Aus dem Institut für Veterinär-Anatomie
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

Anatomical, histological and morphometric comparative study of the cardiovascular and the skeletal system of a male dual purpose and a broiler chicken line

Inaugural-Dissertation
zur Erlangung des Grades eines
Doktors der Veterinärmedizin
an der
Freien Universität Berlin

vorgelegt von
George Harash
Tierarzt aus Hama, Syrien

Berlin 2020
Journal-Nr.: 4224

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Gedruckt mit Genehmigung

des Fachbereichs Veterinärmedizin

der Freien Universität Berlin

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Dritter Gutachter:	**Univ.-Prof. Dr. Jürgen Zentek**

Deskriptoren (nach CAB-Thesaurus): Broilers, chickens, breeds, animal breeding, histology, morphometry, skeleton, cardiovascular system

Tag der Promotion: 11.08.2020

Meiner Familie

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List of abbreviations

BMD	Bone mineral density
Ct.Th	Cortical thickness
LD	Lohmann Dual
M_b	Body mass
M_h	Heart mass
M-fracture	Fracture bending moment
Micro-CT	Microcomputed tomography
M-Max	Maximum bending moment
Ross	Ross 308
Tb.N	Trabecular number
Tb.Th	Trabecular thickness

1 Introduction

1.1 The culling of male chickens and the alternatives

In response to the increasing demand for safe and affordable food in sufficient quantities, intensification of poultry farming started in the mid-twentieth century (Leenstra et al., 2010). Genetic selection enabled increased egg production by layer type chickens and increased meat production using specialized meat type chickens. Subsequently, because of their slow growth rate, male layer type chickens became less profitable for meat production (Leenstra et al., 2009). Using techniques, which make it possible to distinguish males from females immediately after hatching, it became common practice to kill the above mentioned male chicks on day one (Damme et al., 2015).

Alternatives to the killing of male chickens are the rearing of males from layer-lines to a live weight of approximately 600 g and market them as an alternative for quails, or to a live weight of approximately 2,000 g and market them as an alternative to broiler chickens (Koenig et al., 2009). Sex determination in ovo is one possibility which is currently investigated (Krautwald-Junghanns et al., 2018; Galli et al., 2018). Unfortunately, most methods for sex determination in ovo affect hatchability (reduction of hatching rate of about 10%) and chick health negatively (Krautwald-Junghanns et al., 2018).

Dual-purpose chickens scored best out of all the possible alternatives (Leenstra et al., 2011). Whilst hens of dual-purpose chickens are intended to lay eggs, the males shall provide meat (Icken and Schmutz, 2013). Using crosses of meat and layer lines, commercial breeding companies produce dual-purpose chicken lines, such as Lohmann Dual (LD), which is rated to sufficiently perform in meat and egg production (Damme et al., 2015).The novel dual-purpose chicken line Lohmann Dual (LD) is characterized by a balanced fitness for egg as well as meat production (Icken and Schmutz, 2013). LD chickens have better growth and feed efficiency parameters when compared with other dual-purpose chicken lines (Damme et al., 2015; Mueller et al., 2018). Furthermore, the LD show a lower pecking behaviour (Giersberg et al., 2017), less foot pad dermatitis and less pollution of breast plumage (Habig et al., 2016).

1.2 The effect of genetic selection on the cardiovascular and the skeletal system

The high selection for improved meat production has changed the biology of broiler chickens (Tickle et al., 2014). Mounting evidence suggests that selection for such economically desirable traits in the modern broiler chicken has been accompanied by reduced animal welfare (Knowles et al., 2008) and increased mortality (Havenstein et al., 2003).

Research is being directed towards understanding health problems such as the multitude of heart and bone pathologies that may affect broiler chickens (Paxton et al., 2010: Tickle et al., 2014). Understanding the relative growth and size of organs is important as it can help to understand the diseases. For example, unlike in ancestral breeds, with increasing age the broiler heart became progressively smaller in relation to body mass (Schmidt et al., 2009). Furthermore, sudden death syndrome and ascites in chickens are related to morphological changes of the heart and aorta (Decuypere et al., 2000).

Skeletal disorders are one of the primary welfare and economic problems in poultry production. Welfare declines due to pain and stress associated with lameness that impairs movement or causes bone breakage during catching and transportation (Julian, 2005). Leg disorders, such as tibiotarsus dyschondroplasia and angular bone deformity (valgus-varus deformity), are one of the most expensive diseases in terms of costs at the farm and the processing plant (Julian, 1998). Production expenses are increased through costs of treatment. Moreover, during processing carcass condemnations occur because of bone fragility and porosity which causes bone fragments in the meat and discoloration due to blood leakage that is less attractive to the consumer (Gregory and Wilkins, 1992; Pattison, 1992; Bennet et al., 2002; Whitehead et al., 2004). In order to understand skeletal diseases, it is important to study bone strength and internal structure through studying mechanical properties via the bone mechanical test (Štofaníková et al., 2011: Jebsen et al., 2015) and analysing densitometric and geometric parameters using micro computed tomography (micro-CT) (Charuta et al., 2013).

1.3 Essentials of bird anatomy

1.3.1 The heart

Birds of a given body mass have a significantly heavier heart compared mammals. In birds, heart mass scales with respect to body mass as $M_h = 0.014M_b^{0.91}$ where M_h is heart mass and M_b is body mass (Bishop and Butler, 1995). The relationship in mammals is $M_h = 0.0058M_b^{0.98}$ (Prothero, 1979). This may be due to the high cardiac power needed to sustain flight (Dzialowski and Crossley, 2014).

In birds, the heart is located in the cranial part of the common thoracoabdominal cavity, with its long axis slightly to the right of the midline. It is partly enclosed dorsally and laterally by the lobes of the liver (Strunk and Wilson, 2003). The avian heart has two completely divided atria and ventricles. As in mammals, the wall of the avian atria and ventricles consist of an endocardial, a myocardial, and an epicardial layer. The wall of the left ventricle is two to three times thicker than that of the right (Sjaastad et al., 2016; Kubale et al., 2018).

Histologically, the atria and ventricles are quite similar, consisting of the three distinct layers mentioned above. The myocardium, the middle layer, forms the greatest proportion of the heart tissue and is composed mainly from cardiomyocytes (LeGrice et al., 2005). The avian cardiac muscle fibers are much smaller in diameter than mammalian fibers and hence there are many more of them in similarly sized hearts (Kittleson and Kienle, 1998). Avian myocardial cells are typically 2–7 µm in diameter compared with the 10–15 µm diameter of mammalian ones (Smith et al., 2000; Kittleson and Kienle, 1998).

