Anatomical and biomechanical traits of broiler chickens across ontogeny. Part I. Anatomy of the musculoskeletal respiratory apparatus and changes in organ size.

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Genetic selection for improved meat yields, digestive efficiency and growth rates have transformed the biology of broiler chickens. Modern birds undergo a 50-fold multiplication in body mass in just six weeks, from hatching to slaughter weight. However, this selection for rapid growth and improvements in broiler productivity is also widely thought to be associated with increased welfare problems as many birds suffer from leg, circulatory and respiratory diseases. To understand growth-related changes in musculoskeletal and organ morphology and respiratory skeletal development over the standard six-week rearing period, we present data from post-hatch cadaveric commercial broiler chickens aged 0, 2, 4 and 6 weeks. The heart, lungs and intestines decreased in size for hatch to slaughter weight when considered as a proportion of body mass. Proportional liver size increased in the two weeks after hatch but decreased between 2 and 6 weeks. Breast muscle mass on the other hand displayed strong positive allometry, increasing in mass faster than the increase in body mass. Contrastingly, less rapid isometric growth was found in the external oblique muscle, a major respiratory muscle that moves the sternum dorsally during expiration. Considered together with the relatively slow ossification of elements of the respiratory skeleton, it seems that rapid growth of the breast muscles might compromise the efficacy of the respiratory apparatus. Furthermore, the relative reduction in size of the major organs indicates that selective breeding in meat-producing birds has unintended consequences that may bias these birds toward compromised welfare and could limit further improvements in meat-production and feed efficiency.

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ABSTRACT

7   Genetic selection for improved meat yields, digestive efficiency and growth rates have

8   transformed the biology of broiler chickens. Modern birds undergo a 50-fold multiplication in

9   body mass in just six weeks, from hatching to slaughter weight. However, this selection for rapid

10   growth and improvements in broiler productivity is also widely thought to be associated with

11   increased welfare problems as many birds suffer from leg, circulatory and respiratory diseases. To

12   understand growth-related changes in musculoskeletal and organ morphology and respiratory

13   skeletal development over the standard six-week rearing period, we present data from post-hatch

14   cadaveric commercial broiler chickens aged 0, 2, 4 and 6 weeks. The heart, lungs and intestines

15   decreased in size from hatch to slaughter weight when considered as a proportion of body mass.

16   Proportional liver size increased in the two weeks after hatch but decreased between 2 and 6

17   weeks. Breast muscle mass on the other hand displayed strong positive allometry, increasing in

18   mass faster than the increase in body mass. Contrastingly, less rapid isometric growth was found

19   in the external oblique muscle, a major respiratory muscle that moves the sternum dorsally during

20   expiration. Considered together with the relatively slow ossification of elements of the respiratory

21   skeleton, it seems that rapid growth of the breast muscles might compromise the efficacy of the

22   respiratory apparatus. Furthermore, the relative reduction in size of the major organs indicates

23   that selective breeding in meat-producing birds has unintended consequences that may bias these

24   birds toward compromised welfare and could limit further improvements in meat-production and

25   feed efficiency.

26   **INTRODUCTION**

27   Genetic selection in domesticated broiler chickens has brought about significant

28   improvements in the form of increasing meat yields and growth performance. Growth rates in

29   intensively reared industrial broiler chickens have consistently accelerated such that a 300%

30   increase has been engineered in the past 60 years, from 25g per day in the 1950s to 100g per day

31   in the modern bird (Knowles, et al., 2008). Consequently the optimal slaughter mass of

32   approximately 3kg is reached in six rather than 16 weeks (Griffin and Goddard, 1994;

33   Havenstein, et al., 1994a; Govaerts, et al., 2000). Maximising pectoral (breast) muscle mass is a

34   primary target for selection. Compared to ancestral varieties, pectoral hypertrophy in the broiler

35   chicken has resulted in an approximate doubling in muscle size, making up ~20% of total body

36   mass in the modern bird (Havenstein, et al., 2003b; Schmidt, et al., 2009). Mounting evidence

37   suggests that selection for such economically desirable traits in the modern broiler has been

38   accompanied by reduced welfare (Julian, 1998; Knowles, et al., 2008) and increased mortality

39   (Havenstein, et al., 1994a; Havenstein, et al., 2003a). Considerable research is being directed

40   toward understanding welfare problems such as the multitude of leg pathologies that may affect

41   locomotion in broiler chickens (Kestin, et al., 1992; Bradshaw, et al., 2002; Corr, et al., 2003a;

42   Corr, et al., 2003b; Knowles, et al., 2008; Paxton, et al., 2010), cardiac (Wilson, et al., 1988) and

43   pectoral (Randall, 1982) myopathies, pulmonary hypertension (Wideman, 2001) and ascites

44   (Wilson, et al., 1988; Julian, 1993). The prevalence of these conditions indicates that further

45   improvements in industry-efficiencies and meat production may be constrained by the

46   physiological capabilities of broilers because skeletal, cardiac, respiratory and digestive systems

47   appear to be close to their functional limit.

48   Characterising the relationships between body mass and organ, skeleton and muscle size

49   are crucial to our understanding of animal physiology. Physical scaling rules determine the

50   structural and functional consequences of changes in size and therefore exert a profound effect on

51   organismal form (Schmidt-Nielsen, 1984). Understanding the relative growth and size of organs

52   and muscles is important in broilers as it can help to better understand the diseases that they

53   suffer from. For example, the broiler heart and brain become progressively smaller as a

54   proportion of body mass over development, unlike in ancestral breeds (Jackson and Diamond,

55   1996; Schmidt, et al., 2009). In contrast, selection for faster growth and muscle mass is reflected

56   in the proportionally greater intestine mass and accelerated pectoral growth in broiler chickens

57   (Jackson and Diamond, 1996; Konarzewski, et al., 2000; Schmidt, et al., 2009). These

58   relationships demonstrate how artificial selection in the broiler has resulted in developmental

59   trade-offs; reallocation of resources to maximize nutrient absorption and pectoral muscle mass

60   has coincided with a relative decrease in the size of other organs (Havenstein, et al., 1994b;

61   Jackson and Diamond, 1996; Havenstein, et al., 2003b; Schmidt, et al., 2009). To better

62   understand the effects of intensive selection on broilers, more information is required on how

63   increasing body mass and growth rate has shaped their anatomy and physiology across ontogeny.

