FAOSTAT [database on the Internet]. FAO. 2014. Available from: http://faostat.fao.org/default.aspx. Accessed: 20 May 2015.

2. Corr SA, Gentle MJ, McCorquodale CC, Bennett D. The effect of morphology on the musculoskeletal system of the modern broiler. Anim Welf. 2003;12:145-57.

3. Schmidt CJ, Persia ME, Feierstein E, Kingham B, Saylor WW. Comparison of a modern broiler line and a heritage line unselected since the 1950s. Poult Sci.2009;88:2610-9.

4. Knowles TG, Kestin SC, Haslam SM, Brown SN, Green LE, Butterworth A, et al. Leg Disorders in broiler chickens: Prevalence, risk factors and prevention. PLoS One. 2008;3:e1545.

5. Paxton H, Daley MA, Corr SA, Hutchinson JR. The gait dynamics of the modern broiler chicken: a cautionary tale of selective breeding. J Exp Biol. 2013;216:3237-48.

6. Kestin SC, Knowles TG, Tinch AE, Gregory NG. Prevalence of leg weakness in broiler chickens and its relationship with genotype. Vet Rec. 1992;131:190-4.

7. Sanotra GS, Berg C, Lund JD. A comparison between leg problems in Danish and Swedish broiler production. Anim Welf. 2003;12:677-83.

8. Sanotra GS, Lund JD, Ersboll AK, Petersen JS, Vestergaard KS. Monitoring leg problems in broilers: a survey of commercial broiler production in Denmark. Worlds Poult Sci J. 2001;57:55-69.

9. EFSA Panel on Animal Health and Welfare. Scientific opinion on the influence of genetic parameters on the welfare and the resistance to stress of commercial broilers. EFSA J. 2010;8:1666.

10. Yogaratnam V. Analysis of the causes of high rates of carcass rejection at a poultry processing plant. Vet Rec. 1995;137:215-7.

11. Mc Geown D, Danbury TC, Waterman-Pearson AE, Kestin SC. Effect of carprofen on lameness in broiler chickens. Vet Rec. 1999;144:668-71.

12. Danbury TC, Weeks CA, Chambers JP, Waterman-Pearson AE, Kestin SC. Self-selection of the analgesic drug carprofen by lame broiler chickens. Vet Rec. 2000;146:307-11.

13. Caplen G, Colborne GR, Hothersall B, Nicol CJ, Waterman-Pearson AE, Weeks CA, et al. Lame broiler chickens respond to non-steroidal anti-inflammatory drugs with objective changes in gait function: A controlled clinical trial. Vet J. 2013;196:477-82.

14. Vestergaard KS, Sanotra GS. Relationships between leg disorders and changes in the behaviour of broiler chickens. Vet Rec. 1999;144:205-9.

15. Weeks CA, Danbury TD, Davies HC, Hunt P, Kestin SC. The behaviour of broiler chickens and its modification by lameness. Appl Anim Behav Sci. 2000;67:111-25.

16. Kapell DNRG, Hill WG, Neeteson AM, McAdam J, Koerhuis ANM, Avendano S. Twenty-five years of selection for improved leg health in purebred broiler lines and underlying genetic parameters. Poult Sci. 2012;91:3032-43.

17. Jones TA, Dawkins MS. Environment and management factors affecting Pekin duck production and welfare on commercial farms in the UK. Br Poult Sci. 2010;51:12-21.

18. Rodenburg TB, Bracke MBM, Berk J, Cooper J, Faure JM, Guémené D, et al. Welfare of ducks in European duck husbandry systems. Worlds Poult Sci J. 2005;61:633-46.

19. Corr SA, Gentle MJ, McCorquodale CC, Bennett D. The effect of morphology on walking ability in the modern broiler: A gait analysis study. Anim Welf. 2003;12:159-71.

20. Paxton H, Tickle PG, Rankin JW, Codd JR, Hutchinson JR. Anatomical and biomechanical traits of broiler chickens across ontogeny. Part II. Body segment inertial properties and muscle architecture of the pelvic limb. PeerJ. 2014;2:e473.

21. Thorp BH. Skeletal disorders in the fowl - a review. Avian Pathol. 1994;23:203-36.

22. Williams B, Solomon S, Waddington D, Thorp B, Farquharson C. Skeletal development in the meat-type chicken. Br Poult Sci. 2000;41:141-9.

23. Williams B, Waddington D, Murray DH, Farquharson C. Bone strength during growth: Influence of growth rate on cortical porosity and mineralization. Calcif Tissue Int. 2004;74:236-45.

24. Van Wyhe RC, Applegate TJ, Lilburn MS, Karcher DM. A comparison of long bone development in historical and contemporary ducks. Poult Sci. 2012;91:2858-65.

25. Allen V, Elsey RM, Jones N, Wright J, Hutchinson JR. Functional specialization and ontogenetic scaling of limb anatomy in *Alligator mississippiensis*. J Anat. 2010;216:423-45.

26. Paxton H, Anthony NB, Corr SA, Hutchinson JR. The effects of selective breeding on the architectural properties of the pelvic limb in broiler chickens: a comparative study across modern and ancestral populations. J Anat. 2010;217:153-66.

27. Bradshaw RH, Kirkden RD, Broom DM. A review of the aetiology and pathology of leg weakness in broilers in relation to welfare. Avian Poult Biol Rev. 2002;13:45-103.

28. Leterrier C, Nys Y. Composition, cortical structure and mechanical properties of chicken tibiotarsi: Effect of growth rate. Br Poult Sci. 1992;33:925-39.

29. Dial TR, Carrier DR. Precocial hindlimbs and altricial forelimbs: partitioning ontogenetic strategies in mallards (*Anas platyrhynchos*). J Exp Biol. 2012;215:3703-10.

30. Dial TR, Heers AM, Tobalske BW. Ontogeny of aerodynamics in mallards: comparative performance and developmental implications. J Exp Biol. 2012;215:3693-702.

31. Collias NE, Collias EC. A field study of the red jungle fowl in north-central India. Condor. 1967;69:360-86.

32. Provini P, Goupil P, Hugel V, Abourachid A. Walking, Paddling, Waddling: 3D Kinematics Anatidae Locomotion (*Callonetta leucophrys*). J Exp Zool A Ecol Genet Physiol. 2012;317A:275-82.

33. Provini P, Simonis C, Abourachid A. Functional implications of the intertarsal joint shape in a terrestrial (*Coturnix coturnix*) versus a semi-aquatic bird (*Callonetta leucophrys*). J Zool. 2013;290:12-8.

34. Abourachid A, Hackert R, Herbin M, Libourel PA, Lambert F, Gioanni H, et al. Bird terrestrial locomotion as revealed by 3D kinematics. Zoology (Jena). 2011;114:360-8.

35. Gatesy SM. Guineafowl hind limb function. I: Cineradiographic analysis and speed effects. J Morphol. 1999;240:115-25.

36. Prange HD, Schmidt-Nielsen K. The metabolic cost of swimming in ducks. J Exp Biol. 1970;53:763-77.

37. Fish FE. Kinematics of ducklings swimming in formation: consequences of position. J Exp Zool. 1995;273:1-11.

Chapter One – Bookending section

Discussion

Allometric analysis allowed the data on bone morphology and strength and its relationship with growing body mass to be understood in depth. This technique uses body mass to predict each trait within a liner regression. Because body mass is used as the predictive variable, other traits are expected to scale to the inverse of their relationship with body mass. Therefore, a trait which considers geometric length (such as bone length) is expected to scale to body mass$^{0.33}$. A trait which is dependent on area, in other words length^{2}, (such as the stiffness of a bone) is expected to scale to body mass$^{0.67}$. Volumetric measures are expected to scale to body mass^{1} because body mass is also a volumetric measure. Measures which have no basic on geometric measurements (such as angles of torsion) are expected not expected to scale to body mass, so the resulting slope of these regressions is expected to be body mass^{0}. Therefore, for bone stiffness (which is dependent on the cross-sectional area of the bone), the slope of the regression line should be 0.67 if the trait scales isometrically. If the trait scales with positive or negative allometry, then the resulting regression line (and its confidence intervals) will be greater than or less than 0.67, respectively. Both the slope of the regression line and its confidence intervals should be considered when calculating allometry. For example, in Table 1, tibiotarsal cortical area scales to .77 in the layer chicken. This is considered to scale with positive allometry because the confidence intervals around the slope do not overlap the expected value of 0.67. Tibiotarsal stiffness in the Pekin Female Line also scales to 0.77. But here the confidence intervals of the slope do overlap with the expected value of 0.67 so this trait is considered to scale isometrically with body mass.

When considering the effect of lateral curvature of the tibiotarsus in balance, it is important to bear in mind the dynamic position of the bird's centre of mass, as well as the position of the supporting foot. If a lateral curvature of the tibiotarsus (as seen here in duck lines) brings the stance foot to a more medial position, this will increase stability, assuming that the centre of mass does not move laterally to a great extent (as seems to be the case in the mallard). In this situation, the laterally static centre of mass is aligned with and supported by the medially-positioned stance foot. Conversely, the Pekin duck, with its large breast muscle mass, seems to exhibit a wide ranging lateral centre of mass movement. This is anecdotal evidence from observation of the birds' gait, and not based on empirical data. In the case of the Pekin, a medial foot placement could allow the dynamic centre of mass (in its most lateral positions) to exist outside of the zone of support provided by the foot. This situation, which may be very brief, can cause instability. This highlights an important problem with studying gait – because the body is made up of various parts, all moving in complex ways relative to one another, in can be difficult to consolidate the effects of each individual component. This may be why there is still little consensus on what constitutes ideal gait in poultry.

This study is limited to the morphology of the femur and tibiotarsus, due to time-availability. A greater understanding of the differences in hindlimb morphology that occur both within and between species would be possible if the tarsometatarsus and the pelvis had been included in this study. If the study was to be repeated, feed restricted birds of the heavier lines could be added to the analysis to gain a better understanding of the interactions between genetics and environmental factors, although there are ethical issues with feed-restricting birds for this purpose. Gross morphological differences were examined between lines; micro-CT scanning or further bone histology studies may reveal important differences in bone morphology between lines at a sub-structural level. The differences in certain aspect of hindlimb morphology were hypothesised to possibly be swimming-related adaptations. This hypothesis could be investigated in greater depth by comparing bone morphology on a range of species, spanning both swimming and cursorial specialist.

Chapter Two – Bookending section

Context and rational behind the study

Having examined the skeletal leg architecture of the lines studied in Chapter One, gait was objectively measured on the same birds to determine if differences in gait existed between these lines and also to record how gait changes within a line as birds age. Before attempting to improve the visual gait score or to automate it, it was first necessary to collect some baseline data on the average gait for each line at different ages. One of the main challenges to recording gait objectively is to ensure that the bird is walking in a 'normal fashion'. The 'normal' gait of a bird is difficult to define, given that any research setting used to record 'normal' gait will contain differences from the home pen. For example, the gait recording area used in this study had a different substrate and contained a walking area with narrow walled-in sides. Both factors were necessary in order to successfully record gait; however, both factors could also have contributed to altering the 'normal' gait of each individual. This should be kept in mind when interpreting the results of any study on gait.

Corrections and Clarifications

In the introduction, the sentence 'An obvious consequence to selection for high pectoral muscle mass in broiler chickens has been a cranial shift in the body's centre of mass (COM) which has been hypothesised to lead to gait instability related to excess stress on the leg muscles (Corr 03, Hutchinson 14)' should read 'An obvious consequence of selection for high pectoral muscle mass in broiler chickens has been a cranial shift in the body's centre of mass (COM) which has been hypothesised to lead to gait instability related to excess stress on the leg muscles (Corr 03a, Paxton 14)'.

The pressure walkway used to record gait in this chapter was connected to a Dell Latitude E6320 laptop (running Intel Core i5). The walkway was calibrated following manufacturer's guidelines. This involved recording pressures without any weight applied and then, when prompted by the calibration software, placing a fixed weight of 3kg onto each pressure plate (the walkway is made up of two plates). The process was repeated for each plate. The non-slip latex covering on the walkway allowed the bird to maintain grip in a normal fashion as it would in its home pen. There is a possibility that a covering over the sensor would allow the force applied through the foot to be slightly more spread out, thus reducing pressure. However, no difference in recorded pressures was observed when the thin latex sheet was applied, either with live birds or with fixed weights.

Some traits (such as step width or stride length) were measured manually, using recordings from the walkway software. This step was done manually because the software cannot automatically identify avian feet or parts of the avian foot, as it can with humans or dogs or make automated measurements of the distances between steps. Therefore, footprints and the distances between them were measured manually using images of the sequence of footprints recorded by the pressure walkway.

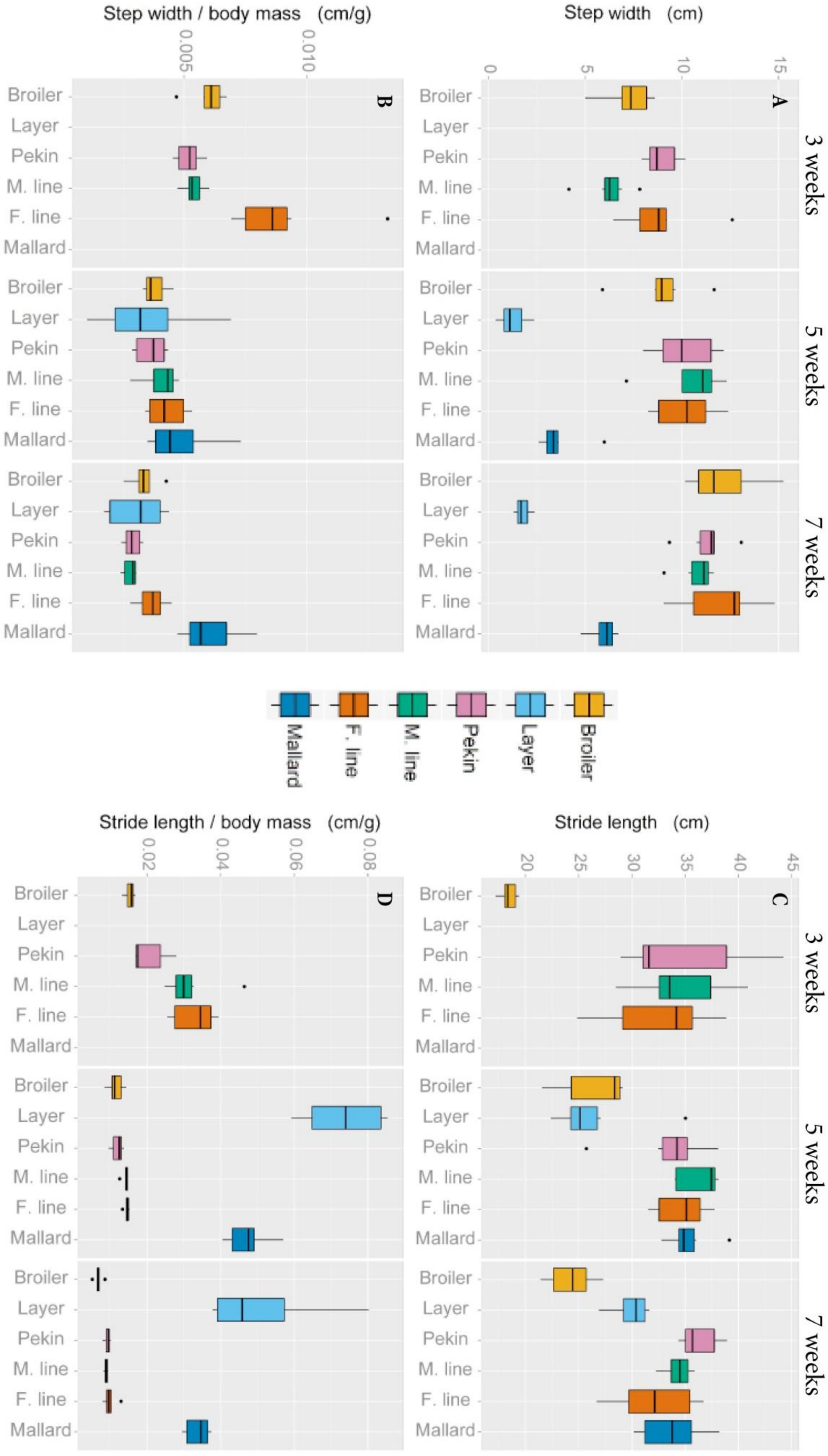
The sentence "The differences in birds' preferred velocity between lines and the variability in velocity within lines was relatively small, suggesting that these values are not just an indication of individual motivation (which would create large variation) but are more likely a result of morphological differences which confine each line to a limited range of 'comfortable' walking speeds." Should read "The differences in birds' preferred velocity between lines was relatively large and the variability in velocity within lines was relatively small, suggesting that these values are not just an indication of individual motivation (which would create large variation within lines) but are more likely a result of morphological differences which confine each line to a limited range of 'comfortable' walking speeds."

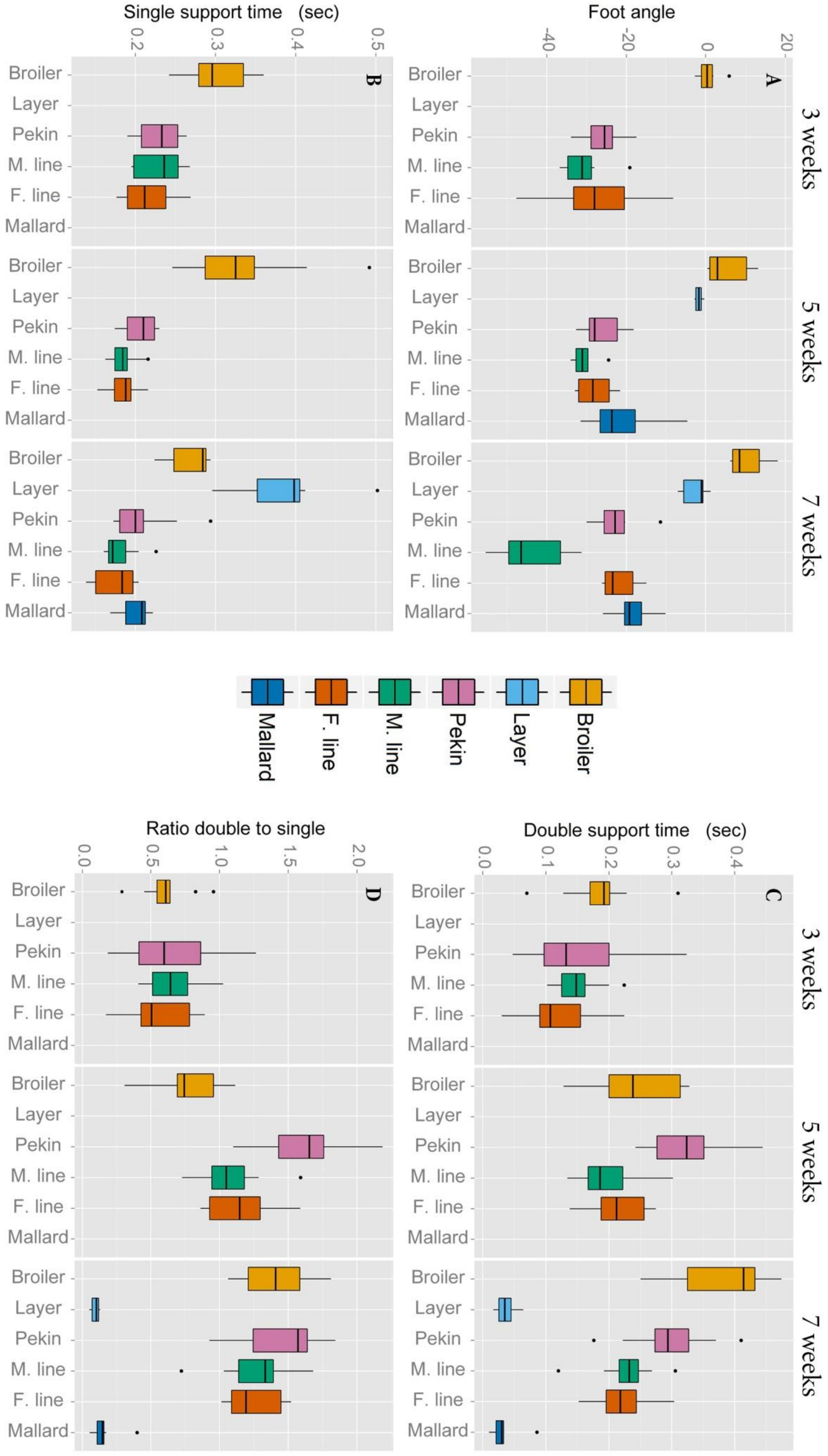
In order to collect data on 12 'satisfactory' walks (as detailed in the methods) more than 12 walks were required for some birds. This number varied between 12 and 20. Birds which were more irregular in their walking ability/motivation, such as broiler chickens at 7 weeks could take up to 40 minutes to complete 12 satisfactory walks. During this time, birds were allowed to rest between walks in order to avoid fatigue. Birds did not receive feed or water while resting outside the pen. Feed and water, as well as access to pen mates, was available at the other end of the walkway and this acted as a motivation for the birds to walk towards the end. With other lines, such as the layer chickens or mallards, motivation to walk was not an issue. Rather, the pace of walking was sometimes too fast, and multiple walks were required to collect data on 12 walks that were at a subjectively acceptable pace. The differences in birds' preferred velocity between lines was relatively large and the variability in velocity within lines was relatively small; coefficients of variation are presented here in Table B1.

