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Anatomical and biomechanical traits of broiler chickens across ontogeny. Part II. Body segment inertial properties and muscle architecture of the pelvic limb

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In broiler chickens, genetic success for desired production traits is often shadowed by welfare concerns related to musculoskeletal health. Whilst these concerns are clear, a viable solution is still elusive. Part of the solution lies in knowing how anatomical changes in afflicted body systems that occur across ontogeny influence standing and moving. Here, to demonstrate these changes we quantify the segment inertial properties of the whole body, trunk (legs removed) and the right pelvic limb segments of five broilers at three different age groups across development. We also consider how muscle architecture (mass, fascicle length and other properties related to mechanics) changes for selected muscles of the pelvic limb. Wholelimb morphology is not uniform relative to body size, with broilers obtaining large thighs and feet between four and six weeks of age. This implies that the energetic cost of swinging the limbs is markedly increased across this growth period, perhaps contributing to reduced activity levels. Hindlimb bone length does not change during this period, which may be advantageous for increased stability despite the increased energetic costs. Increased pectoral muscle growth appears to move the centre of mass cranio-dorsally in the last two weeks of growth. This has direct consequences for ventilation (heavier sterna apparatus must be moved with each breath) and locomotion (potentially greater limb muscle stresses during standing and moving). Our study is the first to measure these changes in the musculoskeletal system across growth in chickens, and reveals how artificially selected changes of the morphology of the pectoral apparatus may cause deficits in locomotion, as well as breathing.

1 **TITLE:**

2 Anatomical and biomechanical traits of broiler chickens across ontogeny. Part II. Body
3 segment inertial properties and muscle architecture of the pelvic limb

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INTRODUCTION:

The poultry industry is a rapidly expanding enterprise (over 870 million broilers slaughtered in the UK; DEFRA 2013 statistics), in which production continues to increase globally by over 130% in some countries (Scanes 2007). In addition to increased production, the broiler chicken has gained an unusual repertoire of anatomical traits, which are repeatedly emphasized in scientific studies and highlight what has developed into a successful breeding programme for obtaining desired production characteristics (e.g. Havenstein et al., 2003; Paxton et al., 2010, 2013). However, a crossroads has been reached where efficient broiler production is haunted by welfare concerns (Julian, 1998; Mench, 2004; Knowles et al., 2008). Broilers may suffer from heart failure and sudden death syndrome (Julian, 1998; Maxwell and Robertson, 1998; Olkowski et al., 2007), reduced adaptive immune function (Cheema et al., 2003), leg weakness (see review; Bradshaw et al., 2002), poor reproductive performance (Siegel and Dunnington, 1987; Hocking, 1993) and appear to be susceptible to suboptimal management of nutrition and their environment (e.g. Vestergaard and Sanotra, 1999; Kestin et al., 2001; Scott, 2002; Brickett et al., 2007; Buijs et al., 2009). Unfortunately, whilst the welfare and economic concerns associated with these issues are clear, there is no optimal evidence-based solution that resolves all concerns surrounding broiler chicken production.

To move toward such a solution, a deeper understanding of how broiler body shape and musculoskeletal function develops during growth and how these changes may influence locomotion is required. Part 1 of this series (Tickle et al., MS) characterized how broiler organ and pectoral muscle growth varies with increasing body mass, with a focus on respiratory system development and changes in organ size, highlighting important repercussions to breathing/ cardiorespiratory performance. Here, we delve deeper into how these and other anatomical changes have affected the overall size and shape of the broiler and investigate how relevant traits have likely influenced their locomotor abilities.

To help achieve our aim, we detail the muscle architectural properties of the major pelvic limb muscles (identified previously by Paxton et al., 2010 as the hip, knee and ankle extensors in broilers) and document how these scale with body size. Skeletal muscle is one of the organs that is most adaptable to environmental change (Lieber, 1986), and an integral component of locomotion (supporting and powering the movement). Muscle mechanical performance is mostly dependent on a few key architectural properties; namely mass, fascicle length and pennation angle. These parameters are typically used to calculate physiological cross-sectional area (PCSA) and thereby estimate muscular capacity for force-generation (PCSA) versus length change (fascicle length) (Powell et al., 1984; Burkholder et al., 1994;

69 Lieber and Friden, 2000). For broilers, muscle architecture data on the broiler currently exist
70 for animals at six weeks of age (see Paxton et al., 2010), but how these properties change
71 across growth in the broiler is unknown. This study therefore focuses on the scaling (i.e., size
72 related, isometric or allometric; Biewener, 1989; Alexander et al., 1981; Alexander and Ker,
73 1990) relationships of select pelvic limb muscles to reveal changes in individual muscle
74 characteristics of individual muscles from hatching to slaughter age (~six weeks). We also
75 consider how the pelvic limb bones (femur, tibiotarsus and tarsometatarsus) scale with body
76 size in the broiler chicken, as effective scaling of the hindlimb bones can reduce the rate at
77 which stress increases with body mass (McMahon, 1973, 1975; Garcia and da Silva, 2004).

78 In addition, differences in limb orientation and motion, and overall gait dynamics,
79 among avian taxa can be partially attributed to variation in body centre of mass (CoM)
80 position (Gatesy and Biewener, 1991; Abourachid, 1993; Hutchinson, 2004). Manion (1984)
81 estimated CoM position for chickens across ontogeny (5-19 days) and noted a cranioventral
82 shift and a subsequent change in limb orientation during standing and walking (more flexed
83 during standing, but more extended during walking). The broiler CoM position has been
84 addressed in preliminary computational analyses by Allen et al (2009) and was found to shift
85 caudodorsally. Other previous research suggests that broilers have a more cranially
86 positioned CoM induced by a large pectoral muscle mass (Abourachid, 1993; Corr et al.,
87 2003a). To better understand the influence of CoM on locomotor ability in broilers, we
88 therefore quantify the variation in CoM position (3D) across ontogeny and relate this
89 variation to anatomical changes documented here and in Part I of this study (Tickle et al.,
90 MS).

91 All broilers used here are considered 'normal' –i.e. no observed lameness, but we
92 document the limb pathologies (identified post mortem) within our study populations. This is
93 essential, because the pathological changes in affected birds do not often relate to walking
94 ability (assessed using gait score and force plate measurements) (Sandiland et al., 2011). We
95 quantify the inertial properties (mass, centre of mass and radius of gyration) of each limb and
96 major body segment (Fig. 1), because these help reveal basic locomotor habits (e.g. limb
97 tapering –i.e. a proximal to distal reduction in muscle mass reflects a specialization for power
98 versus force development; Alexander et al., 1981; Pasi and Carrier, 2003; Hutchinson, 2004,
99 Payne et al., 2005a, 2005b; Smith et al., 2006). Both centre of mass position and radius of
100 gyration are also good descriptors of body area distribution and resistance to rotational
101 movements (Kilbourne, 2013). Importantly, inertial properties in chickens and more
102 specifically broilers are almost completely unstudied. Only a few studies have documented

103 the inertial properties of other ground running birds (e.g., emus, Goetz et al., 2008; guinea
104 fowl, Daley et al., 2007; Rubenson and Marsh, 2009). This study not only provides a novel
105 insight into characteristics that influence broiler locomotor function, but also provides the
106 inertial properties necessary to develop models for dynamic analyses of movement, which
107 have been highly successful in improving our understanding of human pathological gait (e.g.
108 Steel et al., 2012; Fey et al., 2013; Allen et al., 2013).