64   Therefore, in this paper we present data on how organ and muscle growth varies with increasing

65   body mass in a commercial broiler strain.

66   Broiler chickens suffer from respiratory problems that may be related to their rapid

67   musculoskeletal development (Julian, 1998) and their potential to outgrow pulmonary capacity

68   (Wideman, 2001). The avian respiratory system can be considered as a two-part mechanism

69 comprising a pump (musculoskeletal) and gas exchanger (lung). The primary ventilatory

70 mechanism in birds consists of dorsal and ventral movements of the ribs and sternum (Zimmer,

71 1935; Claessens, 2009) that affect air sac volume, thereby facilitating a unidirectional flow of air

72 through the lung (Bretz and Schmidt-Nielsen, 1971; Scheid and Piiper, 1971). Uncinate

73 processes, which are small bones that extend caudodorsally from the vertebral ribs, have been

74 shown to function as levers that assist rib and sternal movements during breathing (Tickle, et al.,

75 2007). Respiratory movements of the skeleton generate pressure changes within the thorax that

76 are necessary to drive inhalation and exhalation, both of which are active processes, driven by

77 respiratory muscle activity (Codd, et al., 2005). Respiratory muscle activity requires metabolic

78 energy consumption. However, studies in guinea fowl (*Numida meleagris*) (Markley and Carrier,

79 2010) have demonstrated that breathing constitutes only 2% of whole-organism metabolism

80 (Markley and Carrier, 2010). In contrast, research on load carrying (Tickle, et al., 2010) and

81 behavioural energetics (Tickle, et al., 2012) indicate a higher cost of breathing in barnacle geese.

82 In particular, carrying extra weight on the sternum, analogous to increased pectoral muscle mass

83 seen in broiler chickens, is energetically expensive compared to an equivalent weight carried on

84 the back (Tickle, et al., 2010). Furthermore, barnacle geese have been shown to achieve

85 metabolic savings by changing posture; when compared to sitting, standing is associated with a

86 25% higher resting metabolic rate (Tickle, et al., 2012). The higher cost of standing has been in

87 part attributed to the energetic cost of moving the heavy weight of the sternum with each breath,

88 which does not occur while sitting (Tickle, et al., 2012). Understanding movements of the

89 sternum are important when considering how selection has shaped morphology in domestic birds

90 due to the selection for enhanced pectoral growth in meat-producing domestic ducks (Gille and

91 Salomon, 1998; Maruyama, et al., 1999), turkeys (Swatland, 1979) and broiler chickens

92 (Havenstein, et al., 1994b; Govaerts, et al., 2000; Havenstein, et al., 2003b; Schmidt, et al.,

93 2009).

94 Only limited information is available on the developmental biology of the avian

95 respiratory skeleton. In the domestic turkey (*Meleagris gallopavo*) a trend for delayed

96 ossification, especially in the uncinate processes, was identified that potentially constrained

97 respiratory performance due to the decreased rigidity of cartilaginous compared to fully ossified

98 bones; cartilaginous uncinate processes will yield under muscle strain before comparable ossified

99 bone would, making them less effective levers (Tickle and Codd, 2009). Furthermore, the growth

100 trajectory of respiratory muscles in the broiler is unknown. Scaling of respiratory muscle growth

101 in proportion to overall body size and, perhaps most importantly when we consider the dorso-

102 ventral breathing movements of the sternum, the pectoral muscles is likely a key factor in
103 determining how effectively and efficiently breathing functions in broiler chickens. To better
104 understand the respiratory problems apparent in broiler chickens, an analysis of how the skeleton
105 develops is necessary. Here we present a description of developmental changes in the
106 musculoskeletal elements of the broiler chicken respiratory system together with an evaluation of
107 how organ size scales with increasing body mass.

108 **MATERIALS AND METHODS**

109 ***Specimens***

110 Broiler chickens from a popular commercial strain were obtained from a commercial supplier.
111 Birds were sampled in a post-hatch growth series between days 1 and 42 (i.e. weeks 0 to 6),
112 corresponding to a 50x range in total body mass (M_b) (Table 1).

113 ***Musculoskeletal growth***

114 M_b, pectoralis major (pectoralis), pectoralis minor (supracoracoideus) and external
115 oblique muscle mass (M_m), wing and ribcage mass, fibre length (L_f) and pennation angle (θ) were
116 measured using an electronic balance (±0.001g), ruler (±1mm) and protractor (±1°). To account
117 for variation within muscle architecture, L_f and θ were calculated as the mean of five
118 measurements in each muscle. Physiological cross-sectional area (**PCSA**) was calculated for each
119 muscle (Eq. 1; Sacks and Roy, 1982):

120
$$\text{PCSA} = M_m * \cos\theta / \rho * L_f \tag{1}$$

121 Density of muscle tissue (ρ) was assumed to be 1.06g cm^{-3} (Mendez and Keys, 1960; Paxton *et al*,
122 2010). Linear measurements of the sternum and uncinate processes were recorded using a ruler
123 (±1mm). Average length of the uncinate processes was calculated from those occurring on ribs 2–
124 5 since these processes were found in all specimens. Girth was measured around the
125 circumference of the thorax, tucked under the wings. One-way ANOVA was used to test for
126 differences in morphology, using mean values of specimens from each developmental stage.
127 Justification for using parametric analysis was based upon the results of a Shapiro-Wilk test and
128 the normal quantile-quantile plot that assessed assumptions of data normality (results displayed in
129 Appendix).