Line	Age (weeks)	Sample size	Mean (cm/sec)	sd (cm/sec)	cv
Broiler	3	12	24.22	6.40	26.41
M. Line	3	12	57.41	8.95	15.58
F. Line	3	12	60.29	14.63	24.26
Layer	3	NA	NA	NA	NA
Mallard	3	NA	NA	NA	NA
Pekin	3	12	53.84	14.34	26.63
Broiler	5	12	28.19	5.80	20.59
M. Line	5	12	64.33	7.93	12.33
F. Line	5	12	55.25	7.21	13.05
Layer	5	NA	NA	NA	NA
Mallard	5	NA	NA	NA	NA
Pekin	5	12	46.41	6.56	14.13
Broiler	7	7	27.32	7.07	25.89
M. Line	7	12	56.56	7.68	13.58
F. Line	7	12	57.62	11.13	19.32
Layer	7	12	36.87	6.53	17.71
Mallard	7	12	77.55	8.54	11.01
Pekin	7	12	52.29	8.98	17.17

Table B1. Means, standard deviations and coefficients of variation for walks recorded in each line at each age. M. Line and F. Line refer to male and female Pekin breeding lines respectively. Data was not collected on layer chickens or mallards at 5 and 7 weeks.

Figures 2 and 3 respectively have been included below in larger size, for easier interpretation.


A
B
C
D
3 weeks
5 weeks
7 weeks
Step width (cm)
0
5
10
15
Step width / body mass (cm/g)
0.005
0.010
Stride length (cm)
20
25
30
35
40
45
Stride length / body mass (cm/g)
0.02
0.04
0.06
0.08
Broiler
Layer
Pekin
M. line
F. line
Mallard


A
Foot angle
20
0
-20
-40
B
Single support time (sec)
0.5
0.4
0.3
0.2
C
Double support time (sec)
0.4
0.3
0.2
0.1
0.0
D
Ratio double to single
2.0
1.5
1.0
0.5
0.0
3 weeks
5 weeks
7 weeks
Broiler
Layer
Pekin
M. line
F. line
Mallard

Line	**Velocity (cm/s)**			**Step width (cm)**			**Stride length (cm)**			**Foot angle (°)**			**Ratio double to single foot support time**		
Experiment 1	*3 wks*	*5 wks*	*7 wks*	*3 wks*	*5 wks*	*7 wks*	*3 wks*	*5 wks*	*7 wks*	*3 wks*	*5 wks*	*7 wks*	*3 wks*	*5 wks*	*7 wks*
Broiler chicken	24.35 (6.40)	28.19 (4.10)	27.11 (2.67)	7.25 (0.53)	8.96 (0.76)	12.20 (0.76)	18.43 (0.33)	26.58 (1.38)	24.29 (0.91)	0.76 (1.23)	5.45 (2.37)	10.44 (2.02)	NA	NA	1.44 (0.103)
Layer chicken	NA	NA	37.00 (4.62)	NA	1.26 (0.30)	1.77 (0.17)	NA	26.50 (1.83)	29.94 (0.73)	NA	-1.68 (0.41)	-2.55 (1.43)	NA	NA	0.09 (0.009)
Pekin hybrid	53.84 (10.14)	46.27 (4.64)	52.42 (8.99)	8.95 (0.37)	10.15 (0.68)	11.33 (0.50)	34.77 (2.59)	33.38 (1.71)	36.32 (0.77)	-25.86 (2.30)	-26.16 (2.28)	-22.22 (2.60)	NA	NA	1.46 (0.090)
SED	3.80 P<0.001 [line]			0.74 P<0.001 [line, age]			2.05 P=<0.001 [line]			2.76 P<0.001 [line]			0.13 P<0.001 [line]		
Experiment 2	*3 wks*	*5 wks*	*7 wks*	*3 wks*	*5 wks*	*7 wks*	*3 wks*	*5 wks*	*7 wks*	*3 wks*	*5 wks*	*7 wks*	*3 wks*	*5 wks*	*7 wks*
Pekin male line	57.34 (6.33)	64.26 (5.61)	56.56 (5.43)	6.23 (0.46)	10.40 (0.82)	10.79 (0.39)	34.56 (1.83)	36.46 (0.84)	34.35 (0.55)	-30.34 (2.61)	-30.81 (1.51)	-43.87 (3.91)	NA	NA	1.28 (0.182)
Pekin female line	60.29 (10.34)	55.25 (5.10)	57.62 (7.87)	8.91 (0.85)	10.18 (0.67)	12.05 (0.87)	32.63 (2.17)	34.68 (1.04)	32.19 (1.62)	-27.47 (5.52)	-27.92 (2.00)	-21.79 (1.92)	NA	NA	1.25 (0.137)
Mallard	NA	NA	77.55 (6.04)	NA	3.65 (0.49)	5.97 (0.29)	NA	35.36 (0.88)	33.76 (1.28)	NA	-21.14 (3.90)	-18.41 (2.19)	NA	NA	0.15 (0.063)
SED	4.08 P<0.001 [line]			0.91 P<0.001 [line, age]			1.96 P=0.255			4.47 P<0.001 [line]			0.09 P<0.001		

Table S3: Tibiotarsal morphology - Least squares means (and standard errors) in all lines at each age. SED = Standard Errors of Difference. P values refer to line*age interactions. Other significant effects are presented in square brackets for each trait.

Chapter Two

Gait in ducks (*Anas platyrhynchos*) and chickens (*Gallus gallus*) – similarities in adaptation to high growth rate

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Author contributions:

Brendan Duggan contributed to all aspects of this work. Paul Hocking and Dylan Clements provided supervision support.

Email addresses:

BMD: Brendan.Duggan@roslin.ed.ac.uk

PMH: Paul.Hocking@roslin.ed.ac.uk

DNC: Dylan.Clements@ed.ac.uk

Abstract

Genetic selection for increased growth rate and muscle mass in broiler chickens has been accompanied by mobility issues and poor gait. There are concerns that the Pekin duck, which is on a similar selection trajectory (for production traits) to the broiler chicken, may encounter gait problems in the future. In order to understand how gait has been altered by selection, the walking ability of divergent lines of high and low growth chickens and ducks was objectively measured using a pressure platform, which recorded various components of their gait.

In both species, lines which had been selected for large breast muscle mass moved at a slower velocity and with a greater step width than their lighter conspecifics. These high-growth lines also spent more time supported by two feet in order to improve balance when compared to their lighter, low-growth conspecifics.

We demonstrate that chicken and duck lines which have been subjected to intense selection for high growth rates and meat yields have adapted their gait in similar ways. A greater understanding of which components of gait have been altered in selected lines with impaired walking ability may lead to more effective breeding strategies to improve gait in poultry.

Introduction

Intense selection for production traits in poultry over approximately 60 generations has led to considerable genetic gain. During this period the body mass of the meat type (broiler) chicken has increased by 300% (Knowles et al. 2008). One unwanted side effect of this genetic gain has been an increased incidence of locomotion (gait) problems (Paxton et al. 2013). Altered gait in livestock is an important welfare issue, causing a reduction in mobility, that may be associated with pain (Mc Geown et al. 1999, Danbury et al. 2000, Caplen et al. 2013) and a reduction in normal behaviours (Vestergaard and Sanotra 1999, Weeks et al. 2000).

Estimates of the prevalence of gait problems in broiler chickens have been reported between 15% and 30% (Kestin et al. 1992, Sanotra et al. 2001, Sanotra et al. 2003, Knowles et al. 2008). The true prevalence of gait problems is difficult to obtain, because of variation between studies in the strains of birds assessed, the gait scoring systems used, the age at which birds are assessed and the management factors at each site (EFSA Panel on Animal Health and Welfare 2010). Whereas recent reliable information on the prevalence of leg weakness in poultry is not available, it is widely accepted that the problem causes economic losses for the producer (Yogaratnam 1995). The scale of gait problems in commercial duck populations is also poorly defined, with the only study (which reported the prevalence of gait abnormalities in 46 flocks of commercial ducks) estimating that 14% of 23 day old and 21% of 41 day old Pekin ducks showed signs of gait abnormality (Jones and Dawkins 2010).

The aetiology of gait problems in poultry is varied and complex. An obvious consequence to selection for high pectoral muscle mass in broiler chickens has been a cranial shift in the body's centre of mass (COM) which has been hypothesised to lead to gait instability related to excess stress on the leg muscles

(Corr 03, Hutchinson 14). Skeletal disorders have also been associated with increased body mass and growth rate, some of which negatively affect gait. These include tibial dyschondroplasia (TD), valgus/varus deformities, bone torsion and bone fractures (Bradshaw et al. 2002). While some of these abnormalities may be painful, others may simply alter gait due to conformational changes (Corr et al. 2003b).

Since gait problems were first reported in broiler chickens (Farm Animal Welfare Council 1992, Kestin et al. 1992), efforts have been made to alleviate gait issues across various species through selection, with varying results. For example, selection has been shown to reduce the incidence of TD in broilers over the course of two decades (Kapell et al. 2012). However, poor gait still remains; perhaps due to the difficulty in measuring gait and low heritability leading to relatively little genetic gain in the trait (Sandilands et al. 2011). The standard method of gait assessment is a visual gait score (Kestin et al. 1992). While this is a rapid and inexpensive method of high-throughput phenotyping, the visual gait score has been reported to have relatively poor reliability, due to the subjective nature of the score (Kestin et al. 1992, Anon 2000, Garner et al. 2002). Previous attempts to improve the objectivity of the visual gait score in broilers have led to more reliable estimates (Garner et al. 2002). The development of a better gait score with improved repeatability may lead to better estimates of heritability and long-term genetic gain for gait-related traits in selection programmes. However, objective gait measurement tools used in research, such as kinematic and kinetic systems (Corr et al. 2007, Sandilands et al. 2011, Caplen et al. 2012, Paxton et al. 2013) are unsuitable for use on breeding farms due to costs and time constraints.

The aim of this study was to objectively identify gait changes which have occurred through selection in chicken and duck lines selected for high growth rates and to compare these to conspecifics which have either not been selected for high growth rates (the layer chicken) or which have undergone no artificial selection (the mallard). We also report how certain gait parameters change within lines during growth to slaughter age. Broiler chickens were used as an example of a line selected for high growth rate and layers to represent a line with a growth rate more similar to their ancestral phenotype, the red junglefowl. In Pekin ducks a commercial hybrid and two breeding lines were used as examples of high growth rate birds; these were compared with their ancestral phenotype, the mallard (*Anas platyrhyncos*). The layer chicken and the mallard were assumed to possess an optimal gait for their respective species. It was expected that heavy lines of both species which have undergone selection for increased meat yield would adapt their gait in similar ways to compensate for their change in morphology. A greater understanding of how gait has changed through selection in these lines may inform a more robust gait scoring system based on objective measurement of key gait components and identify which aspects of gait are indicative of the ideal walk.

Materials and Methods

Animals and Husbandry

The gait of 216 birds of different lines was measured objectively using a pressure-sensitive walkway (Tekscan Animal Walkway, Tekscan, Boston, USA) at three ages in two separate experiments; each experiment used different lines of birds. During the first experiment 36 broiler chickens (Ross 308), 36 layer chickens (Lohman Brown) and 36 Pekin ducks (Cherry Valley commercial hybrid) were raised in walled research pens. The second experiment used the same pens to house 36 heavy male line Pekin ducks, 36 lighter female line Pekin ducks (both Cherry Valley breeding stock) and 36 mallards (Hy-Fly Game Hatcheries, UK). Alongside general health and reproductive traits, the male Pekin line is selected with a greater emphasis on feed efficiency whereas the female Pekin line is selected with a greater emphasis on reproductive traits. These Pekin lines were chosen because they are representative of the breeding stock, which is the target group for improving gait by genetic selection. Both these duck breeding lines contained equal numbers of males and females.

Birds were raised following industry guidelines as much as possible. All birds were initially housed from day of hatch under brooder lamps in a single pen per line to regulate temperature. At seven days, birds were randomly allocated in a randomised block design to two blocks of nine pens. Each pen ($2.16m^2$) contained 4 males and 4 females in an area of 0.27 m^2 per bird, increasing to 0.36 m^2 per bird from 21 days and 0.54 m^2 per bird from 35 days as birds were removed for measurement. The lighting regime was 23 hours light: 1 hour dark at hatch, reducing by one hour light per day for the first seven days and remaining at 16 hours light: 8 hours dark thereafter. The mean light intensity in each pen was 120 lux. Barn temperature was 25°C at two weeks, reducing to 24°C at three weeks, 22°C at four weeks and remained at 20°C from five weeks until termination. Experiment 1 used wood shavings as a substrate as this is the industry norm for chickens. Experiment 2 used straw as a substrate, as is the case on most UK duck farms. All birds were fed *ad libitum* and water was provided ad libitum in suspended bell drinkers. Broilers were given a commercial starter feed for the first 10 days, grower from day 11-35 and finisher from day 36 onwards. Layers were fed on a commercial starter feed for the first 35 days before transferring to a grower feed from day 36 onwards. All duck lines in both experiments were fed on a starter feed until day 10 and on a grower feed thereafter: both duck diets were supplied by the breeding company.

The study was approved by the Veterinary Ethical Review Committee at the University of Edinburgh.

Data Collection

At three ages (21, 35 and 49 days) two randomly selected birds (one male and one female) from each pen (six males and six females per line) were walked repeatedly over a Tekscan pressure walkway (Tekscan, Boston, MA, USA). The walkway consisted of two sensing tiles connected together to form a single low-profile 1 m x 0.5 m pressure walkway which recorded at a resolution of 1.4 sensing elements per cm^2. Two 'Tekscan EH-2 Evolution' handles were used to connect this system to a laptop computer, allowing kinetic data to be analysed using proprietary software (Tekscan Walkway, v7.02). The walkway was calibrated as per manufacturer guidelines, using pressures which were appropriate for the weight of the birds to be recorded. Proprietary equilibration files (10 PSI and 20 PSI) were used when gathering data. In order to capture information on the entire walk of each bird, the pressure walkway recorded at a frequency of 62.5 Hz. This sampling frequency, while lower than usual for studies of this kind, was necessary due to memory restrictions of the software. Birds were motivated to walk in a straight line over the pressure walkway by placing 50 cm high plywood boards on each side parallel to the walkway. The walkway was covered by a 1 mm thick latex sheet to ensure the birds did not slip. Each bird was released at one end of the walkway and allowed to walk freely (away from the camera) towards two pen-mates which were held in a pen at the other end of the walkway. As a standardisation check, each walk was recorded using a video camera (Microsoft LifeCam Studio), which linked simultaneously to the pressure data collected by the Tekscan software. At least 12 'satisfactory' walks were recorded for each bird. A walk was deemed satisfactory if the bird moved at a steady pace in a straight line without slipping or stumbling. Birds were allowed to walk at their own preferred speed. After 12 walks had been recorded, each bird was euthanatised and dissected to assess leg morphology (Duggan et al. 2015). The data from each walk was analysed using Tekscan software. Each walk was checked again for pausing, stumbling and straightness by viewing the recorded video clips, which afforded an alternate view (from behind, at the level of the birds' head). Any walks which did not capture four successive steps in a straight line on the recording area of the pressure platform, or which showed pausing/stumbling on video, were discarded. A custom script (Python) was used for the remaining walks of each bird to select the five walks which deviated least in velocity. An 'ideal' velocity for all birds was not chosen as birds differed in their average velocity depending on age, line and behavioural traits such as shyness or fear and because forcing animals to walk at a particular speed may lead to inconsistent gaits as has been observed in other species (Voss et al. 2010). The five walks which deviated least in their velocity were considered to be most representative of each bird's comfortable walking speed. Data from these five walks were averaged for each bird to obtain measures of velocity, step width, stride length, foot angle (whether the middle, third, toe is internally or externally rotated during ground contact), peak vertical force (PVF, the force applied through the ground during stance time), vertical impulse (a product of the vertical force and the time over which it is applied), support time (the time spent supported by either a single foot or both feet) and duty factor (the proportion of a single gait cycle during which one foot is in contact with the ground). Step width is the distance between the lines of progression of the left and right feet. The line of progression of each foot was determined by drawing a line from the point most posterior to the middle toe of the foot for consecutive steps of that foot. Stride length is the distance measured parallel to the line of progression of a foot, between the posterior heel points of two consecutive footprints of the foot in question. Although 12 birds from each line at each age were walked over the pressure walkway, only seven broiler chickens at seven weeks of age were capable of displaying ‘normal’ gait. Gait data from the remaining five ‘lame’ broilers were not included in the analysis at this age.

Analysis

Gait data collected by the pressure platform were analysed by a split-plot statistical model using REML, with effects for pen nested within block and treatment effects of genetic line, age and sex. The resulting variance components were used to ascertain differences between each line by t-test (at a significance level of $P<0.01$). Certain traits (step width, stride length and foot angle were measured manually from the trace of foot pressures left on the walkway to avoid measurement errors from the proprietary software’s automated measuring system due to its inability to correctly identify the foot pressure pattern consistently. As manual measurement of these traits is labour intensive, six birds from each line at each age (with the exception of 3 week layers and mallards) were selected randomly for measurement. Because birds were selected randomly for these measurements, blocking effects were not included in the statistical model for analysing these traits. Separate REMLs were performed to compare the lines from Experiment 1 (the broiler chicken, the layer chicken and the Pekin commercial hybrid) and the lines from Experiment 2 (the male Pekin line, the female Pekin line and the mallard).

Single support time, double support time and the ratio of double to single support time were analysed by general ANOVA as these traits were only measured at one age (seven weeks) when layer chickens and mallards provided large enough pressures for accurate measurement of these traits. Tukey post hoc tests (at a significance level of $P<0.01$) were performed to ascertain differences between lines.

Certain gait traits were also analysed allometrically by assessing their scaling relationships with body mass; the log of each trait was regressed against the log of body mass using a reduced major axis regression. The slope of the resulting regression equation was compared to the expected scaling component for that trait. Length measurements were expected to scale to body mass$^{0.33}$ and non-dimensional traits (such as duty factor) were expected to scale to body mass^{0}.

Results

Least squares means and standard errors of treatment difference for gait traits in all lines and ages are presented in Appendix IV.

