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

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. All broilers used had no observed lameness, but we document the limb pathologies identified post mortem, since these two factors do not always correlate, as shown here. The most common leg disorders, including bacterial chondronecrosis with osteomyelitis and rotational and angular deformities of the lower limb, were observed in chickens at all developmental stages. Whole limb 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 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.

1 **TITLE:**

2 Anatomical and biomechanical traits of broiler chickens across ontogeny. Part II. Body segment

3 inertial properties and muscle architecture of the pelvic limb

5 **AUTHORS:**

6 Heather Paxton[1], Peter G. Tickle[2], Jeffery W. Rankin[1], Jonathan R. Codd[2] and John R.

7 Hutchinson[1]

9 **AFFILIATIONS:**

10 [1]Structure & Motion Laboratory, Department of Comparative Biomedical Sciences, The Royal

11 Veterinary College, University of London, Hatfield, Hertfordshire, UK

12 [2]Faculty of Life Sciences, University of Manchester, Manchester, UK

14 **CORRESPONDING AUTHOR:**

15 Heather Paxton, Structure & Motion Laboratory, Department of Comparative Biomedical

16 Sciences, The Royal Veterinary College, University of London, Hatfield, Hertfordshire, UK, +44

17 (0) 1707 666 422, hpaxton@rvc.ac.uk

INTRODUCTION:

36   The poultry industry is a rapidly expanding enterprise (over 870 million broilers

37 slaughtered in the UK; DEFRA 2013 statistics), in which production continues to increase

38 globally by over 130% in some countries (Scanes 2007). In addition to increased production, the

39 broiler chicken has gained an unusual repertoire of anatomical traits, which are repeatedly

40 emphasized in scientific studies and highlight what has developed into a successful breeding

41 programme for obtaining desired production characteristics (e.g. Havenstein et al., 2003; Paxton

42 et al., 2010, 2013). However, a crossroads has been reached where efficient broiler production is

43 haunted by welfare concerns (Julian, 1998; Mench, 2004; Knowles et al., 2008). Broilers may

44 suffer from heart failure and sudden death syndrome (Julian, 1998; Maxwell and Robertson,

45 1998; Olkowski et al., 2007), reduced adaptive immune function (Cheema et al., 2003), leg

46 weakness (see review; Bradshaw et al., 2002), poor reproductive performance (Siegel and

47 Dunnington, 1987; Hocking, 1993) and appear to be susceptible to suboptimal management of

48 nutrition and their environment (e.g. Vestergaard and Sanotra, 1999; Kestin et al., 2001; Scott,

49 2002; Brickett et al., 2007; Buijs et al., 2009). Unfortunately, whilst the welfare and economic

50 concerns associated with these issues are clear, there is no optimal evidence-based solution that

51 resolves all concerns surrounding broiler chicken production.

52   To move toward such a solution, a deeper understanding of how broiler body shape and

53 musculoskeletal function develops during growth and how these changes may influence

54 locomotion is required. Part 1 of this series (Tickle et al., MS) characterized how broiler organ

55 and pectoral muscle growth varies with increasing body mass, with a focus on respiratory system

56 development and changes in organ size, highlighting important repercussions to breathing/

57 cardiorespiratory performance. Here, we delve deeper into how these and other anatomical

58 changes have affected the overall size and shape of the broiler and investigate how relevant traits

59 have likely influenced their locomotor abilities.

60   To help achieve our aim, we detail the muscle architectural properties of the major pelvic

61 limb muscles (identified previously by Paxton et al., 2010 as the hip, knee and ankle extensors in

62 broilers) and document how these scale with body size. Skeletal muscle is one of the organs that

63 is most adaptable to environmental change (Lieber, 1986), and an integral component of

64 locomotion (supporting and powering the movement). Muscle mechanical performance is mostly

65 dependent on a few key architectural properties; namely mass, fascicle length and pennation

66 angle. These parameters are typically used to calculate physiological cross-sectional area (PCSA)

67 and thereby estimate muscular capacity for force-generation (PCSA) versus length change

68 (fascicle length) (Powell et al., 1984; Burkholder et al., 1994; Lieber and Friden, 2000). For

69 broilers, muscle architecture data on the broiler currently exist for animals at six weeks of age

70 (see Paxton et al., 2010), but how these properties change across growth in the broiler is

71 unknown. This study therefore focuses on the scaling (i.e., size related, isometric or allometric;

72 Biewener, 1989; Alexander et al., 1981; Alexander and Ker, 1990) relationships of select pelvic

73 limb muscles to reveal changes in individual muscle characteristics of individual muscles from

74 hatching to slaughter age (~six weeks). We also consider how the pelvic limb bones (femur,

75 tibiotarsus and tarsometatarsus) scale with body size in the broiler chicken, as effective scaling of

76 the hindlimb bones can reduce the rate at which stress increases with body mass (McMahon,

77 1973, 1975; Garcia and da Silva, 2004).

78   In addition, differences in limb orientation and motion, and overall gait dynamics, among

79 avian taxa can be partially attributed to variation in body centre of mass (CoM) position (Gatesy

80 and Biewener, 1991; Abourachid, 1993; Hutchinson, 2004). Manion (1984) estimated CoM

81 position for chickens across ontogeny (5-19 days) and noted a cranioventral shift and a

82 subsequent change in limb orientation during standing and walking (more flexed during standing,

83 but more extended during walking). The broiler CoM position has been addressed in preliminary

84 computational analyses by Allen et al., (2009) and was found to shift caudodorsally. Other

85 previous research suggests that broilers have a more cranially positioned CoM induced by a large

86 pectoral muscle mass (Abourachid, 1993; Corr el al., 2003a). To better understand the influence

87 of CoM on locomotor ability in broilers, we therefore quantify the variation in CoM position

88 (3D) across ontogeny and relate this variation to anatomical changes documented here and in Part

89 I of this study (Tickle et al., MS).

90   All broilers used here are considered 'normal' –i.e. no observed lameness, but we

91 document the limb pathologies (identified post mortem) within our study populations. This is

92 essential, because the pathological changes in affected birds do not often relate to walking ability

93 (assessed using gait score and force plate measurements) (Sandiland et al., 2011). We quantify

94 the inertial properties (mass, centre of mass and radius of gyration) of each limb and major body

95 segment (Fig. 1), because these help reveal basic locomotor habits (e.g. limb tapering –i.e. a

96 proximal to distal reduction in muscle mass reflects a specialization for power versus force

97 development; Alexander et al., 1981; Pasi and Carrier, 2003; Hutchinson, 2004, Payne et al.,

98 2005a, 2005b; Smith et al., 2006). Both centre of mass position and radius of gyration are also

99 good descriptors of body area distribution and resistance to rotational movements (Kilbourne,

100 2013).   Importantly, inertial properties in chickens and more specifically broilers are almost

101 completely unstudied. Only a few studies have documented the inertial properties of other

102 ground running birds (e.g., emus, Goetz et al., 2008; guinea fowl, Daley et al., 2007; Rubenson

103 and Marsh, 2009; quail, Andrada et al., 2013; lapwing, Nyakatura et al., 2012). This study not

104 only provides a novel insight into characteristics that influence broiler locomotor function, but

105 also provides the inertial properties necessary to develop models for dynamic analyses of

106 movement, which have been highly successful in improving our understanding of human

107 pathological gait (e.g. Steel et al., 2012; Fey et al., 2013; Allen et al., 2013).