109

110

MATERIALS AND METHODS:

111 *Birds*

112 Fresh male cadavers of a commercial broiler strain at different ages (days 1, 13, 29, 32
113 and 40; Table 1) were used for the hind limb muscle analysis and then a further five broilers
114 of the same commercial strain at approximately 14, 28 and 42 days of age (2, 4 and 6 weeks
115 old) were used to calculate the centre of mass (CoM) of the whole body, trunk and the pelvic
116 limb segments --i.e. the thigh, drumstick, shank and foot (Fig. 1). Pelvic limb bone
117 dimensions (femur, tibiotarsus and tarsometatarsus) were also recorded at 2, 4 and 6 weeks of
118 age. These data are from the same chickens used in Part I (Tickle et al., MS), which focuses
119 on anatomy of the musculoskeletal respiratory apparatus and changes in body and organ size
120 across ontogeny. As our data are cross-sectional rather than longitudinal, this study
121 approximates an ontogenetic analysis, via inference from comparisons among individuals.
122 All chickens were raised in a commercial setting under similar management guidelines and
123 were not outwardly influenced by a laboratory setting. All experiments are ethically
124 approved.

125

126 *Pathology*

127 All birds were macroscopically evaluated to establish the incidence of leg pathologies
128 (marked as present/ absent unless otherwise stated; Table 2). Each chicken was examined for
129 bacterial chondronecrosis with osteomyelitis (BCO) in the femur and tibiotarsus, tibial
130 dyschondroplasia (TD), tibial rotation (RT) and valgus/varus deformities (VVD). Tibial
131 rotation above 20° was considered abnormal. Similar to other studies (e.g. Shim et al., 2012)
132 VVD was classified as mild, moderate or severe following the methods described by Leterrier
133 and Nys (1992).

134

135 *Muscle Architecture*

136 The left pelvic limb from each individual (refer to Table 1) was dissected, with
137 fourteen specific muscle-tendon units (see Table 3 for muscle names and abbreviations) being
138 identified and systematically removed. Muscles identified included the main hip, knee and
139 ankle extensors, which are involved in limb support during the stance phase and have been
140 shown to have either massive, short-fibred muscles favouring greater force-generating
141 capacity or have long parallel-fibred muscles for fast contraction (Paxton et al., 2010), as in
142 many other terrestrial species. Architectural measurements taken included muscle mass (M_m ;
143 tendon removed), which was measured on an electronic balance ($\pm 0.001\text{g}$), muscle fascicle
144 length (L_f ; $\pm 1\text{mm}$), and fibre pennation angle (θ), where appropriate ($\pm 1^\circ$). In order to
145 account for variation of fascicle length and pennation angle within a muscle, L_f and θ were
146 calculated as the mean of five measurements made across each muscle. Physiological cross-
147 sectional area (PCSA) was calculated for each muscle (Eq. 1; Sacks and Roy, 1982) from
148 these measurements according to the equation:

$$\text{PCSA} = M_m \cos \theta (\rho L_f)^{-1} \quad (1)$$

151
152 Muscle tissue density (ρ) was assumed to be 1.06g cm^{-3} , the standard value for
153 mammalian and avian muscle (Mendez and Keys, 1960; Paxton et al., 2010).

154 155 *Centre of Mass and Inertial Properties*

156 Computed tomography (CT) scans were acquired of five male bird cadavers from each
157 group. The cadavers were scanned with a GE Lightspeed 8-detector scanner at 100mA and
158 120 kVp X-ray beam intensity using a 1mm CT slice thickness. In order to minimise postural
159 effects on CoM estimates, the birds were placed in the same upright position with their left
160 side resting on a radiolucent cushion for scanning and limbs posed as similarly as possible
161 (Fig. 1a). Mimics 14.12 imaging software (Materialise; Leuven, Belgium) was then used to
162 segment the resulting DICOM image files, creating a 3D representation of the skeleton, body
163 and the pelvic limb segments (trunk, thigh, drumstick, shank and the foot; Fig. 1b). We used
164 predefined thresholds set for bone and flesh, with some manual adjustment from those
165 baseline values as appropriate to ensure smooth, plausible rendering. Custom software
166 (Hutchinson et al., 2007; Allen et al., 2009)) was then used to estimate the whole body/
167 segment CoM. This method allows accurate specification of the CoM relative to any user
168 defined point on the body or segment. These points are easily identified using bony

169 landmarks visible on the CT images and are essential for putting CoM position in the context
170 of gross morphology, which is necessary for biomechanical analyses. Using the 3D model
171 representations of the skeleton, whole body CoM position was quantified relative to the right
172 hip, trunk CoM was taken relative to the pelvis (centre point between the hips on the pelvis),
173 and for the remaining segments, CoM position was expressed relative to the proximal end of
174 the bone (Fig. 2). Three-dimensional coordinates (x [craniocaudal], y [dorsoventral] and z
175 [mediolateral] co-ordinates for the CoM were then recorded. Whole body CoM position was
176 expressed as a percentage of femur length (see Allen et al., 2009).

177 Segment anatomical properties that we measured and present here include segment
178 mass (m; as % body mass), segment length (L; see Fig. 2), centre of mass (as % segment
179 length), and radius of gyration (r; as % segment length), which are essential information
180 required to calculate the moments of inertia (kgm^2 ; Eq. 3) and subsequently complete the set
181 of inertial properties required for biomechanical analyses:

$$R = (I m^{-1})^{0.5} \quad (2)$$

182
183
184
185 The radius of gyration (% segment length; Eq. 2) was calculated using the principal
186 moment of inertia (I) and mass of the segment, both estimated using the custom software.
187 The mean difference between the dissected segment mass recorded and that estimated were
188 small (< 5%). These data also provide further information on muscle mass distribution within
189 the limb.

$$I = m(r L)^2 \quad (3)$$

193 ***Bone Scaling Dataset***

194 Data from Part I of this study were also used for this analysis. The individual bone
195 lengths of the left pelvic limb were recorded ($\pm 1\text{mm}$) for each of four age groups: day 1
196 (n=10), ~14 days (n=20), ~28 days (n=19), ~42 days (n=19). Total leg length was defined as
197 the sum of the individual pelvic limb bone lengths. Limb bone proportions were calculated as
198 a percentage of total leg length.

200 ***Statistical Analysis***

201 All of our analyses take into account differences in body size across age groups. To
202 analyse the muscle architecture data, the linear relationship between log-transformed body

203 mass and muscle mass, fascicle length and PCSA were examined using the reduced major-
204 axis regression (RMA) function in the statistical program PAST (Hammer *et al.*, 2001). RMA
205 regression was appropriate for analysis of these morphological characters because error in
206 both x and y variables is considered (Rayner, 1985; Sokal and Rohlf, 1995). However, errors
207 in our body mass values were far smaller than errors in the independent variables, so ordinary
208 least squares regression might have been satisfactory (Sokal and Rohlf, 1995). Upper and
209 lower 95% confidence intervals (CIs) and the R^2 value for each regression line were
210 calculated to quantify the variation around the mean (Fig. 3, Table 4). Isometric scaling was
211 assumed when the scaling exponent \pm 95% CIs overlapped the expected value. Reduced
212 major-axis regression was also used to examine the linear relationship between log-
213 transformed body mass and bone length. This was done using custom Matlab (The
214 Mathworks, Nattick, MA, USA) software code. The 95% CIs and the R^2 value for each
215 regression line were also calculated (Fig. 4). Similar to the statistical analysis used for the
216 muscle architecture data, isometric scaling was assumed when the scaling exponent \pm 95%
217 CIs overlapped the expected value.