130   The scaling relationships between musculoskeletal characters and body mass were

131   determined using reduced major axis (**RMA**) linear regression, a method that is suitable since it

132   takes into account variation in both x and y axes ([Rayner, 1985](#); [Sokal and Rohlf, 1995](#)). All

133   regression analyses were performed on log10-transformed data to establish allometric equations

134   in the form:

135

136                     $$\log y = \log a + b \log x \qquad\qquad (1)$$

137   where a represents the intercept and the exponent b represents the slope of the line equation.

138   Upper and lower 95% confidence intervals (**CI**) and the R^2 value were calculated for each

139   regression line slope. RMA analyses were performed in the PAST statistical program ([Hammer,](#)

140   [et al., 2001](#)). Assuming geometric similarity (i.e., isometry) over ontogeny, all dimensions are

141   expected to scale in proportion to each other meaning that lengths should scale to $M_b^{0.33}$, areas to

142   $M_b^{0.67}$ and masses to $M_b^{1.00}$. Isometric scaling was assumed where the regression slope ±95% CI

143   overlapped the expected value.

144   ***Histological staining***

145   The ossification pattern of the thoracic skeleton over ontogeny was examined using the

146   histochemical staining protocol of Tickle and Codd (2009). All muscle tissue was removed using

147   dissection, the preparation cleaned by immersion in a 1% potassium hydroxide (KOH) solution

148   and then the skeleton was treated with solutions of alcian blue (uptake corresponds to cartilage)

149   and alizarin red (uptake corresponds to bone). Photographs of stained specimens were taken

150   using a light microscope (Leica MZ9s; Leica Microsystems, Milton Keynes, UK) and

151   subsequently analysed in Leica image analysis software. For comparison of structural properties,

152   relative area of bone and cartilage was calculated for the uncinate process that projects from the

153   fourth vertebral rib in all specimens ([Tickle and Codd, 2009](#)).

154   **RESULTS**

155   ***Organ Development***

156   Heart and lung mass follow a negative allometric growth pattern, decreasing relative to

157   body mass over the six-week growth period (Tables 1 and 2; Fig. 1A). Heart mass decreases from

158   0.74% to 0.53% of body mass over the growth period, while proportional lung mass reduces by

159   almost half, decreasing from 1.04% to 0.55% (Tables 1 and 2; Fig. 1B). Proportional liver mass

160   significantly increased between 0 and 2 weeks, reached a peak of 3.63% on day 14, then

161   decreased between 2 and 6 weeks when it accounted for 2.48% of M_b (Table 1; Fig. 1C). Taking

162   all data into account indicated that overall liver mass followed an isometric growth pattern; i.e.,

163   in direct proportion to increasing M_b (Table 2). Repeating the scaling analysis for birds only aged

164   between 0 and 2 weeks found positive allometric growth, $M_b^{1.10}$ while birds aged between 2 and 6

165   weeks had negative allometric growth, $M_b^{0.76}$ (Table 2; Fig. 1C). Total intestine mass was found to

166   strongly decline as a proportion of M_b over growth with a negative allometric regression slope of

167    $M_b^{0.75}$ (Tables 1 and 2; Fig. 1D).

168   *Carcass Parts*

169   No significant difference was detected between proportional wing masses with increasing age,

170   indicating a directly proportional relationship with M_b. After accounting for variation due to body

171   mass, girth significantly increased during growth (Table 3), reflecting a relative lateral expansion

172   of the thorax. In contrast, as a proportion of M_b, ribcage mass was significantly lower at day 28

173   than at days 14 or 42 (Table 3) whereas normalised keel length displayed a trend for increased

174   length, being highest in 42-day birds (Table 3).

175   *Thoracic Anatomy*

176   Pectoralis major and minor (i.e., M. pectoralis and M. supracoracoideus) M_m increased as a

177   proportion of M_b over development, showing strong positively allometric growth (Table 4 and 5).

178   The growth of these muscles was defined by two phases: an initial rapid increase in M_m between

179   weeks 0 and 2 was followed by a relatively slower increase between weeks 2 and 6 (Table 5; Fig.

180   2). Sternal keel length and depth developed with positive allometry, increasing above the

181   expected geometric scaling exponent ($M_b^{0.33}$) (Table 6), while mean uncinate process length

182   scaled to $M_b^{0.30}$, indicating reduced length with increasing body mass. Growth of external oblique

183   muscle was found to increase in direct proportion to increasing body mass (Table 6).

184   *Skeletal Development*

185   Ossification of the uncinate processes commences at around the time of hatch; 15-day embryos

186   have entirely chondrified processes whereas one-day old chicks show 40% ossification (Table 7;

187   Fig. 3). Uncinate process bone synthesis proceeds from the midpoint in proximal and distal

188   directions and overall bone area plateaus at ~77% of total uncinate process area at 40 days old.