108

109   **MATERIALS AND METHODS:**

110 *Birds*

111   Fresh male cadavers of a commercial broiler strain at different ages (days 1, 13, 29, 32

112 and 40; Table 1) were used for the hind limb muscle analysis and then a further five broilers of

113 the same commercial strain at approximately 14, 28 and 42 days of age (2, 4 and 6 weeks old)

114 were used to calculate the centre of mass (CoM) of the whole body, trunk and the pelvic limb

115 segments --i.e. the thigh, drumstick, shank and foot (Fig. 1). Hatchlings (day 1) are not included

116 in these CoM data, since our focus is in later stages of their development when breast muscle

117 growth is more evident and leg health issues are apparent. These chickens had been previously

118 killed by cervical dislocation. Pelvic limb bone dimensions (femur, tibiotarsus and

119 tarsometatarsus) were also recorded at 2, 4 and 6 weeks of age. These data are from the same

120 chickens used in Part I (Tickle et al., MS), which focuses on anatomy of the musculoskeletal

121 respiratory apparatus and changes in body and organ size across ontogeny. As our data are cross-

122 sectional rather than longitudinal, this study approximates an ontogenetic analysis, via inference

123 from comparisons among individuals. All chickens were raised in a commercial setting under

124 similar management guidelines and were not outwardly influenced by a laboratory setting. Full

125 ethical approval for this experiment was granted by the RVC Ethics Committee (approval URN

126 No. 2008-0001) under a Home Office license.

127

128 *Pathology*

129   All birds were macroscopically evaluated to establish the incidence of leg pathologies

130 (marked as present/ absent unless otherwise stated; Table 2). Each chicken was examined for

131 bacterial chondronecrosis with osteomyelitis (BCO) in the femur and tibiotarsus, tibial

132 dyschondroplasia (TD), tibial rotation (RT) and valgus/varus deformities (VVD). Tibial rotation

133 above 20º was considered abnormal. Similar to other studies (e.g. Shim et al., 2012) VVD was

134 classified as mild, moderate or severe following the methods described by Leterrier and Nys

135 (1992).

136

137 *Muscle Architecture*

138   The left pelvic limb from each individual (refer to Table 1) was dissected, with fourteen

139 specific muscle-tendon units (see Table 3 for muscle names and abbreviations) being identified

140 and systematically removed. Muscles identified included the main hip, knee and ankle extensors,

141 which are involved in limb support during the stance phase and have been shown to have either

142 massive, short-fibred muscles favouring greater force-generating capacity or have long parallel-

143 fibred muscles for fast contraction (Paxton et al., 2010), as in many other terrestrial species.

144 Architectural measurements taken included muscle mass (M_m; tendon removed), which was

145 measured on an electronic balance (± 0.001g), muscle fascicle length (L_f; ± 1mm), and fibre

146 pennation angle (θ), where appropriate (± 1º). In order to account for variation of fascicle length

147 and pennation angle within a muscle, L_f and θ were calculated as the mean of five measurements

148 made across each muscle. Physiological cross-sectional area (PCSA) was calculated for each

149 muscle (Eq. 1; Sacks and Roy, 1982) from these measurements according to the equation:

150

151
$$PCSA = M_m \cos\theta ¿ \frac{\rho L_f ¿^{-1}}{}$$
    (1)

152

153   Muscle tissue density (ρ) was assumed to be 1.06g cm^{-3}, the standard value for

154 mammalian and avian muscle (Mendez and Keys, 1960; Paxton et al., 2010).

155

156 *Centre of Mass and Inertial Properties*

157   Computed tomography (CT) scans were acquired of five male bird cadavers from each

158 group. The cadavers were scanned with a GE Lightspeed 8-detector scanner at 100mA and 120

159 kVp X-ray beam intensity using a 1mm CT slice thickness. In order to minimise postural effects

160 on CoM estimates, the birds were placed in the same upright position with their left side resting

161 on a radiolucent cushion for scanning and limbs posed as similarly as possible (Fig. 1a). Mimics

162 14.12 imaging software (Materialise; Leuven, Belgium) was then used to segment the resulting

163 DICOM image files, creating a 3D representation of the skeleton, body and the pelvic limb

164 segments (trunk, thigh, drumstick, shank and the foot; Fig. 1b). We used predefined thresholds

165 set for bone and flesh, with some manual adjustment from those baseline values as appropriate to

166 ensure smooth, plausible rendering. Custom software (Hutchinson et al., 2007; Allen et al.,

167 2009)) was then used to estimate the whole body/ segment CoM. This method allows accurate

168 specification of the CoM relative to any user defined point on the body or segment. These points

169 are easily identified using bony landmarks visible on the CT images and are essential for putting

170 CoM position in the context of gross morphology, which is necessary for biomechanical analyses.

171 Using the 3D model representations of the skeleton, whole body CoM position was quantified

172 relative to the right hip, trunk CoM was taken relative to the pelvis (centre point between the hips

173 on the pelvis), and for the remaining segments, CoM position was expressed relative to the

174 proximal end of the bone (Fig. 2). Three-dimensional coordinates (x [craniocaudal], y

175 [dorsoventral] and z [mediolateral]) for the CoM were then recorded. Whole body CoM position

176 was expressed as a percentage of femur length (see Allen et al., 2009).

177 Segment anatomical properties that we measured and present here include segment mass

178 (m; as % body mass), segment length (L; see Fig. 2), centre of mass (as % segment length), and

179 radius of gyration (r; as % segment length), which are essential information required to calculate

180 the moments of inertia (kgm^2; Eq. 3) and subsequently complete the set of inertial properties

181 required for biomechanical analyses:

182

183 $$R = (I\ m^{-1})^{\ 0.5} \qquad\qquad (2)$$

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. The

187 mean difference between the dissected segment mass recorded and that estimated were small (<

188 5%). These data also provide further information on muscle mass distribution within the limb.