218 For CoM values and segment anatomical properties, the statistics used followed those
219 set out in Part I of this study. In brief, a Shapiro-Wilk normality test, in combination with
220 considering subsequent PP and QQ plots of the data, and Levene's test for equal variances
221 were used to test the assumptions of a one-way analysis of variance (ANOVA). An ANOVA
222 test was then conducted with a subsequent Bonferroni post-hoc test to check for differences
223 among the three age groups. If equal variances were violated, the Welch statistics are
224 reported in conjunction with the results of a subsequent Games-Howell post-hoc test (Table
225 8).

226

227

RESULTS:

Pathology

229 There were a number of pathological changes in the study population at all stages of
230 development (see Table 2). Bacterial chondronecrosis with osteomyelitis (BCO) was present
231 in all populations both in the femur and proximal tibia. There was an increased incidence of
232 BCO in the femur as the broilers aged, with 88% of the six week old chickens affected. Tibial
233 dyschondroplasia was present in all populations with no apparent correlation with age
234 (average 41%). Rotated tibia was more prevalent in the younger birds (~33% of the study
235 population). Valgus angulation of the lower limb was seen only in the six-week old chickens,
236 with approximately 45% of these chickens affected. No varus deformities were observed.

237

238 ***Muscle architecture***

239 Across ontogeny, the masses of the major hip, knee and ankle extensor muscles
240 generally scale with positive allometry, increasing in relative size as broilers grew (Table 4).
241 The exceptions were the FMTM (M. femorotibialis medialis), TCF (M. tibialis cranialis caput
242 femorale), PIF (M. puboischiofemorale) and IC (M. iliotibialis cranialis), which did not
243 change significantly with body mass. Interestingly, fascicle length, in general, did not change
244 with body mass, but scaled isometrically. However, the IL (M. iliotibialis lateralis) muscle
245 showed an increase in fascicle length, whereas the GM (M. gastrocnemius medialis) showed a
246 decrease in fascicle length. Despite these changes in fascicle length, PCSA appeared to
247 increase allometrically across ontogeny in the majority of the pelvic limb muscles. The IC
248 and TCF, however, scaled more in accordance with isometry.

249

250 ***Whole Body Centre of Mass***

251 Whole body CoM moved caudodorsally until 28 days of age. By 42 days of age there
252 was a significant cranial shift in CoM position, moving ~10% cranially and ~30% more
253 dorsally in the last two weeks approaching slaughter age (Table 5).

254

255 ***Segment Properties***

256 There was a 50-fold range in body mass of our sample of broiler chickens (Table 1),
257 ranging from ~0.04kg at one day old to ~2.4kg at 40 days of age. Trunk mass contribution to
258 whole body mass increased across ontogeny and was ~5% larger at 42 days of age relative to
259 the youngest group. Similarly, a significantly larger relative thigh muscle mass was found in
260 older birds (by ~3% body mass) compared to the younger broilers. Drumstick mass increased
261 significantly with age whereas shank mass remained unchanged. Between 28 and 42 days,
262 the chickens' foot mass also increased significantly by ~30% (see Table 6).

263 Trunk CoM moved caudally between 28 and 42 days (i.e., moving more caudally),
264 and the thigh, drumstick and shank CoM moved to more proximal positions (Table 5). In
265 contrast, the foot's CoM moved more distally at 42 days of age.

266 The radii of gyration about the axes of long-axis rotation (Table 6) experienced an
267 increase in the foot segments of the six week old broilers and remained unchanged in the
268 thigh, drumstick and shank. However, the radius of gyration of the whole trunk segment
269 showed a decrease for long-axis rotation – i.e. a lower resistance to yaw. The radii of
270 gyration in the parasagittal plane, decreased in the drumstick and shank across broiler

271 ontogeny, but progressively increased in the foot. There was also a relative reduction of $r\%$
272 about the axis of abduction/ adduction rotation. Radii of gyration for the thigh remained
273 unchanged.

274

275 ***Bone Scaling***

276 Tibiotarsus and tarsometatarsus length scaled isometrically with body mass, whereas
277 femur length scaled with slight negative allometry (Fig. 4). As a result, there was a relative
278 increase in total leg length from 14 days to 28 days. Limb length remained unchanged
279 between four and six weeks. The femur accounts for ~30% of total leg length and the
280 tibiotarsus accounts for ~41% of leg length. The tarsometatarsus is relatively shorter than the
281 other pelvic limb bones accounting for ~29% of total leg length.

282

283

283 **DISCUSSION**

284 The genetic success of the modern broiler and the subsequent changes to the
285 morphology of broiler chickens have been well documented, in order to determine the lines'
286 commercial performance (e.g. Gous et al., 1999) and compare both growth responses and
287 physiological adaptations resulting from distinctive selection pressures (e.g. Havenstein et al.,
288 2003a, b; Reddish and Lilburn, 2004; Schmidt et al., 2009). A marked change in total
289 pectoral muscle mass of the commercial broiler is a common finding of all these prior studies.
290 Similarly, we found this mass to represent ~20% of total body mass in slaughter age chickens
291 (see Part I; Tickle et al., MS). Part I revealed how enlarged pectoral muscle mass, among
292 other anatomical changes, may compromise the efficacy of the respiratory apparatus. Here we
293 show how these changes influence the locomotor ability of the broiler.

294

295 ***Pathology***

296 Leg weakness in broilers comprises not only nonspecific gait problems and lower
297 activity levels, but also a wide range of disorders that are generally classified as infectious,
298 degenerative, or developmental (for a review see Bradshaw et al., 2002). The most common
299 disorders include bacterial chondronecrosis with osteomyelitis (BCO), angular and torsional
300 deformities (e.g. valgus-varus (VVD) and rotated tibia (RT)) and tibial dyschondroplasia
301 (TD). With the exception of VVD, these conditions were observed in birds at all
302 developmental stages in this study. BCO was common in the broilers (Table 2), far exceeding
303 previous estimates of infection in commercial flocks (approximately 0.5% (McNamee, et al.,
304 1998)). Incidence of BCO in the femur peaked at 88% of 42-day-old birds. This variation is

305 likely due to differences in pathological examination procedure. Following Wideman et al.
306 (2013), we concur that epiphyseolysis should be attributed to underlying traumatic
307 (osteochondrosis) or infectious (osteomyelitis) femoral head pathology. Femoral head
308 separation dominated in our study's birds (FHS; epiphyseolysis). Furthermore, the high
309 percentage of birds we found to have BCO may reflect a predisposition to the condition or
310 differences in husbandry practices (McNamee and Smyth, 2000). Increasing occurrence of
311 BCO over development is consistent with previous results that identified peak incidence at
312 around five weeks of age (McNamee 1999a). Increasing incidence over development may
313 reflect increased stresses acting on the bone, which are thought to contribute to BCO
314 (Wideman, 2013). Similarly, BCO in the tibiotarsus was widespread and increased with age,
315 showing a peak at 42 days (Table 2). BCO causes lesions in the load-bearing growth plates of
316 the femur and tibiotarsus, so rapid growth and weight gain may be an aggravating factor when
317 bacterial infection is present. Considering the widespread incidence of BCO in birds that
318 were otherwise deemed healthy, bone lesions are a significant problem affecting welfare
319 standards in broiler chickens.