The divergence in growth rate and body mass between lines selected for carcass traits and 'unselected' lines is displayed in Figure 1A. Figure 1B shows the comfortable velocity ranges of each line, at three, five and seven weeks of age. The layer chicken moved at a significantly faster speed than the broiler and the Pekin commercial hybrid walked significantly faster than both chicken lines ($P<0.005$). In Experiment 2, the mallard walked significantly faster than the Pekin breeding lines ($P<0.001$). In each species, the lines unselected for high muscle mass (the layer chicken and mallard) both walked with a significantly narrower step width than their heavier conspecifics (the broiler chicken and Pekin duck respectively) ($P<0.001$). Both body mass and the length of the tibiotarsus (a proxy for leg length) were initially included as covariates in the analysis of step width but had no effect. There was a line by age interaction in Experiment 1 ($P=0.021$); step width increased substantially after five weeks in both chicken lines whereas no substantial increase in step width was observed after five weeks in the Pekin commercial hybrid (Fig 2A). The ratio of step width to body mass is presented in Fig 2B. In Experiment 1, the stride length differed between lines ($P<0.001$). Tibiotarsal length and body mass were included in an initial statistical model as covariates but had no effect. The layer had a longer stride than the Pekin hybrid, which had a longer stride than the broiler chicken ($P<0.001$). There was a line by age interaction ($P=0.012$); broiler stride length decreased after five weeks but the stride lengths of the layer and Pekin hybrid increased. In Experiment 2, there was no difference in stride length between duck lines (Fig 2C). The ratio of stride length to body mass is presented in Fig 2D.

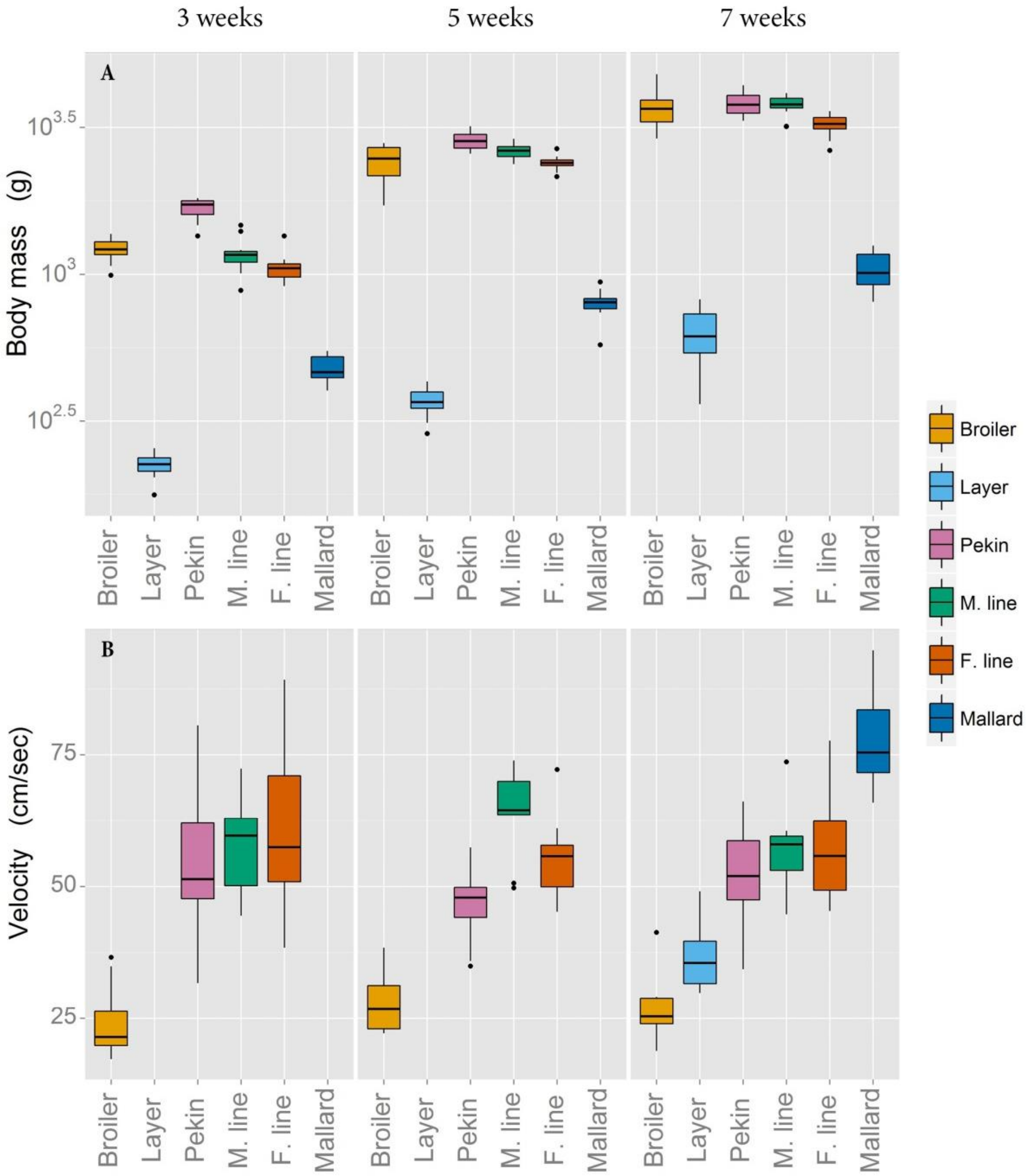

Fig 1. Body mass and comfortable walking velocity. Body mass (A) and comfortable walking velocity (B) in the broiler and layer chicken, the Pekin commercial hybrid ("Pekin"), the Pekin male breeding line ("M.Line"), the Pekin female breeding line ("F. line") and the mallard. Body mass is presented on a log scale for clarity. For velocity, each value represents the mean velocity of five walks from a single bird. Velocities of the layer chicken and mallard were not recorded at three and five weeks of age due to limited sensitivity of the pressure walkway at these body masses.

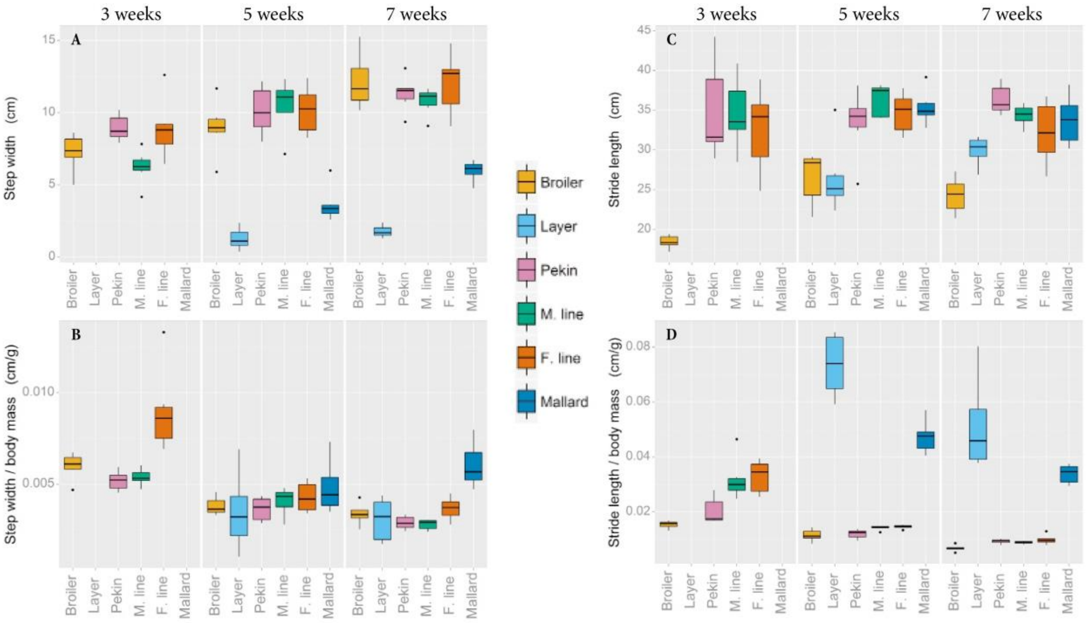

Fig 2. Step width and stride length, presented as raw means (A and C, respectively) and as ratios of body mass (B and D, respectively). Data was not recorded at three weeks of age in the layer chicken or mallard line due to limited sensitivity of the pressure walkway at this body mass.

The angles at which the feet were placed during walking were significantly different between broiler and layer chickens in Experiment 1 (Fig 3A); layers' feet aligned sagittally to the direction of travel whereas those of broilers were externally rotated (P<0.001). The feet of the Pekin commercial hybrid were internally rotated compared with both chicken lines (P<0.001). In Experiment 2 there was no difference in foot angle between the mallard and the female Pekin breeding line (which both displayed similar means and variation of foot angle to the Pekin commercial cross in Experiment 1); however the foot angle of the male Pekin line was more internally rotated (P=0.001) compared with the female line and the mallard (Fig 3a). The foot became more internally rotated after 5 weeks of age in the male Pekin line whereas the feet of the mallard and female line both became less internally rotated after this age, leading to a line by age interaction (P=0.008).

In both species, heavy lines spent more time being supported by two legs during walking when velocity was accounted for as a covariate (P<0.001) (Fig 3C). The ratio of double support time to single support time (with walking velocity accounted for as a covariate) was greater in heavier lines of both species compared to their lighter conspecifics (P<0.001) (Fig 3D).

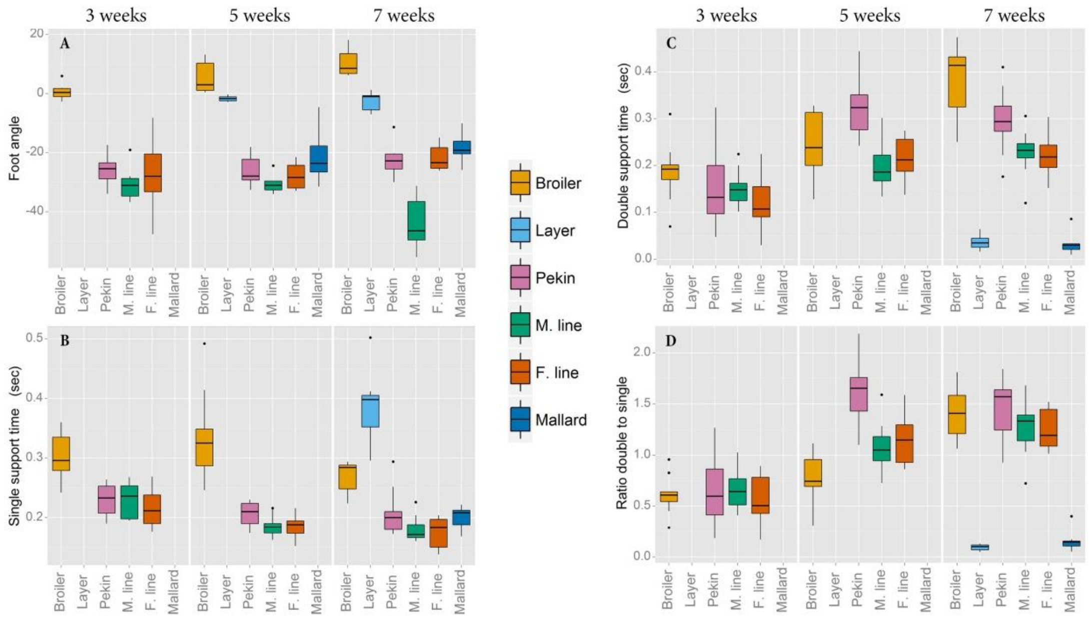

Fig 3. Foot angle (A), single foot support time (B), double foot support time (C) and the ratio of double to single foot support time (D) at three five and seven weeks. A positive foot angle represents externally rotated feet while a negative value indicated internal rotation. Single support time (B) is the proportion of the gait cycle during which the bird has only one foot in contact with the ground. Double support time (C) is the proportion of the gait cycle during which both feet are in contact with the ground. Foot angle data for the layer chicken and mallard at three weeks of age and support time data for the same lines at three and five weeks of age were ommitted due to limited sensitivity of the pressure walkway at these body masses.

No differences were found in PVF between the lines in Experiment 1 (Fig 4A). However, PVF significantly decreased with age (P<0.001). In Experiment 2 PVF was also found to decrease with age (P<0.001). Significant differences were seen between lines in Experiment 2; the male and female Pekin lines produced higher PVFs than the mallard (P<0.005). Vertical impulse (Fig 4B) was greater in the broiler chicken than in both the layer chicken and Pekin commercial hybrid (P<0.01). In Experiment 2 both the male and female Pekin lines produced a higher vertical impulse than the mallard (P<0.001). In both experiments vertical impulse increased with age (P<0.001 in Experiment 1 and P=0.029 in Experiment 2).

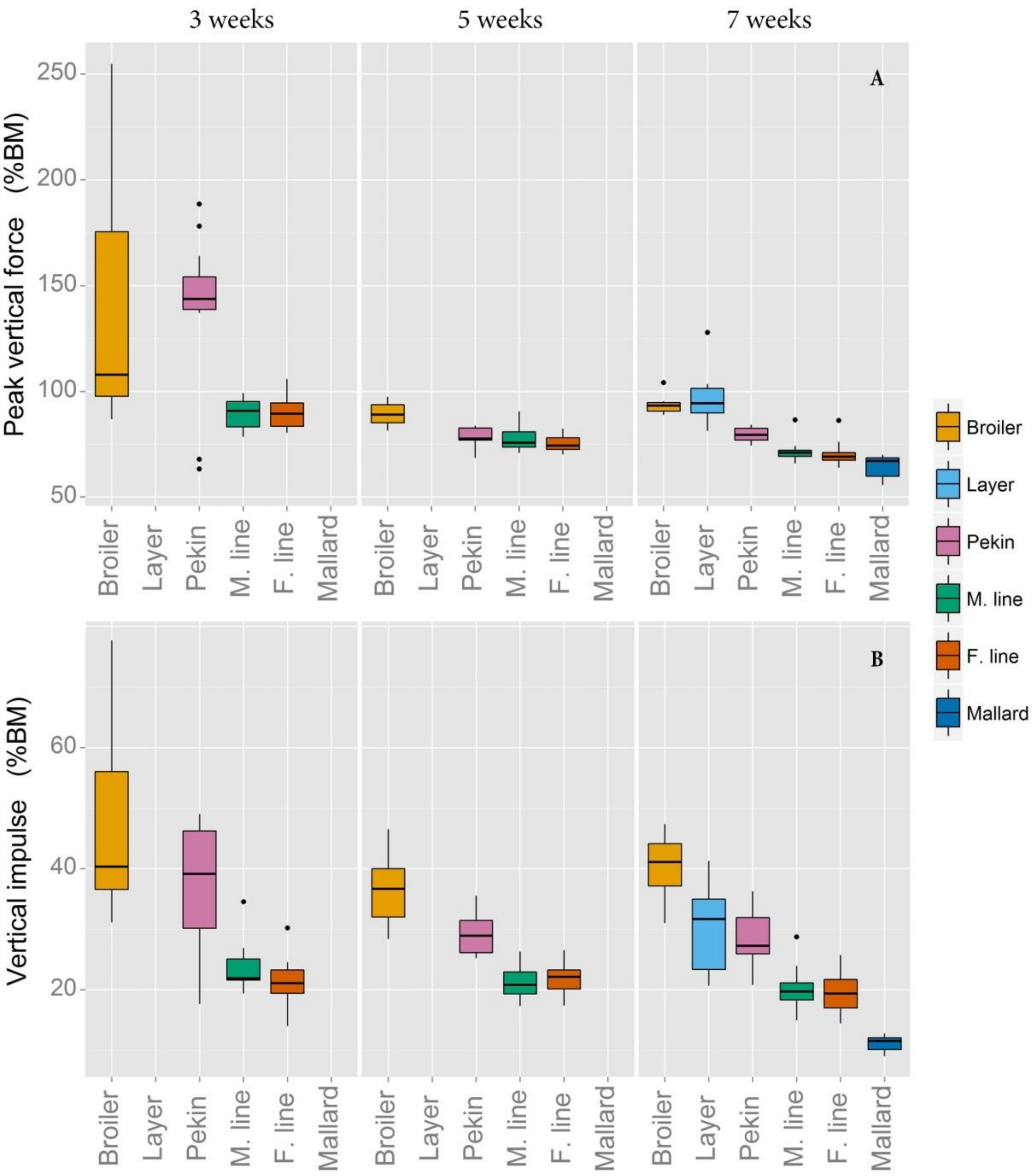

Fig 4. Mean peak vertical forces (A) and vertical impulse (B) values expressed as a percentage of body mass. Data for the layer chicken and mallard at three and five weeks of age were ommitted due to limited sensitivity of the pressure walkway at these body masses.

Step width changed with both positive allometry and isometry, depending on line (Table 1). No relationship with body mass was observed with stride length, with the exception of the broiler chicken; in this line, stride length scaled isometrically. The ratio of double to single foot support time scaled with positive allometery in all lines, with the exception of the mallard, in which no relationship with body mass was observed for this trait. The allometry of both step width and the ratio of double to single foot support time are presented for all lines in Figure 5A and 5B respectively.

Trait	Expected slope	Slope	Lower CI	Upper CI	R^2
Step width					
Broiler	0.33	0.59 (+)	0.45	0.77	0.72
Layer	0.33	1.54	0.83	2.87	0.12[1]
Pekin hybrid	0.33	0.44 (=)	0.30	0.66	0.40
Pekin male line	0.33	0.57 (+)	0.44	0.74	0.77
Pekin female line	0.33	0.43 (=)	0.28	0.65	0.31
Mallard	0.33	1.63 (+)	0.98	2.70	0.43
Stride length					
Broiler	0.33	0.37 (=)	0.26	0.54	0.49
Layer	0.33	0.38	0.21	0.67	0.27[1]
Pekin hybrid	0.33	0.36	0.22	0.60	0.01[1]
Pekin male line	0.33	0.15	0.09	0.26	0.00[1]
Pekin female line	0.33	0.24	0.15	0.40	0.00[1]
Mallard	0.33	0.37	0.20	0.70	0.07[1]
Support time ratio					
Broiler	0	1.01 (+)	0.78	1.31	0.53
Layer	0	1.48 (+)	0.87	2.50	0.47
Pekin hybrid	0	1.66 (+)	1.31	2.11	0.55
Pekin male line	0	0.74 (+)	0.60	0.90	0.68
Pekin female line	0	1.07 (+)	0.87	1.32	0.63
Mallard	0	3.38	1.91	5.97	0.27[1]

Duty Factor					
Broiler	0	0.17 (+)	0.13	0.22	0.54
Layer	0	0.10 (+)	0.06	0.16	0.48
Pekin hybrid	0	0.27 (+)	0.21	0.34	0.56
Pekin male line	0	0.12 (+)	0.10	0.15	0.68
Pekin female line	0	0.17 (+)	0.14	0.21	0.65
Mallard	0	0.33	0.19	0.60	0.25[1]

Table 1. Slopes and R^2 values for gait traits, along with their 95% confidence intervals.

All regressions presented here are significant ($P<0.05$) with the exception of those marked [1]. Length measurements regressed against body mass have an expected slope of 0.33 and non-dimensional measurements have an expected slope of 0. The symbols next to each slope indicate positive allometry (+) or isometry (=).