189

190 $$I = m(r\ L)^{\ 2} \qquad\qquad (3)$$

191

192 ***Bone Scaling Dataset***

193 Data from Part I of this study were also used for this analysis. The individual bone

194 lengths of the left pelvic limb were recorded (± 1mm) for each of four age groups: day 1 (n=10),

195 ~14 days (n=20), ~28 days (n=19), ~42 days (n=19). Total leg length was defined as the sum of

196 the individual pelvic limb bone lengths. Limb bone proportions were calculated as a percentage

197 of total leg length.

198

Statistical Analysis

200   All of our analyses take into account differences in body size across age groups. To
201   analyse the muscle architecture data, the linear relationship between log-transformed body mass
202   and muscle mass, fascicle length and PCSA were examined using the reduced major-axis
203   regression (RMA) function in the statistical program PAST (Hammer et al., 2001). RMA
204   regression was appropriate for analysis of these morphological characters because error in both x
205   and y variables is considered (Rayner, 1985; Sokal and Rohlf, 1995). Upper and lower 95%
206   confidence intervals (CIs) and the R^2 value for each regression line were calculated to quantify
207   the variation around the mean (Fig. 3, Table 4). Isometric scaling was assumed when the scaling
208   exponent ± 95% CIs overlapped the expected value. Reduced major-axis regression was also
209   used to examine the linear relationship between log-transformed body mass and bone length.
210   This was done using custom Matlab (The Mathworks, Nattick, MA, USA) software code. The
211   95% CIs and the R^2 value for each regression line were also calculated (Fig. 4). Similar to the
212   statistical analysis used for the muscle architecture data, isometric scaling was assumed when the
213   scaling exponent ± 95% CIs overlapped the expected value.

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

221

222   **RESULTS:**

Pathology

224   There were a number of pathological changes in the study population at all stages of
225   development (see Table 2). Bacterial chondronecrosis with osteomyelitis (BCO) was present in
226   all populations both in the femur and proximal tibia. There was an increased incidence of BCO
227   in the femur as the broilers aged, with 88% of the six week old chickens affected. Tibial
228   dyschondroplasia was present in all populations with no apparent correlation with age (average
229   41%). Rotated tibia was more prevalent in the younger birds (~33% of the study population).

230   Valgus angulation of the lower limb was seen only in the six-week old chickens, with

231   approximately 45% of these chickens affected. No varus deformities were observed.

232

233   *Muscle architecture*

234   Across ontogeny, the masses of the major hip, knee and ankle extensor muscles generally

235   scale with positive allometry, increasing in relative size as broilers grew (Table 4). The

236   exceptions were the FMTM (M. femorotibialis medialis), TCF (M. tibialis cranialis caput

237   femorale), PIF (M. puboischiofemoralis) and IC (M. iliotibialis cranialis), which did not change

238   significantly with body mass. Interestingly, fascicle length, in general, did not change with body

239   mass, but scaled isometrically. However, the IL (M. iliotibialis lateralis) muscle showed an

240   increase in fascicle length, whereas the GM (M. gastrocnemius medialis) showed a decrease in

241   fascicle length. Despite these changes in fascicle length, PCSA appeared to increase

242   allometrically across ontogeny in the majority of the pelvic limb muscles. The IC and TCF,

243   however, scaled more in accordance with isometry.

244

245   *Whole Body Centre of Mass*

246   Whole body CoM moved caudodorsally until 28 days of age. By 42 days of age there

247   was a significant cranial shift in CoM position, moving ~10% cranially and ~30% more dorsally

248   in the last two weeks approaching slaughter age (Table 5).

249

250   *Segment Properties*

251   There was a 50-fold range in body mass of our sample of broiler chickens (Table 1),

252   ranging from ~0.04kg at one day old to ~2.4kg at 40 days of age. Trunk mass contribution to

253   whole body mass increased across ontogeny and was ~5% larger at 42 days of age relative to the

254   youngest group. Similarly, a significantly larger relative thigh muscle mass was found in older

255   birds (by ~3% body mass) compared to the younger broilers. Drumstick mass increased

256   significantly with age whereas shank mass remained unchanged. Between 28 and 42 days, the

257   chickens' foot mass also increased significantly by ~30% (see Table 6).

258   Trunk CoM moved caudally between 28 and 42 days, and the thigh, drumstick and shank

259   CoM moved to more proximal positions (Table 5). In contrast, the foot's CoM moved more

260   distally at 42 days of age.

261   The radii of gyration about the axes of long-axis rotation (Table 6) experienced an

262   increase in the foot segments of the six week old broilers and remained unchanged in the thigh,

263   drumstick and shank. However, the radius of gyration of the whole trunk segment showed a

264   decrease for long-axis rotation – i.e. a lower resistance to yaw. The radii of gyration in the

265   parasagittal plane, decreased in the drumstick and shank across broiler ontogeny, but

266   progressively increased in the foot. There was also a relative reduction of *r%* about the axis of

267   abduction/ adduction rotation. Radii of gyration for the thigh remained unchanged.

268

269   ***Bone Scaling***

270   Tibiotarsus and tarsometatarsus length scaled isometrically with body mass, whereas

271   femur length scaled with slight negative allometry (Fig. 4). As a result, there was a relative

272   increase in total leg length from 14 days to 28 days. Limb length remained unchanged between

273   four and six weeks. The femur accounts for ~30% of total leg length and the tibiotarsus accounts

274   for ~41% of leg length. The tarsometatarsus is relatively shorter than the other pelvic limb bones

275   accounting for ~29% of total leg length.

276

277   **DISCUSSION**

278   The genetic success of the modern broiler and the subsequent changes to the morphology

279   of broiler chickens have been well documented, in order to determine the lines' commercial

280   performance (e.g. Gous et al., 1999) and compare both growth responses and physiological

281   adaptations resulting from distinctive selection pressures (e.g. Havenstein et al., 2003a, b;

282   Reddish and Lilburn, 2004; Schmidt et al., 2009). A marked change in total pectoral muscle mass

283   of the commercial broiler is a common finding of all these prior studies. Similarly, we found this

284   mass to represent ~20% of total body mass in slaughter age chickens (see Part I; Tickle et al.,

285   MS). Part I revealed how enlarged pectoral muscle mass, among other anatomical changes, may

286   compromise the efficacy of the respiratory apparatus. Here we show how these changes influence

287   the locomotor ability of the broiler.

288

289   ***Pathology***

290   Leg weakness in broilers comprises not only nonspecific gait problems and lower activity

291   levels, but also a wide range of disorders that are generally classified as infectious, degenerative,

292   or developmental (for a review see Bradshaw et al., 2002). The most common disorders include

293   bacterial chondronecrosis with osteomyelitis (BCO), angular and torsional deformities (e.g.