320 TD commonly leads to growth plate abnormalities, infections and tibial deformation
321 (Lynch et al., 1992) but, similar to BCO, TD does not necessarily induce lameness of
322 sufficient severity to impair walking ability (Pattison, 1992). TD has been found to occur
323 between 2 and 8 weeks of age (Edwards, 1984; Lynch, et al., 1992) and our findings are
324 consistent with this observation (Table 2). However, considerable variation exists in the
325 reported prevalence of TD, ranging in 42 day old birds from approximately 2% (Shim, et al.,
326 2012; Siller, 1970) to 50% (Prasad, et al., 1972; Sauveur and Mongin, 1978; Vaiano et al.,
327 1994) of total flock population. Incidence of TD in this study was relatively high and
328 occurred in all age groups, peaking at 28 days (57%). However, determining why the
329 incidence of TD is high in this study is difficult because the condition reflects a complicated
330 interaction of contributing factors, including dietary deficiencies, toxins, genetic
331 predisposition and rapid growth rate (Edwards, 1984; Julian, 1998; Orth and Cook, 1994;
332 Shim, et al., 2012).

333 Valgus-varus deformities (VVD) were observed in 42-day-old birds, but was not
334 present in younger broilers (Table 2). Comparable reports indicate that VVD occurs with
335 varying incidence, affecting as few as 0.5% (Julian, 1984) to 30-40% of birds in a flock
336 (Leterrier and Nys, 1992; Shim, et al., 2012). The prevalence of VVD in this study fell near
337 the high end of the reported range, with 45% of birds at 42 days of age observed to have mild
338 or moderate VVD. Our observation that symptoms of VVD occur only in older broilers is

339 consistent with the progressive nature of this deformity (Julian, 1984; Julian, 2005; Shim, et
340 al., 2012). However, no deformity was seen in 28-day-old birds, which is perhaps surprising
341 because this is approximately the age that VVD often becomes prominent (Julian, 1998;
342 Julian, 2005; Randall and Mills, 1981), although the timing of onset is known to vary
343 (Randall and Mills, 1981).

344 Rotated Tibia (RT) occurred in birds at all developmental stages at a higher than
345 expected rate (i.e., value) compared to previous work (0.2%, Bradshaw *et al*, 2002)).
346 However, Bradshaw *et al* reported a reduced proportion of RT in older broilers, which
347 perhaps indicates that affected birds were culled because the condition becomes clearly
348 obvious around 21 days of age (Riddell and Springer, 1985). The exact aetiology of RT is
349 unknown; however TD and VVD may exacerbate the incidence of RT (Bradshaw, et al.,
350 2002), thereby contributing to the relatively high proportion of birds with an outward torsion
351 of the tibial shaft.

352 Overall, there is a clear need to monitor the leg health of flocks, not only to aid
353 breeders to make adjustments to management practices or genetics when necessary, but also
354 to quickly identify lame birds for euthanasia on welfare grounds. The high incidence of leg
355 pathologies highlights the problem of maintaining high growth rates and breast muscle mass
356 (M_b) at the expense of broiler anatomy and physiology. In addition, lameness represents a
357 significant economic cost to the industry as birds with leg weakness are prematurely culled or
358 have an increased incidence of mortality. Efforts to improve the health of growing broilers
359 will have the twin benefit of improving both welfare standards and productivity.

360

361 ***Muscle Architecture***

362 Architectural properties used to calculate the effective physiological cross-sectional
363 area (PCSA) (Gans and Bock, 1965) of muscle take into account the effect of pennate
364 fascicles on maximizing force per unit area. PCSA is thus greater in pennate muscles and is
365 directly proportional to its force generation capacity (Burkholder et al., 1994; Lieber and
366 Friden, 2000). In broilers, the PCSA of the major hip, knee and ankle extensors (essential for
367 supporting body mass and maintaining an upright standing posture; Gatesy 1999; Reilly,
368 2000; Hutchinson, 2004), scale with positive allometry – i.e., these muscles have a greater
369 force-generating capacity (reflected in their relatively larger PCSAs) as the broiler develops
370 (Table 4, Fig. 3). As a result, muscular force production capacity in broiler chickens should
371 increase with age, likely as a direct consequence of weight vs. force scaling constraints
372 imposed by resisting gravity and inertia (e.g., Corr et al., 2003).

373 Broiler chickens appear to generally increase the PCSA of their pelvic limb muscles
374 by increasing muscle mass, rather than by increasing fascicle length, which scaled
375 isometrically (Table 4, Fig. 3). This increase in mass is likely due to increased hypertrophy
376 (increase in muscle fibre size), which is well known to occur in broiler skeletal muscle and is
377 the assumed dominant model for postnatal growth (Aberle and Stewart, 1983; Soike and
378 Bergman, 1998; Remignon et al., 1994; Goldspink and Yang, 1999). In addition, muscular
379 force production is invariant to muscle fascicle length, but longer fascicles exact a metabolic
380 cost because a larger volume of muscle is activated for each Newton of force (Kram and
381 Taylor, 1990; Roberts et al., 1998). Thus, the isometric scaling of fascicle length we observed
382 in this study avoids such added costs.

383 Interestingly, the PCSAs of the M. iliotibialis cranialis (IC) and M. tibialis cranialis
384 caput femorale (TCF) scale more in accordance with isometry. The relative force-generating
385 capacity of these muscles therefore remains unchanged throughout the growth of the broiler.
386 The TCF is a knee extensor and ankle flexor and is assisted by other muscles that also serve
387 as knee extensors and ankle flexors (e.g., M. femorotibialis and M. extensor digitorum
388 longus). Similarly, the IC is also a knee extensor, but additionally acts as a hip flexor,
389 supported by the M. iliopsoas (ITC) to flex and medially rotate the femur.
390 The additional support of these muscles may help to explain why the IC and TCF scale in
391 unusually isometric ways. The IC and TCF may be redundant. However, biomechanical
392 analyses of *in vivo* function are needed to test how much their function alters with growth in
393 broilers.

394

395 ***Centre of Mass and Inertial Properties***

396 At the youngest age (14 days old) studied here, chickens' trunk mass accounted for
397 ~75% of total body mass. At slaughter weight (around 42 days of age) total trunk mass had
398 increased to ~80% total body mass. The ~5% increase in trunk mass is largely attributable to
399 pectoral muscle growth, which occurred at a relatively faster rate than body mass (see Part I
400 Tickle et al., MS).

401 Interestingly, relative hind limb segment mass (summed segment masses; muscle and
402 bone mass combined) did not decrease during growth, accounting for ~15% of total body
403 mass at slaughter age and thus representing a total 5% body mass increase across ontogeny.
404 The proportion of bone mass contributing to total segment mass is likely small because the
405 muscle to bone ratio is known to be high in commercial broilers (Ganabadi et al., 2009). The
406 increase in leg mass was instead incurred by increases in drumstick and thigh muscle mass.

407 Drumstick segment mass increased across ontogeny, becoming relatively larger at each age
408 category, whereas thigh segment mass only had substantial changes during the last two weeks
409 of growth (from 28 – 42 days old). Thigh muscle mass increased by ~3% of total body mass
410 during this period. Changes in thigh and drumstick segment mass are expected, as these
411 segments yield the most meat and are the most consumed portions (alongside breast meat) on
412 the market (Broadbent et al., 1981). However, the increase in hind limb segment mass is
413 striking. Previous studies typically show an ontogenetic reduction in the investment of
414 metabolic resources towards pelvic limb muscle growth (e.g. Berri et al., 2007; Schmidt et al.,
415 2009) and the main drivers of selection in broiler chickens are still a greater yield of breast
416 muscle mass and a faster post-hatch growth rate (Arthur and Albers, 2003). Thus, changes in
417 leg muscle mass may not reflect a direct difference in selection pressures. However, a relative
418 increase in hind limb muscle mass may reflect a functional demand for larger hip and knee
419 extensors to support their increasing body mass. Corr et al. (2003a) studied two strains of
420 birds (relaxed and selected) raised on two different feeding regimes and suggested that the
421 large pectoral muscle mass of the broiler has displaced their CoM cranially. Similarly,
422 Abourachid (1993) suggested that increased stresses on the pelvic limbs of heavier broad-
423 breasted turkeys were induced by a more cranially positioned CoM. We found that broilers
424 show a change in whole body CoM position consistent with these previous findings, shifting
425 from a caudodorsal to a craniodorsal location between 28 and 42 days of age, which would
426 increase demand for muscular force production to balance it.