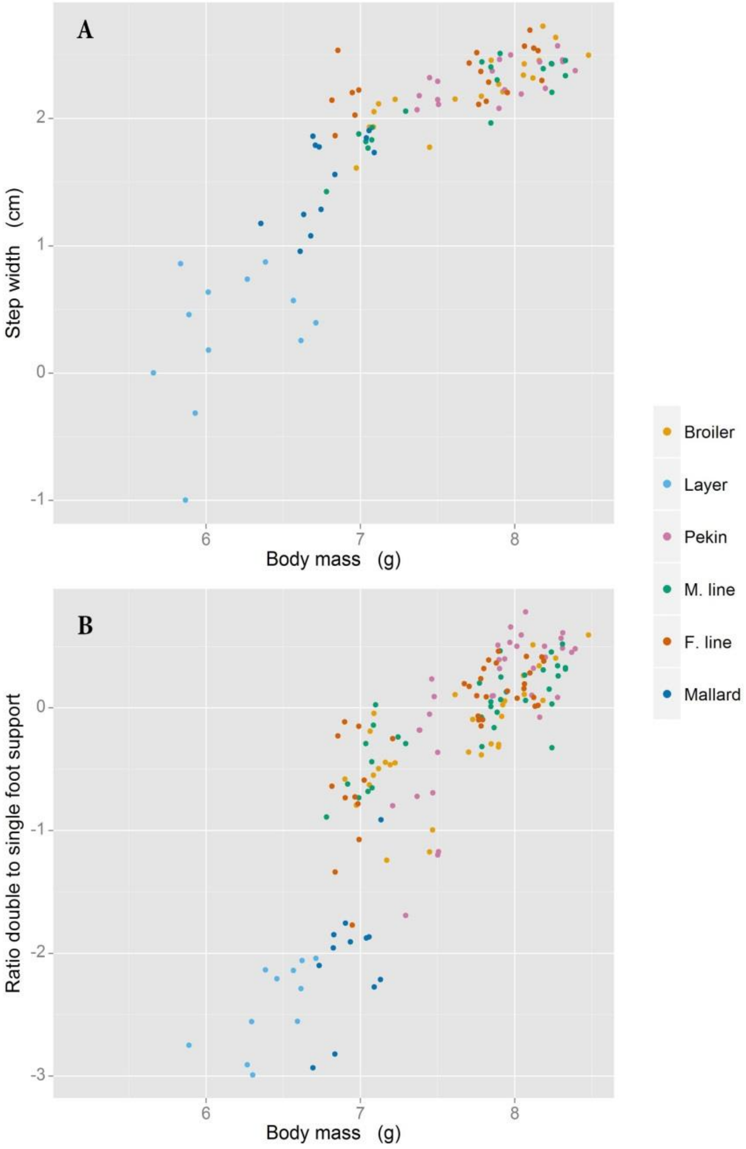

Fig 5. Step width (A) and the ratio of double foot support time to single foot support time (B) regressed against body mass. All values are logged. Data at all ages is included; however data for the layer chicken and mallard at three weeks of age were ommitted due to limited sensitivity of the pressure walkway at these body masses.

Discussion

These results demonstrate that gait variables, both within and between bird species, change throughout growth. They also highlight the effects that intense selection for rapid growth has had on the gait of modern broiler chickens and ducks.

Velocity

The measures of velocity used in this study represent the birds' comfortable walking speeds. The speed recorded from each bird is an average of the five walks that were closest to each other in velocity. The 'preferred' walking speeds of duck lines were greater than those of chickens. Also, within each species, lines selected for high growth rate and meat yield were slower than their 'unselected' conspecifics (Fig. 1B).

The fact that chickens do not walk as quickly as ducks is not unexpected – ducks undergo relatively early leg development, reaching adult leg size by five weeks of age, whereas the legs of chickens continue to grow in size after slaughter age at seven weeks (Dial and Carrier 2012, Duggan et al. 2015). Therefore it is unsurprising that ducks find it easier to achieve higher walking speeds compared with chickens. Why layer chickens and mallards, both of which have not been selected for high growth rate, reach faster walking speeds than their much larger conspecifics is less obvious. The markedly different hindlimb architecture of the heavier lines combined with a cranial shift in the body's COM due to a disproportionally large increase in breast muscle may have led to an imbalanced gait which requires lower speeds (and higher duty factors) to improve stability. These differences in body morphology have been highlighted as a cause for altered gait in broiler chickens (Corr et al. 2003b, Paxton et al. 2013).

Velocity is not just a measure of an individual's ability to move at a certain speed but also their motivation. We found that each bird had a range of speeds at which it could move. The differences in birds' preferred velocity between lines and the variability in velocity within lines was relatively small, suggesting that these values are not just an indication of individual motivation (which would create large variation) but are more likely a result of morphological differences which confine each line to a limited range of 'comfortable' walking speeds.

Step width and stride length

Step width was greater in heavier lines compared with their lighter conspecifics (Fig. 2A). It is expected that a wider step width, while providing a good base of support during standing, will lead to poor balance during walking. If the stance is wide when the swing leg is lifted during walking then the body's COM will not be aligned with the centre of pressure of the supporting foot. The COM will begin to move away from the supporting leg until the swing leg is grounded to provide stability once again. This process leads to a large lateral movement of the body's COM during walking, which may be energetically expensive and could lead to stumbling. The necessity to ground the swing leg quickly to ensure the COM does not fall to the ground potentially explains why the heavy broiler chickens have a shorter stride than the layers; however the Pekin lines and the mallard have a similar stride length (Fig. 2C). The mallard's step width (when expressed as a ratio of body mass), although narrower than the heavier Pekin is relatively wider than other lines (Fig. 2B), perhaps due to its naturally wide hull-shaped body. This may explain the lower than expected stride lengths observed in the ancestral line. A relatively shorter stride length (Fig. 2D) may contribute to the lower velocities seen in these heavy lines compared with the lighter lines. Conversely, in the layer chicken and mallard lines, the step width is narrower and the body's COM is closer to the vertical axis of the supporting foot during walking. This balanced support allows these slow-growth lines to spend less time supported by both feet during walking as their COM is relatively stable above a single supporting foot.

Ratio of double to single foot support

The ratio of double foot support to single foot support is a temporal measure of limb placement which may be an indicator of balanced gait, as suggested by Corr (2003b). Theoretically, a bird with an imbalanced gait will spend more time supporting its weight across both feet; therefore, a low ratio of double to single foot support is indicative of a bird with a well-balanced gait. The current data show that, at seven weeks of age, both layer chickens and mallards have much lower ratios of double to single foot support than their heavier conspecifics, suggesting that these lines, which are unselected for high muscle mass, have better balanced gaits (Fig. 3D). This lack of balance in high growth lines may result from an increase in pectoral muscle mass which has led to a cranial shift in the COM of broiler chickens (Corr et al. 2003a, b, Paxton et al. 2014). While it was not possible to measure temporal foot placement in layers and mallards at three and five weeks of age, data from the high-growth lines also suggests that younger (lighter) birds, which have less pectoral muscle mass, have lower ratios of double to single foot support and thus are better balanced when walking. Also, for heavier birds, the greatest period of stress on the leg bones occurs when the entire body mass is supported through one foot; distributing body mass across both feet by increasing double foot support time would reduce the likelihood of bone damage, as suggested by Caplen et al (2012).

Foot angle

The angle at which the foot is placed during the stance phase of walking can affect balance by moving the base of support to a position either more or less medially aligned with the body's COM. In seven week old broilers, the feet are externally rotated (pointing outwards). This has previously been reported in heavy broiler chickens (Corr et al. 2003b). Theoretically, this would allow the middle toe to extend laterally away from the body's COM, thus providing a wider base of support, extending the 'safe zone' in which the body's COM can move laterally out of alignment with the supporting foot without causing instability. This is important as broilers shift their COM laterally while walking to ensure that the COM is medially aligned with the supporting foot before lifting the swing foot (Corr et al. 2003b). Turkeys employ a similar movement (Abourachid 1991). In contrast, the feet of all duck lines were internally rotated (Fig. 3A). In theory, pointing the toes inward would partially counteract the wide stance seen in heavy lines, which leads to shorter stride lengths and hence lower velocities. An internally rotated foot position would align the toe more medially to the body's COM, improving stability during single foot support, but also reducing the safe zone in which the COM can move without causing instability during walking. That this internal foot rotation is also seen in the mallard suggests that this trait has not developed due to rapid growth or increased body mass but rather is an adaptive trait in the wild phenotype. By seven weeks, the distal end of the tibiotarsus has rotated internally (Duggan et al. 2015), and this may partially explain foot placement in ducks. However, previous studies in broiler chickens have found limited evidence for a link between bone torsion and foot rotation (Corr et al. 2003b, a). It is not clear why the feet of the male Pekin line are rotated internally to a much greater extent than the other duck lines. It is possible that torsion of the tarsometatarsus, as has been observed to occur in the broiler chicken (Duff and Thorp 1985) may play a role. Subjectively, the male Pekin line did not display noticeably worse gait than the other Pekin lines.

Peak Vertical Force and Impulse

Mean peak vertical forces and vertical impulses applied through the ground during walking are plotted in Fig. 4, where both are expressed as a percentage of body mass. While pressure platform systems are generally known to provide different values of forces compared to measurements made by force plates, the values are reliable to use for comparisons between individual animals (Lascelles et al. 2006). The lighter lines used for this study were of a mass which was close to the limits of detection for this pressure walkway and the data from three and five week layer chickens and mallards for certain traits were not analysed. At seven weeks of age, layers and broilers did not differ in the peak vertical forces (expressed as a percentage of body mass) they applied through the ground when walking (Fig. 4A). However, mallards at this age produced lower peak vertical forces (as a percentage of body mass) than the heavier Pekin lines. In commercial lines relative peak vertical forces decreased as the birds grew. At three weeks, broiler chickens and Pekin ducks can subjectively be described as having clumsy gaits. Neural control of foot placement and leg muscle function may not be fully developed at this age and it is possible that rapid leg acceleration is responsible for these higher ground reaction forces in certain younger birds. Birds at this age are growing rapidly and these allometric changes may lead to difficulty judging both distances of anatomical points in relation to the rest of the body and muscle force output (Carrier 1996). The large variation in peak vertical force values observed in the broiler chicken and Pekin commercial cross at three weeks suggests that some birds are maturing earlier than others; some early maturing birds may have already developed more complete neural control of leg movements by this age and so may not display large ground reaction forces. Any interpretations of peak vertical force measurements should take into account the sampling frequency, which in this study was 62.5Hz. A higher frequency allows more accurate determination of peak vertical force events. During this study it was not possible to measure at a higher frequency; memory restrictions dictated that lower sampling frequency be used in order to capture information on each birds entire walk. While this frequency was considered to be adequate for birds walking at this pace, the possibility remains that some peak vertical force events may not have been detected.

Vertical impulse (force, as a percentage of body mass, applied across time) values do not change as the birds age (Fig. 4B). Although the peak vertical forces (as a percentage of body mass) do not change between five and seven weeks, the actual peak force acting on the bones is increasing, because body mass is increasing during this time. As the greatest stress on the leg bones occurs during single foot support, it is possible that, as birds grow heavier, they increase double foot support time to counteract these increases in peak vertical forces and thus a constant vertical impulse is maintained. At seven weeks, the smaller layer chicken and mallard lines produce lower vertical impulses than their heavier conspecifics, most likely due to lower double foot support times in the gait of layers and mallards. The relatively large values observed in the layer line are most likely the result of the high peak vertical forces produced by these birds (Fig. 4A).

Allometry

The ratio of double foot support time to single foot support time and duty factor both scaled with positive allometry for all lines except the mallard, for which no relationship with body mass was observed. Duty factor is another way of expressing the double to single support time ratio and so it is expected that the two scale with a similar allometry (although the scaling exponent of each trait differs as one is a proportion and the other a ratio). When the entire mass of the bird is supported by one leg (during single support) the strain on the leg bones is likely to be at its greatest and the heavy lines which have a wide step width are likely to be unbalanced. Increasing the double support time alleviates the impact of these issues on mobility. As birds become heavier it is possible that they increase their double support time above the lower limit that is required to prevent them becoming unbalanced, which in turn leads to positive allometry as observed in these traits. Step width scaled either with isometry or with positive allometry in different lines whereas stride length did not scale to body mass.

Conclusions

Intense selection for economic traits such as breast muscle mass and growth rate have been accompanied by dramatic changes in several components of gait in both chickens and ducks. The heavy lines of both species have diverged to a similar extent from their ‘unselected’ conspecifics for certain gait traits, suggesting the use of similar strategies to deal with instability due to increased growth or breast muscle mass. Certain traits, such as foot angle, also differ between ‘unselected’ lines, indicating different evolutionary pressures acted on these species prior to domestication. These data can be used to improve the objectivity of gait scoring: by focusing on certain gait components which are likely to play a key role in balanced gait (such as step width or stride length), it may be possible to improve heritability estimates for gait traits and increase selection success.

References

Abourachid, A. 1991. Comparative gait analysis in two strains of turkey, *Meleagris gallopavo*. British Poultry Science **32**:271-277.

Anon. 2000. The welfare of chickens kept for meat production (broilers). European Commission, Brussels, Belgium.

Bradshaw, R. H., R. D. Kirkden, and D. M. Broom. 2002. A review of the aetiology and pathology of leg weakness in broilers in relation to welfare. Avian and Poultry Biology Reviews **13**:45-103.

Caplen, G., G. R. Colborne, B. Hothersall, C. J. Nicol, A. E. Waterman-Pearson, C. A. Weeks, and J. C. Murrell. 2013. Lame broiler chickens respond to non-steroidal anti-inflammatory drugs with objective changes in gait function: A controlled clinical trial. The Veterinary Journal **196**:477-482.

Caplen, G., B. Hothersall, J. C. Murrell, C. J. Nicol, A. E. Waterman-Pearson, C. A. Weeks, and G. R. Colborne. 2012. Kinematic Analysis Quantifies Gait Abnormalities Associated with Lameness in Broiler Chickens and Identifies Evolutionary Gait Differences. Plos One **7**.

Carrier, D. R. 1996. Ontogenetic limits on locomotor performance. Physiological Zoology **69**:467-488.

Corr, S. A., M. J. Gentle, C. C. McCorquodale, and D. Bennett. 2003a. The effect of morphology on the musculoskeletal system of the modern broiler. Animal Welfare **12**:145-157.

Corr, S. A., M. J. Gentle, C. C. McCorquodale, and D. Bennett. 2003b. The effect of morphology on walking ability in the modern broiler: A gait analysis study. Animal Welfare **12**:159-171.

Corr, S. A., C. McCorquodale, J. McDonald, M. Gentle, and R. McGovern. 2007. A force plate study of avian gait. Journal of Biomechanics **40**:2037-2043.

Danbury, T. C., C. A. Weeks, A. E. Waterman-Pearson, S. C. Kestin, and J. P. Chambers. 2000. Self-selection of the analgesic drug carprofen by lame broiler chickens. Veterinary Record **146**:307-311.

Dial, T. R. and D. R. Carrier. 2012. Precocial hindlimbs and altricial forelimbs: partitioning ontogenetic strategies in mallards (*Anas platyrhynchos*). Journal of Experimental Biology **215**:3703-3710.

Duff, S. and B. Thorp. 1985. Patterns of physiological bone torsion in the appendicular skeletons of domestic fowl. Research in Veterinary Science **39**:307-312.

Duggan, B. M., P. M. Hocking, T. Schwarz, and D. N. Clements. 2015. Differences in hindlimb morphology of ducks and chickens: effects of domestication and selection. Genetics, Selection, Evolution : GSE **47**:88.

EFSA Panel on Animal Health and Welfare. 2010. Scientific opinion on the influence of genetic parameters on the welfare and the resistance to stress of commercial broilers. EFSA Journal **8**:1666.

Farm Animal Welfare Council. 1992. Report on the welfare of broiler chickens. Surbiton : FAWC, Surbiton.

Garner, J. P., C. Falcone, P. Wakenell, M. Martin, and J. A. Mench. 2002. Reliability and validity of a modified gait scoring system and its use in assessing tibial dyschondroplasia in broilers. British Poultry Science **43**:355-363.

Jones, T. A. and M. S. Dawkins. 2010. Environment and management factors affecting Pekin duck production and welfare on commercial farms in the UK. British Poultry Science **51**:12-21.

Kapell, D. N. R. G., W. G. Hill, A. M. Neeteson, J. McAdam, A. N. M. Koerhuis, and S. Avendano. 2012. Twenty-five years of selection for improved leg health in purebred broiler lines and underlying genetic parameters. Poultry Science **91**:3032-3043.

Kestin, S., T. Knowles, A. Tinch, and N. Gregory. 1992. Prevalence of leg weakness in broiler chickens and its relationship with genotype. Veterinary Record **131**:190-194.

Knowles, T. G., S. C. Kestin, S. M. Haslam, S. N. Brown, L. E. Green, A. Butterworth, S. J. Pope, D. Pfeiffer, and C. J. Nicol. 2008. Leg disorders in broiler chickens: prevalence, risk factors and prevention. Plos One **3**:e1545.

Lascelles, B. D. X., S. C. Roe, E. Smith, L. Reynolds, J. Markham, D. Marcellin-Little, M. S. Bergh, and S. C. Budsberg. 2006. Evaluation of a pressure walkway system for measurement of vertical limb forces in clinically normal dogs. American Journal of Veterinary Research **67**:277-282.

Mc Geown, D., T. C. Danbury, A. E. Waterman-Pearson, and S. C. Kestin. 1999. Effect of carprofen on lameness in broiler chickens. Veterinary Record **144**:668-671.

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

Paxton, H., P. G. Tickle, J. W. Rankin, J. R. Codd, and J. R. Hutchinson. 2014. Anatomical and biomechanical traits of broiler chickens across ontogeny. Part II. Body segment inertial properties and muscle architecture of the pelvic limb. Peerj **2**.

Sandilands, V., S. Brocklehurst, N. Sparks, L. Baker, R. McGovern, B. Thorp, and D. Pearson. 2011. Assessing leg health in chickens using a force plate and gait scoring: how many birds is enough? The Veterinary record **168**:77.

Sanotra, G. S., C. Berg, and J. D. Lund. 2003. A comparison between leg problems in Danish and Swedish broiler production. Animal Welfare **12**:677-683.

Sanotra, G. S., J. D. Lund, A. K. Ersboll, J. S. Petersen, and K. S. Vestergaard. 2001. Monitoring leg problems in broilers: a survey of commercial broiler production in Denmark. Worlds Poultry Science Journal **57**:55-69.

Vestergaard, K. S. and G. S. Sanotra. 1999. Relationships between leg disorders and changes in the behaviour of broiler chickens. Veterinary Record **144**:205-209.

Voss, K., L. Galeandro, T. Wiestner, M. Haessig, and P. M. Montavon. 2010. Relationships of Body Weight, Body Size, Subject Velocity, and Vertical Ground Reaction Forces in Trotting Dogs. Veterinary Surgery **39**:863-869.

Weeks, C. A., T. D. Danbury, H. C. Davies, P. Hunt, and S. C. Kestin. 2000. The behaviour of broiler chickens and its modification by lameness. Applied Animal Behaviour Science **67**:111-125.

Yogaratnam, V. 1995. Analysis of the causes of high rates of carcass rejection at a poultry processing plant. Veterinary Record **137**:215-217.

Chapter Two – Bookending section

Discussion

Stability in gait is dependent on supporting the mass of the body through the supporting limbs, which at certain points in the gait cycle must be lifted off the ground and swung forward to move the entire body in a certain direction. Gravity acts on the mass of the body through a point called the centre of mass (COM). During walking, the force of gravity acts on the mass of the body through the supporting foot (assuming only one foot is grounded). This point of pressure (the force spread over the area of the foot) is called the centre of pressure. Stability in gait is achieved when the centre of pressure (the supporting foot) is located directly beneath the COM of the body. This is the case when both feet are grounded, even in birds with a wide stance, because the centre of pressure is located at the midpoint between the two supporting feet. Thus, the force of gravity is acting in a straight line between the COM and the ground. If the COM moved medially or laterally relative to the centre of pressure, it would no longer be directly supported by the centre of pressure. This could occur if the body position moved to one side of the centre of pressure or if the centre of pressure moved to one side (as happens when a foot is lifted into swing phase; the remaining supporting foot in stance phase becomes the new location for the centre of pressure). In this situation (single foot support time), the force of gravity acts on the COM in a straight line between the COM (now moved relative to the centre of pressure) and a point on the ground located medially or laterally to the (remaining grounded) foot position. Gravity will act to pull the COM towards this point on the ground. In lay terms, the body will begin to fall over to the side. This falling can be halted if the other foot (at this point raised off the ground in the swing phase) is grounded beneath the COM, or in a position that centres the COM between both feet. In this latter scenario (double foot support time), the centre of pressure will be located between the two grounded feet, directly beneath the COM. This creates stability once more, until the other foot is lifted into the swing phase and centre of pressure becomes the supporting foot once more. If the foot is not grounded quickly enough, the body will fall over entirely. In birds with a wide step width, there are two ways in which the foot can be lifted, swung forward and grounded again to avoid falling; to limit the length of the stride or move the swinging foot quickly through a long stride. In birds with a narrow step width, a short stride length or fast movement of the swinging foot are not needed as the COM will remain relatively closely aligned with the centre of pressure when it alternate position between times of single and double foot support.