The cardiomyocytes are supplied by a dense capillary network from the right and left coronary arteries originating from the ascending aorta. This capillary network is three-dimensionally arranged along the longitudinal axis of the cardiomyocytes (Anderson and Anderson, 1980). Histologically, the capillaries are composed of a one-cell thickness layer of endothelial cells, whereas the muscular and adventitial layers are absent (Abdul-Aziz and Fletcher, 2016).

The increased heart mass with age is accompanied by increased cardiac capillary density in an effort to meet the oxygen requirements (Anversa et al., 1986). Cardiac capillary growth can be studied based on capillary counts, either per mm² (capillary density) or expressed as capillary:cardiomyocyte ratio. These counts are taken from cross-sections (Hudlická, 1982).

1.3.2 The aorta

The aorta is a major artery acting as an elastic reservoir, storing blood transiently during systolic ejection, and providing flow to the periphery during diastole (Dzialowski and Crossley, 2014). The aortic wall is composed of three layers, i.e., the tunica intima, tunica media, and tunica adventitia. The tunica intima consists of a single layer of endothelial cells supported by subendothelial connective tissue. The tunica media of elastic arteries is broad and consists of concentrically fenestrated sheaths of elastic fibers (lamellar units) separated by collagenous tissue and few muscle fibers (Abdul-Aziz and Fletcher, 2016). The tunica adventitia is composed of loose connective tissue of predominantly collagen fibers with fewer elastic fibers; the cellular component of this layer consists mainly of fibroblasts. The tunica adventitia of the aorta contains vasa vasorum, which provide nutrients to the aortic wall (Abdul-Aziz and Fletcher, 2016). The arteries tend to distribute the stress, imposed to the vessel wall by the specific conditions applying to a particular vessel (blood pressure, vessel radius). The lamellar units of the tunica media share the mechanical work, where a single lamellar unit presents elastic properties, allowing it to withstand a certain range of mechanical load (Faury, 2001).

1.3.3 The skeleton

The functional adaptation to flying and running led to characteristic changes in the body structure of birds (Schwarze and Schröder, 1972). The adjustments include; concentration of the weight and thus the center of gravity in the middle of the body, lack of a urinary bladder, a special respiratory and reproductive system and the formation of feathers (Scholtyssek and Doll, 1978). The skeleton of birds differs from that of mammals (Salomon, 1993). The structure of the skeleton in different bird species is comparable (Maierl et al., 2001).

The skeletal system is divided into the appendicular skeleton and axial skeleton. The appendicular skeleton, comprised of the long bones, consists primarily of compact (cortical or lamellar) bone arranged in concentric layers around Haversian canals. The axial skeleton includes the spinal column and flat bones of the skull and is composed of cancellous bone (Abdul-Aziz and Fletcher, 2016).

In terms of body mass, the skeleton of birds is significantly lighter than that of mammals, it makes up only 4.5% of the total weight of the bird's body. Weight reduction is due to pneumatization, which varies according to different species and bones. In addition, a bird's body cavity has 9 air sacs that extend into some hollow bones (Schwarze and Schröder, 1972).

Bone strength refers to the amount of loading force required to cause the material to fail under a certain loading condition (Petit et al., 2005). Bone strength is influenced by its morphology (shape, size and mass), mechanical properties (rigidity and bone breaking strength) and microstructure (cortical and trabecular BMD) (McDevitt et al., 2006; Yair et al., 2017).

1.4 Aims and hypotheses

My study was designed to investigate the macro- and microstructure of the cardiovascular and the skeletal system of a new dual-purpose chicken line, Lohmann Dual, and compare results with those of a fast-growing chicken line, Ross 308, throughout the period from time of hatching until chickens reached their market body weight.

For assessment of the cardiovascular system, the following parameters were determined:

1) Heart: mass and relative mass of the heart, the thickness of the ventricular myocardial wall, cardiomyocyte size, density of myocardial capillaries.

2) Aortic wall: aortic wall thickness, aortic diameter, number of aortic elastic lamellae, and elastic fiber percentage.

Investigation of the skeleton aimed at assessing bone strength by measuring the following parameters of the tibiotarsus:

1) Morphological parameters: weight, relative weight, length, relative length, mass per unit of length and width.

2) Mechanical properties: rigidity, maximum bending moment and fracture bending moment.

3) Trabecular and cortical bone properties.

2 Heart ventricular histology and microvasculature together with aortic histology and elastic lamellar structure: A comparision of a novel dual-purpose to a broiler chicken line

Published in:

PLOS ONE, March 21, 2019
Public Library of Science

Please find the original article via the following digital object identifier:
https://doi.org/10.1371/journal.pone.0214158

PLOS ONE

RESEARCH ARTICLE

Heart ventricular histology and microvasculature together with aortic histology and elastic lamellar structure: A comparison of a novel dual-purpose to a broiler chicken line

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Citation: Harash G, Richardson KC, Alshamy Z, Hünigen H, Hafez HM, Plendl J, et al. (2019) Heart ventricular histology and microvasculature together with aortic histology and elastic lamellar structure: A comparison of a novel dual-purpose to a broiler chicken line. PLoS ONE 14(3): e0214158. https://doi.org/10.1371/journal.pone.0214158

Editor: Arda Yildirim, Tokat Gaziosmanpasa University, TURKEY

Received: December 12, 2018

Accepted: March 7, 2019

Published: March 21, 2019

Data Availability Statement: All relevant data are within the manuscript and its Supporting Information files.

Funding: The project is supported by funds of the German Government's Special Purpose Fund held at Landwirtschaftliche Rentenbank (grant number 28RZ372049). The authors acknowledge support by the German Research Foundation and the Open Access Publication Fund of the Freie Universität Berlin. The funders had no role in study design,

Abstract

The use of dual-purpose chickens is a strategy to avoid killing one-day-old male chicks of egg laying lines. Lohmann Dual (LD) is a novel dual-purpose chicken line created by the crossbreeding of layer and broiler lines. However, many of the cardiovascular diseases of broilers are likely to be associated with intensive genetic selection for growth and feed conversion efficiency. This study aimed to compare the macroscopic and microscopic structure of the heart and the aorta of the LD chicken line with that of the broiler chicken line, Ross 308 (Ross) under typical husbandry conditions for meat production. Eighty, one-day-old male chicks of each line were housed for 5 weeks (Ross) and 9 weeks (LD). Six birds of each line were sampled weekly. Heart mass, thickness of ventricular walls, cardiomyocyte size and blood capillary density as well as aortic diameter and thickness, number of elastic lamellae and elastic fiber percentage in the aortic wall were determined. The growth patterns of the heart were the same in the two lines. Although LD chickens had a lower absolute heart mass than that of Ross chickens, the relative heart mass in both lines was similar. The cardiomyocytes of LD chickens were larger than those of Ross's of the same body weight (BW), nevertheless both lines had similar thicknesses of their ventricular walls. The blood capillary density was greater in the LD heart than in that of the Ross heart. The aorta of LD chickens had proportionally; a greater aortic lumen radius, larger numbers of elastic lamellae and more elastic fibers than in Ross chickens. Our results suggest that the heart and aorta of the LD chickens have not been disadvantaged by their intensive genetic selection; furthermore, LD chickens have a better myocardial capillary supply and better aortic mechanical properties than those of Ross chickens.