427 In all cases, the craniocaudal CoM location in broilers was far more cranial than
428 previous estimates. Allen et al. (2009) estimated CoM cranial position to be ~38% of femur
429 length, compared to the 70-90% femur length estimated in this study. On the other hand,
430 dorsoventral estimates broadly corresponded to literature values (Allen et al., 2009). The
431 difference in craniocaudal CoM position likely relates directly to pectoral muscle growth.
432 The pectoral muscle mass of the broilers used in this study yielded an additional ~4% of total
433 body mass in comparison to the broilers used by Allen et al. (2009). One limitation of our
434 study is that whole body CoM was normalized by femur length, which did scale with slight
435 negative allometry (see discussion below). Using femur length may introduce a slight bias to
436 our results, but normalization by other factors including total limb length have been shown to
437 yield the same result (Allen et al., 2009).

438 There are also substantial changes in the distal segment of the pelvic limb between 28
439 and 42 days. Foot mass increases by ~30% and foot CoM moves distally. Large feet may
440 serve to improve the apparent stability reported in the broiler (e.g. Corr et al., 2003b) during

441 the stance phase of gait. However, larger feet will influence the broiler's ability to accelerate
442 and decelerate the limb during swing, which in turn can affect the metabolic cost of
443 locomotion (Kilbourne and Hoffman, 2013). Both an increase in mass and a more distal shift
444 in the limb's mass distribution will effectively increase a limb's moment of inertia, (i.e.,
445 resistance to angular acceleration, Steudel, 1990; Wickler et al., 2004; Kilbourne and
446 Hoffman, 2003). Broiler pelvic limbs would therefore require more metabolic energy to
447 accelerate and decelerate them as the birds grow. However, the influence of mass is much
448 smaller than the influence of the distribution of the mass (radii of gyration reported here) on
449 the moment of inertia. Doubling mass would essentially double the moment of inertia,
450 whereas doubling the radii of gyration would increase the moment of inertia four-fold (Eq. 3).
451 The radii of gyration in the parasagittal plane and about the axes of long-axis rotation
452 increased in the foot segments of the ~42 day old broiler. Thus, increased radii of gyration in
453 the foot segment of the broiler contribute significantly to the limb's moment of inertia.

454 We found that broilers' whole pelvic limb morphology changed across ontogeny, with
455 the main changes in the thigh and foot segments. These segments had increased muscle mass
456 and a more distal mass distribution (rather than having relatively longer limbs; see bone
457 scaling discussion), resulting in relatively larger moments of inertia. The changes in limb
458 morphology are likely to assist in supporting the increased supportive forces required by a
459 more cranially positioned CoM and to help improve stability during locomotion. However,
460 these changes also likely exact a relatively higher metabolic cost to locomotion.

461

462 ***Bone Scaling***

463 We have shown that femur length scaled with slight negative allometry, whereas the
464 lengths of the tibiotarsus and tarsometatarsus scale with isometry across broiler ontogeny
465 (Fig. 4). Regardless, total relative limb length remained unchanged from four to six weeks in
466 growing broilers may be an adaptation related to their apparent instability (Paxton et al.,
467 2013). Maintaining shorter limbs may act to moderate the lateral motion of the CoM and aid
468 balance (Bauby and Kuo, 2000). However, short limbs also likely lead to an increased
469 energetic cost (Steudel-Numbers and Tilken, 2004).

470 Here we have considered how pelvic limb morphology changes during broiler
471 ontogeny, in coordination with other changes such as pectoral muscle mass. Together, these
472 changes have influenced broiler morphology across ontogeny, which may have influenced
473 locomotor ability as well. The relative force-generating capacity of the hind limb muscles is
474 greater in older broilers, and is primarily achieved through increasing muscle mass but

475 maintaining a constant fascicle length. Increases in thigh segment mass and a relative
476 increase in the moment of inertia of the distal limb (due mainly to increased foot size) may
477 reflect adaptations to cope with the apparent instability and a more cranially positioned CoM
478 as broilers grow. Although the architectural changes we have observed have obvious
479 advantages for maintaining an upright posture and forward progression of broiler chickens,
480 these morphological changes likely have a negative impact on locomotion, exacting relatively
481 higher metabolic costs during growth, which may have knock-on consequences for activity
482 levels and even overall health.

483

484

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Table 1 – Subject data

Age (days)	Sample size (n)	Body mass (kg)
1	10	0.044 ± 0.01
13	10	0.431 ± 0.08
29	5	1.542 ± 0.05
32	5	1.738 ± 0.08
40	7	2.452 ± 0.09

Data represented here are for the broiler chickens used only for muscle architecture and are means ± standard deviation. These data form part of the mean data presented in Table 2, Part I of this study.

Table 2 – Incidence of pathology across growth in broiler chickens

Age (days)	BCO (femur)	BCO (tibiotarsus)	TD (present)	VVD				RT (abnormal)
				normal	mild	moderate	severe	
14	63	53	43	100	0	0	0	33
28	75	93	57	100	0	0	0	15
42	88	97	24	55	42	3	0	16

Data are presented as percentage of total study population. Bacterial chondronecrosis (BCO) and tibial dyschondroplasia (TD) were marked as present or absent. The severity of these abnormalities was not considered. Where present, valgus/ varus deformities (VVD) were classified as mild (10-25°), moderate (25-45°) or severe (>45°) following the methods described by Leterrier and Nys (1992). Tibial rotation (RT) above 20° was considered abnormal. Pathologies may have been recorded in one pelvic limb or both pelvic limbs of the individual birds; this is not distinguished here.

Table 3 – The major muscles of the chicken pelvic limb

Muscle	Abbreviation
M. iliotibialis cranialis	IC
M. iliotibialis lateralis	IL
M. gastrocnemius pars lateralis	GL
M. gastrocnemius pars medialis	GM
M. fibularis longus	FL
M. ilirotrochantericus caudalis	ITC
M. femorotibialis medialis	FMT
M. iliofibularis	ILFB
M. flexor cruris lateralis pars pelvica	FCLP
M. flexor cruris medialis	FCM
M. caudofemoralis pars caudalis	CFC
M. tibialis cranialis caput femorale	TCF
M. puboischiofemoralis pars medialis	PIFM
M. puboischiofemoralis pars lateralis	PIFL