294   valgus-varus (VVD) and rotated tibia (RT)) and tibial dyschondroplasia (TD). With the

295   exception of VVD, these conditions were observed in birds at all developmental stages in this

296 study. BCO was common in the broilers (Table 2), far exceeding previous estimates of infection

297 in commercial flocks (approximately 0.5%). Incidence of BCO in the femur peaked at 88% of

298 42-day-old birds. This variation is likely due to differences in how these estimates of infection

299 were determined. Femoral head separation (FHS; epiphyseolysis) dominated in our study's birds,

300 which we concur should be attributed to underlying traumatic (osteochondrosis) or infectious

301 (osteomyelitis) femoral head pathology (following Wideman et al., (2013)). FHS is often shown

302 separately in studies to show the progression of the disease (e.g. Wideman et al., 2012; Wideman

303 and Pevzner, 2012; Wideman et al., 2013), but here we are purely interested in the presence or

304 absence of the condition. Furthermore, the high percentage of birds we found to have BCO may

305 reflect a predisposition to the condition or differences in husbandry practices . Increasing

306 occurrence of BCO over development is consistent with previous results that identified peak

307 incidence at around five weeks of age (McNamee 1999a). Increasing incidence over

308 development may reflect increased stresses acting on the bone, which are thought to contribute to

309 BCO (Wideman, 2013). Similarly, BCO in the tibiotarsus was widespread and increased with

310 age, showing a peak at 42 days (Table 2). BCO causes lesions in the load-bearing growth plates

311 of the femur and tibiotarsus, so rapid growth and weight gain may be an aggravating factor when

312 bacterial infection is present. Considering the widespread incidence of BCO in birds that were

313 otherwise deemed healthy, bone lesions are a significant problem affecting welfare standards in

314 broiler chickens.

315   TD commonly leads to growth plate abnormalities, infections and tibial deformation

316 (Lynch et al., 1992) but, similar to BCO, TD does not necessarily induce lameness of sufficient

317 severity to impair walking ability (Pattison, 1992). TD has been found to occur between 2 and 8

318 weeks of age and our findings are consistent with this observation (Table 2). However,

319 considerable variation exists in the reported prevalence of TD, ranging in 42 day old birds from

320 approximately 2% to 50% of total flock population. Incidence of TD in this study was relatively

321 high and occurred in all age groups, peaking at 28 days (57%). However, determining why the

322 incidence of TD is high in this study is difficult because the condition reflects a complicated

323 interaction of contributing factors, including dietary deficiencies, toxins, genetic predisposition

324 and rapid growth rate .

325   Valgus-varus deformities (VVD) were observed in 42-day-old birds, but was not present

326 in younger broilers (Table 2). Comparable reports indicate that VVD occurs with varying

327 incidence, affecting as few as 0.5% to 30-40% of birds in a flock . The prevalence of VVD in

328 this study fell near the high end of the reported range, with 45% of birds at 42 days of age

329 observed to have mild or moderate VVD. Our observation that symptoms of VVD occur only in

330 older broilers is consistent with the progressive nature of this deformity . However, no deformity

331 was seen in 28-day-old birds, which is perhaps surprising because this is approximately the age

332 that VVD often becomes prominent , although the timing of onset is known to vary .

333 Rotated Tibia (RT) occurred in birds at all developmental stages at a higher than expected

334 rate (i.e., value) compared to previous work (0.2%, Bradshaw et al., 2002)). However, Bradshaw

335 et al., reported a reduced proportion of RT in older broilers, which perhaps indicates that affected

336 birds were culled because the condition becomes clearly obvious around 21 days of age . The

337 exact aetiology of RT is unknown; however TD and VVD may exacerbate the incidence of RT ,

338 thereby contributing to the relatively high proportion of birds with an outward torsion of the tibial

339 shaft.

340 Overall, there is a clear need to monitor the leg health of flocks, not only to aid breeders

341 to make adjustments to management practices or genetics when necessary, but also to quickly

342 identify lame birds for euthanasia on welfare grounds. The high incidence of leg pathologies

343 highlights the problem of maintaining high growth rates and breast muscle mass (M_b) at the

344 expense of broiler anatomy and physiology. In addition, lameness represents a significant

345 economic cost to the industry as birds with leg weakness are prematurely culled or have an

346 increased incidence of mortality (Mench, 2004).   Efforts to improve the health of growing

347 broilers will have the twin benefit of improving both welfare standards and productivity.

348

349 ***Muscle Architecture***

350 Architectural properties used to calculate the effective physiological cross-sectional area

351 (PCSA) (Gans and Bock, 1965) of muscle take into account the effect of pennate fascicles on

352 maximizing force per unit area. PCSA is thus greater in pennate muscles and is directly

353 proportional to its force generation capacity (Burkholder et al., 1994; Lieber and Friden, 2000).

354 In broilers, the PCSA of the major hip, knee and ankle extensors (essential for supporting body

355 mass and maintaining an upright standing posture; Gatesy 1999; Reilly, 2000; Hutchinson, 2004),

356 scale with positive allometry – i.e., these muscles have a greater force-generating capacity

357 (reflected in their relatively larger PCSAs) as the broiler develops (Table 4, Fig. 3). As a result,

358 muscular force production capacity in broiler chickens should increase with age, likely as a direct

359 consequence of weight vs. force scaling constraints imposed by resisting gravity and inertia (e.g.,

360 Corr et al., 2003). However, these muscles still have smaller force-generating capabilities and

361 shorter, presumably slower-contracting muscles than their wild counterpart, the Giant Junglefowl

362   (Paxton et al., 2010). Broiler chickens appear to generally increase the PCSA of their pelvic limb

363   muscles by increasing muscle mass, rather than by increasing fascicle length, which scaled

364   isometrically (Table 4, Fig. 3). This increase in mass is likely due to increased hypertrophy

365   (increase in muscle fibre size), which is well known to occur in broiler skeletal muscle and is the

366   assumed dominant model for postnatal growth (Aberle and Stewart, 1983; Soike and Bergman,

367   1998; Remignon et al., 1994; Goldspink and Yang, 1999). In addition, muscular force production

368   is invariant to muscle fascicle length, but longer fascicles exact a metabolic cost because a larger

369   volume of muscle is activated for each Newton of force (Kram and Taylor, 1990; Roberts et al.,

370   1998). Thus, the isometric scaling of fascicle length we observed in this study avoids such added

371   costs.

