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

Heather Paxton, Peter G Tickle, Jeffery W Rankin, Jonathan R Codd, John R Hutchinson

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 andmoving). 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.

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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 el 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 20º 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 (± 0.001g), muscle fascicle

144   length (L_f; ± 1mm), and fibre pennation angle (θ), where appropriate (± 1°). 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:

149

150    $$PCSA = M_m \cos\theta \, (\rho L_f)^{-1} \hspace{3cm} (1)$$

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:

182

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

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.

190

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

192

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 (± 1mm) 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.

199

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

6

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 ± 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 ± 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:**

228   ***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. puboischiofemoralis) 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   **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 epiphyeolysis 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. iliotrochantericus caudalis (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

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

14

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

488

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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. 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

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	*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 ($p < 0.05$).

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
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	$78.0 \pm 1.8^{a,b}$	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.

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.
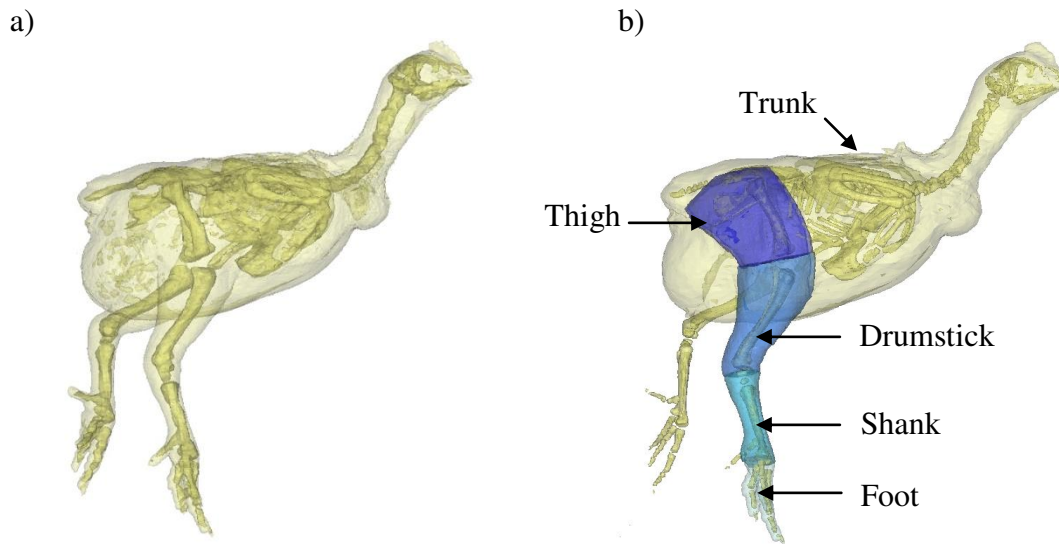
a)

b)