data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Introduction

In response to the increasing demand for safe and economically priced food in large quantities, intensification of the worldwide poultry industry began in the mid-twentieth century. Efficiency was enabled by advances in the breeding, housing and feeding of the birds and an increased knowledge of avian veterinary medicine. Over time, intensive genetic selection resulted in significantly higher egg production by layer type chickens and greater meat yields from broiler chickens. However, with the layer type poultry lines, meat production from male birds is inefficient and uneconomic. Consequently it became common practice, worldwide, to kill one-day-old male hatchlings [1]. For example in Germany, about 46 million one-day-old male chicks from layer genetic lines are killed annually [2].

An alternative to killing one-day-old male layer chickens is to farm dual-purpose chickens, where females lay eggs and males produce meat. Historically traditional dual-purpose breeds are inferior in both egg and meat production to the genetically designed specialized layer and broiler lines [3]. Recently commercial breeding companies have developed new dual-purpose breeds using crosses between meat and layer lines to create breeds such as the Lohmann Dual (LD) that exceed the performance of the late nineteenth and twentieth century dual-purpose breeds such as the Malines and the Schweizerhuhn, particularly in meat production [4].

Genetic selection of domesticated chickens for meat production has produced startling improvements in their growth rates and meat yields. Over the past 60 years growth rates of intensively reared broiler chickens have increased steadily such that a 300% increase in body weight has been engineered from 25 g per day in the 1950s to 100 g per day in the modern bird [5]. Simultaneously, the slaughter age of contemporary broiler chickens has reduced from 9 to 5 weeks and slaughter weight exceeds 2.2 kg [6].

In order to meet the metabolic demands for high muscle mass growth in meat producing birds an optimally functioning cardiovascular system that delivers adequate oxygen to all body cells is critical [7]. The cardiovascular capacity of chickens and of other commercial poultry such as quail and turkeys has been affected greatly by the genetic selection for rapid and high meat production [8]. For example, the hearts of broiler chickens have become progressively smaller in relation to the body mass when compared to their ancestral breeds such as the heritage line from the University of Illinois at Urbana-Champaign (UIUC) [8] and jungle fowl [9]. In addition, modern meat breeds have significantly greater susceptibility to sudden death syndrome and ascites diseases, both strongly linked to morphological changes of the heart and aorta [10]. For instance, the quantity and architecture of the elastic and collagen fibers within the aortic wall directly determines its elasticity and strength. Any significant alteration to these fibers can lead to mechanical and functional changes associated with aortic disease [11].

This study compared the anatomy of the heart and the aorta throughout postnatal growth of a highly selected fast-growing conventional broiler line (Ross 308) to that of the recently developed genetic line of a dual-purpose chicken (Lohmann Dual, LD). For this the thickness of ventricular myocardial walls, cardiomyocyte size, density of myocardial capillaries, aortic wall thickness, aortic diameter, number of aortic elastic lamellae and elastic fiber percentage in the aortic wall were measured.

Material and methods

Animals and husbandry

Eighty birds of a broiler line (Ross 308) and 80 birds of a dual-purpose line (Lohmann Dual, LD), were obtained from Lohmann Tierzucht, Cuxhaven, Germany and were kept separately under the same husbandry conditions as described previously [12]. Briefly, chickens had *ad*

libitum access to a mash diet and water for the duration of the study. A starter diet (231.5 g protein and 12.6 MJ ME/kg) was fed from hatching to day 14 and then they were fed a grower diet (214.4 g protein and 13 ME/kg) from day 15 to the end of the study.

The study ended when chickens of both lines had reached a live body weight of 2000g, i.e. day 35 for Ross chickens and 63 for LD chickens. The study was authorized by the Animal Welfare Committee: Landesamt für Gesundheit und Soziales, Berlin, Germany, ID: 0236/15.

Sample collection

On each sample day, for Ross on days 1, 7, 14, 19, 21, 25, 28, 32 and 35, and for LD on days 1, 7, 14, 21, 28, 32, 35, 42, 49, 56 and 63, six birds from each line were selected at random and their live body weight (BW) was measured to an accuracy of 0.1 g using a mechanical scale (Sartorius, Göttingen, Germany). Then the birds were killed by decapitation according to Germany's animal welfare standards.

Morphometric analysis of heart

Immediately after death, the heart was dissected free from the carcass, weighed to an accuracy of 0.01 g on an electronic laboratory balance (Sauter-Cumulus, Freiburg, Germany) and the relative heart mass (g/100 g BW) determined. A one-centimeter-thick cross section sample was dissected from the midpoint between base and apex of the heart and prepared for morphometric examination (Fig 1). Specimens were washed in 0.9% sodium chloride solution, fixed in phosphate buffered formalin (4%, pH 7, 24 h, room temperature), and then dehydrated in a graded series of ethyl alcohol and embedded in paraffin wax. Eight 5 μm thick transverse serial sections were cut for each bird. Four were stained with Mayer's hematoxylin and eosin (H&E) for general morphological examination. The other four transverse sections were labelled with the endothelial marker Arachis hypogaea lectin (Peanut agglutinin, PNA) that binds to galactosyl (ß-1,3) N-acetylgalactosamine [13] to examine the capillary network. Two-dimensional morphometric analysis was carried out using an optical microscope (Axioskop, Carl Zeiss, Jena, Germany) and an image analysis system, NIS-Elements AR (Nikon Instruments Inc., U.S.A.).

Of the four sections stained with H&E the best section that had no artifacts, such as regional tissue shrinkage, wrinkles or cracks was selected and the following measurements were taken:

- The thickness of the left ventricular wall (LVW) was measured at five different locations at a magnification of 12.5× (Fig 2A). Subsequently, the average value of the 5 measurements was calculated. The right ventricular wall (RVW) was measured in the same way (Fig 2A).
- The relative LVW and RVW thicknesses (mm/100g BW) were expressed relative to the bird's body weight (BW).
- The ratio of the thicknesses of the ventricular walls (right:left).
- Cardiomyocyte size was determined by measuring the cross-sectional area and the diameter of 25 cardiomyocytes in the left ventricular wall, and then the average calculated. The measurement was determined at the level of the nucleus at a magnification of 400× (Fig 2B). Here the outline of each cell was circumscribed manually using the NIS-Elements AR software then the system calculated automatically the diameter (μm) and the cross-sectional area (μm^2).