The maintenance of balance during walking will be influenced by speed. For example, assuming other gait parameters remain the same, a bird walking quickly can afford to use a wider step width than a bird walking slowly, because the fast pace of the fast bird’s feet will mean that although its COM and centre of pressure will be relatively misaligned during single support time, the single support time will be short, so the body will not deviate very much from upright balance before returning to the stability of double support time. The lines studied in this Chapter were allowed to walk at their own natural velocity, so their gaits will, to some extent, be influenced by this. It is not possible to account for the effect of velocity by attempting to restrict each to walk at the same velocity and this will result in an unnatural gait. While it is clear that this variation in velocity will affect various aspects of gait (It has been demonstrated in quadrupeds that it is feasible to use a large variation in speeds without significantly affecting the ground reaction forces measured (Hans et al. 2014). A potential solution to any variance in gait parameters that may be encountered when different lines walk at a range of velocities is to correct each velocity by height; this technique has been successful in dogs (Volstad et al. 2016).

In Figure 2, step width when presented as a proportion of body mass shows more similar values in relation to other lines than when presented as a stand-alone trait. When presented as a proportion of body mass, the trait is divided by a different value for each line, so the relative relationship between lines will change. Body mass was not significant when fitted as a covariate in the analysis of step width. Fitting body mass as a covariate would send the regression through the mean whereas dividing the trait by body mass would force the regression through zero. Clearly the effect of using a ratio (to body mass) highlighted differences between the step length of ancestral lines and their conspecifics, rather than their step width. Both results support our conclusion that chicken and duck lines selected for high growth rates and meat yields have adapted their gait in similar ways when compared to their ancestral conspecifics. As step width and length are likely to be related to each other (linearly) and to mass (non-linearly), evaluation of the ratio of step width to step length may have negated the effects of mass on the different species and lines evaluated.

References

Hans, E. C., B. Zwarthoed, J. Seliski, B. Nemke, and P. Muir. 2014. Variance associated with subject velocity and trial repetition during force platform gait analysis in a heterogeneous population of clinically normal dogs. Vet J **202**:498-502.

Volstad, N., B. Nemke, and P. Muir. 2016. Variance associated with the use of relative velocity for force platform gait analysis in a heterogeneous population of clinically normal dogs. Vet J **207**:80-84.

Chapter Three

The development of a prototype tool to measure tibiotarsal curvature in live ducks

Abstract

The gait of a bird is directly linked to its leg morphology. Pekin ducks exhibit a characteristic lateral curvature in the distal tibiotarsus that may have an influence on the bird's gait. As gait scores can be subjective and unreliable, the ability to objectively measure aspects of the morphology that affect gait, such as leg bone curvature, may help to predict which individuals are likely to have sub-optimal gait within a breeding programme. The aim of this study was to develop and evaluate the use of a tool that can objectively measure the lateral curvature of the distal tibiotarsus in Pekin ducks in the accurate and efficient manner necessary for implementation within a breeding programme.

A tool to measure bone curvature was developed by adapting two digital callipers. Measurement trials were conducted on batches of approximately 170 Pekin ducks (Cherry Valley male breeding line) and three ages (21, 28 and 42 days) and within each trial, repeatability tests were conducted on a small sample of approximately 30 birds. Repeatability was assessed by calculating the Pearson correlation coefficient of the 1st and 2nd curvature measurements within each bird.

Numerous issues were encountered during the trials, both in the design of the tool and in the design of the implement used to hold the birds still for measurement. Also, morphological features of the Pekin leg made measurement of bone curvature extremely difficult. Correlation coefficients for bone curvature measured with this tool were low.

A tool of this design is not suitable for the measurement of tibiotarsal curvature in Pekin ducks. Some improvement may be made if the tool was fixed to a pivot to avoid movement of the tool position during measurement. However, more success in measuring bone curvature is likely with other technologies such as computed tomography.

Introduction

Gait and leg architecture are intrinsically linked; the way in which an animal uses its legs to move across a terrain will depend on the morphology of those legs and how they interact with both the body and the ground through muscles, tendons and ligaments. Initial analysis of the leg bone morphology and gait of ducks and chickens (Chapter 1 and 2) suggested that tibiotarsal lateral curvature may affect gait, specifically the ratio of single support time to double support time. Leg bone curvature (such as valgus and varus deformities) have been linked to poor gait (Bradshaw et al. 2002). It was hypothesised that the lateral curvature seen in the distal tibiotarsus of Pekin lines may adversely affect gait. In these birds, morphology was measured using CT analysis. As CT analysis of morphology is an expensive and time consuming technique and impossible to perform on a farm setting without significant investment in equipment, the aim of this study was to develop a tool which may be used to measure bone curvature quickly and easily in the Pekin duck. To our knowledge, tools have not been used to objectively measure leg bone curvature within poultry breeding companies, although curvature-related deformities, such as valgus and varus deformities, are identified subjectively by eye during the selection process (Kapell et al. 2012, Kapell et al. 2017). The successful development of such a tool would allow any link between bone curvature and gait score to be investigated on a much larger scale on farm. If successful, such a tool may lend itself well to use as part of commercial breeding programmes, assuming a link exists between leg bone curvature and gait. The avian hindlimb is made of three main bones – the femur, the tibiotarsus and the tarsometatarsus. As the tarsometatarsus was not studied in chapters 1 and 2, it was not considered for measurement using this tool. The femur is surrounded by the musculature of the leg and so measurement of the curvature of this bone using an external tool is impossible. Since a large portion of the tibiotarsus is not covered by muscle, this bone was chosen as the most feasible bone on which to measure curvature.

As this is a pilot study using a novel technique, a large amount of trial and error was involved. Therefore, the methods of data collection did not follow a rigid methodology across ages but rather the methodology evolved as the limitations of the tool and the technique became apparent. The main aim of this study was to develop the use of the tool as a means of data collection and assess whether it could be used reliably, rather than to record the relationship between gait and hindlimb morphology, which was a secondary aim.

Methods and Results

Tool development

It was decided that the most accurate and practical method to measure curvature in the live bird was to measure the length perpendicular (in the lateral direction) and parallel to the direction of the tibiotarsus and the use Pythagoras theorem to calculate the angles of curvature. As straight lines were to be measured, digital calipers were used. Two calipers (Duratool D00377 carbon fibre digital caliper) were purchased and cut to size. Industrial adhesive tape and nuts and bolts were used to fix one of the calipers at a perpendicular angle to the other. This allowed the tool to measure the length of the bone as well as the degree to which the bone deviated from ‘straightness’ at a given point, which enabled the curvature of the bone to be calculated. The point at which lateral displacement of the bone would be measured (by the perpendicular caliper) was 33mm from the distal reference point at the distal end of the tibiotarsus. This point was chosen as this was the point of greatest lateral displacement in the tibiotarsi measured in Chapter 1. The tool was designed to measure the lateral curvature of the left tibiotarsus. To do this, a bird would have to be held in supine position (on its back) with its legs straight (Fig. 1). As the bone is not fully visible at any age due to leg muscle mass and feathering, a bony landmark at the proximal tibiotarsus had to be palpated and used as a reference point. An initial pilot study was carried out using three adult layer chickens at Easter Bush to ascertain which bony landmarks were suitable to use. The easiest and most reliable point to palpate was the tibiotarsal tuberosity (cnemial crest) on the cranial side of the bone. It was not necessary to palpate for a landmark on the distal end as there was no overlying muscle present at this point, and the *depressio epicondylaris lateralis* (DEP) was easily identified as the distal reference point.

Fig. 1. The curvature of the tibiotarsus is measured while the bird is laid in supine position with its legs held and fixed points.

The tool (Fig. 2) was designed based on the idea that, rather than using a protractor-based design to directly measure curvature, it would be more accurate and repeatable to measure both the tibiotarsal length, and how much a certain point along the shaft (33mm from the distal end) deviated from a straight line and then to use Pythagoras' theorem to work out the angle of curvature. With this in mind, a tool was designed which combined two digital callipers – one to record bone length and the other to measure how much the shaft deviated from a straight line at a certain point. Some thought was given to adding a protractor to measure bone torsion but this was found to be unfeasible given the difficulty in moving the foot through a repeatable plane of motion accounting for rotation at both the stifle and hock joints.

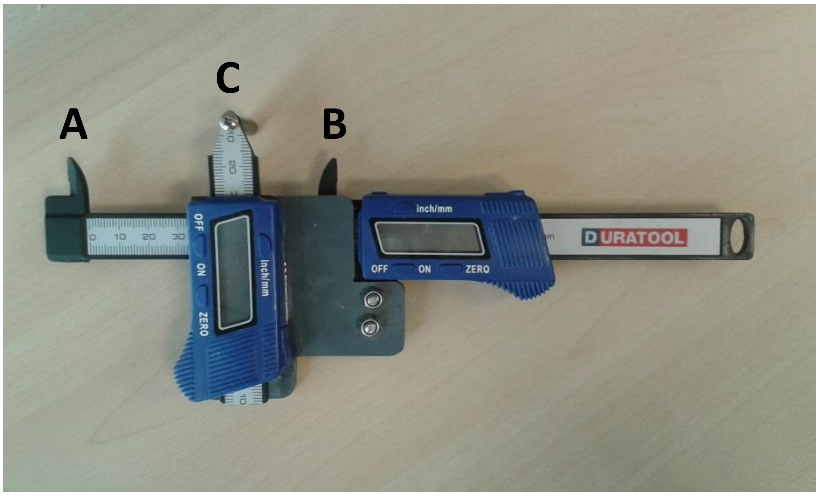

Fig. 2. The bone curvature tool, consisting of two digital callipers, fixed perpendicular to each other. Tibiotarsal length (a proxy, see text for details) is measured as the distance between points A and B. The degree of lateral bending of the tibiotarsal shaft is measured at a fixed point (33mm from B) as the distance between C and a line joining A and B. These distances are then used to estimate the overall bone curvature (using Pythagoras' theorem).

The tool was tested on adult layers at the Roslin Institute, University of Edinburgh. At this point it was realised that the way in which the tool was held while making the measurement would affect the result. For instance, slightly tilting the tool would mean that while the length measurement remained the same, the deviation of the shaft from a straight line would be more or less, depending on which way the tool was tilted. It was decided that the tool would be held as flat as possible (parallel to the table surface on which the bird is held) when making measurements.

The 'length' calliper was used to measure the distance between the tibial tuberosity and the DEP and this distance was used as a proxy for overall tibiotarsal length. As the length calliper was held at these landmarks, the 'curve' calliper was extended until further extension was halted by the bone. Some firmness was applied to the 'curve' calliper to ensure its extension had been stopped by bone and not skin.

These two measurements, in theory, could be used to accurately estimate bone curvature.

Data collection

The tool was used to measure curvature in the left tibiotarsus of male line Pekin ducks (male line) at three ages on farm at Cherry Valley's Highfield trial site. Tibiotarsal curvature, weight and gait were recorded at three ages: 21 days, 28 days and 42 days. These ages where chosen to represent the standard life span of the production bird. The birds were held in place using a duck holding device (previously developed at Cherry Valley). The device requires approximately eight seconds to get the bird restrained and once the bird is in place it does not need to be held. The device consists of a flat board, on one end of which a metal crook is attached to hold the neck and on the other end a bar is attached under which the legs are held. The bird is restrained in supine position (on its back). This leg bar obstructed the tool so it was discarded and replaced with a member of staff who held both legs (Fig. 1). A putty mould (which took the shape of the left leg and the abdomen side) was instead used to ensure the body and legs were held in the same position for each measurement.

A total of 172 birds were measured at 21 days, 185 birds at 28 days and 158 birds at 42 days of age. Numbers varied due to staff availability and ease of measurement at each age. At the end of each batch of measurements, a number of birds from the batch were measured again to assess the repeatability of the tool. Gait was also scored again to estimate the repeatability of the gait score. At 21 days the tibial tuberosity was difficult to locate by palpation which made it difficult to accurately measure the tibial length. Also, the putty, which worked well for most birds, was not suitable for the largest birds which did not fit into the mould formed by its predecessors. This mould also became dry over time and lost shape. Therefore it was decided that the mould would not be used in future and instead the legs would be held extended, with the tarsometatarsus positioned at marked points on the duck holding board. At 28 days, with the legs held extended, it was easier to locate to tibial tuberosity; it felt as it had become bigger but this may have been an effect of extension of the legs which would reduce the muscle belly around the bone. However, at this age the abdomen had also become bigger and was obstructing the tool, forcing the tool to tilt during measurement, which would lead to inaccuracies measuring the lateral displacement of the bone shaft. Therefore it was decided to use wooden blocks at the end of the duck-holding device for the next measurement (42 days) in order to raise the legs up so that the abdomen would not obstruct the tool. The repeatability of bone curvature measured by the tool at 28 days using Pearson's correlation coefficient was poor (r^2=0.41, p≤0.001, n=22). At 42 days the blocks worked well to reduce the obstruction caused by the abdomen. However, at this age, the musculature around the proximal tibiotarsus had grown to a point where the tibial tuberosity was very difficult to palpate. This led to inaccuracies in measuring the tibial length. The repeatability of bone curvature measured by the tool at 42 days using Pearson's correlation coefficient was poor (r^2=0.38, p≤0.0001, n=32).

From these trials it was decided that the most reliable measurements would be made at approximately 28 days, with the legs extended and raised on blocks.

At a later date (approximately nine months after the initial trial above), when male line birds were available, another repeatability test was carried out (this time using 26 day old birds rather than 28 day old birds due to staff availability). The raised wooden blocks were used, and the abdomen only noticeably obstructed the tool for one bird. However, repeatability using Pearson's correlation coefficient was very poor (r^2=0.046, p=0.257, n=30). It was expected that the repeatability at 26 days would be close to the repeatability previously estimates at 28 days, or even better due to the addition of the wooden blocks to limit obstruction from the abdomen. This was not the case, possibly because far fewer birds were measured at 26 days so the tool user was out of practice. The testing of the curvature measurement tool was discontinued as it was no longer of interest to the breeding company.

Discussion

Tibiotarsal curvature is difficult to measure in ducks using a caliper-style tool, partly because the proximal tibiotarsus is obscured by the muscle mass of the leg; only the distal half of the bone is ‘visible’. These results suggest that a hand-held tool will always be subject to tilting by the user and imprecision in the location of the proximal landmark, leading to inaccuracy and reducing the reliability of the tool. As the tool measurements were unreliable, no comparison was made between bone curvature and gait scores for these birds. The tibial tuberosity is also sometimes difficult to find by palpation and this time-consuming step limits the tool’s potential to integrate into current selection procedures. However, it is likely that this skeletal curvature (which seems to be unique to ducks) plays some role in the birds’ gait; therefore it is important to understand the adaptive origin of this bone curvature and its role in gait. Skeletal morphology has been suggested to affect gait in various studies (Kestin et al. 1992, Bradshaw et al. 2002, Garner et al. 2002, Knowles et al. 2008, Kapell et al. 2012, Kapell et al. 2017). The system described above, in its current state, is not suitable for the accurate measurement of tibiotarsal curvature in the Pekin duck. However, a similar system may be successful if the tool was fixed on a pivot which could swing into position to make a measurement. This would eliminate the issue of tool-tilting, which likely plays a major role in reducing the repeatability of the results. An alternate, if more expensive, option is to take an X-ray image of the leg of each bird and use automated software to measure the angle of curvature. This could form part of a general leg health X-ray assessment which may also include bone density and TD measurements. However, the angle at which the bone is positioned for X-ray scanning will affect the curvature angle measured. The advantage of X-ray (and X-ray-based imaging methods such as computed tomography is that measurements can be made from the very centre point of the bone, rather than from the outer surface of the bone as occurs with the tool developed in this pilot-study. By measuring from the bone centre, the true functional curvature of the bone can be recorded. Computed tomography (CT, described in Chapter 1) remains the most reliable, if most labour-intensive and expensive, method for measuring bone curvature in intact birds. However, in commercial setting, labour and cost may not be an issue, as CT is now routinely used as part of commercial breeding programmes to measure yield. Since CT scans already exist for each selection candidate as part of this yield-measurement process, the issues of labour and cost become negligible. In theory, it should be possible to develop automated software to identify the tibiotarsus in each scan (birds are always scanned in the same confirmation to ensure uniformity of measurements). The wide variety of potential traits which CT can measure suggests that this technology will play a growing role in phenotype measurement within commercial breeding programmes, possibly leading to a shift away from the use of hand-held tools to measure phenotypes.

There were many limitations to this study. As it was initially conceived as a simple pilot study, the work was carried out on an *ad hoc* basis. Staff availability to help with data collection was very limited, which meant that data collection dates were chosen based on staff availability, rather than on development ages of the birds, and on any given day, the number of birds that could be recorded was limited. The availability of staff was limited partly by the low priority of this exploratory study within the company, and partly by biosecurity restrictions at the study site; staff taking part in this study were restricted from visiting other farms within the breeding company for a number of days afterwards due to routine biosecurity protocols. Staff availability also limited the number of birds that could be re-tested to calculate repeatability of the tool. If staff were available for more hours on testing days, more birds could be measured. Given the low priority of the work, limited staff availability and the limited success of initial trails, the study was ended relatively early in the tool development process. A greater investment of time, staff and equipment may have improved the success of this tool development.

Conclusions

A hand-held tool such as the device tested in this chapter is unsuitable to accurate measurement of bone curvature in live birds. Further progress in measuring this trait is more likely to be made from imaging software, such as that associated with CT scanning.

References

Bradshaw, R. H., R. D. Kirkden, and D. M. Broom. 2002. A review of the aetiology and pathology of leg weakness in broilers in relation to welfare. Avian and Poultry Biology Reviews **13**:45-103.

Garner, J. P., C. Falcone, P. Wakenell, M. Martin, and J. A. Mench. 2002. Reliability and validity of a modified gait scoring system and its use in assessing tibial dyschondroplasia in broilers. British Poultry Science **43**:355-363.

Kapell, D. N. R. G., W. G. Hill, A. M. Neeteson, J. McAdam, A. N. M. Koerhuis, and S. Avendano. 2012. Twenty-five years of selection for improved leg health in purebred broiler lines and underlying genetic parameters. Poultry Science **91**:3032-3043.

Kapell, D. N. R. G., P. M. Hocking, P. K. Glover, V. D. Kremer, and S. Avendano. 2017. Genetic basis of leg health and its relationship with body weight in purebred turkey lines. Poultry Science **96**:1553-1562.

Kestin, S., T. Knowles, A. Tinch, and N. Gregory. 1992. Prevalence of leg weakness in broiler chickens and its relationship with genotype. Veterinary Record **131**:190-194.

Knowles, T. G., S. C. Kestin, S. M. Haslam, S. N. Brown, L. E. Green, A. Butterworth, S. J. Pope, D. Pfeiffer, and C. J. Nicol. 2008. Leg disorders in broiler chickens: prevalence, risk factors and prevention. Plos One **3**:e1545.

Chapter Four

Higher heritabilities for gait components compared with overall gait scores can improve mobility in ducks

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Author contributions:

Brendan Duggan contributed to all aspects of this work. Paul Hocking, Dylan Clements and Anne Rae provided supervision support.

Email addresses:

BMD: Brendan.Duggan@roslin.ed.ac.uk

AMR: Anne.Rae@cherryvalley.co.uk

DNC: Dylan.Clements@ed.ac.uk

PMH: Paul.Hocking@roslin.ed.ac.uk

Abstract

Genetic progress in selection for higher body mass and meat yield in poultry has been associated with an increase in gait problems which are detrimental to productivity and welfare. The incidence of suboptimal gait in breeding flocks is controlled through the use of a visual gait score, a subjective assessment of walking ability in each bird. The subjective nature of the visual gait score has led to concerns over its effectiveness in reducing the incidence of suboptimal gait in poultry through breeding. The aims of this study were to assess the reliability of the current visual gait scoring system in ducks and to develop a more objective method of selecting for better gait. A more objective system for recording gait would give breeding companies the opportunity to more accurately assess the gait of each individual and calculate accurate breeding values for walking ability in each individual bird.