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**
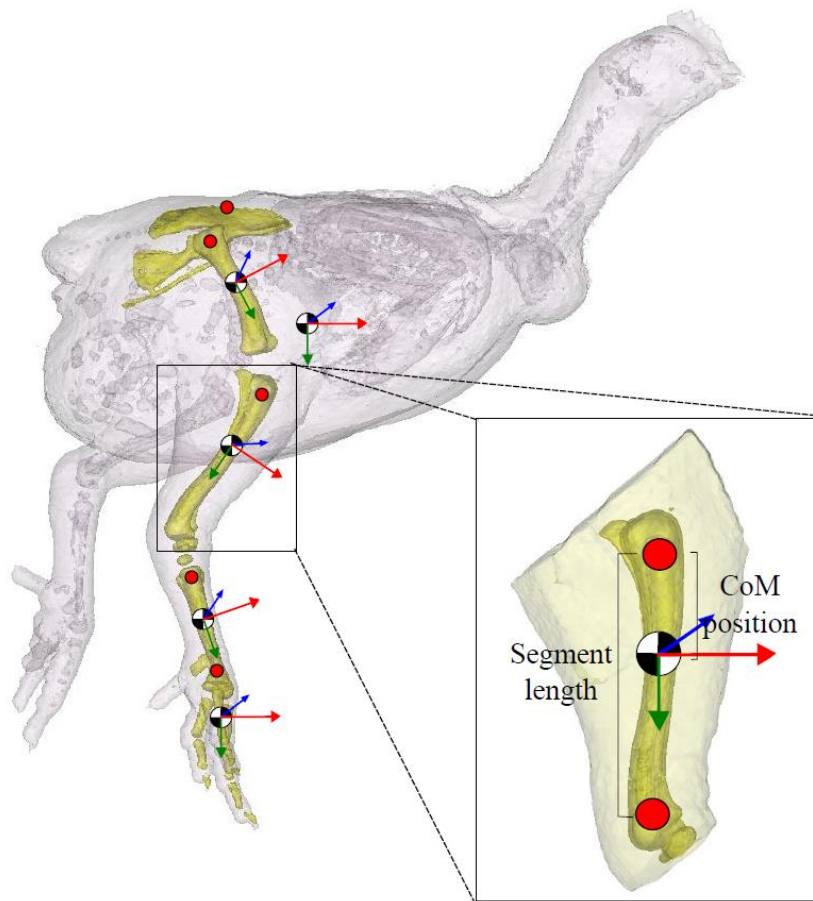
The pelvis, femur, tibiotarsus, tarsometatarsus and the bones of the foot are highlighted in this 3D model. Centre of mass (CoM) position is shown (black/ white circle; approximate position given). Trunk CoM was taken relative to the pelvis (centre point between the hips on the pelvis), and for the remaining segments, CoM 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
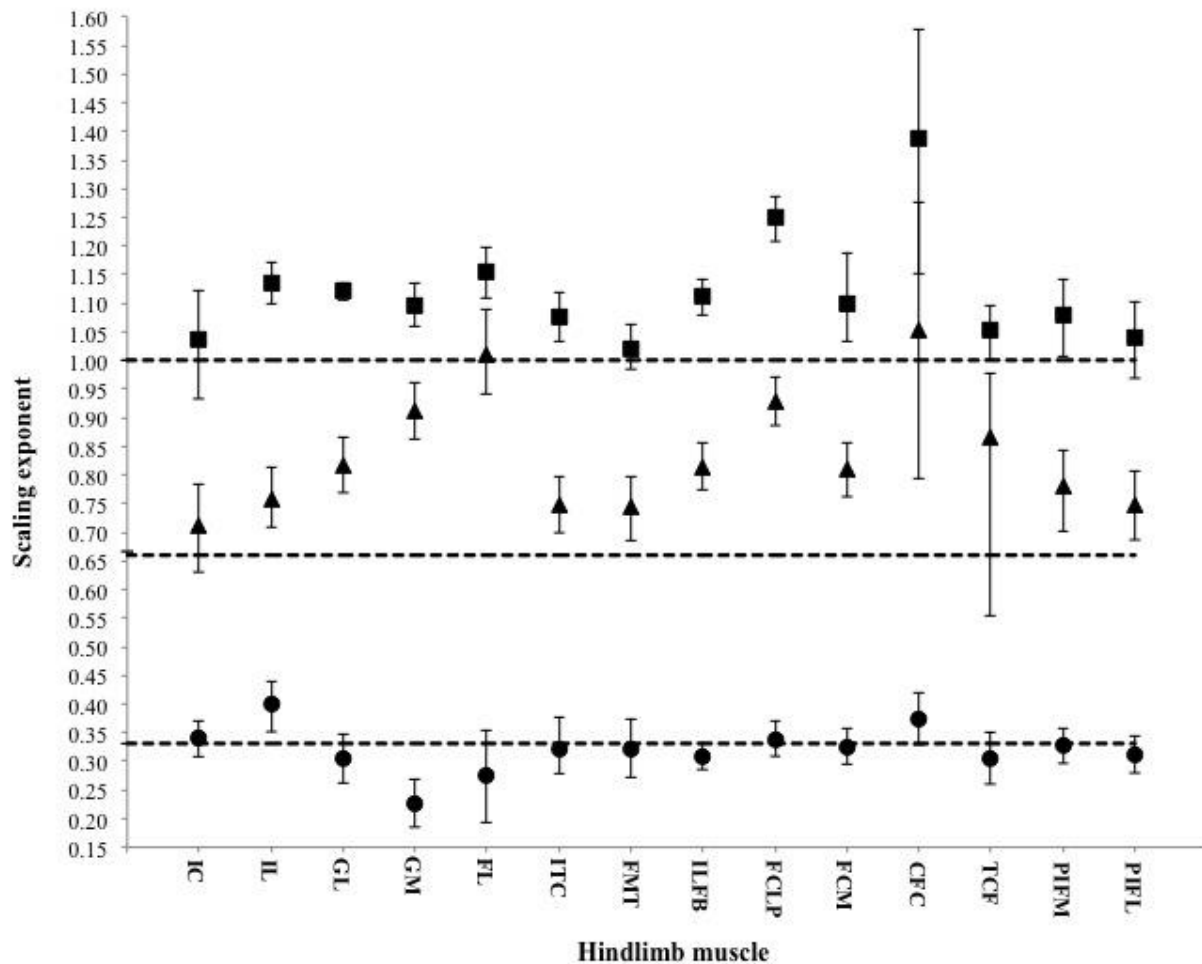
Symbols indicate the regression slope for muscle mass (squares), physiological cross-sectional area (PCSA; triangles) and