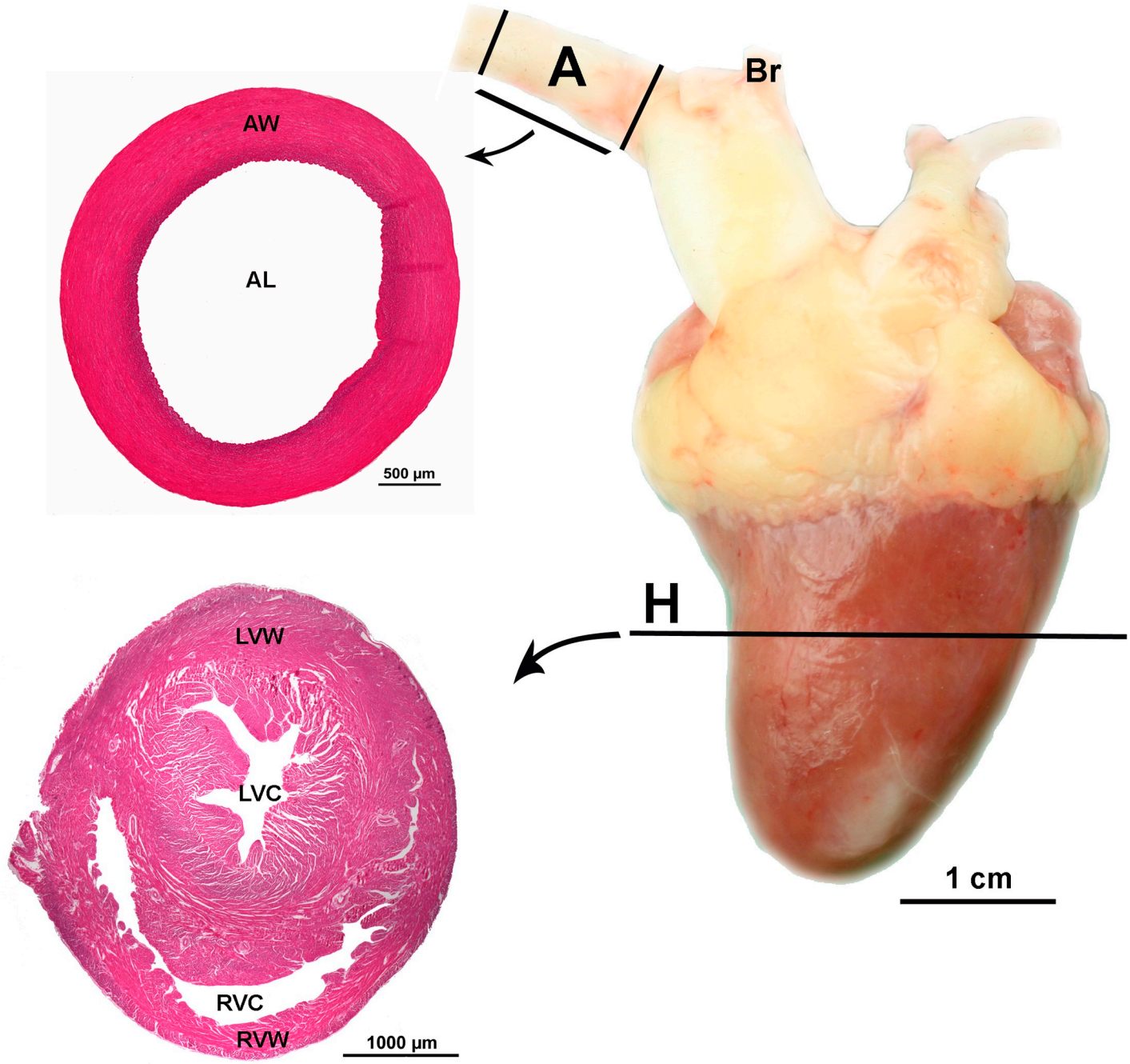

Fig 1. Location of sample collection. Position of the cross section samples from the heart (H) and from the aorta (A); (AL) aortic lumen, (AW) aortic wall, (Br) brachiocephalic arteries, (LVC) left ventricular cavity, (LVW) left ventricular wall, (RVC) right ventricular cavity and (RVW) right ventricular wall of a 63-day-old Lohmann Dual chicken.

https://doi.org/10.1371/journal.pone.0214158.g001

Of the four sections labeled with the lectin PNA the best section without artifacts was selected and 5 visual fields (each field = 33000 µm^2) examined. The following parameters were measured at a magnification of 400×:

- Capillary density:

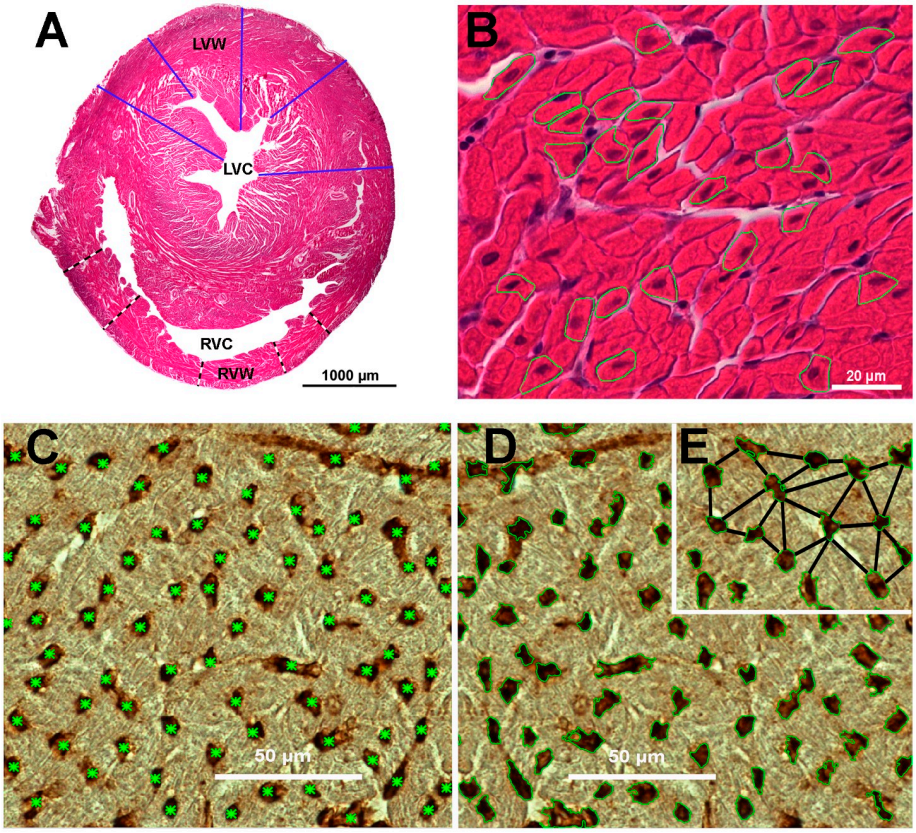

Fig 2. Transverse section of heart ventricular walls of a 63-day-old Lohmann Dual chicken. (A) ventricular wall thicknesses (black-white lines are of the right ventricular wall; blue lines are of the left ventricular wall; (LVC) left ventricular cavity; (LVW) left ventricular wall; (RVC) right ventricular cavity and (RVW) right ventricular wall), (B) representative cardiomyocytes outlined in green, (C) blood capillaries are each indicated by a green asterisk, (D) the area occupied by capillaries is each outlined in green, (E) intercapillary distances; the capillary distances between the nearest neighboring capillaries. A and B stained with H&E. C, D and E stained with Arachis hypogaea Lectin.

https://doi.org/10.1371/journal.pone.0214158.g002

1. The number of blood capillary profiles per mm^2 of field of view. Each capillary profile in the field of view was identified and marked manually by a green asterisk using the imaging software, then the system automatically calculated the total number of the capillary profiles (Fig 2C).
2. The percentage of the area occupied by capillaries per field of view (Fig 2D).

- The number of the cardiomyocytes per capillary. The cardiomyocytes (only those with a complete nucleus) and the blood capillaries were counted in each visual field, then the number of cardiomyocytes was divided by the number of blood capillary profiles.
- Intercapillary distance (μm) was determined by measuring 25 intercapillary distances in each sample, starting with the capillary located nearest to the center of the field of view. The shortest distance from the outer circumference of this capillary wall was drawn manually to the neighbouring capillaries and from these radially outwards until 25 distances were measured, and the average value was determined according to Al Masri et al, (2017) [14] (Fig 2E).

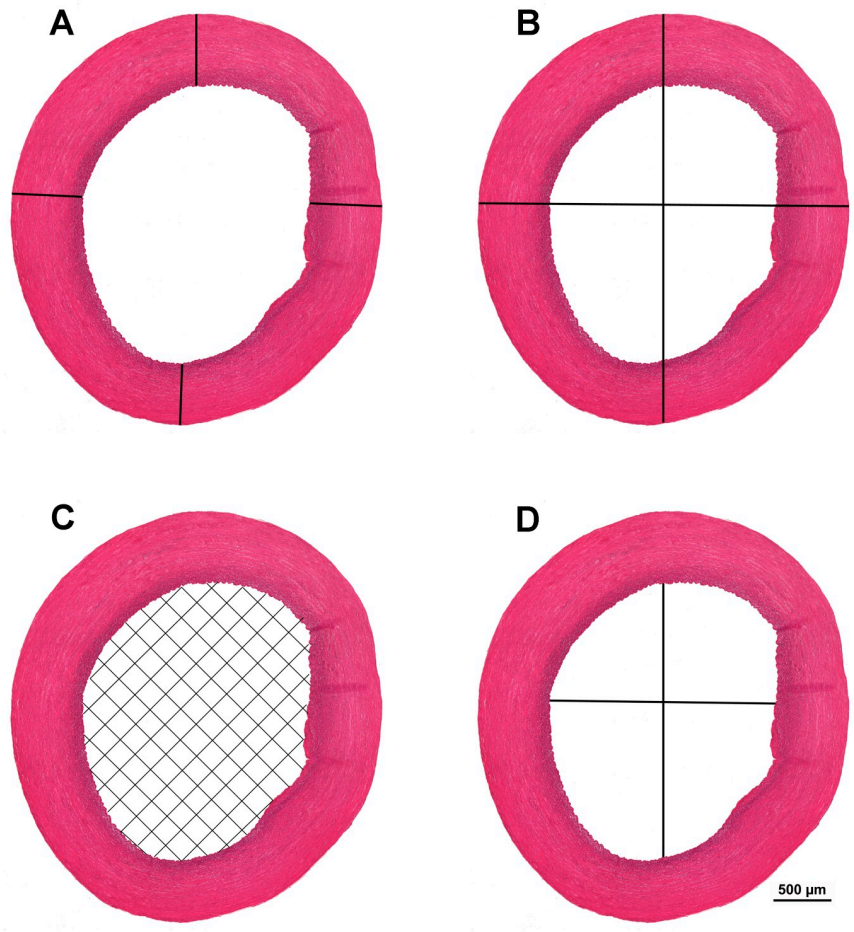

Fig 3. Cross-section of aortic wall of a 7-day-old Lohmann Dual chicken. (A) aortic wall thickness, (B) aortic diameter, (C) cross sectional area of aortic lumen, (D) diameter of aortic lumen. H&E.

https://doi.org/10.1371/journal.pone.0214158.g003

Morphometric analysis of aorta

For each bird, a one centimeter wide aortic ring sample was dissected from the aorta immediately distal to the brachiocephalic arteries origin (Fig 1). The specimens were washed in 0.9% sodium chloride solution, fixed in phosphate buffered formalin (4%, pH 7, 24 h, room temperature), dehydrated in a graded series of ethyl alcohol and then embedded in paraffin wax. Eight serial 5 μm thick transverse sections of each sample were cut and four sections stained with H&E and four sections with Weigert's Resorcin Fuchsin [13]. Two-dimensional morphometric analysis was carried out using an optical microscope (Axioskop, Carl Zeiss, Jena, Germany) and an image analysis system, NIS-Elements AR (Nikon Instruments Inc., U.S.A.).

Of the four sections stained with H&E the best section that was free of artifacts was selected and the following parameters were determined for each bird at a magnification of 12.5×:

- Aortic wall thickness (mm) was measured in each sample at four different locations and the mean of the values was calculated (Fig 3A).
- Aortic diameter (mm) was calculated as the mean of the largest and smallest external aortic diameter values (Fig 3B).

- Cross sectional area of the aortic lumen (mm^2). The aorta inner surface was selected using a colour tool. Based on this selection, the program calculated the surface area of the aortic lumen automatically (Fig 3C).
- Aortic lumen diameter (mm) (Fig 3D).
- Aortic lumen diameter/aortic wall thickness ratio was determined.