372   Interestingly, the PCSAs of the M. iliotibialis cranialis (IC) and M. tibialis cranialis caput

373   femorale (TCF) scale more in accordance with isometry. The relative force-generating capacity

374   of these muscles therefore remains unchanged throughout the growth of the broiler. The TCF is a

375   knee extensor and ankle flexor and is assisted by other muscles that also serve as knee extensors

376   and ankle flexors (e.g., M. femorotibialis and M. extensor digitorum longus). Similarly, the IC is

377   also a knee extensor, but additionally acts as a hip flexor, supported by the M. iliotrochantericus

378   caudalis (ITC; known to be significantly larger in the broiler [Paxton et al., 2010])) to flex and

379   medially rotate the femur. The additional support of these muscles may help to explain why the

380   IC and TCF scale in unusually isometric ways. The IC and TCF may be redundant, especially

381   when limb motion in these broilers is likely to be 1) knee-driven (e.g. Gatesy, 1999; Reilly, 2000;

382   Hutchinson and Allen, 2009), requiring action of the major knee flexors (*M. iliofibularis,*

383   *M.flexor cruris lateralis),* and 2) three-dimensional, demanding large supportive forces at the hip

384   for the considerable mediolateral forces they experience when they walk (Paxton et al., 2013).

385   However, biomechanical analyses of *in vivo* function are needed to test how much their function

386   alters with growth in broilers.

387

388   ***Centre of Mass and Inertial Properties***

389   At the youngest age (14 days old) studied here, chickens' trunk mass accounted for ~75%

390   of total body mass. At slaughter weight (around 42 days of age), total trunk mass had increased

391   to ~80% total body mass. The ~5% increase in trunk mass is largely attributable to pectoral

392   muscle growth, which occurred at a relatively faster rate than body mass (see Part I Tickle et al.,

393   2014).

394   Interestingly, relative hind limb segment mass (summed segment masses; muscle and

395   bone mass combined) did not decrease during growth, accounting for ~15% of total body mass at

396   slaughter age and thus representing a total 5% body mass increase across ontogeny. The

397   proportion of bone mass contributing to total segment mass is likely small because the muscle to

398   bone ratio is known to be high in commercial broilers (Ganabadi et al., 2009). The increase in leg

399   mass was instead incurred by increases in drumstick and thigh muscle mass. Drumstick segment

400   mass increased across ontogeny, becoming relatively larger at each age category, whereas thigh

401   segment mass only had substantial changes during the last two weeks of growth (from 28 – 42

402   days old). Thigh muscle mass increased by ~3% of total body mass during this period. Changes

403   in thigh and drumstick segment mass are expected, as these segments yield the most meat and are

404   the most consumed portions (alongside breast meat) on the market (Broadbent et al., 1981).

405   However, the increase in hind limb segment mass is striking and comparable to a progenitor

406   population (total limb muscle mass ~16%) at the same approximate physiological mass and

407   indeed larger than its wild counterpart at the same age by ~4% body mass (see Paxton et al.,

408   2010). Previous studies typically show an ontogenetic reduction in the investment of metabolic

409   resources towards pelvic limb muscle growth (e.g. Berri et al., 2007; Schmidt et al., 2009) and

410   the main drivers of selection in broiler chickens are still a greater yield of breast muscle mass and

411   a faster post-hatch growth rate (Arthur and Albers, 2003). Thus, changes in leg muscle mass may

412   not reflect a direct difference in selection pressures. However, a relative increase in hind limb

413   muscle mass may reflect a functional demand for larger hip and knee extensors to support their

414   increasing body mass. Corr et al., (2003a) studied two strains of birds (relaxed and selected)

415   raised on two different feeding regimes and suggested that the large pectoral muscle mass of the

416   broiler has displaced their CoM cranially. Similarly, Abourachid (1993) suggested that increased

417   stresses on the pelvic limbs of heavier broad-breasted turkeys were induced by a more cranially

418   positioned CoM. We found that broilers show a change in whole body CoM position consistent

419   with these previous findings, shifting from a caudodorsal to a craniodorsal location between 28

420   and 42 days of age, which would increase demand for muscular force production to balance it.

421   Interestingly, the centre of mass of the Giant Junglefowl (a representative progenitor population)

422   has been shown to move caudodorsally, not craniodorsally, across ontogeny. This cranial shift in

423   broilers may therefore be the direct result of increased pectoral muscle mass growth between four

424   and six weeks of age.

425   In all cases, the craniocaudal CoM location in broilers was far more cranial than previous

426   estimates. Allen et al., (2009) estimated CoM cranial position to be ~38% of femur length,

427   compared to the 70-90% femur length estimated in this study. On the other hand, dorsoventral

428   estimates broadly corresponded to literature values (Allen et al., 2009). The difference in

429   craniocaudal CoM position likely relates directly to pectoral muscle growth. The pectoral muscle

430   mass of the broilers used in this study yielded an additional ~4% of total body mass in

431   comparison to the broilers used by Allen et al., (2009). One limitation of our study is that whole

432   body CoM was normalized by femur length, which did scale with slight negative allometry (see

433   discussion below). Using femur length may introduce a slight bias to our results, but

434   normalization by other factors including total limb length have been shown to yield the same

435   result (Allen et al., 2009).

436   There are also substantial changes in the distal segment of the pelvic limb between 28

437   and 42 days. Foot mass increases by ~30% and foot CoM moves distally. Large feet may serve

438   to improve the apparent stability reported in the broiler (e.g. Corr et al., 2003b) during the stance

439   phase of gait. However, larger feet will influence the broiler's ability to accelerate and decelerate

440   the limb during swing, which in turn can affect the metabolic cost of locomotion (Kilbourne,

441   2013; Kilbourne and Hoffman, 2013). Both an increase in mass and a more distal shift in the

442   limb's mass distribution will effectively increase a limb's moment of inertia, (i.e., resistance to

443   angular acceleration, Steudel, 1990; Wickler et al., 2004; Kilbourne and Hoffman, 2003). Broiler

444   pelvic limbs would therefore require more metabolic energy to accelerate and decelerate them as

445   the birds grow. However, the influence of mass is much smaller than the influence of the

446   distribution of the mass (radii of gyration reported here) on the moment of inertia. Doubling

447   mass would essentially double the moment of inertia, whereas doubling the radii of gyration

448   would increase the moment of inertia four-fold (Eq. 3). The radii of gyration in the parasagittal

449   plane and about the axes of long-axis rotation increased in the foot segments of the ~42 day old

450   broiler. Thus, increased radii of gyration in the foot segment of the broiler contribute

451   significantly to the limb's moment of inertia.

452   We found that broilers' whole pelvic limb morphology changed across ontogeny, with the

453   main changes in the thigh and foot segments. These segments had increased muscle mass and a

454   more distal mass distribution (rather than having relatively longer limbs; see bone scaling

455   discussion), resulting in relatively larger moments of inertia. The changes in limb morphology

456   are likely to assist in supporting the increased supportive forces required by a more cranially

457   positioned CoM and to help improve stability during locomotion. However, these changes also

458   likely exact a relatively higher metabolic cost to locomotion.