Table 4 – RMA regression analyses

Muscle	M_m expected slope: 1.00				PCSA expected slope: 0.67				L_f expected slope: 0.33			
	Slope	Lower 95% CI	Upper 95% CI	R ²	Slope	Lower 95% CI	Upper 95% CI	R ²	Slope	Lower 95% CI	Upper 95% CI	R ²
IC	1.038 (=)	0.933	1.123	0.961	0.712 (=)	0.630	0.783	0.938	0.342 (=)	0.308	0.372	0.923
IL	1.136 (+)	1.099	1.171	0.994	0.759 (+)	0.709	0.814	0.968	0.399 (+)	0.352	0.440	0.938
GL	1.121 (+)	1.104	1.136	0.998	0.817 (+)	0.769	0.868	0.977	0.306 (=)	0.261	0.349	0.859
GM	1.095 (+)	1.059	1.136	0.992	0.912 (+)	0.861	0.963	0.977	<i>0.226 (-)</i>	0.185	0.268	0.801
FL	1.156 (+)	1.109	1.199	0.988	1.009 (+)	0.940	1.089	0.945	0.274 (=)	0.193	0.354	0.420
ITC	1.075 (+)	1.032	1.118	0.989	0.746 (+)	0.699	0.796	0.969	0.322 (=)	0.279	0.377	0.844
FMTM	1.021 (=)	0.985	1.062	0.984	0.743 (+)	0.685	0.796	0.948	0.322 (=)	0.271	0.375	0.758
ILFB	1.112 (+)	1.078	1.143	0.994	0.815 (+)	0.773	0.856	0.981	0.307 (=)	0.286	0.330	0.961
FCLP	1.250 (+)	1.208	1.286	0.992	0.929 (+)	0.887	0.969	0.979	0.339 (=)	0.309	0.371	0.924
FCM	1.101 (+)	1.032	1.187	0.958	0.811 (+)	0.762	0.857	0.974	0.326 (=)	0.294	0.358	0.938
CFC	1.387 (+)	1.151	1.578	0.926	1.054 (+)	0.793	1.277	0.869	0.374 (=)	0.329	0.419	0.881
TCF	1.052 (=)	1.000	1.097	0.979	0.867 (=)	0.553	0.977	0.887	0.306 (=)	0.260	0.352	0.720
PIFM	1.079 (=)	1.007	1.143	0.969	0.779 (+)	0.701	0.843	0.933	0.327 (=)	0.297	0.358	0.893
PIFL	1.040 (=)	0.969	1.102	0.975	0.748 (+)	0.687	0.808	0.952	0.311 (=)	0.280	0.343	0.918

Data here are the RMA regression analyses that were used to determine the linear relationships between pelvic limb muscle architecture and body mass. Expected regression slopes for isometric growth are given for each parameter. Symbols next to each calculated regression slope indicate isometric growth (=), positive allometry (+; emphasized in bold) or negative allometry (-; emphasized in italics). All regressions were significant ($p < 0.05$).

Table 5 – Whole body centre of mass position

Age	CoM Position (% femur length)	
Group	Craniocaudal	Dorsoventral
14 days	90.6 ± 10.7 ¹	89.8 ± 13.1 ¹
28 days	68.3 ± 5.0 ^{1,2}	55.4 ± 17.7 ²
42 days	76.6 ± 12.2 ²	28.2 ± 19.5 ²

Data represented are means ± standard deviation. Centre of mass (CoM) position is calculated as a percentage of femur length. Craniocaudal and dorsoventral positions are shown. Data with no common superscript differ significantly at the 0.05 level.

Table 6 – Pelvic limb segment inertial properties

Segment	Age Group	Segment Mass (% body mass)	CoM Position (% segment length)	Radius of Gyration (% segment length)		
				x	y	z
Trunk	14 days	74.6 ± 1.7 ^a	19.5 ± 2.5	23.2 ± 1.0	41.1 ± 0.3 ^b	35.0 ± 1.9 ^a
	28days	78.0 ± 1.8 ^{a,b}	20.1 ± 4.3	24.0 ± 1.2	40.0 ± 1.7 ^b	37.1 ± 1.8 ^{a,b}
	42 days	81.7 ± 3.2 ^b	15.3 ± 2.7	33.3 ± 12.0	37.1 ± 1.8^a	37.8 ± 0.8 ^b
Thigh	14 days	5.19 ± 1.4 ^a	43.7 ± 4.3^a	47.2 ± 8.5	44.1 ± 7.4	49.6 ± 14.5
	28days	5.22 ± 0.4 ^a	38.1 ± 2.0^b	48.3 ± 6.2	40.6 ± 4.2	52.1 ± 8.8
	42 days	8.21 ± 1.0^b	28.7 ± 2.4^c	46.2 ± 7.0	42.5 ± 4.3	52.3 ± 7.1
Drumstick	14 days	3.9 ± 0.3^a	30.1 ± 14.2	49.1 ± 6.3	24.9 ± 2.9	51.0 ± 6.1^a
	28days	4.6 ± 0.5^b	32.7 ± 2.3	40.0 ± 5.3	25.6 ± 4.0	40.2 ± 7.1 ^{a,b}
	42 days	5.66 ± 0.2^c	24.2 ± 4.2	34.0 ± 12.4	21.2 ± 11.7	34.3 ± 12.2^b
Shank	14 days	0.98 ± 0.1 ^a	36.1 ± 10.3^a	50.5 ± 1.2 ^a	18.1 ± 0.2	50.8 ± 1.1 ^a
	28days	1.02 ± 0.1 ^{a,b}	17.3 ± 5.5 ^b	49.8 ± 2.3 ^a	17.2 ± 1.2	50.2 ± 2.5 ^a
	42 days	1.23 ± 0.2 ^b	22.6 ± 6.3 ^b	38.0 ± 4.1^b	23.3 ± 20.7	37.1 ± 2.7^b
Foot	14 days	0.63 ± 0.03 ^a	38.6 ± 4.3 ^a	34.3 ± 0.9	35.2 ± 1.8 ^b	23.3 ± 1.4
	28days	0.61 ± 0.07 ^a	28.4 ± 7.3 ^a	31.0 ± 2.3	21.2 ± 6.3^a	28.0 ± 4.5
	42 days	0.91 ± 0.12^b	51.9 ± 9.1^b	38.7 ± 16.4	29.9 ± 3.9 ^b	29.7 ± 21.4

Data represented are means ± standard deviation. Centre of mass position (CoM) is located relative to the proximal end of the segment (trunk CoM is relative to the centre line between the hips), and is shown along the craniocaudal (for trunk) or proximodistal (for limbs) axis (Fig. 2). Data in a column with no common superscript differ significantly at the 0.05 level.

Table 7 – Pelvic limb bone segment dimensions

Age Group	Sample size	Leg Length(cm)	Individual bones (% leg length)		
			Femur	Tibiotarsus	Tarsometatarsus
1 day	10	7.5 ± 0.44^a	30.4 ± 0.68	41.5 ± 1.8	28.0 ± 1.4
14 days	19	14.9 ± 0.93 ^b	29.9 ± 0.6	40.9 ± 1.1	29.2 ± 1.0
28 days	19	21.4 ± 1.3 ^b	29.3 ± 1.1	40.4 ± 1.5	30.2 ± 1.1
42 days	20	26.9 ± 2.2 ^b	29.0 ± 1.1	42.0 ± 1.4	29.0 ± 0.7

Data presented here are for the left pelvic limb only, and are means ± standard deviation. Total leg length is the sum of the individual pelvic limb bones. Absolute values for leg length are presented here, but normalized values (divided by body mass^{1/3}) were used for the statistical analysis to compare how leg length changed across ontogeny (see Fig. 4 for scaling relationship). Data in a column with no common superscript differ significantly at the 0.05 level.