189 The remaining 23% remains cartilaginous, shared between tip and base (Table 7). At hatch the

190 sternum exhibits ossification in the most proximal portion in addition to centres of ossification in

191 the caudolateral processes. While bone growth extends distally along the sternum, at slaughter

192 age ossification of the sternal keel remains incomplete (Fig. 3)

193   **DISCUSSION**

194 ***Organ Development***

195   Our observations confirm a decrease in heart and lung mass relative to body mass over

196 development (Tables 2 and 3). These findings mirror the reduction in relative heart (Havenstein,

197 et al., 1994b; Govaerts, et al., 2000; Thaxton, 2002; Havenstein, et al., 2003b; Schmidt, et al.,

198 2009) and lung mass (Havenstein, et al., 1994b; Govaerts, et al., 2000; Havenstein, et al., 2003b)

199 seen over ontogeny in Ross-type broiler breeds. While absolute mass of the heart is higher than

200 found in unselected lines, proportional mass is lower at hatching and the difference progressively

201 increases over development (Schmidt, et al., 2009). Similarly, lung mass progressively declines

202 as a proportion of body mass over development in our broiler strain, mirroring the negative

203 allometric lung growth in Ross broilers ($M_b^{0.84}$ compared to $M_b^{0.86}$ in this study) (Govaerts, et al.,

204 2000). Considering our results together with previous reports indicates that reduced circulatory

205 and respiratory capacity is an unintended consequence of genetic selection for rapid growth and

206 high M_b. The reduction in broiler heart and lung mass compared to slower-growing, lighter breeds

207 can be considered to be a contributing factor in the increased mortality and disease in modern

208 broilers (Wideman, 2001; Havenstein, et al., 2003b). Further developments in genetic selection

209 for increasing growth and improving breast yields may be constrained by limited respiratory and

210 circulatory functional capacity. The increased incidence of physiological problems and mortality

211 (Havenstein, et al., 1994a; Havenstein, et al., 2003a) in modern broilers indicates that these

212 systems are already working close to maximal levels to satisfy the physiological demands of

213 growth.

214   A complex pattern of liver growth was revealed, with organ mass growing proportionally

215 faster than overall body mass growth in the two weeks after hatch, followed by a proportional

216 decrease in mass between two weeks and six weeks post-hatch. Liver development in Ross-type

217 broilers follows a similar pattern of proportionally decreasing after a period of rapid growth soon

218 after hatch (Govaerts, et al., 2000; Schmidt, et al., 2009). Furthermore, Ross broiler peak liver

219   mass as a proportion of M_b was reached on day 7 (3.80%) (Schmidt, et al., 2009) and day 8

220   (3.38%) (Govaerts, et al., 2000), although the findings presented here do not include birds

221   sampled at these stages. Nevertheless, proportional liver mass at day 14 is similar between our

222   study's and Ross birds (3.15% (Govaerts, et al., 2000); or approximately 3.5% (Schmidt, et al.,

223   2009) of M_b. The relatively fast pace of growth in the first two weeks, followed by proportional

224   decline, indicates that the liver matures rapidly, ready for the transition from fat-rich yolk stores

225   to a predominantly carbohydrate diet (Schmidt, et al., 2009). Genetic selection for digestive

226   efficiency in broilers may directly influence the developmental profile of the liver due to the

227   important function it plays in carbohydrate and fat metabolism.

228   Total intestine mass declined relative to M_b during growth, which is similar to previous

229   reports of proportional decline in intestine mass over development in Ross-type broilers (Iji, et

230   al., 2001; Schmidt, et al., 2009). Absolute and proportional values of the intestine are higher in

231   this study, potentially in part due to a larger intestine in this commercial broiler strain and also in

232   part due to methodological differences; here we report intestine mass and contents rather than the

233   empty intestine mass (Iji, et al., 2001). Exhaustion of the yolk sac contents presumably accounts

234   for the large (6.9%) decline in proportional intestine mass during the first 14 days of post hatch

235   development (Viera and Moran, 1999). After this period, relative intestine mass decreases by

236   approximately 2-2.5% every 14 days (Table 1), which is similar to previous reports (calculations

237   using the data provided in table 2 of Iji *et al.* (2001) and figure 5 of Schmidt *et al* (2009) indicate

238   an approximate decrease of 2.4%). Schmidt *et al* (2009) reported complicated post-hatch

239   intestinal growth, with positive allometric growth in the first seven days followed by a sustained

240   period of negative allometric growth, possibly reflecting an early maturation of the digestive

241   system (Govaerts, et al., 2000). To balance this relative mass loss and maintain high feed

242   efficiency, morphometric changes occur during this period to increase surface area for absorption

243   of nutrients from ingested food (Schmidt, et al., 2009).

244   ***Carcass Parts***

245   While proportional wing mass did not significantly change over growth the overall

246   average yield of 7.0% is below that reported in Ross broilers (8.6%: Havenstein*,* et al, 1994b;

247   7.9%: Havenstein, et al, 2003b). The absence of significant change in wing mass is consistent

248   with the absence of flight as a locomotor mode and the reliance on leg muscles for terrestrial

249   locomotion, and occurs despite steep increases in pectoral muscle mass. Girth in 42-day-old

250   broilers was very similar to a recent report for pureline and broiler birds (Paxton, et al., 2013).

251 Presumably the rapid increase in pectoral muscle mass is a factor in the reduced proportional
252 ribcage mass over development. Normalised sternal keel length was found to increase over
253 growth, indicating that the area available for pectoral muscle attachment is greater as the birds
254 reach slaughter weight, consistent with the rapid proportional increase in pectoral mass.

255 ***Thoracic Anatomy***

256   The relationship between increasing pectoral mass and body mass was positively
257 allometric, indicating that the breast muscles became proportionally much larger over the six-
258 week growth period. Allometric relationships were stronger than those reported for Ross broilers
259 (Govaerts, et al., 2000; Schmidt, et al., 2009), indicating that the birds in this study were able to
260 lay down breast muscle more rapidly during development. Overall breast muscle size as a
261 proportion of M_b was similar to the values reported for Ross (Govaerts, et al., 2000; Havenstein,
262 et al., 2003b; Schmidt, et al., 2009) and similar commercial strain (Paxton, et al., 2013) broilers.