459

Bone Scaling

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

Here we have considered how pelvic limb morphology changes during broiler ontogeny, in coordination with other changes such as pectoral muscle mass. Together, these changes have influenced broiler morphology across ontogeny, which may have influenced locomotor ability as well. The relative force-generating capacity of the hind limb muscles is greater in older broilers, and is primarily achieved through increasing muscle mass but maintaining a constant fascicle length. Increases in thigh segment mass and a relative increase in the moment of inertia of the distal limb (due mainly to increased foot size) may reflect adaptations to cope with the apparent instability and a more cranially positioned CoM as broilers grow. Although the architectural changes we have observed have obvious advantages for maintaining an upright posture and forward progression of broiler chickens, these morphological changes likely have a negative impact on locomotion, exacting relatively higher metabolic costs during growth, which may have knock-on consequences for activity levels and even overall health.

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Figure 1

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.
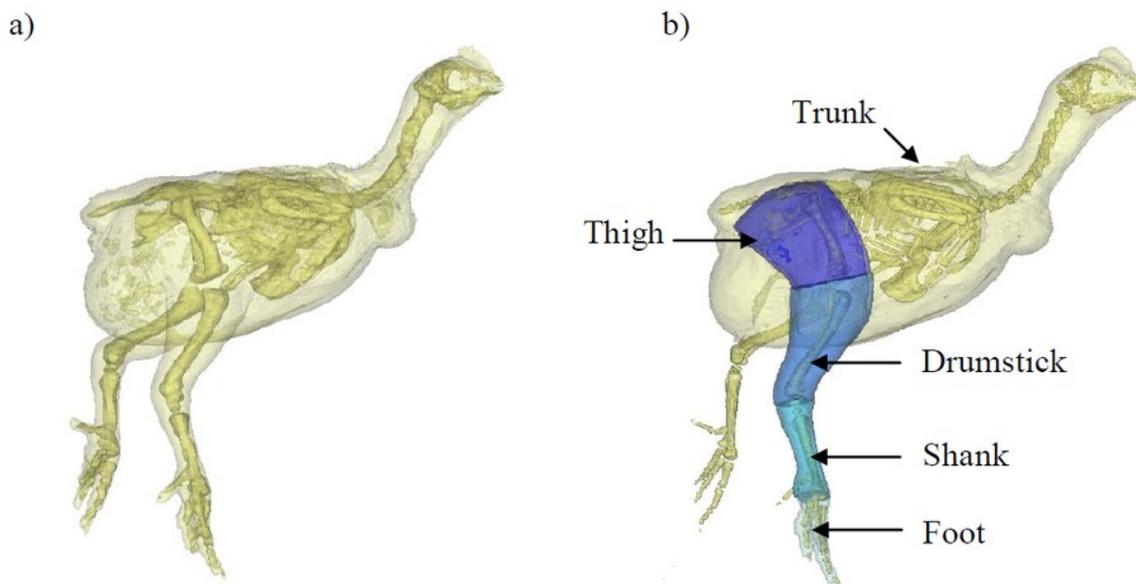
Figure 2

Figure 2 – Segment inertial properties

The pelvis, femur, tibiotarsus, 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 positionwas 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.