Of the four sections stained with Weigert's Resorcin Fuchsin the best section without artifacts was selected and the following parameters were determined:

- The thicknesses of the tunica intima (400×), media (50×) and adventitia (400×) of the aorta were measured at four different locations of the histological section. The mean of the measured values was calculated (Fig 4).
- The ratio of the thicknesses of the aortic layers (intima:media:adventitia) was calculated.
- The number of elastic lamellae of the tunica media was counted manually at four different locations of the tunica media under 100× magnification for each bird, then the mean of the measured values was calculated (Fig 5).
- The relative number of elastic lamellae was scaled to a 1 mm aortic wall thickness to allow a comparison across genetic lines.
- The aortic area occupied by elastic fibers (%) was measured by the image analysis system, NIS-Elements AR in 4 randomly chosen visual fields (each field = 0.138 mm^2, 200 × magnification). The mean of the 4 values was calculated.

Statistical analysis

Statistical analyses were performed using the statistical package program IBM SPSS Statistics 23 (IBM Corporation, New York, USA). The graphs were generated using the statistical package program JMP Pro 13 (SAS Institute Inc., Cary, USA). Continuous variables are presented as mean ± standard error of the mean (SEM). Comparisons of the two lines of the same age groups were performed using the Mann–Whitney U test. One-way analysis of variance (ANOVA) with a post hoc Least Significant Difference test (LSD) were performed to evaluate the effect of age on the relative mass of the heart and the relative thickness of ventricular walls. To explore the effect of chicken line and BW on heart and aorta measurements, all data collected were regressed against the chicken line and the BW using the log-log regression model. Due to the relationship between some of the data and body weight being non-linear, the body weight and the data were log10-transformed prior to analysis. All statistical analyses were two-sided with significance defined as a p-value of < 0.05.

Results

Live body weight

While the broiler line (Ross 308) grew to an average weight of 2.13 kg within 5 weeks post hatching, the dual-purpose chicken line LD took 9 weeks to reach the same average weight. From d 1 to d 35 post hatching, the Ross chickens grew at a rate of 57.7 g/d, and the LD chickens grew at a rate of 22.04 g/d. From d 1 to d 63 post hatching LD grew at a rate of 31.8 g/d. The BW of the Ross chickens significantly exceeded that of the LD chickens at all sampling intervals from d 1 until d 35 (U-test, $p < 0.05$) (Table 1).

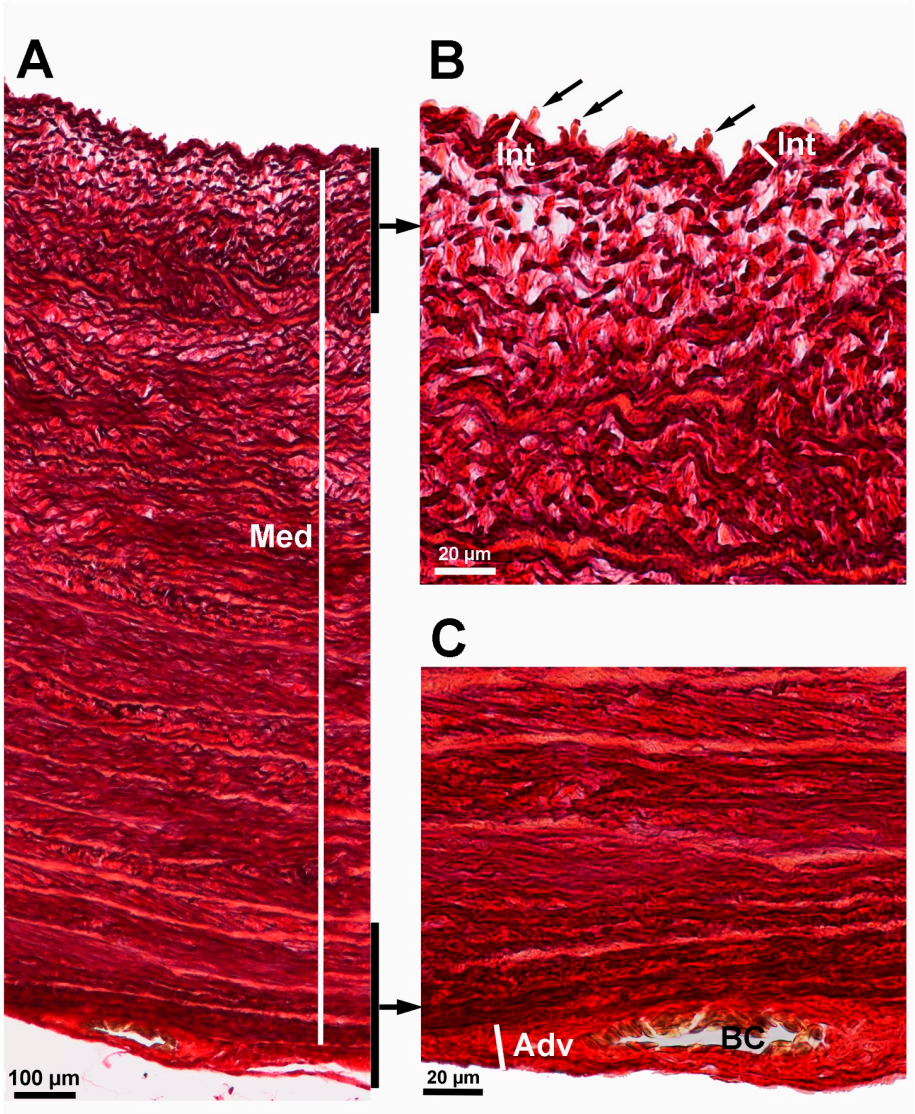

Fig 4. Aortic wall layers of a 35-day-old Lohmann Dual chicken. (A) entire aortic wall with tunica media (Med), (B) luminal aspect of the aortic wall showing the tunica intima (Int) and endothelial cells (arrows), (C) outer aspect of the aortic wall showing the tunica adventitia (Adv) and blood capillary (BC). Weigert's Resorcin Fuchsin.

https://doi.org/10.1371/journal.pone.0214158.g004

Macroscopic examination of the heart

Heart mass and relative heart mass. The heart mass increased steadily with age in both lines. From d 1 to d 35 post hatching, the Ross birds' heart grew at a rate of 0.344 g/d, whereas the LD birds' heart grew by 0.16 g/d over the same period, and by a rate of 0.188 g/d from d 1 to d 63. The mass of the heart in LD chickens was significantly lower than that of Ross chickens in all comparable age groups between d 1 and d 35 post hatching (U-test, $p < 0.05$) (Table 1). When heart mass was expressed relative to body weight, LD chickens had a higher relative heart mass than Ross chickens only at d 35 (U-test, $p = 0.004$). The relative heart mass in both lines decreased with increasing age. The relative heart mass of LD chickens decreased significantly between d 7 and d 14 as well as between d 21 and d 28 by 20% (LSD, $p \leq 0.001$). In