263   Allometric scaling was found for sternal keel dimensions; this proportional increase in the
264 size of the sternum over development presumably enables fast growth of the breast muscles. In
265 contrast, average uncinate process length was found to decrease over growth, scaling with a slight
266 negative allometry. Uncinate processes act as levers for movement of the ribs and sternum during
267 breathing, and overall length is an important factor that determines the magnitude of this leverage
268 (Tickle, et al., 2007). Therefore, the relative shortening of uncinate processes might correspond to
269 a decrease in ventilatory performance during growth.

270   Considering that sternal mass (i.e. breast muscle, (Table 6)) increases with positive
271 allometry during growth it was expected that the external oblique muscle would undergo similar
272 growth to maintain functional efficacy. This muscle attaches to the base of uncinate processes and
273 the sternal margin and is active during the exhalation phase of breathing, when it elevates the
274 sternum dorsally (Codd, et al, 2005). Our data indicate however that the external oblique muscle
275 becomes progressively smaller in comparison to the breast muscles as the birds age, and may be
276 less able to maintain adequate expiration. Further evidence is provided by the isometric growth of
277 L_f and PCSA, since maximum force-generating capacity increases in direct proportion to muscle
278 area (Lieber and Friden, 2000). To our knowledge, no comparable data on respiratory muscle
279 development exist, so it is difficult to establish whether this pattern of growth is unique to the
280 broiler or more widespread among ancestral fowl breeds and other species. Coupled with the
281 decreased proportional heart and lung masses, however, selection for meat-producing traits
282 appears to have the undesirable effect of compromising the engine of aerobic metabolism; i.e.,

283 the respiratory system. Clearly more research into the respiratory anatomy in birds is required to
284 understand the potential trade-offs between breast growth and breathing performance.

285 ***Skeletal Development***

286   The broiler skeleton ossifies following a pattern similar to other domestic poultry (Hogg,
287 1980; Maxwell, 2008; Tickle and Codd, 2009). Ossification of skeletal characters, however, is
288 seen to develop at different time points. For example, the long bones of the leg and wing are
289 almost entirely ossified at hatch, while the sternum, tip of the scapula and proximal and distal
290 portions of the ribs ossify after hatch (Fig. 3). Ossification of uncinate processes begins around
291 the time of hatch, in agreement with an earlier report in the chicken (Hogg, 1980), when bone
292 replacement of cartilage becomes apparent in the midpoint of the shaft (Table 7; Fig. 3). Rapid
293 synthesis of bone tissue must occur in the days immediately prior to hatch, considering that the
294 processes are entirely chondrified six days before hatch (Table 7). Onset of ossification therefore
295 coincides with the initial *in ovo* respiratory skeleton movements and transition from
296 chorioallontoic gas exchange to pulmonary ventilation (Menna and Mortola, 2002); perhaps this
297 mechanical stimulus prompts remodeling of uncinate process structure toward ossified tissue.
298 Uncinate process growth in the broiler is similar to development in the domestic turkey (Tickle
299 and Codd, 2009) in that ossification remains incomplete, with a plateau in bone growth at around
300 80% of total process area. An interesting difference between domestic turkey and chicken is the
301 lack of a bony symphysis between the base of the process and adjoining rib in the broiler (Tickle
302 and Codd, 2009). A cartilaginous base may have significant implications for the functional
303 efficacy of the uncinate process because they act as force-amplyfying levers for muscle
304 contractions during rib movement (Tickle, et al., 2007). The cartilaginous portion of uncinate
305 processes has a lower elastic modulus, or is less stiff, than the ossified area which might
306 compromise the lever function of the uncinate processes (Tickle and Codd, 2009). It is possible
307 that an ossified connection between uncinate process and rib would form later in development, as
308 is seen in the domestic turkey between days 64 and 94 post-hatch (Tickle and Codd, 2009),
309 suggesting that immaturity of the skeleton even at slaughter weight may have a functional impact
310 on breathing in the broiler.

311   Ossification of the sternum is incomplete over the six-week period, with specifically the
312 distal portion of the keel remaining chondrified. The cartilaginous distal portion of the sternum is
313 common to meat-producing and laying strains (Breugelmans, et al., 2007) and presumably
314 reflects the reduced or negligible importance of flight as a means of locomotion in domestic fowl;

315 without need for a sturdy anchor to accommodate and dissipate the strong contractions of
316 pectoral muscles during flapping flight, it seems the energetic resources are directed to growing
317 muscle tissue rather than synthesis of bone for the sternum.

318   The rapid development of body mass in broilers is not mirrored by accelerated
319 ossification of the respiratory skeleton. Instead the skeleton appears to develop in a pattern
320 similar to other poultry, indicating that this process is tightly conserved within galliform birds.
321 Given the potential compromise in respiratory performance due to cartilaginous skeletal elements
322 coupled with a large sternal weight, selection for faster and increased ossification may provide a
323 benefit to broiler health.

324   Here we have considered the how the growth trajectories of major organs and respiratory
325 elements are affected by increasing body mass in broilers. In contrast to the strong pectoral
326 growth, major organs become proportionally smaller with age and development of the respiratory
327 pump is delayed. Our findings indicate that selection for rapid growth and large breast muscles
328 could have the unintended consequence of negatively affecting broiler physiology by
329 compromising the function capacity of cardiovascular and respiratory systems.

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Internal organ growth over development.

Anatomical and biomechanical traits of broiler chickens across ontogeny. 1. Anatomy of the musculoskeletal respiratory apparatus and changes in organ size. Internal organ mass as a proportion of total body mass (M_b) over development. Data are mean ± standard deviation. Asterisk (*) denotes significant differences at the 0.05 level.