Table 8 - Levene's test and ANOVA results

	Levene's test	Degrees of freedom	F	P
Bone lengths:				
Total Leg Length	< 0.001	3,34.7	968.5	< 0.001
Segment properties:				
Whole Body CoM (cranial-caudal)	0.435	2,12	6.629	0.011
Whole Body CoM (Dorsal-ventral)	0.475	2,11	16.729	< 0.001
Trunk:				
Mass	0.295	2,12	11.638	0.002
CoM position	0.193	2,12	3.227	0.076
Radius of gyration (x)	<0.001	2,7.1	2.088	0.194
Radius of gyration (y)	0.004	2,4.881	9.732	0.020
Radius of gyration (z)	0.171	2,12	4.551	0.034
Thigh:				
Mass	0.180	2,12	13.65	0.001
CoM position	0.547	2,12	30.675	< 0.001
Radius of gyration (x)	0.555	2,11	0.108	0.899
Radius of gyration (y)	0.508	2,12	0.514	0.611
Radius of gyration (z)	0.103	2,12	0.099	0.907
Drumstick:				
Mass	0.002	2,6.885	69.702	< 0.001
CoM position	0.054	2,12	1.261	0.318
Radius of gyration (x)	0.358	2,12	3.902	0.050
Radius of gyration (y)	0.270	2,12	1.024	0.388
Radius of gyration (z)	0.441	2,12	4.533	0.034
Shank:				
Mass	0.583	2,12	4.820	0.029
CoM position	0.503	2,12	7.985	0.006
Radius of gyration (x)	0.129	2,12	31.746	< 0.001
Radius of gyration (y)	0.012	2,5.542	1.297	0.695
Radius of gyration (z)	0.301	2,12	59.342	<0.001
Foot:				
Mass	0.295	2,12	18.969	<0.001
CoM position	0.502	2,12	13.332	0.001
Radius of gyration (x)	0.027	2,6.047	4.376	0.465
Radius of gyration (y)	0.189	2,12	1.920	0.001
Radius of gyration (z)	0.027	2,5.831	2.367	0.717

Degrees of freedom = (df_{between} , df_{within}). Where the assumption of equal variances cannot be met (significant Levene's test result), the Welch statistics are reported.

Figure 1 - A 3D model representation of the broiler showing the body and pelvic limb segments.

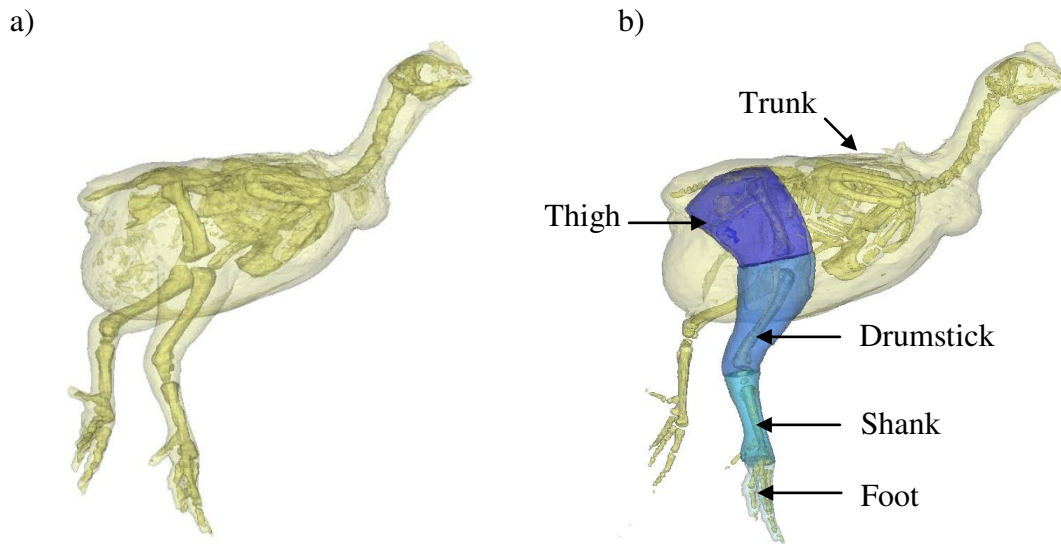
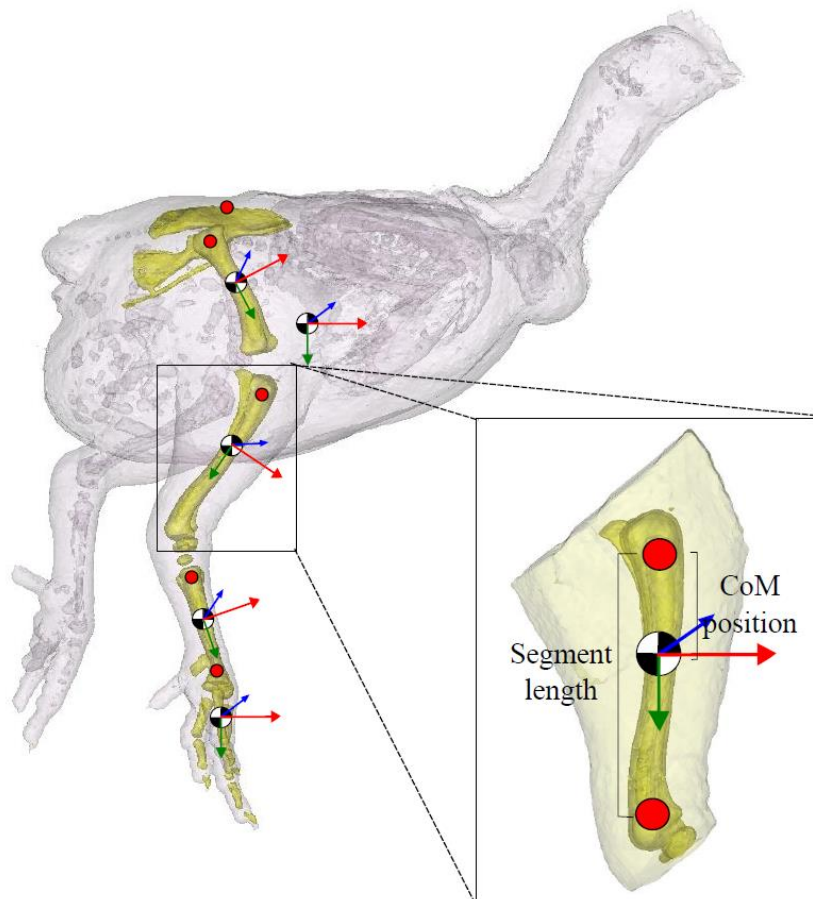