Experienced gait scorers assessed short video clips of walking ducks to estimate the reliability of the current visual gait scoring system. In order to develop a more objective scoring system, gait components were visually scored on 5000 pedigreed Pekin ducks and genetic parameters were estimated for these components. It was expected that measurements of gait components would be more objective than those of the overall visual gait score.

Heritability estimates of the more objective gait components were as good as, or better than, those of the standard visual gait score. The recording of gait components can potentially be automated, which may increase accuracy further. Genetic correlations were generally low and suggest that it is possible to use gait components to select for an overall improvement in both economic traits and gait as part of a balanced breeding programme.

Introduction

Increases in growth rate and breast muscle mass which have been achieved through selective breeding of poultry have been associated with welfare problems, notably an increased incidence of poor gait (which includes ‘leg weakness’) (Farm Animal Welfare Council 1992, Bradshaw et al. 2002, Knowles et al. 2008, Jones and Dawkins 2010, Paxton et al. 2013). Birds with leg weakness may suffer pain and have difficulty reaching food and water (McGeown et al. 1999, Danbury et al. 2000, Bradshaw et al. 2002, Caplen et al. 2013) leading to economic losses for the producer and possible starvation for the animals. Gait problems were first reported in turkeys and broiler chickens (Nestor 1984, Kestin et al. 1992) although early studies focussed mainly on the emergence of skeletal leg defects rather than gait itself (Mercer and Hill 1984, Nestor 1984). Poor gait has since been observed in other heavy meat-producing birds (Abourachid 1991, Martrenchar 1999, Jones and Dawkins 2010, Da Costa et al. 2014, Makagon et al. 2015). Although poor gait has not been reported to as great an extent in Pekin ducks, there is concern that gait problems may manifest in the future if selection for production traits continues along its current trajectory, mirroring their emergence in other poultry species. It is important to consider that while gait problems may be associated with pain, sub-optimal gait may also be simply a functional consequence of an altered morphology in lines which have been heavily selected for increased muscle mass (Corr et al. 2003, Duggan et al. 2015).

Traditionally, in chickens and ducks, gait is assessed and selected against using a visual gait score (Kestin et al. 1992, Kestin et al. 2001), an ordinal score given to each bird based on a visual assessment of how that individual walks. Although efforts have been made to refine the visual gait score (Garner et al. 2002), it remains a subjective measure of walking ability and thus is prone to error. Previous studies have found relatively moderate kappa coefficients between 0.6 and 0.8 in ducks and chickens (Webster et al. 2008, Makagon et al. 2015). This may suffice for flock-level welfare assessments but is below the accuracy required for selection. A recent EU report on the welfare of broiler chickens acknowledges the subjective nature of the gait scoring system and highlights the need to develop a more objective system of assessing gait (Anon 2000).

Gait is a complex trait requiring the integration of sensory input, balance, conformation and fine motor control and heritability estimates for poultry gait tend to be low (Whitehead et al. 2003, EFSA Panel on Animal Health and Welfare 2010, Kapell et al. 2016). Similarly low heritability estimates have been published for visual gait scores in other species (Chapinal et al. 2012). Additionally, as the visual assessment of gait is a subjective measure (Garner et al. 2002) heritability estimates may be low, limiting the potential genetic progress when selecting for such a trait. Attempts have been made to circumvent this problem of low heritability estimates by focusing selection on objectively measured traits such as tibial dyschondroplasia or bone deformity (Kapell et al. 2012), although it remains unclear how these phenotypes affect the overall walking ability of birds. However, some gait components, such as step width will certainly affect the overall walking ability of an animal and have not as yet been genetically evaluated.

The aim of this study was to estimate the reliability, heritability and genetic parameters of the visual gait score which is currently used in Pekin ducks and to compare this to heritability estimates for particular components of gait that are scored visually. It was hypothesised that heritability estimates for these components of gait would be more heritable than the overall gait score. This was previously found to be the case in dairy cattle (Chapinal et al. 2012). Components were chosen for ease of measurement as well as for their hypothesised influence on overall gait. This study focusses on two gait components; step width, which influences balance during the stride, and body roll, which is a proxy for centre of mass movement during walking. There may be other components of gait which are more central to the overall movement of the bird; these components were chosen due to their ease of measurement. The components were also chosen on the basis of our previous findings that poultry lines selected for breast muscle mass ambulate with a wider step width and at a slower velocity (which is likely to increase body roll for a given step width) (Duggan et al. 2016). The purpose of this study was to ascertain the suitability of selecting for gait components, rather than to identify which components in particular should become the focus of future selection programmes. The benefit of assessing the suitability of gait components is that these components lend themselves more readily to automated (and thus objective) measurement than the current (subjective) overall gait assessment. The development of automated and objective measurement of gait components can potentially increase the accuracy of our assessments of gait and facilitate an improvement in the walking ability of poultry populations through selective breeding. Such automated systems (for example pressure platforms linked to analysis software) have been developed for gait analysis in humans and other species.

Methods

Assessment of gait score

In order to assess the reliability of the standard visual gait score in ducks, seven-week-old Pekin ducks were gait scored by four industry gait scorers. Scorers were shown three video sequences of 36 birds walking over a runway. The video camera (Microsoft LifeCam Studio, recording at 30 frames per second) was placed behind each bird at a height of 15 cm. The video sequence contained 144 walks - four walks (including one duplicate) from each bird. Each walk lasted approximately 3 seconds in order to replicate the high throughput of birds during assessments on breeding farms. Scorers were asked to rate each walk with a score of 1 (very poor gait) to 5 (perfect gait). None of the scorers were informed that the sequences contained duplicate recordings or multiple walks from the same birds. Agreement between and within scorers was assessed using Kendall's coefficient of concordance using Minitab software (Minitab version 17, Minitab Inc.).

Measurement of gait components

Over the course of eight weeks, on one day per week, a total of over 5000 Pekin ducks were visually scored for gait. On average, 650 birds were visually scored in each week. Two breeding lines (A and B) of Pekin duck were used, alternating each week. In total, data was collected from four hatches of each line (a different hatch was measured each week). These breeding lines are grandparent stock of the standard Cherry Valley commercial hybrid duck. Line A forms part of the maternal grandparent stock and Line B forms part of the paternal grandparent stock. All birds were hatched in the same hatchery and raised according to the Cherry Valley published guidelines. Water and feed (standard industry rations) were provided *ad lib*. The photoperiod was 23 hours light on day one, reducing by one hour per day until day six when the photoperiod was 18 hours of light and this was maintained to the end of the trial. Gait scoring took place as part of standard phenotypic measurement procedures which form the normal selection procedures of the breeding company. All phenotypic measurements took place at a single measurement station on the same breeding farm. After corralling birds at six weeks of age into a small area adjacent to the measurement station, each bird was weighed and its (ultrasonic) breast muscle depth was recorded. The birds were subsequently placed on a custom-built walkway (1.2 m wide and 4.8 m long) and allowed to walk away at their own pace, during which time each bird's overall gait and gait components were scored (during normal selection procedures, birds are gait scored while walking over loose straw bedding). The walkway consisted of a wooden base (6mm thick plywood) which was covered by a sheet of 7mm green artificial turf in order to provide grip and to create a contrast so as to make the birds' feet easier to see. Perspex sheeting (30 cm high) was fixed to the sides of the walkway to ensure the birds walked straight to the end of the walkway. Gait was assessed using a visual gait score (which forms part of the company's routine phenotypic measurement). Gait scores for Lines A and B were recorded by two different members of staff (each line was scored by only one individual), both of whom were experienced at scoring gait.

The visual gait score used by the breeding company spans a 1 to 5 scale, with 1 representing a bird which is markedly lame and 5 representing perfect gait. The score for a bird was downgraded if, when walking, that individual displayed bowed or splayed legs, medially or laterally rotated feet, or if the angle of the back to the floor was outside the 35-65 degree range. Birds which were lame, immobile or walked on their hocks were given a score of 1. Most ducks were assigned scores between 2 and 4 (in this trial, 1% were given a gait score of 1; 29% a score of 2; 61% a score of 3 and 8% a score of 4).

An experienced farm staff member scored the overall visual gait score for each line. In addition to the overall visual gait score, two gait components were recorded simultaneously (i.e.) step width and body roll. Different members of staff recorded the visual scores on each line, whereas the same person scored components of gait for both lines. Step width was scored visually as the estimated distance (perpendicular to the direction of travel) between the most posterior parts of the feet on a 1 to 3 scale, a score of 1 denoting the feet as being very close together (or overlapping) and a score of 3 denoting that the feet were widely spaced during walking. Body roll (also on a 1 to 3 scale) was recorded as the degree of rolling of the shoulders during walking. This was considered an approximation of centre of mass movement, since the position of centre of mass was impossible to ascertain visually. A score of 1 represented very little rolling of the shoulders whereas a score of 3 was given to birds which rolled their shoulders to a large degree while walking. In addition to standard phenotypic measures of breast depth and body mass, feed conversion ratios (FCRs) for each bird were calculated by automated measurement of each bird's individual feed intake and body mass. Data collected at the phenotypic measurement station was collated with information of the FCR of each bird. The pedigree of all birds was known, stretching back 15 generations. Phenotypic information was not available for any of the previous generations.

Genetic analysis

Variance components resulting from univariate and bivariate mixed models of restricted maximum likelihoods were used to estimate heritability of the visual gait score and the gait component scores as well as to calculate the genetic correlations between traits using ASReml (ASReml-W, version 3, VSN International Ltd.). A total of six traits were analysed using the following model which included fixed effects of sex and hatch and random effects of animal, pen and the permanent environment effect of the dam. The model terms were:

$$\mathbf{y = Xb + Za + Vp + Wd + e},$$

where **y** is the vector of trait measurements, **b** is a vector of the fixed effects accounting for the interaction between the hatch and the sex of each bird, **a** the vector of additive genetic effects, **p** is a vector of the pen effects, **d** the vector of permanent environmental effects of the dam and **e** is the vector of residuals. **X**, **Z, V** and **W** are incidence matrices which relate the vectors **b**, **a**, **p** and **d** with **y**. The variance/covariance structure was assumed to be:

$$V\begin{bmatrix}\mathbf{a}\\\mathbf{p}\\\mathbf{d}\\\mathbf{e}\end{bmatrix}\begin{bmatrix}\mathbf{A}\otimes\mathbf{G} & \mathbf{0} & \mathbf{0} & \mathbf{0}\\\mathbf{0} & \mathbf{I}\otimes\mathbf{P} & \mathbf{0} & \mathbf{0}\\\mathbf{0} & \mathbf{0} & \mathbf{I}\otimes\mathbf{C} & \mathbf{0}\\\mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}\otimes\mathbf{R}\end{bmatrix},$$

where **A** and **I** are the additive genetic relationship matrix and identity matrix, respectively. **G**, **P**, **C** and **R** represent the variance - covariance matrices of additive genetic effects, pen effects, permanent environmental effects of the dam and residual effects, respectively. Breast muscle depth and body mass were not included as covariates in order to assess how gait traits correlate to these important economic traits. Breast depth was measured by ultrasound and while this can be used as a proxy for overall breast muscle volume, this is not a measure of the overall breast size (as it is measured only in a plane perpendicular to the breast width). Breast muscle depth may be a partial covariate, and we would expect the breast width to grow in all planes, but not necessarily proportionally. Both breast size and body mass are important economic traits which are selected for in their own right._The pedigree and data structures are summarised in Tables 1 and 2 respectively.

Line	Individuals in pedigree	Generations in pedigree	Sires	Sires of sires	Dams of sires	Dams	Sires of dams	Dams of sires
A	120,031	15	1078	364	577	4039	663	1418
B	81,765	15	1078	377	535	3622	699	1349

Table 1. Pedigree structure for Lines A and B.

Line	Phenotyped Males	Phenotyped Females	Gait score	Step width	Body roll	Finish weight (g)	Breast depth (mm)	FCR
A	1375	1254	2.80 (0.66) {23.6} [0]	2.13 (0.46) {21.6} [229]	2.08 (0.53) {25.5} [230]	3760 (290) {7.7} [0]	152 (15.3) {10.1} [0]	1.90 (0.17) {8.9} [887]
B	1342	1280	2.70 (0.56) {20.7} [1]	2.10 (0.43) {20.5} [269]	2.06 (0.47) {22.8} [271]	3362 (297) {8.8} [0]	146 (16.7) {11.4} [0]	2.02 (0.23) {11.4} [69]

Table 2. Means (and standard deviations) and {coefficients of variation} for all traits measured in Lines A and B. Phenotypes were recorded at six weeks of age. The number of missing values for each trait are presented in square parentheses.

Results

Kendall's coefficient of concordance, calculated between four experienced observers scoring gait in short video clips, was 0.49 (df = 132, $p<0.001$). The Kendall's coefficient of concordance within observers (scoring duplicate videos) was 0.75 (df = 135, $p<0.001$). No clear observer drift effect was detected – scorers deviated to a similar degree when scoring the first 60 walks compared to the last 60.

Heritability estimates with genetic and phenotypic correlations for Lines A and B are displayed in Tables 3 and 4 respectively. The heritability estimates of the standard gait score were low and standard errors in the female line were high. Body roll had similar heritability estimates to gait score whereas step width was estimated to have higher heritabilities than both gait score and body roll. Economic traits (finish weight, breast depth and FCR) had moderate heritabilities, with the exception of breast depth in Line A.

Phenotypic correlations between traits varied between lines. Generally, gait traits had very low phenotypic correlations with economic traits and correlations between economic traits were also low, with the exception of finish weight and breast depth (Tables 3 and 4). As this study used relatively low sample sizes, estimates for genetic correlations between traits were associated with relatively high standard errors. Most genetic correlations between gait traits and economic traits were not significant ($p>0.05$), with the exception of line B, where significant genetic correlations were observed between step width and breast depth ($t=2.16$, $p<0.05$), and between gait score and FCR ($t=3.26$, $p<0.01$). The standard gait score had moderate to good genetic correlations with other gait traits (-0.51 to -0.69; 0.56 to 0.57). The significant r_G between economic traits were moderate (0.23 to 0.61).

Trait	Gait score	Step width	Body roll	Finish weight	Breast depth	FCR
Gait score	**0.061** **(0.055)**	-0.346 (0.202)	-0.690 (0.146)	-0.703 (0.373)	-0.374 (0.319)	0.095 (0.303)
Step width	*-0.162* *(0.034)*	**0.238** **(0.074)**	0.561 (0.227)	0.217 (0.167)	0.066 (0.165)	-0.111 (0.181)
Body roll	*-0.337* *(0.025)*	*0.282* *(0.029)*	**0.079** **(0.034)**	0.160 (0.215)	-0.033 (0.222)	-0.379 (0.218)
Finish weight	*-0.039* *(0.030)*	*0.069* *(0.034)*	*0.020* *(0.029)*	**0.274** **(0.091)**	0.452 (0.145)	0.609 (0.135)
Breast depth	*0.056* *(0.040)*	*0.092* *(0.028)*	*0.065* *(0.037)*	*0.439* *(0.028)*	**0.151** **(0.074)**	0.205 (0.172)
FCR	*0.1226* *(0.037)*	*0.007* *(0.036)*	*-0.037* *(0.032)*	*0.067* *(0.036)*	*0.079* *(0.031)*	**0.272** **(0.096)**

Table 3. Heritability estimates (in bold) of gait and other major economic traits for Line A, along with their genetic correlations (above diagonal) and phenotypic correlations (in italics, below diagonal). Standard errors for all estimates are in parentheses.

Trait	Gait score	Step width	Body roll	Finish weight	Breast depth	FCR
Gait score	**0.115 (0.058)**	0.138 (0.199)	-0.506 (0.170)	0.126 (0.176)	-0.022 (0.186)	0.442 (0.136)
Step width	*-0.016 (0.028)*	**0.166 (0.058)**	0.571 (0.155)	0.029 (0.150)	-0.326 (0.151)	-0.156 (0.160)
Body roll	*-0.156 (0.028)*	*0.314 (0.023)*	**0.112 (0.047)**	-0.164 (0.163)	0.059 (0.173)	-0.136 (0.175)
Finish weight	*0.186 (0.025)*	*0.048 (0.027)*	*0.010 (0.025)*	**0.401 (0.090)**	0.230 (0.112)	0.303 (0.127)
Breast depth	*0.074 (0.024)*	*-0.034 (0.025)*	*0.046 (0.024)*	*0.390 (0.024)*	**0.295 (0.046)**	0.077 (0.140)
FCR	*0.071 (0.026)*	*-0.016 (0.027)*	*0.004 (0.026)*	*-0.079 (0.028)*	*-0.074 (0.025)*	**0.294 (0.048)**

Table 4. Heritability estimates (in bold) of gait and other major economic traits for Line B, along with their genetic correlations (above diagonal) and phenotypic correlations (in italics, below diagonal). Standard errors for all estimates are in parentheses.

Discussion

Gait problems are a major animal welfare issue facing modern poultry in intensive production systems. The present results suggest that a more targeted approach to assessing gait by focussing on gait components has the potential to improve progress in selecting for better gait in breeding birds.

The pilot study using limited data suggests that the current visual gait scoring system, while showing some level of agreement between scorers, is not adequately reliable for long-term use in breeding programmes, but can be improved. The Kendall's coefficient of concordance suggests that low concordance exists between scorers. A coefficient of concordance of at least 0.7 is desirable (Martin and Bateson 2007) and it could be argued that within a breeding programme, the acceptable level of concordance should be higher than this. Indeed, when scoring video clips of the same walks (using the standard visual gait score described above), all four scorers agreed 28% of the time and three of the four scorers agreed 74% of the time. Individual scorers failed to allocate the same score to two duplicate walks 26% of the time. Some of these inconsistencies may be due to the short duration of each video recording. Short recordings were chosen so as to replicate conditions during assessments on farm; however for certain birds on farm, the scorer will observe a walk for longer than three seconds before allocating a score for that bird. The viewing angle of the camera, which was chosen to give a clearer view of the birds' gait, is also different from the viewpoint used when scoring during selection on farm, which is from a standing position.

The suboptimal reliability of the visual gait score recorded using these video clips suggest that an alternate and more rigorous method of gait assessment is required to make progress on selecting for optimal gait as weight increases. Previous work on gait in cows suggests that assessing components of gait may yield better heritability estimates (Chapinal et al. 2012). Certain gait components such as step width and the ratio of double to single support time are known to have changed to a similar extent in both ducks and chickens which have undergone selection for increased growth weight and meat yield (Duggan et al. 2016) and selection decisions based on these components may yield greater progress than the current subjective gait scoring system.

This study estimated genetic parameters for components of gait and compared these to those of the overall visual gait score. The data gathered on economic traits had coefficients of variation (CVs) in the range expected for economic traits (Table 2). Data for gait traits had CVs in a similar range to objectively measured gait data in Pekin lines in Chapter 2. The heritability of step width was greater than that of the original gait score in both lines and standard errors were approximately the same for both estimates. This is to be expected; the gait score is a subjective measurement based on a visual assessment of overall body movements, without any tangible reference points. Although many gait scoring systems refer to reference points in their categorisation of each score (such as a splay of the legs or the angle of the back to the floor), there are often so many parameters and reference points within the overall gait score that it can be difficult to refer to these while scoring birds in a short space of time. Step width on the other hand is a simpler score based on only one aspect of foot placement and therefore one would expect this score to be more objective. Additionally, the recorder measuring step width can make use of reference points on the ground to compare successive birds. The heritability of body roll was similar to that of the gait score, probably because unlike step width, the assessment of body roll is a more subjective assessment. However, it is likely that body roll and step width are not fully independent traits. A bird with greater step width is expected to exhibit more body roll at a given speed when walking, given that once it lifts a foot during the stride phase, its centre of mass will be less aligned with its point of support (the remaining grounded foot) in birds with greater step width travelling at the same velocity. Therefore the body will begin to roll until such time as the swinging foot is grounded again. By increasing speed, the swinging foot can be grounded quicker, leaving less time for the body to roll. Momentum may also play a role in reducing body roll at higher speeds. Heritability estimates for other economic traits (finish weight, breast depth and FCR) were in the range expected, with some differences observed between lines. For example, the mean estimate for the heritability of body weight in this study (0.34) is in a similar range to heritabilities of 0.28 to 0.45 which have been estimated in other recent poultry studies (Mignon-Grasteau et al. 1998, Rekaya et al. 2013, Bailey et al. 2015, Kapell et al. 2016). Heritability estimates presented in this study were calculated from one phenotyped generation; it is expected that these heritabilities could be estimated with more accuracy if more generations had been phenotyped, as is the case within commercial breeding programmes. Some relatively large differences exist between lines in heritabilities for certain traits. For example, Line A (a female line) has lower heritabilities for finish weight and breast depth than Line B (a male line). Greater selection emphasis is likely to be placed on these traits in the male line than in the female line. But given that only one generation of data has been phenotyped in each line, sampling bias may have occurred, where the accuracy of the heritabilities may differ because the variation seen for a given trait in this small dataset is not representative of the variation in that line as a whole. A larger dataset may result in heritability estimates that are more closely aligned between lines.