Segment length

CoM position
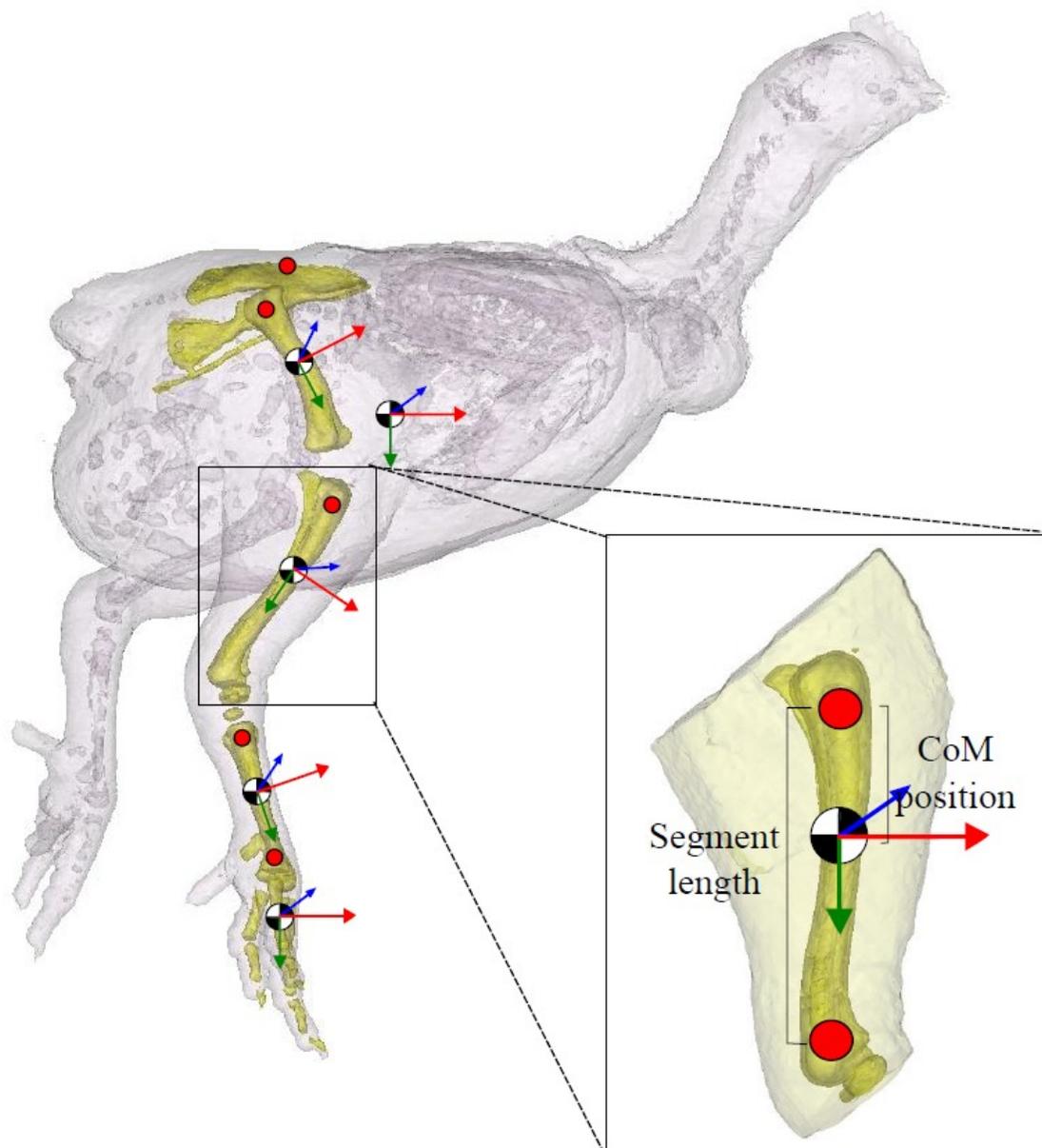
Figure 3

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 barsrepresent 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).
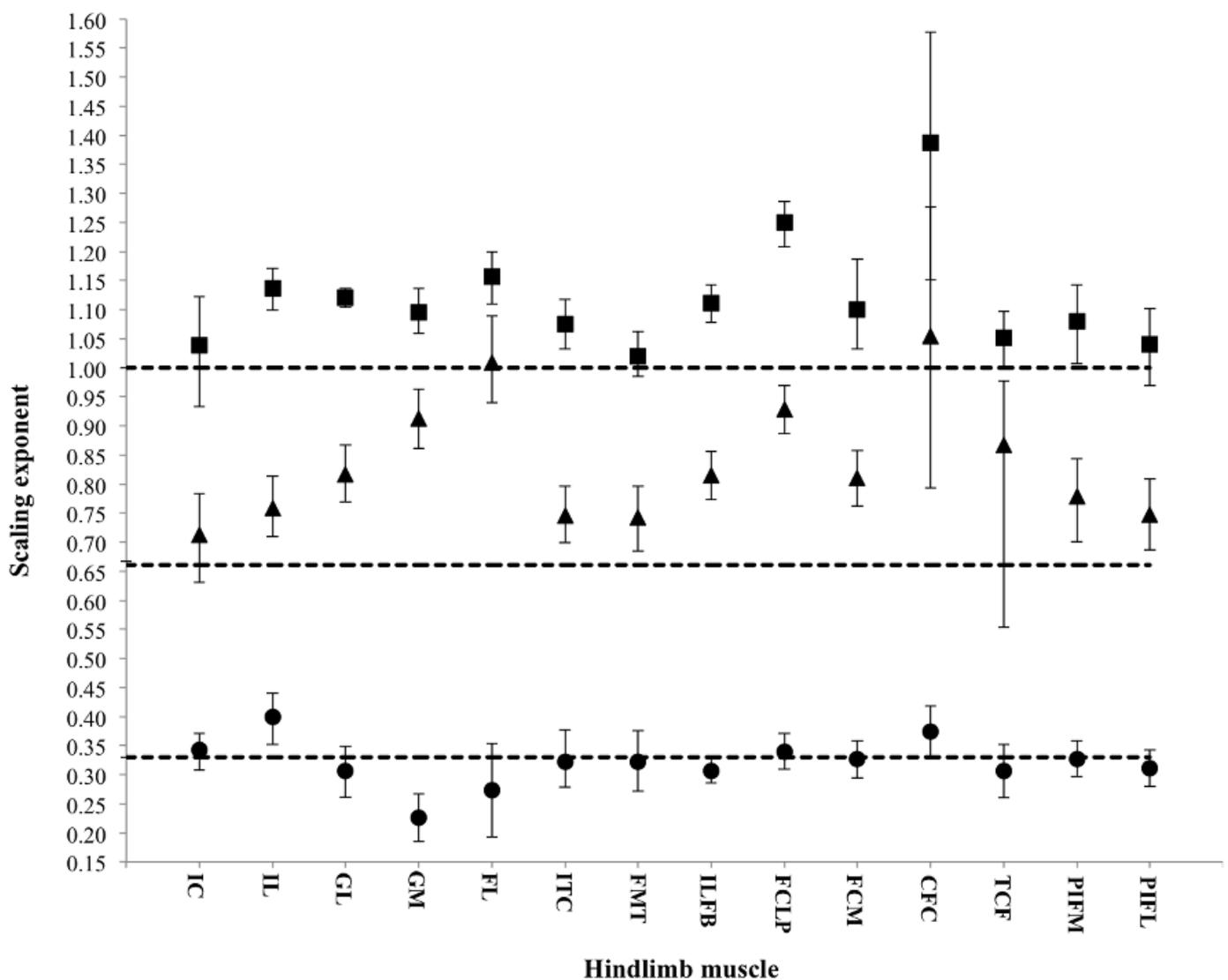
Figure 4

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 (scalingexponent ± 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.
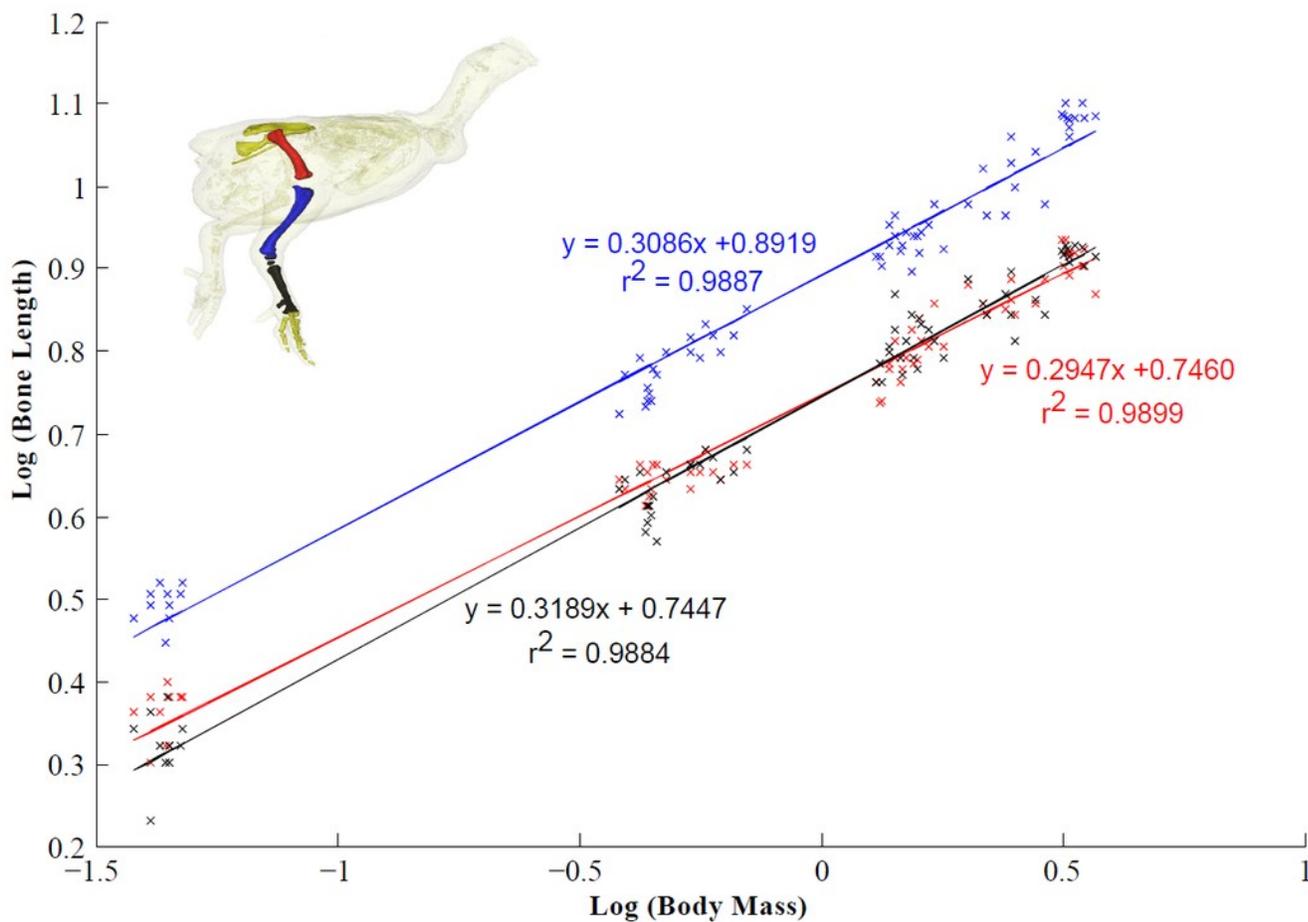
Table 1 – Subject data