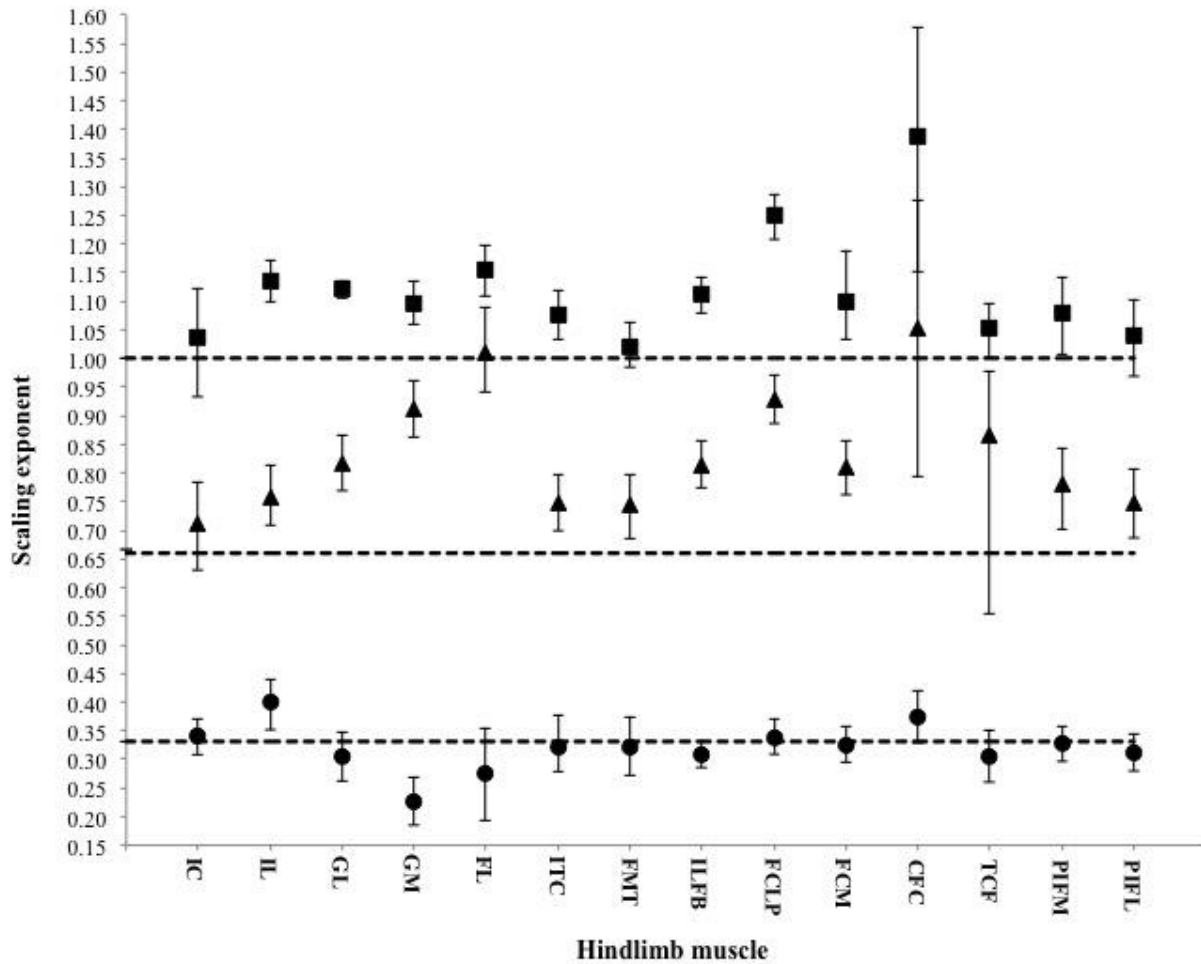
Fig. 1 shows the 3D model representation of the broiler's body and its corresponding segments created within MIMICS software. Each chicken was placed in the same upright position with their left side resting on a radiolucent cushion during scanning. Fig. 1a shows the whole body and skeleton produced within this software and Fig. 1b shows the trunk (leg flesh removed) and pelvic limb segments. The translucent outline represents the 'virtual flesh' of the models used to estimate the inertial properties (mass, centre of mass and radius of gyration) of each segment.

Figure 2 – Segment inertial properties



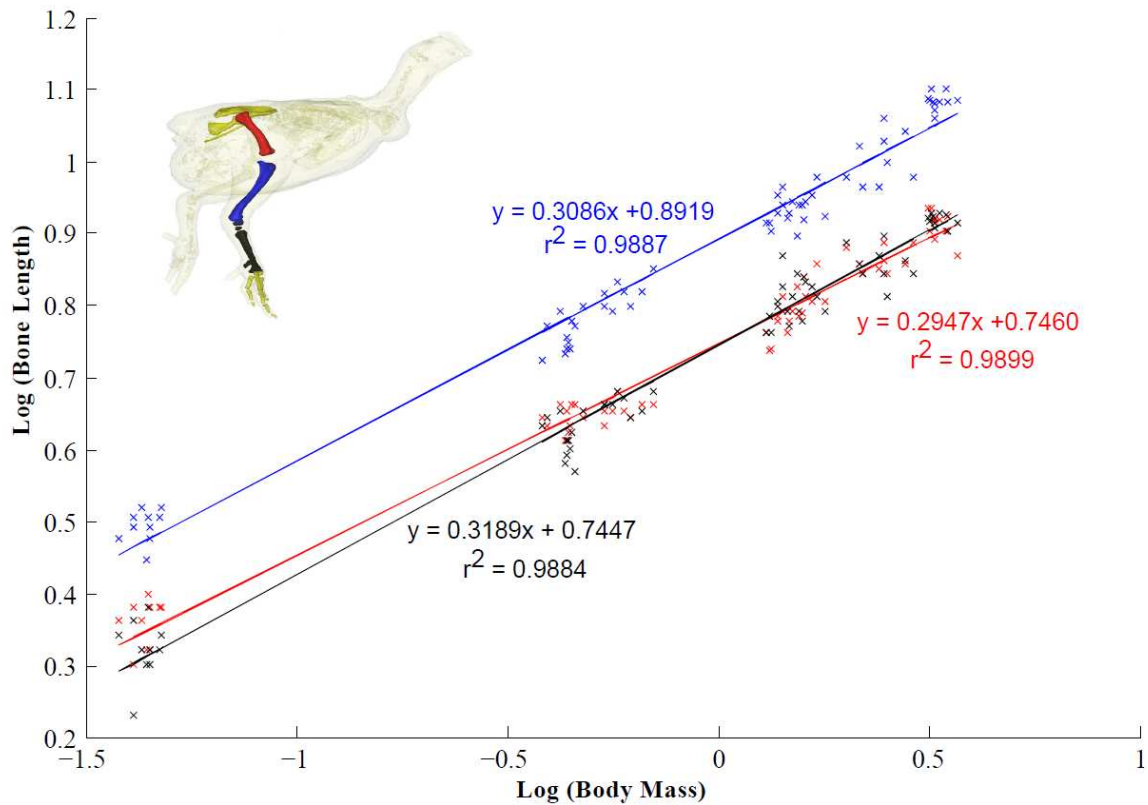
The pelvis, femur, tibia, tarsometatarsus and the bones of the foot are highlighted in this 3D model. Centre of mass (CoM) position is shown (black/ white circle; approximate position given). Trunk CoM was taken relative to the pelvis (centre point between the hips on the pelvis), and for the remaining segments, CoM position was expressed relative to the proximal end of the bone (red markers shown). The local anatomical coordinate system for each segment is given (x (red), y (green) and z (blue)). Segment length (excluding the pelvis) is defined as the distance between the proximal and distal marker on the segment, as shown.

Figure 3 - Scaling exponents of pelvic limb muscle properties as a function of increasing body mass



Symbols indicate the regression slope for muscle mass (squares), physiological cross-sectional area (PCSA; triangles) and muscle fascicle length (circles). Error bars represent upper and lower 95% confidence intervals. Dashed lines represent expected values for isometric (directly proportional) scaling of muscle properties with body mass ($y = 1.0$: muscle mass, $y = 0.67$: PCSA, $y = 0.33$: muscle fascicle length).

Figure 4 – Scaling relationship of the pelvic limb bones (femur, tibiotarsus and tarsometatarsus)



95% confidence intervals (CIs) for the femur, tibiotarsus and tarsometatarsus are 0.2947 ± 0.0104 , 0.3086 ± 0.0114 and 0.3189 ± 0.0120 respectively (scaling exponent \pm CI). Isometric scaling of the tibiotarsus and tarsometatarsus is concluded because the 95% CIs overlapped the expected value (0.33). The femur scaled with slight negative allometry.