Table 1

Age (days)	n	M_b (kg)	Heart (% M_b)	Lung (% M_b)	Liver (% M_b)	Intestine (% M_b)
1	10	0.04 ± 0.003*	0.74 ± 0.05	1.04 ± 0.20*	2.99 ± 0.34*	17.1 ± 2.71*
14	10	0.59 ± 0.06*	0.71 ± 0.10	0.75 ± 0.16*	3.63 ± 0.30	9.89 ± 1.05*
28	9	1.40 ± 0.09*	0.66 ± 0.06	0.72 ± 0.09*	3.10 ± 0.32	8.27 ± 0.90*
42	13	3.27 ± 0.21*	0.53 ± 0.07*	0.55 ± 0.12*	2.48 ± 0.22	5.34 ± 0.51*

Scaling relationship between organ and body mass.

The regression slope indicates the proportional change of organ mass with increasing body mass, and 95% confidence intervals are shown (95% CL). Isometric (=) and negative allometric (-) growth are indicated by symbols.

Table 2

	N	Slope	Lower 95% CL	Upper 95% CL	R^2
Heart	69	0.91 (-)	0.89	0.94	0.98
Lungs	62	0.86 (-)	0.83	0.90	0.97
Liver (wks. 0 - 6)	69	0.95 (-)	0.92	1.00	0.98
Liver (wks. 0 - 2)	30	1.10 (+)	1.06	1.15	0.99
Liver (wks. 2 - 6)	59	0.76 (-)	0.72	0.80	0.95
Intestine	69	0.75 (-)	0.72	0.78	0.98

Morphological examination of the external body.

Data represented are mean ± standard deviation. Following the principles of geometric similarity (Alexander et al., 1981), girth and keel length are normalised by body mass$^{1/3}$ to negate the effect of body size on our data. Asterisk (*) denotes significant differences at the 0.05 level.

Table 3

Age (days)	n	Wings (% M_b)	Girth	Keel length	Rib Cage (% M_b)
14	10	7.5 ± 0.6	0.21 ± 0.01*	0.08 ± 0.004*	10.0 ± 1.1
28	9	7.8 ± 0.5	0.23 ± 0.01*	0.07 ± 0.004*	7.49 ± 0.9*
42	12	5.8 ± 0.4	0.25 ± 0.01*	0.10 ± 0.005 *	8.9 ± 0.7

Breast muscle growth over development.

Anatomical and biomechanical traits of broiler chickens across ontogeny. 1. Anatomy of the musculoskeletal respiratory apparatus and changes in organ size. Breast muscle mass (% M_b) over development. Data are mean ± standard deviation. Asterisk (*) denotes significant differences at the 0.05 level.

Table 4

Age (days)	Pectoralis major (% M_b)	Pectoralis minor (% M_b)
1	0.58 ± 0.05*	0.12 ± 0.02*
14	8.65 ± 0.98*	1.88 ± 0.27*
28	12.12 ± 1.12*	2.51 ± 0.31*
42	14.50 ± 1.71*	3.10 ± 0.37*

Positive allometric growth of breast M_m.

Table 5

	Age (weeks)	N	Slope	Lower 95% CL	Upper 95% CL	R^2
pectoralis major (M. pectoralis)	0 – 6	69	1.60	1.56	1.66	0.98
	0 – 2	30	1.83	1.77	1.88	0.99
	2 - 6	59	1.29	1.24	1.34	0.98
pectoralis minor (M. supracoracoideus)	0 - 6	68	1.62	1.57	1.70	0.98
	0 – 2	30	1.87	1.79	1.95	0.98
	2 - 6	58	1.29	1.22	1.36	0.96

Table 6_(on next page)

Scaling relationships of thoracic musculoskeletal parameters and body mass.

The regression slope indicates proportional change with increasing body mass. Isometric (=), positive (+) and negative (-) allometric growth are indicated by symbols.

Table 6

		n	Slope	Lower 95% CL	Upper 95% CL	R^2
pectoralis major	M_m	69	1.60 (+)	1.56	1.66	0.98
	L_f	37	0.46 (+)	0.42	0.50	0.95
	PCSA	37	1.23 (+)	1.19	1.27	0.99
pectoralis minor	M_m	68	1.62 (+)	1.57	1.70	0.98
	L_f	34	0.55 (+)	0.50	0.62	0.89
	PCSA	29	1.17 (+)	1.09	1.25	0.97
external oblique	M_m	25	0.97 (=)	0.84	1.09	0.89
	L_f	15	0.31 (=)	0.12	0.48	0.32
	PCSA	15	0.90 (=)	0.72	1.09	0.86
sternal keel	length	34	0.48 (+)	0.44	0.51	0.97
	depth	34	0.55 (+)	0.51	0.59	0.95
uncinate process	length	31	0.30 (-)	0.28	0.32	0.94

Ossification of the uncinate processes.

Anatomical and biomechanical traits of broiler chickens across ontogeny. 1. Anatomy of the musculoskeletal respiratory apparatus and changes in organ size. Structural changes in uncinate process 4 over development from embryo (6 days before hatch) to slaughter age; presence of cartilage and bone as derived from stained specimens. Data presented as mean ± standard deviation (SD).