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 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.

Table2 – Incidence of pathology across growth in broiler chickens

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 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.

Table3 – The major muscles of the chicken pelvic limb

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. iliotrochantericus 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

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 4 – RMA regression analyses

Muscle	M_m expected slope: 1.00 Slope	Lower 95% CI	Upper 95% CI	R^2	PCSA expected slope: 0.67 Slope	Lower 95% CI	Upper 95% CI	R^2	L_f expected slope: 0.33 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	*0.226 (-)*	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 ($p < 0.05$).

Table5 – Whole body centre of mass position

Data represented are means ± standard deviation. Centre of mass (CoM) position is calculated as a percentage of femur length and is expressed relative to the right hip joint of the chicken. Craniocaudal and dorsoventral positions are shown. Data with no common superscriptdiffer significantly at the 0.05 level.

Table 5 – Whole body centre of mass position

| Age Group | CoM Position (% femur length) | |
	Craniocaudal	Dorsoventral
14 days	90.6 ±10.7[1]	**89.8 ± 13**[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 and is expressed relative to the right hip joint of the chicken. 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

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 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	$78.0 \pm 1.8^{a,b}$	20.1 ± 4.3	24.0 ± 1.2	40.0 ± 1.7^b	$37.1 \pm 1.8^{a,b}$
	42 days	81.7 ± 3.2^b	15.3 ± 2.7	33.3 ± 12.0	$\mathbf{37.1 \pm 1.8^a}$	37.8 ± 0.8^b
Thigh	14 days	5.19 ± 1.4^a	$\mathbf{43.7 \pm 4.3^a}$	47.2 ± 8.5	44.1 ± 7.4	49.6 ± 14.5
	28days	5.22 ± 0.4^a	$\mathbf{38.1 \pm 2.0^b}$	48.3 ± 6.2	40.6 ± 4.2	52.1 ± 8.8
	42 days	$\mathbf{8.21 \pm 1.0^b}$	$\mathbf{28.7 \pm 2.4^c}$	46.2 ± 7.0	42.5 ± 4.3	52.3 ± 7.1
Drumstick	14 days	$\mathbf{3.9 \pm 0.3^a}$	30.1 ± 14.2	49.1 ± 6.3	24.9 ± 2.9	$\mathbf{51.0 \pm 6.1^a}$
	28days	$\mathbf{4.6 \pm 0.5^b}$	32.7 ± 2.3	40.0 ± 5.3	25.6 ± 4.0	$40.2 \pm 7.1^{a,b}$
	42 days	$\mathbf{5.66 \pm 0.2^c}$	24.2 ± 4.2	34.0 ± 12.4	21.2 ± 11.7	$\mathbf{34.3 \pm 12.2^b}$
Shank	14 days	0.98 ± 0.1^a	$\mathbf{36.1 \pm 10.3^a}$	50.5 ± 1.2^a	18.1 ± 0.2	50.8 ± 1.1^a
	28days	$1.02 \pm 0.1^{a,b}$	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	$\mathbf{38.0 \pm 4.1^b}$	23.3 ± 20.7	$\mathbf{37.1 \pm 2.7^b}$
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	$\mathbf{21.2 \pm 6.3^a}$	28.0 ± 4.5
	42 days	$\mathbf{0.91 \pm 0.12^b}$	$\mathbf{51.9 \pm 9.1^b}$	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.

Table7 – Pelvic limb bone segment dimensions

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 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.

Table8 - Levene's test and ANOVA results

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.

	Levene's test	Degrees of freedom	F	P	Table 8 -
Bone lengths:					**Levene's**
Total Leg Length	< 0.001	3,34.7	968.5	< 0.001	**test and**
Segment properties:					**ANOVA**
Whole Body CoM (cranial-caudal)	0.435	2,12	6.629	0.011	**results**
Whole Body CoM (Dorsal-ventral)	0.475	2,11	16.729	< 0.001	
Trunk:					Degrees of
Mass	0.295	2,12	11.638	0.002	freedom =
CoM position	0.193	2,12	3.227	0.076	($df_{between}$,
Radius of gyration (x)	<0.001	2,7.1	2.088	0.194	df_{within}).
Radius of gyration (y)	0.004	2,4.881	9.732	0.020	Where the
Radius of gyration (z)	0.171	2,12	4.551	0.034	assumption
Thigh:					of equal
Mass	0.180	2,12	13.65	0.001	variances
CoM position	0.547	2,12	30.675	< 0.001	cannot be
Radius of gyration (x)	0.555	2,11	0.108	0.899	met
Radius of gyration (y)	0.508	2,12	0.514	0.611	(significant
Radius of gyration (z)	0.103	2,12	0.099	0.907	Levene's test
Drumstick:					result), the
Mass	0.002	2,6.885	69.702	< 0.001	Welch
CoM position	0.054	2,12	1.261	0.318	statistics are
Radius of gyration (x)	0.358	2,12	3.902	0.050	reported.
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	