Phenotypic correlations of the gait score with the production traits were generally low, which suggests that the gait score is indeed a measure of gait, rather than a proxy measure of body mass or breast depth. The gait score had a low to moderate r_P with the components of gait, whereas r_P between each component of gait was generally moderate. Due to the relatively low sample size, genetic correlations were generally associated with relatively large standard errors that were of a similar magnitude to the correlation estimates. These correlations will not be discussed here because, due to these high standard errors, the existence of a biologically genuine correlation is uncertain. The notable exception was the favourable genetic correlations between gait score, step width and body roll in both lines A and B. In Line B, breast depth (considered a proxy for pectoral muscle mass) is negatively correlated with step width; continued selection for greater breast depth may lead to a narrower step width. The effect of this narrower step width on balance will depend on the degree to which the body's centre of mass moves laterally during gait. It is important to remember that breast muscle depth is a proxy measure and may not be an accurate representation of overall breast size. In addition, we cannot say for certain whether selection for larger breast muscle mass would indeed decrease the step width. Our understanding of how various traits relate to each other to affect gait (or particular components of gait such as step width) is still very rudimentary. If we assume that a larger breast muscle size reduces stability in the walking bird due to a more erratic or expansive displacement of the centre of mass, then the position of the centre of pressure in relation to the centre of mass becomes more important in maintaining stability. A narrow step width may ensure that during the swing phase (when only one foot is grounded), the centre of mass is more closely aligned with the centre of pressure (ie - the grounded foot). Conversely a wide step width, which may seem more stable during standing, could increase the misalignment of the centre of pressure with the centre of mass during the swing phase of walking. However, these are theoretical assumptions and more work is needed to clarify the relationships between step width, breast muscle size and gait.

In Line B, a positive genetic correlation exists between FCR and gait score. Birds with a high FCR may be more likely to have a lower body mass and less breast muscle, which would increase the likelihood that these birds would have a higher gait score. Genetic correlations between gait components and production traits in this study were generally low and the data suggest that selection for improved gait may not be compromised by negative responses in economic traits. The basis of these genetic correlations may be linkage disequilibrium – genes which control one trait may be located close to genes controlling the correlated trait, so that these genes remain linked during recombination. Pleiotropy may also play a role, where the same genes may control multiple traits. An example may be a gene for increase body mass. This same gene is also likely to lead to a lower gait score, since a greater mass may reduce balance when walking.

Poor gait is acknowledged to have arisen as a consequence of selection for production traits such as growth rate and body mass and it is difficult to select for an improvement in both traits simultaneously. However, this emergence of gait problems may be due to the extreme emphasis historically placed on production traits within breeding programmes. Also, it is possible that inaccuracies in measuring a trait such as gait subjectively may have hindered progress in selecting towards improvements in both gait and production traits simultaneously. The use of gait components, which are potentially more accurate that the traditional overall gait score may lead to progress in selecting both gait and production traits simultaneously. Poor gait can be caused by leg health defects such as long bone deformities, foot pad dermatitis and crooked toes (Julian 1984, Bradshaw et al. 2002, Da Costa et al. 2014). In chickens, these components of gait have been shown to have low heritabilities and low to moderately unfavourable correlations with body mass, an important economic trait (Kapell et al. 2012). However, long term balanced selection has led to reductions in the incidences of those defect traits that negatively affect gait, suggesting that similar changes are also possible for populations selected for gait components other than those related to leg defects.

These results should be viewed in the context of the data available. Some correlations have likely been missed due to a lack of data (only one generation was phenotyped). Therefore, the significant correlations and also the heritability estimates reported here may be different if a larger data set had been available. Also, these data do not represent the true variation present in this population. Some birds were excluded from the dataset due to splayed legs, internally rotated feet or valgus/varus long bone deformities as these birds are eliminated as selection candidates during data screening. Inclusion of these defective birds in the dataset would likely affect the variation in step width, body roll and overall gait scores recorded and thus affect heritability and correlation estimates for this population. A limitation of this study is the relevance of these results when compared with the everyday gait of birds on standard commercial farms. As part of routine phenotypic data collection within the breeding programme, gait was scored immediately after each bird was weighed, leg health-checked and an ultrasound reading of breast depth had been recorded. Each bird was then placed on the farm bedding and walked away from the recording station at its own pace, during which time gait was visually measured, both as an overall gait score and as gait components. The disorienting effect of the previous trait measurements (birds are turned upside down during body weight and leg health measurements) should not be under-estimated. While this does not affect the validity of comparing the overall gait score to components in this instance, each bird may not exhibit exactly the same gait had it not undergone phenotypic measurement immediately prior to walking. For example, the gait component ‘body roll’ may be more exaggerated in this setting due to a temporary reduction in balance and orientation. To more accurately measure the true gait of a bird, either using the overall gait score or gait components, birds should not be subject to phenotypic measurement or excessive handling immediately prior to gait scoring. Another potential limitation is the choice of substrate used during scoring. The straw bedding on the farm was too irregular and uneven to give a fair representation of gait for each bird (as each may chose a slightly different path over the irregularities of the surface provided by the straw bedding). The artificial grass walkway was used to provide a level walking surface on which all birds could be assessed. Birds will walk differently on this novel surface depending on how familiar and comfortable they are with it. In order to habituate the birds to the artificial grass surface, the walkway was left in the home pen on the birds for two days; this was judged a sufficient amount of time for birds to habituate to the surface and they showed no sign of unease towards the artificial grass surface during data recording.

These data demonstrate that the visual assessment of gait components during selection is both feasible and yields promising heritability estimates. The use of gait components holds promise for future improvements in selection for improved gait in ducks; as they are simpler traits, the assessment of gait components can be automated, for example by using pressure sensing technology as in Duggan *et al* (2016). Automation of measurement has the potential to bring about greater objectivity and increase breeding success. It is important to note however, that although the gait components that are the focus of this paper can be measured satisfactorily and carry reasonable heritability estimates, it is not yet known which components should be selected to improve gait. For example, it could be argued that a wide step width would be beneficial to a bird with large lateral displacement of the centre of mass whereas a narrow step width would be beneficial to a bird with little lateral centre of mass movement. However, it is also difficult to differentiate cause and effect associations between step width and lateral body movement. A more thorough understanding of how gait components are integrated to effect overall locomotion is therefore necessary before recommendations can be made upon which particular gait components should be used in breeding programmes. It is likely that most improvement will be achieved using a selection index which combines weighted measurement of various gait components. Indeed, current overall gait scoring methods use a combination of components which are subconsciously weighted in different ways depending on the observers’ opinions of what optimal gait entails. By solely focussing on the measurement of gait components, this differential weighting among observers can be avoided.

Conclusions

Scoring overall gait visually is a subjective measure which generally generates low (but useable) heritabilities. We demonstrate that focussing on gait components, rather than overall gait, can generate heritability estimates which are equal to or better than the conventional visual gait score in ducks. The benefit of using components of gait is that their measurement can be automated to generate greater accuracy and easily combined to create an index score of overall gait. Genetic correlations, while difficult to ascertain, are generally low; therefore it is possible to use gait components to select for an overall improvement in both economic traits and gait as part of a balanced breeding programme.

References

Abourachid, A. 1991. Comparative gait analysis in two strains of turkey, *Meleagris gallopavo*. British Poultry Science **32**:271-277.

Anon. 2000. The welfare of chickens kept for meat production (broilers). European Commission, Brussels, Belgium.

Bailey, R. A., K. A. Watson, S. F. Bilgili, and S. Avendano. 2015. The genetic basis of pectoralis major myopathies in modern broiler chicken lines. Poultry Science **94**:2870-2879.

Bradshaw, R. H., R. D. Kirkden, and D. M. Broom. 2002. A review of the aetiology and pathology of leg weakness in broilers in relation to welfare. Avian and Poultry Biology Reviews **13**:45-103.

Caplen, G., G. R. Colborne, B. Hothersall, C. J. Nicol, A. E. Waterman-Pearson, C. A. Weeks, and J. C. Murrell. 2013. Lame broiler chickens respond to non-steroidal anti-inflammatory drugs with objective changes in gait function: A controlled clinical trial. The Veterinary Journal **196**:477-482.

Chapinal, N., A. Sewalem, and F. Miglior. 2012. Short communication: Estimation of genetic parameters for gait in Canadian Holstein cows. Journal of Dairy Science **95**:7372-7376.

Corr, S. A., M. J. Gentle, C. C. McCorquodale, and D. Bennett. 2003. The effect of morphology on walking ability in the modern broiler: A gait analysis study. Animal Welfare **12**:159-171.

Da Costa, M. J., J. L. Grimes, E. O. Oviedo-Rondon, I. Barasch, C. Evans, M. Dalmagro, and J. Nixon. 2014. Footpad dermatitis severity on turkey flocks and correlations with locomotion, litter conditions, and body weight at market age. Journal of Applied Poultry Research **23**:268-279.

Danbury, T. C., C. A. Weeks, A. E. Waterman-Pearson, S. C. Kestin, and J. P. Chambers. 2000. Self-selection of the analgesic drug carprofen by lame broiler chickens. Veterinary Record **146**:307-311.

Duggan, B. M., P. M. Hocking, and D. N. Clements. 2016. Gait in ducks (*Anas platyrhynchos*) and chickens (*Gallus gallus*): similarities in adaptation to high growth rate. Biology Open **5**:1077.

Duggan, B. M., P. M. Hocking, T. Schwarz, and D. N. Clements. 2015. Differences in hindlimb morphology of ducks and chickens: effects of domestication and selection. Genetics, Selection, Evolution : GSE **47**:88.

EFSA Panel on Animal Health and Welfare. 2010. Scientific opinion on the influence of genetic parameters on the welfare and the resistance to stress of commercial broilers. EFSA Journal **8**:1666.

Farm Animal Welfare Council. 1992. Report on the welfare of broiler chickens. Surbiton : FAWC, Surbiton.

Garner, J. P., C. Falcone, P. Wakenell, M. Martin, and J. A. Mench. 2002. Reliability and validity of a modified gait scoring system and its use in assessing tibial dyschondroplasia in broilers. British Poultry Science **43**:355-363.

Jones, T. A., and M. S. Dawkins. 2010. Environment and management factors affecting Pekin duck production and welfare on commercial farms in the UK. British Poultry Science **51**:12-21.

Julian, R. J. 1984. Valgus-varus deformity of the intertarsal joint in broiler chickens Canadian Veterinary Journal-Revue Veterinaire Canadienne **25**:254-258.

Kapell, D. N. R. G., W. G. Hill, A. M. Neeteson, J. McAdam, A. N. M. Koerhuis, and S. Avendano. 2012. Twenty-five years of selection for improved leg health in purebred broiler lines and underlying genetic parameters. Poultry Science **91**:3032-3043.

Kapell, D. N. R. G., P. M. Hocking, J. Ralph, V. Kremer, P. Valentin, P. Glover, A. N. M. Koerhuis, M. Swalander, A. M. Neeteson, and S. Avendano. 2016. Genetic basis of leg health and its relationship with body weight in purebred turkey lines. Poultry Science:In Press.

Kestin, S., T. Knowles, A. Tinch, and N. Gregory. 1992. Prevalence of leg weakness in broiler chickens and its relationship with genotype. Veterinary Record **131**:190-194.

Kestin, S. C., S. Gordon, G. Su, and P. Sorensen. 2001. Relationships in broiler chickens between lameness, liveweight, growth rate and age. Veterinary Record **148**:195-197.

Knowles, T. G., S. C. Kestin, S. M. Haslam, S. N. Brown, L. E. Green, A. Butterworth, S. J. Pope, D. Pfeiffer, and C. J. Nicol. 2008. Leg disorders in broiler chickens: prevalence, risk factors and prevention. Plos One **3**:e1545.

Makagon, M. M., R. Woolley, and D. M. Karcher. 2015. Assessing the waddle: An evaluation of a 3-point gait score system for ducks. Poultry Science **94**:1729-1734.

Martin, P., and P. Bateson. 2007. Measuring behaviour - An introductory guide. Cambridge University Press.

Martrenchar, A. 1999. Animal welfare and intensive production of turkey broilers. Worlds Poultry Science Journal **55**:143-152.

McGeown, D., T. C. Danbury, A. E. Waterman-Pearson, and S. C. Kestin. 1999. Effect of carprofen on lameness in broiler chickens. Veterinary Record **144**:668-671.

Mercer, J. T., and W. G. Hill. 1984. Estimation of genetic parameters for skeletal defects in broiler chickens. Heredity **53**:193-203.

Mignon-Grasteau, S., C. Beaumont, J.-P. Poivey, and H. de Rochambeau. 1998. Estimation of the genetic parameters of sexual dimorphism of body weight in 'label' chickens and Muscovy ducks. Genetics Selection Evolution **30**:1-11.

Nestor, K. E. 1984. Genetics of growth and reproduction in the turkey. 9. Long-term selection for increased 16-week body weight. Poultry Science **63**:2114-2122.

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

Rekaya, R., R. L. Sapp, T. Wing, and S. E. Aggrey. 2013. Genetic evaluation for growth, body composition, feed efficiency, and leg soundness. Poultry Science **92**:923-929.

Webster, A. B., B. D. Fairchild, T. S. Cummings, and P. A. Stayer. 2008. Validation of a Three-Point Gait-Scoring System for Field Assessment of Walking Ability of Commercial Broilers. The Journal of Applied Poultry Research **17**:529-539.

Whitehead, C. C., R. H. Fleming, R. J. Julian, and P. Sørensen. 2003. Skeletal problems associated with selection for increased production. Pages 29-52 *in* M. W. a. A. SE, editor. Poultry Genetics, breeding and Biotechnology. CABI, Oxon, UK.

General Discussion

Summary

The overall aims of this thesis were to understand how intense selection for faster growth rates and larger body masses has changed morphology and gait in poultry and whether a more objective alternative to the standard visual gait score could be developed as a means to improve poultry gait through selection. Gait problems have not yet been widely reported in ducks but a concern exists that sub-optimal gait may develop in the future if the Pekin duck continues on its current selection trajectory towards a larger body mass and faster growth rate. As gait problems have been widely reported in the broiler chicken, this thesis therefore considered the chicken as a model to compare with the duck, given that both species share similar selection practices in recent times. Choosing the most appropriate model species for use as comparisons can be difficult. Each line or species has benefits and disadvantages; the rational for using the chosen lines in this thesis is explored later in this Discussion.

The first chapter reported how the morphology of both chickens and ducks has changed over the course of intense selection for higher growth rates and body masses. In this study, the heavy broiler chicken was compared to its lighter conspecific, the layer chicken, and the heavy Pekin duck was compared to its lighter conspecific (and ancestral phenotype), the mallard. The ancestral phenotype of the broiler chicken is the red jungle-fowl. The layer chicken has obviously undergone intense selection when compared with the jungle-fowl; however, selection in layer lines has focused on health and reproductive traits, rather than growth rate or body mass and therefore, since the layer chicken was more easily available than the red jungle-fowl, it was deemed appropriate to be used here as a comparison. It should be noted however, that the layer chicken has undergone some selection for leg and bone health traits. All duck lines developed adult-sized legs at an earlier stage than the chicken lines. This finding agrees with previous studies on the ontogeny of legs and wings in mallards (Dial and Carrier 2012, Dial et al. 2012), demonstrating that this species has evolved to prioritise leg development in early life before functional capabilities of the wing are developed. This has advantages, especially in predator escape, which relies heavily on the legs (running and swimming) in ducks. Whereas size does not equate to skeletal maturity, as the duck legs did not grow after five weeks of age, the bones may have had more opportunity to remodel in order to tolerate the loads imposed on them. This may explain why the duck tibiotarsi were relatively stronger than those of chickens by seven weeks of age. Another interesting finding from this chapter was the lateral curvature observed in the tibiotarsi of all duck lines. This bone curvature complements findings on joint anatomy in another duck species, the ringed teal (Provini et al. 2013). This curvature would hypothetically move the feet to a more medial position during swimming and walking. When swimming, this adaptation would reduce diagonal motion across a body of water and thus most likely reduce the energy expenditure associated with swimming. Other work has reported that ducks will swim in formations that reduce energy expenditure (Prange and Schmidt-Nielsen 1970, Fish 1995), suggesting that a strong selection pressure exists in order for this behaviour to evolve. Therefore it is conceivable that such a selection pressure may also affect limb morphology, as seems to be the case here. However, a more medial placement of the feet during walking would increase the risk of falling over, if the ducks' centre of mass tended to sway laterally to a large degree during the gait cycle, as seems to be the case. This lateral sway would bring the centre of mass outside of the zone of stability provided by the medially positioned supporting foot.

Conversely, in the mallard, where such a lateral movement of the centre of mass is not as evident, the medially positioned supporting foot can increase stability, as is also suggested in another study on teal (Provini et al. 2012). Thus, is seems that this lateral curvature seen in ducks may create instability in birds who’s centre of mass ranges laterally during walking, such as the relatively heavy-breasted Pekin lines but may benefit birds with less dynamic lateral centre of mass movement.

The second chapter reported how gait has changed through selection in these same lines. A pressure walkway was used to measure various components of the birds’ gait. It was clear that although they belonged to two separate species with very different gaits, the heavy lines of both the chickens and ducks have adapted their gait in similar ways to deal with the increased loads they are required to carry. The hypothesised effect of more medial foot placements (mentioned above) was not observed when step width was measured in these walking birds. Indeed, the step width of the Pekin lines was similar to that of the broiler chicken. Gait is an extremely complex trait, composed of intersections between the morphology of various body parts, behaviour, sensory input, processing and physiology. This thesis examines only some of the many morphological aspects of gait; therefore it is not surprising that gait observed in Chapter 2 may not match the hypothesised predictions based on morphology measurements from Chapter 1. For example, leg muscle sizes and force-generating capabilities have changes greatly through selection in chickens (Paxton et al. 2010) (and likely in ducks) but muscle morphology/physiology has not been examined in detail in this thesis. Both the Pekin and broiler chicken move their centre of mass in different ways and the step width of the Pekin is not wide enough to cater for the range of its centre of mass movements. The lateral motion of the Pekins during the gait cycle, which is also seen in broiler chickens (Paxton et al. 2013), likely causes their centre of mass to stray outside the ‘zone of safety’ established by the positioning of the feet during stance phase. A greater understanding is required of how tibiotarsal curvature affects foot positioning and how this relates to the ‘zone of safety’ within which the centre of mass can move in Pekin ducks. If this is achieved then it may be possible to reduce the incidence of falls and birds ‘on their backs’ by selecting breeding birds based on leg curvature. Data from other studies on this topic is not available since this distal tibiotarsal curvature has not previously been reported.