Table 7

Age (days)	Bone		Cartilage at base		Cartilage at tip	
	% total area	SD	% total area	SD	% total area	SD
-6	0	0	100	0	100	0
1	39.5	16.8	35.0	9.4	25.5	9.6
13	72.6	8.4	23.5	8.1	3.9	0.2
29	72.6	7.4	13.4	7.7	14.0	0.3
40	76.8	8.6	9.1	5.1	14.2	2.5

Figure 1

Breast muscle growth during development

Scatter plots showing log transformed pectoralis major and minor masses against log body mass over development from 0 - 2-weeks (solid line) and 2 – 6-weeks old (dashed line). The equations describing lines of best fit were: (A) pectoralis major 0 – 2 weeks: $M_b^{1.83} - 3.28$ ($n = 30$, $r^2 = 0.99$; $p < 0.001$); 2 – 6 weeks: $M_b^{1.29} - 1.83$ ($n = 59$; $r^2 = 0.98$; $p < 0.001$). (B) pectorals minor 0 - 2 weeks: $M_b^{1.87} - 4.06$ ($n = 30$, $r^2 = 0.98$; $p < 0.001$); 2 – 6 weeks: $M_b^{1.28} - 2.48$ ($n = 58$; $r^2 = 0.96$; $p < 0.001$).
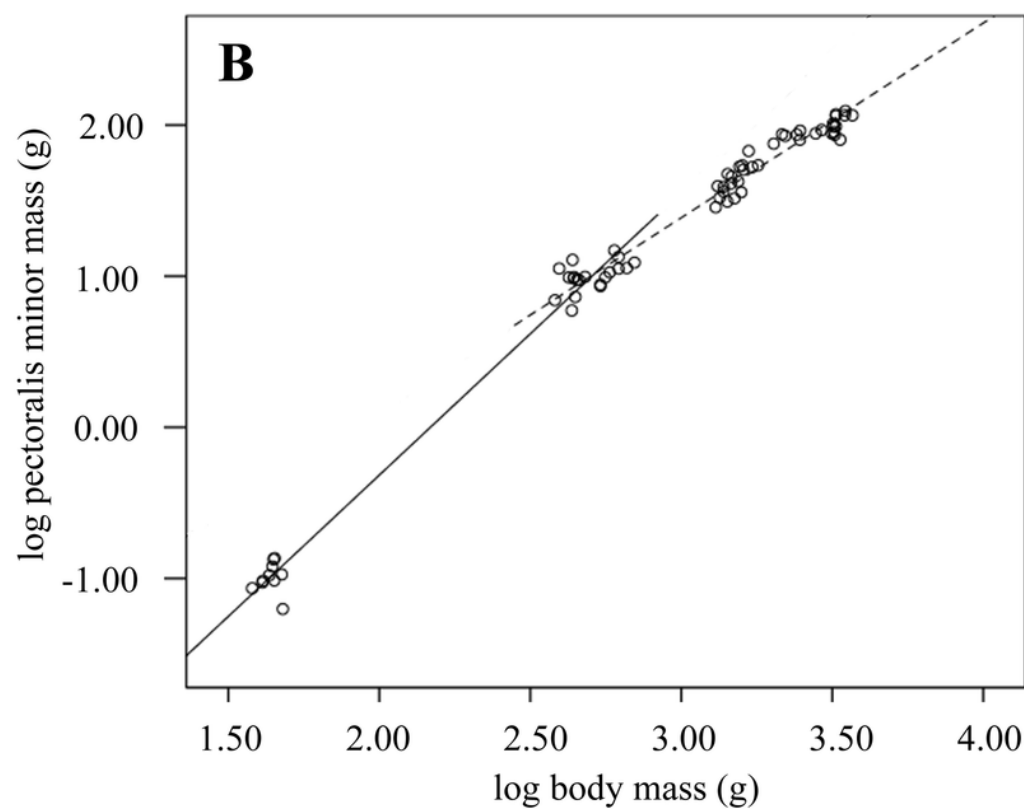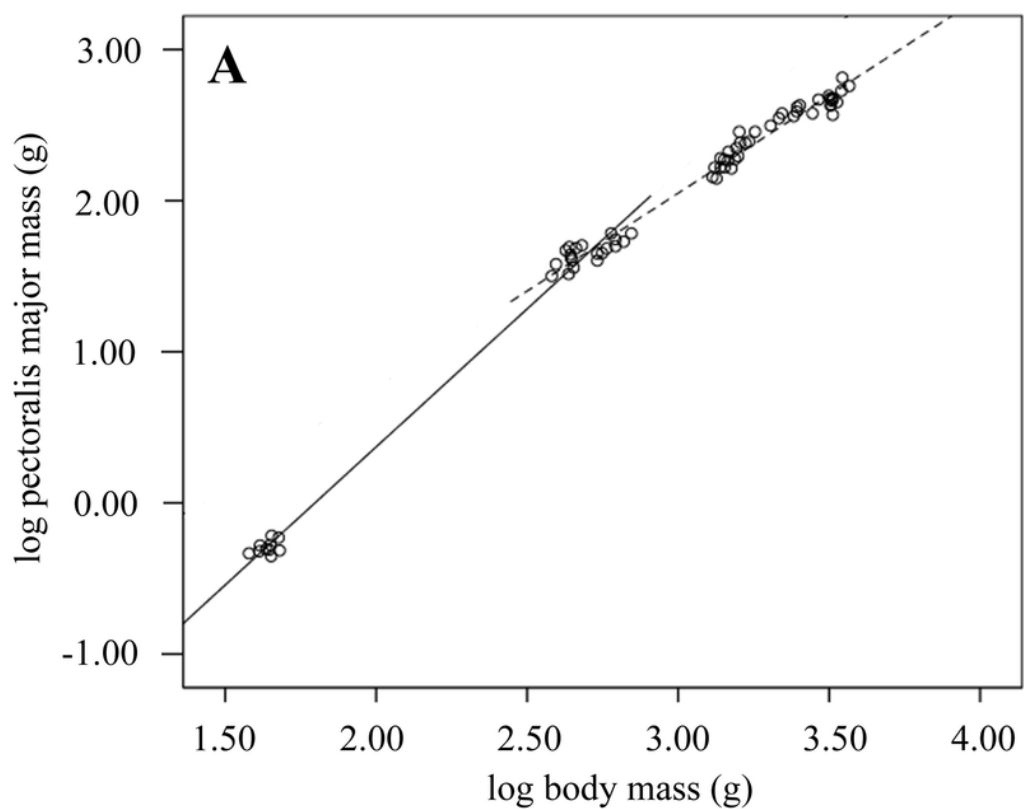
Figure 2