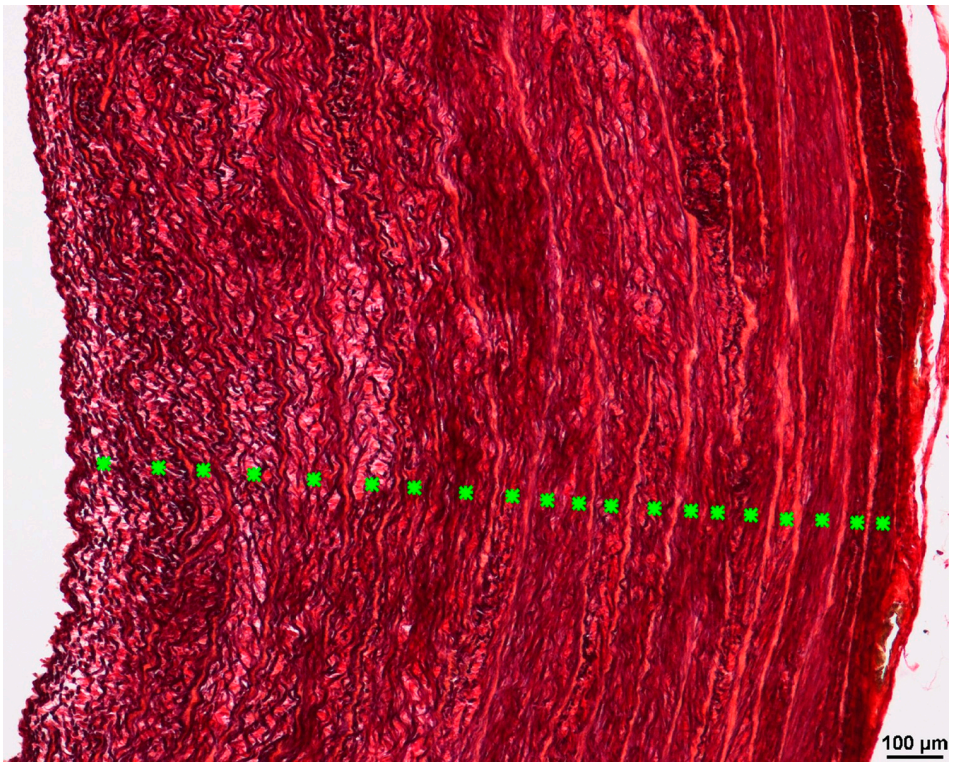

Fig 5. Internal elastic lamellae in the aortic wall of a 35-day-old Lohmann Dual chicken. Each individual elastic lamella is indicated by a green asterisk. Weigert's Resorcin Fuchsin.

https://doi.org/10.1371/journal.pone.0214158.g005

Ross chickens, the decrease in the relative heart mass was between d 7 and d 14 by 15% (LSD, $p = 0.011$) and by 18% between d 14 and d 19 (LSD, $p = 0.009$) (Fig 6, Table 1). Regression analysis showed that when the birds were of the same BW, the chicken line had no effect on the heart mass ($p = 0.091$), whereas the body weight had an effect on the heart mass ($p < 0.001$), adjusted $R^2 = 0.99$.

Ventricular myocardial walls. In both chicken lines, the absolute ventricle wall thicknesses increased with each time interval, however the relative thickness of the ventricle walls decreased with age. The thickness of the LVW increased similarly in both lines up to d 14 without any differences between LD and Ross chickens. From d 21 onwards, the LVW was found to be significantly thinner in LD chickens compared to that of Ross chickens (U-test, $p < 0.05$). However, except at hatching, Lohmann Dual chickens had a thicker relative LVW than Ross chickens did in all age groups (U-test, $p < 0.05$).

The RVW was thinner in LD than in Ross chickens only at d 14 and d 32 (U-test, $p < 0.05$). At all other times the thickness did not differ between the two chicken lines. However, the relative thickness of RVW was greater in LD chickens than in Ross's in all age matched groups (U-test, $p < 0.05$), except at d 14 and d 28 (Table 2). Regression analysis showed that when the birds had an equal BW, the chicken line had no effect on ventricular wall thicknesses (LVW and RVW). However, body weight had an effect ($p < 0.001$), adjusted $R^2 = 0.89$ and 0.40 for LVW and RVW, respectively. The ratio of the thickness of the ventricular walls (right:left) at d 1 and d 35 was significantly lower in LD chickens (1:2.5, 1:2.9) than in Ross chickens (1:3.4, 1:4.1). The overall ratio was 1:4.1 for Ross and 1:3.7 for LD (Table 2).

Table 1. Live body weight, heart mass and relative heart mass of LD and Ross chicken lines versus day post hatching.

Age (days)	Line (n)	BW (g)	Heart mass (g)	Heart mass (g) per 100g BW
		Mean ± SEM	Mean ± SEM	Mean ± SEM
1	Ross (6)	52.26 ± 0.94	0.53 ± 0.03	1 ± 0.05
	LD (6)	42.45 ± 1.25	0.42 ± 0.02	0.98 ± 0.03
7	Ross (6)	169.47 ± 7.92	1.65 ± 0.08	0.97 ± 0.03
	LD (6)	101.2 ± 3.32	1 ± 0.04	0.99 ± 0.06
14	Ross (6)	435.35 ± 11.44	3.7 ± 0.19	0.85 ± 0.03
	LD (6)	224.77 ± 5.31	1.85 ± 0.04	0.82 ± 0.02
19	Ross (6)	640.73 ± 45.22	4.62 ± 0.36	0.72 ± 0.02
21	Ross (6)	746.58 ± 23.81	6.2 ± 0.44	0.83 ± 0.05
	LD (6)	329.17 ± 18.8	2.89 ± 0.14	0.89 ± 0.04
25	Ross (6)	1191.67 ± 37.78	8.5 ± 052	0.71 ± 0.04
28	Ross (6)	1221 ± 44.5	8.94 ± 0.6	0.73 ± 0.03
	LD (6)	575.33 ± 25.54	4.25 ± 0.29	0.74 ± 0.02
32	Ross (6)	1677.83 ± 70.52	11.01 ± 0.7	0.65 ± 0.02
	LD (6)	754.17 ± 38.3	5.49 ± 0.27	0.74 ± 0.04
35	Ross (6)	2013.17 ± 58.26	12.58 ± 0.26	0.63 ± 0.01
	LD (6)	791.67 ± 23.85	6.03 ± 0.21	0.76 ± 0.02
42	LD (6)	1130.5 ± 24.15	7.7 ± 0.18	0.68 ± 0.02
49	LD (6)	1522.5 ± 46.02	10.49 ± 0.58	0.69 ± 0.03
56	LD (6)	1817.33 ± 54.79	11.77 ± 0.53	0.65 ± 0.03
63	LD (6)	2011.83 ± 74.66	12.27 ± 0.59	0.61 ± 0.02