muscle fascicle length (circles). Error bars represent upper and lower 95% confidence intervals. Dashed lines represent expected values for isometric (directly proportional) scaling 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)
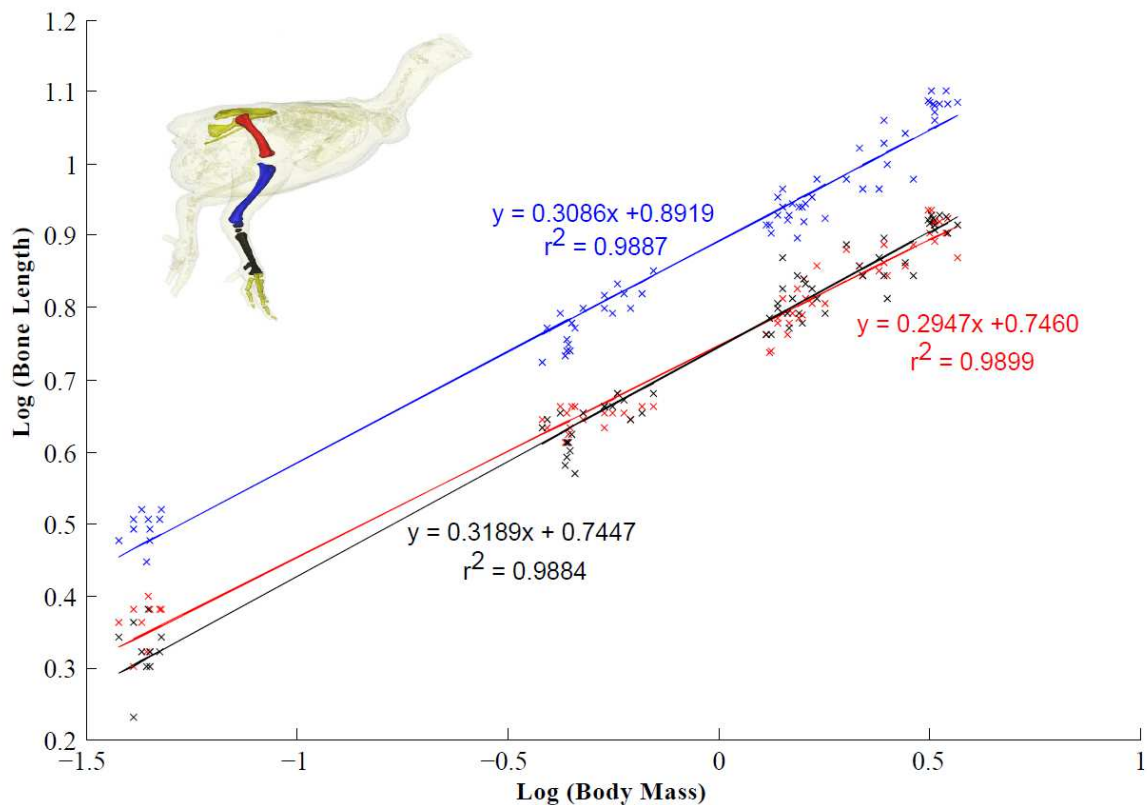
95% confidence intervals (CIs) for the femur, tibiotarsus and tarsometatarsus are 0.2947 ± 0.0104, 0.3086 ± 0.0114 and 0.3189 ± 0.0120 respectively (scaling exponent ± CI). Isometric scaling of the tibiotarsus and tarsometatarsus is concluded because the 95% CIs overlapped the expected value (0.33). The femur scaled with slight negative allometry.