Ossification of the thoracic skeleton

Representative stained skeletons showing the progression of ossification in the vertebral ribs, uncinate processes and sternum. Blue areas correspond to cartilage and red areas to bone. Ossification of ribs and uncinate processes are shown for the hatchling (A and B), 2-week old (C and D) and 6-week old (E and F) birds panels. Ossification of the uncinate processes and sternum remain incomplete at slaughter age. Scale bars represent 10mm.
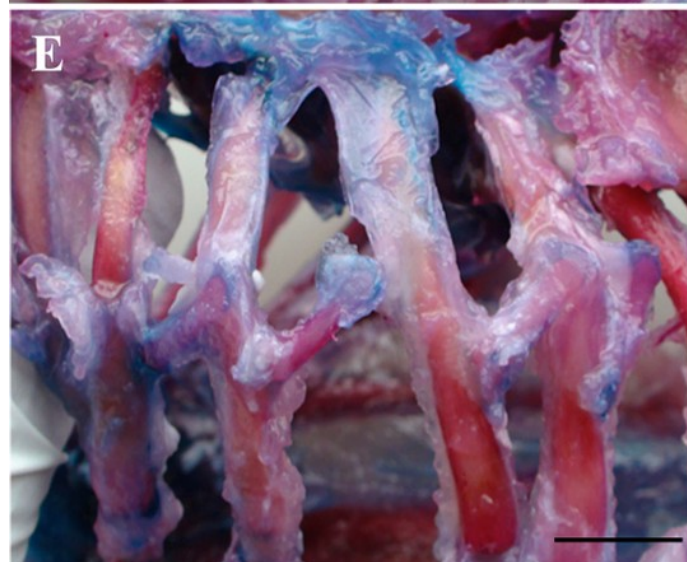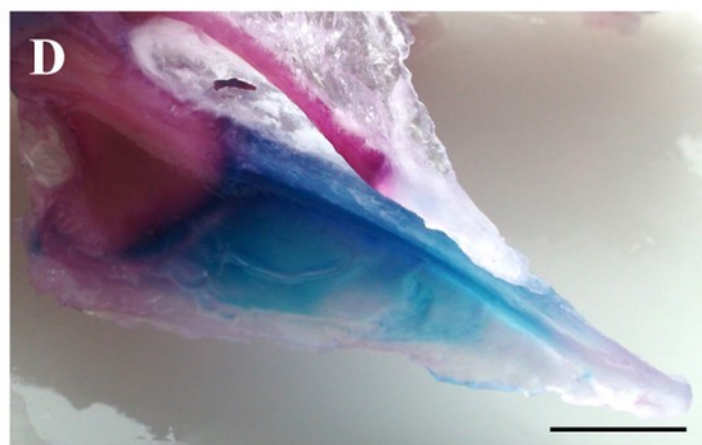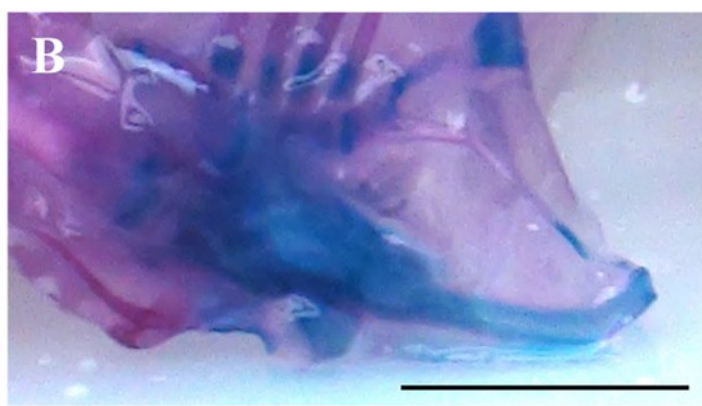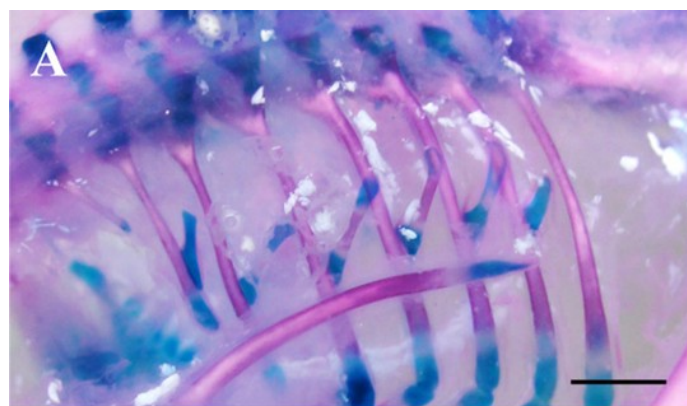
Figure 3

Organ growth during development

Scatter plots showing log transformed organ masses against log body mass over development from hatch to 6-weeks old. The equations describing lines of best fit were: (A) heart: $M_b^{0.91} - 1.96$; ($n = 69$; $r^2 = 0.98$; p < 0.001); (B) lung: $M_b^{0.86} - 1.76$ ($n = 62$; $r^2 = 0.97$; p < 0.001); (C) liver: $0 - 2$ weeks old (solid line): $M_b^{1.10} - 1.70$ ($n = 30$; $r^2 = 0.99$; p < 0.001); $2 - 6$ weeks old (dashed line): $M_b^{0.76} - 0.77$ ($n = 59$; $r^2 = 0.95$; p < 0.001); (D) intestine: $M_b^{0.75} - 0.34$ ($n = 69$; $r^2 = 0.98$; p < 0.001).