The second chapter also reported a difference in foot angle observed between species. Chickens generally pointed their feet in the direction of travel during the stance phase of the gait cycle (with seven week broilers pointing their feet slightly outward) as has been seen elsewhere (Corr et al. 2003b). All duck lines, on the other hand, pointed their feet medially (inward) when walking. This may be linked to the bone curvature or torsion observed in duck lines in Chapter One. However, many factors not measured here, such as muscling, tendon or ligament position may play a role in foot angle during gait and therefore it would be speculative to suggest a cause for this foot angle with the data that is available. Provini *et al* also report a medially orientated foot position in teal and suggest it may be due to the position of the tarsus and metatarsus but do not offer a further explanation (Provini et al. 2012).

The third chapter investigated the feasibility of incorporating bone curvature measurements into the current phenotyping system of Pekin ducks on Cherry Valley selection farms and concluded that manual objective assessment of bone curvature using a hand-held tool would be difficult; alternate solutions (such as X-ray or CT) may prove more useful. While the tool could measure curvature in a dissected out bone, it was not useful for live birds, as the muscle around the bone made accurate measurements of bone curvature impossible. Previous studies on measuring long bone curvature in live poultry (or other species) were not found, perhaps due to the inherent difficulties involved in this type of measurement, as detailed within the chapter. Bone curvature is an important trait and previous work has shown that leg bone morphology can be related to gait (Bradshaw et al. 2002, Corr et al. 2003a, Provini et al. 2013, Robison et al. 2015).

The fourth chapter attempted to draw on the results of the preceding chapters on morphology and gait changes to identify components of gait which could be scored as an alternative to the standard visual gait score. The visual gait score currently used to assess gait in birds under selection in duck breeding systems is subjective and therefore prone to error. The need to develop an objective measure of poultry gait has been acknowledged by the scientific community (Anon 2000). An initial repeatability test (based on video clips which, however, do not exactly match the scoring conditions on breeding farms) demonstrated the lack of concordance which exists using the visual gait score. The aim of this study was to develop a more objective score by focussing on simpler, easier to measure gait components, rather than attempting to judge the overall walking ability of a bird. A study on dairy cattle has found that using gait components can yield higher heritabilities that using an overall visual gait score (Chapinal et al. 2012). The original gait score and the new gait components score were used to assess approximately 5000 pedigreed Pekin ducks as part of Cherry Valley’s selection procedures. The gait components had an equal or higher heritability to the visual gait score and the components did not show any unfavourable genetic correlations with important economic traits. This very positive development demonstrates the potential benefits of focussing on gait components rather than on an overall gait score and furthermore the recording of these components could be automated using the pressure walkway used in Chapter Two. These automated measures will likely yield more accurate heritability estimates as the error in recording them will be reduced. Selection can then be performed on those objectively measured components which are deemed most important, or the components can be weighted and combined to form an objective gait score. As our knowledge of how gait components interact is still rather limited (especially in ducks), a greater understanding of gait is required before appropriate gait components can be chosen on which to practice selection within breeding programmes. Many studies have addressed this issue (Abourachid 1991, Corr et al. 2003b, a, Usherwood et al. 2008, Paxton et al. 2010, Abourachid et al. 2011, Clark and Higham 2011, Caplen et al. 2012, Paxton et al. 2013, Robison et al. 2015) and further works such as these will help to develop a picture of how gait components interact to influence overall gait. The findings in Chapters One and Two of this thesis will add to that body of information on morphology and gait dynamics in poultry and how they interact. Chapter Four also adds to the neglected area of the genetics of gait. Along with recently published heritability estimates for gait in turkeys (Kapell et al. 2017) and previous work on the genetics of leg health traits which affect gait in broiler chickens (Kapell et al. 2012), this study can help breeding companies to gain a better understanding of the genetic control of gait in poultry and work towards the development of breeding strategies to achieve an optimal gait for broiler birds.

Limitations

While the three chapters presented here contain interesting results, there are aspects of the work which could have been improved. An immediate criticism may be the omission in Chapters One and Two of the red jungle-fowl as the ancestral phenotype in chickens. Instead, the layer chicken was used to represent a conspecific of the broiler which had not undergone intense selection for high growth rate and body mass. There are various reasons for choosing the layer chicken. Firstly, jungle-fowl are rare in the UK and true jungle-fowl chicks are very expensive; the layer chicks used in this study were free. Secondly, jungle-fowl are much flightier than the fully-domestic layer chicken. This is an important consideration, especially with regard to the gait work presented in Chapter Two; in order to collect gait data representative of the normal walking behaviour of each line, birds must remain calm and not display evasive behaviours during locomotion. It is likely that jungle-fowl would have displayed an aversion towards handlers which may have affected the data collected. Finally, it is unknown whether true jungle-fowl still exist. The habitat of the jungle-fowl in South-east Asia has been home to human settlers for millennia. Domestic chickens were part of almost all human settlements which were distributed throughout the jungle-fowl's natural range. Therefore, since it is likely that gene transfer occurred numerous times between the wild jungle-fowl population and the domesticated chicken over many generations, it is unlikely that 'true' jungle-fowl (which are fully representative of the ancestral phenotype of the broiler chicken) exist today, although the currently available jungle-fowl would still be the most ancestral species available. The layer chicken has not undergone selection for growth rate and body mass to the same extent as the broiler chicken. However, the layer chicken has undergone some selection towards leg health, especially towards improving bone mineral content in the leg, as bone fractures have been a long term issue in laying hens. This is an important point since the layer chicken here is being used to represent a proxy of the ancestral 'unselected' form when, in fact, the layer chicken has undergone considerable selection for leg health that may affect traits such as gait. Bone mineral density is unlikely to have a great effect on gait in healthy birds but the selection process for improving bone mineral density may have led to other structural changes in the architecture of the layer chicken leg that can affect gait. This should be considered when interpreting the results on skeletal morphology in Chapter 1 and the results on gait in Chapter 2.

An alternative to using a different line to represent slow growing conspecifics if to use the same line as the line of interest but to feed restrict birds so that they represent a slower-growing version of the bird of interest. This approach has been used previously (Williams et al. 2004) and has both advantages and drawbacks. On one hand, the study benefits from the reduced variability in lines. If *ad lib* fed Pekin ducks were compared to feed-restricted of the same line, any differences seen in a trait (for example leg bone morphology or gait) could be considered to be due to the difference in mass between the two lines, suggesting that selection for high body mass may be responsible for an altered bone morphology or gait in the modern broiler bird. This would not be possible if two totally separate lines were used (such as the modern Pekin and the mallard) as difference seen in a trait could be due to body mass but could also be due to the differing selective histories of each line. However, the use of feed-restricted birds for comparison is not ideal. Feed restriction will not only affect the mass of the feed-restricted bird, but will also affect its physiology and development. Feed-restricted birds may not reach sexual or physiological maturity at the same time as *ad lib* birds of the same line. Feed restriction may also affect behaviour. For example, it may lead to pecking or cannabilism and increased activity (Williams et al. 2004), which can affect traits such as gait. There are also serious welfare concerns associated with hunger and feed-restriction in broiler birds which are genetically pre-disposed to high feed intake. For these reasons, the decision was made to use slow-growing conspecifics of a different line, rather than to feed-restrict individuals of the same line for comparison. But it should be acknowledged that neither system is an ideal method to compare the effects of selection for body mass in broiler birds.

The data presented in Chapter One and Chapter Two was collected from birds in two experiments. Broiler chickens, layer chickens and Pekin commercial hybrids were raised in Experiment One in pens located in a converted pig shed. Pekin male line birds, Pekin female line birds and mallards were raised in Experiment Two, which was located in the same pens four months later. There was not enough space to raise all lines together at the same time. The gap in time between the two experiments was due to availability; this shed was also used for farrowing and the bird experiments were run between farrowings. A dedicated poultry research facility was not available for use due to biosecurity concerns. Ideally, all six lines would have been raised at the same time in the same shed, had a suitable location been available. There is a possibility that seasonal differences may have affected the internal environment in each experiment but no evidence was found of this.

Most of the results presented in Chapter Two relate to temporal and spatial aspects of the birds' gait, rather than the forces applied through the ground as the birds walk. This is because the pressure walkway was not sensitive enough to accurately record ground reaction forces or pressures at all ages, particularly in the lighter lines (the layer chicken and the mallard). A smaller walkway, which is more sensitive and records at a higher spatial resolution, can be used but this smaller walkway would not capture the necessary number of steps for most of the birds measured in this study. The purchase of both plates was not financially feasible. The technology can be custom made at a level of sensitivity and in a size which would suit this study but this option was not available at the time of purchase.

Chapter Three was an exploratory proof-of-concept trial to develop a tool for measuring lateral curvature of the tibiotarsus in live Pekin ducks. The trial would have been improved had staff availability been greater, as it would have reduced time limitations on test days and increased numbers of birds being tested. Also, as this trial was deemed a low priority in relation to the rest of the project, limited time was available for tool design and development.

The fourth chapter demonstrated that heritability estimates for components of gait can be higher than those for the visual gait score. Genetic correlations however were difficult to estimate in some cases, as the standard errors of the estimate were almost as large as the estimates themselves. This is due to the sample size of birds measured; for this pilot study, only approximately 2,500 birds were measured in each line. Over 3000 individuals would usually be required to estimate genetic correlations which were significant. The genetic correlations which were significant in this study were low but favourable, which is promising. The study also made no indication of which gait components should be used in future selection, or indeed what type of step width or body roll is desirable. This is outside the scope of the present work. It is likely that an intermediate step width and minimal body roll will lead to a more balance gait; however, the ideal step width will depend on the centre of mass movements. A far greater understanding of how gait components interact to form the overall gait of each bird is necessary before particular components can be identified as playing a central role in ‘good gait’. Indeed, the concept of ‘good gait’ has yet to be properly defined. There is the possibility of a circular argument here – how can we select for ideal gait if we are unsure of what the ideal is? And how can a new system of recording gait be validated if the current system that is used for comparison is not in itself reliable? A solution may be to view the ancestral phenotype as an example of the ideal gait for that species, before the effects of artificial selection have changed this ‘ideal gait’. For example, the gait of the mallard with a narrow step with and minimal lateral movement of the centre of mass may be viewed as an optimal gait to select toward in all duck lines. However, the morphology of the mallard and the modern Pekin have diverged considerably and so a gait that is optimal for the mallard’s morphology may not be optimal for the very different morphology of a broiler bird such as the Pekin. It has been suggested that the gait of the modern broiler chicken (greatly altered from its ancestral phenotype) may indeed be the optimal gait for moving this new broiler phenotype with its proportionally larger breast muscle mass and altered centre of mass position, although that is not to say it is without welfare issues (Corr et al. 2003b). The solution may be to use the ancestral gait as the ideal to aim towards, but to allow for some deviation from this ideal due to the altered morphology of the modern broiler bird. The validation of a new system of gait scoring (such as the use of certain gait components) is difficult if the current gait scoring system is not considered reliable way of measuring gait. It may be necessary to validate the new measures by rigorous modelling of the effects of selecting towards this new gait score over generations. For example, one could attempt to estimate various moments and ground reaction forces that make up gait and model how these would change between different gaits. For example, how much lateral movement of the centre of mass would occur if the feet were placed in a narrow step width during walking, or in a wide step width? Similar modelling studies have been carried out to model dinosaur or robotic gait (Hutchinson 2004b, a, Hugel et al. 2011, Allen et al. 2013).

Implications

The results presented in this thesis can make a genuine impact in improving the welfare of poultry raised for meat production while also making production more profitable for producers and breeding companies alike. The work, which focusses in particular on the Cherry Valley Pekin duck, will enable this breeding company to maintain its reputation for sound gait. Selection against the lateral curvature of the tibiotarsus (reported in Chapter One) and the medial rotation of the foot (reported in Chapter Two) may improve stability in birds which tend to fall over when making quick turns. It is however important to firstly test the theory of lateral tibiotarsal curvature causing a reduction in the size of the ‘zone of safely’ and secondly the consequences of altering the skeletal architecture must be examined before any selection takes place. Measurement of lateral tibiotarsal curvature in live birds using a physical tool is unlikely to be successful, as shown in Chapter Three. A greater potential for success in this area lies in the use of CT scanning and software development for automated measurement of bone curavtures.

The entire work of the thesis builds towards the development of a more objective gait score. Chapter Two outlines particular components of gait, such as step width and support time ratios which have changed due to selection for larger body mass. Chapter Four looks at the suitability of the current visual gait score in ducks. The less that optimal reliability between scorers highlights some of the limitations in using a categorical scoring system for such a subjective trait. Since birds of gait score 1 are completely lame and cannot walk, and even birds with excellent gait are almost never given a score of 5 (perhaps this is because subjective gait scoring involves looking out for faults so there may be a hesitancy for scorers to give even good birds a perfect score of 5), the gait scoring system used on this farm is essentially a three-score system; almost all birds receive a score of 2, 3 or 4 (in this trial, 1% were given a gait score of 1; 29% a score of 2; 61% a score of 3 and 8% a score of 4). When analysing this categorical gait score, there is a statistical assumption that the categorical scores represent an underlying continuous trait. With a 3-score system, the likelihood that the categorical scale accurately represents the underlying continuous scale is low. A possible improvement on this scoring system would be to create more scoring categories, to bring the categorical scale closer to the underlying continuous scale. The data presented in Chapter Four also demonstrates that by focusing on gait components, rather than on the traditional visual gait score, higher heritabilities are observed, which can lead to greater genetic progress if selecting those traits. Only relatively minor development of the proprietary software is required to fully automate the system for high-throughput use in poultry, thus making the use of gait components more objective and accurate than the visual gait score. The proprietary software can currently automatically recognise and analyse the steps of cats, dogs and humans. Automating analysis for another biped species, especially one with as simple a foot shape as ducks, would require a small training set of data. A greater challenge is to ensure that a normal gait is measured on each bird in the breeding programme is a quick and efficient manner. Birds which hesitate, stumble or do not walk in a straight line will not provide the normal gait recording that is necessary for an automated system such as this. In a research setting, walks can be repeated in order to gather clean, usable data but within the industrial setting, repeating a walk may not be feasible due to time constraints associated with measuring a large number of traits on many birds as part of a breeding programme. A possible solution to this may be to measure birds at younger ages when walking ability is better and the birds are more likely to walk uninterrupted to the end of the recording walkway. Gait at this younger age may be an adequate predictor of gait at a later age. A greater understanding of how gait components relate to the overall walking ability of the bird is required in order to establish which gait components should be selected for to achieve the breeding goal of an ideal gait. Morphological traits, such as leg length or hip angle, can also be incorporated into a model of ideal gait. This study has demonstrated the feasibility of eschewing the traditional visual gait score and adopting gait components as a more objective and potentially more accurate method of improving gait in all species of poultry. Welfare concerns surrounding gait have plagued the poultry industry for decades and while some progress has been made on reducing leg health problems (Kapell et al. 2012), poor gait still remains. The development of an objective gait scoring technique that can be used in high-throughput phenotyping within breeding companies has the potential to increase genetic progress of gait traits in a range of poultry species and improve the welfare of billions of individual birds.

References

Abourachid, A. 1991. Comparative gait analysis in two strains of turkey, *Meleagris gallopavo*. British Poultry Science **32**:271-277.

Abourachid, A., R. Hackert, M. Herbin, P. A. Libourel, F. Lambert, H. Gioanni, P. Provini, P. Blazevic, and V. Hugel. 2011. Bird terrestrial locomotion as revealed by 3D kinematics. Zoology **114**:360-368.

Allen, V., K. T. Bates, Z. H. Li, and J. R. Hutchinson. 2013. Linking the evolution of body shape and locomotor biomechanics in bird-line archosaurs. Nature **497**:104-107.

Anon. 2000. The welfare of chickens kept for meat production (broilers). European Commission, Brussels, Belgium.

Bradshaw, R. H., R. D. Kirkden, and D. M. Broom. 2002. A review of the aetiology and pathology of leg weakness in broilers in relation to welfare. Avian and Poultry Biology Reviews **13**:45-103.

Caplen, G., B. Hothersall, J. C. Murrell, C. J. Nicol, A. E. Waterman-Pearson, C. A. Weeks, and G. R. Colborne. 2012. Kinematic Analysis Quantifies Gait Abnormalities Associated with Lameness in Broiler Chickens and Identifies Evolutionary Gait Differences. Plos One **7**.

Chapinal, N., A. Sewalem, and F. Miglior. 2012. Short communication: Estimation of genetic parameters for gait in Canadian Holstein cows. Journal of Dairy Science **95**:7372-7376.

Clark, A. J., and T. E. Higham. 2011. Slipping, sliding and stability: locomotor strategies for overcoming low-friction surfaces. The Journal of Experimental Biology **214**:1369-1378.

Corr, S. A., M. J. Gentle, C. C. McCorquodale, and D. Bennett. 2003a. The effect of morphology on the musculoskeletal system of the modern broiler. Animal Welfare **12**:145-157.

Corr, S. A., M. J. Gentle, C. C. McCorquodale, and D. Bennett. 2003b. The effect of morphology on walking ability in the modern broiler: A gait analysis study. Animal Welfare **12**:159-171.

Dial, T. R., and D. R. Carrier. 2012. Precocial hindlimbs and altricial forelimbs: partitioning ontogenetic strategies in mallards (*Anas platyrhynchos*). Journal of Experimental Biology **215**:3703-3710.

Dial, T. R., A. M. Heers, and B. W. Tobalske. 2012. Ontogeny of aerodynamics in mallards: comparative performance and developmental implications. Journal of Experimental Biology **215**:3693-3702.

Fish, F. E. 1995. Kinematics of ducklings swimming in formation: coonsequences of position. Journal of Experimental Zoology **273**:1-11.

Hugel, V., R. Hackert, and A. Abourachid. 2011. Kinematic modeling of bird locomotion from experimental data. Ieee Transactions on Robotics **27**:185-200.

Hutchinson, J. R. 2004a. Biomechanical modeling and sensitivity analysis of bipedal running ability. II. Extinct taxa. Journal of Morphology **262**:441-461.

Hutchinson, J. R. 2004b. Biornechanical modeling and sensitivity analysis of bipedal running ability. I. Extant taxa. Journal of Morphology **262**:421-440.

Kapell, D. N. R. G., W. G. Hill, A. M. Neeteson, J. McAdam, A. N. M. Koerhuis, and S. Avendano. 2012. Twenty-five years of selection for improved leg health in purebred broiler lines and underlying genetic parameters. Poultry Science **91**:3032-3043.

Kapell, D. N. R. G., P. M. Hocking, P. K. Glover, V. D. Kremer, and S. Avendano. 2017. Genetic basis of leg health and its relationship with body weight in purebred turkey lines. Poultry Science **96**:1553-1562.

Paxton, H., N. B. Anthony, S. A. Corr, and J. R. Hutchinson. 2010. The effects of selective breeding on the architectural properties of the pelvic limb in broiler chickens: a comparative study across modern and ancestral populations. Journal of Anatomy **217**:153-166.

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

Prange, H. D., and K. Schmidt-Nielsen. 1970. The metabolic cost of swimming in ducks. Journal of Experimental Biology **53**:763-&.

Provini, P., P. Goupil, V. Hugel, and A. Abourachid. 2012. Walking, Paddling, Waddling: 3D Kinematics Anatidae Locomotion (*Callonetta leucophrys*). Journal of Experimental Zoology Part a-Ecological Genetics and Physiology **317A**:275-282.

Provini, P., C. Simonis, and A. Abourachid. 2013. Functional implications of the intertarsal joint shape in a terrestrial (*Coturnix coturnix*) versus a semi-aquatic bird (*Callonetta leucophrys*). Journal of Zoology **290**:12-18.

Robison, C. I., M. Rice, M. M. Makagon, and D. M. Karcher. 2015. Duck gait: Relationship to hip angle, bone ash, bone density, and morphology. Poultry Science.

Usherwood, J. R., K. L. Szymanek, and M. A. Daley. 2008. Compass gait mechanics account for top walking speeds in ducks and humans. Journal of Experimental Biology **211**:3744-3749.

Williams, B., D. Waddington, D. H. Murray, and C. Farquharson. 2004. Bone strength during growth: Influence of growth rate on cortical porosity and mineralization. Calcified Tissue International **74**:236-245.