BW: live body weight; LD: Lohmann Dual; Line: genetic line; n: animal number; Ross: Ross 308; SEM: standard error of the mean.

https://doi.org/10.1371/journal.pone.0214158.t001

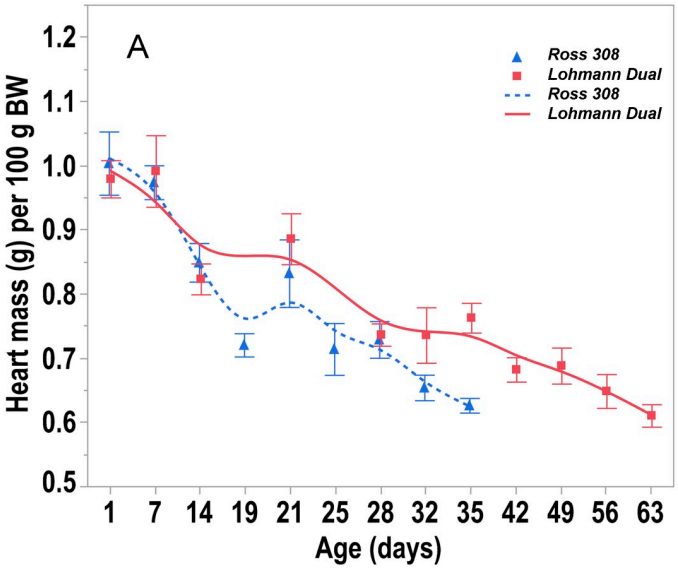

Fig 6. Trendlines of the changes in relative heart mass versus day post hatching. Bars refer to mean ± standard error of the mean of the chicken samples at each time interval.

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Table 2. Ventricular wall measurements over time of both chicken lines.

Age (days)	Line (n)	RVW thickness (mm)	LVW thickness (mm)	RVW thickness (mm) per 100g BW	LVW thickness (mm) per 100g BW	LVW:RVW ratio
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
1	Ross (6)	0.46 ± 0.06	1.44 ± 0.08	0.87 ± 0.12	2.77 ± 0.19	3.4 ± 0.34
	LD (6)	0.51 ± 0.02	1.27 ± 0.06	1.32 ± 0.1	3 ± 0.06	2.5 ± 0.17
7	Ross (6)	0.4 ± 0.02	1.79 ± 0.12	0.24 ±0.02	1.06 ± 0.08	4.51 ± 0.24
	LD (6)	0.45 ± 0.03	1.62 ± 0.15	0.45 ± 0.02	1.6 ± 0.13	3.65 ± 0.41
14	Ross (6)	0.94 ± 0.11	2.08 ± 0.35	0.21 ± 0.02	0.47 ± 0.08	2.37 ± 0.48
	LD (6)	0.62 ± 0.05	2.01 ± 0.05	0.28 ± 0.02	0.89 ± 0.03	3.32 ± 0.2
19	Ross (6)	0.67 ± 0.06	2.55 ± 0.24	0.11 ± 0.01	0.41 ± 0.04	4.1 ± 0.64
21	Ross (6)	0.68 ± 0.07	3.02 ± 0.17	0.09 ± 0.01	0.4 ± 0.02	4.64 ± 0.48
	LD (6)	0.54 ± 0.05	2.26 ± 0.09	0.16 ± 0.01	0.7 ± 0.04	4.28 ± 0.3
25	Ross (6)	0.86 ± 0.17	3.5 ± 0.08	0.07 ± 0.02	0.3 ± 0.01	4.67 ± 0.65
28	Ross (6)	0.89 ± 0.15	3.59 ± 0.24	0.07 ± 0.01	0.29 ± 0.01	4.45 ± 0.54
	LD (6)	0.74 ± 0.12	2.7 ± 0.05	0.13 ± 0.03	0.47 ± 0.02	4.13 ± 0.6
32	Ross (6)	0.79 ± 0.05	3.44 ± 0.1	0.05 ± 0	0.2 ±0.01	4.44 ± 0.31
	LD (6)	0.55 ± 0.03	2.27 ± 0.08	0.07 ± 0	0.3 ± 0.02	4.17 ± 0.3
35	Ross (6)	1.05 ± 0.06	4.28 ± 0.06	0.05 ± 0	0.21 ± 0.01	4.14 ± 0.25
	LD (6)	1.07 ± 0.1	2.92 ± 0.15	0.14 ± 0.01	0.37 ± 0.03	2.9 ± 0.4
42	LD (6)	0.94 ± 0.07	2.78 ± 0.19	0.08 ± 0.01	0.25 ± 0.02	3.06 ± 0.35
49	LD (6)	0.82 ± 0.06	3.18 ± 0.1	0.05 ± 0	0.21 ± 0.01	3.95 ± 0.31
56	LD (6)	0.9 ± 0.11	3.13 ± 0.23	0.05 ± 0	0.17 ± 0.02	3.75 ± 0.65
63	LD (6)	0.81 ± 0.05	3.68 ± 0.16	0.04 ± 0	0.18 ± 0.01	4.61 ± 0.26

LD: Lohmann Dual; LVW: left ventricular wall; Line: genetic breed; n: animal number; Ross: Ross 308; RVW: right ventricular wall; SEM: standard error of the mean.

https://doi.org/10.1371/journal.pone.0214158.t002

Microscopic examination of the heart

Size of the cardiomyocytes. From day 1 to day 35, the cardiomyocytes' diameter increased by 20% for LD chickens and 26.35% for Ross chickens whilst the cardiomyocytes' cross-sectional area increased by 43% (LD) and 59.5% (Ross) (Fig 7A and 7B).

No differences between the two chicken lines were found at any age. However, regression analysis showed that the cardiomyocyte diameter was 1.2% greater ($p = 0.006$) (Fig 7C) and their cross sectional area was about 2.3% larger ($p = 0.007$) (Fig 7D) in LD chickens compared to those of Ross chickens of the same body weight, $R^2 = 0.65$ and 0.65, respectively.

Myocardial capillary density. In comparisons of the myocardium in both chicken lines at hatching, the percentage area occupied by capillaries and the number of capillary profiles in the myocardium, were greater in LD chickens than in Ross chickens (U-test, $p = 0.009$). From d 1 to d 7, the means of these values did not change in LD chickens, whereas they increased in Ross chickens (LSD, $p \leq 0.001$) resulting in no difference between LD and Ross chickens at d 7. Subsequently, from d 14 to d 28 the values did not change in each chicken line and there was no difference between LD and Ross chickens. At 32 and 35 days the myocardium of LD chickens had larger areas occupied by capillaries as well as greater number of capillary profiles

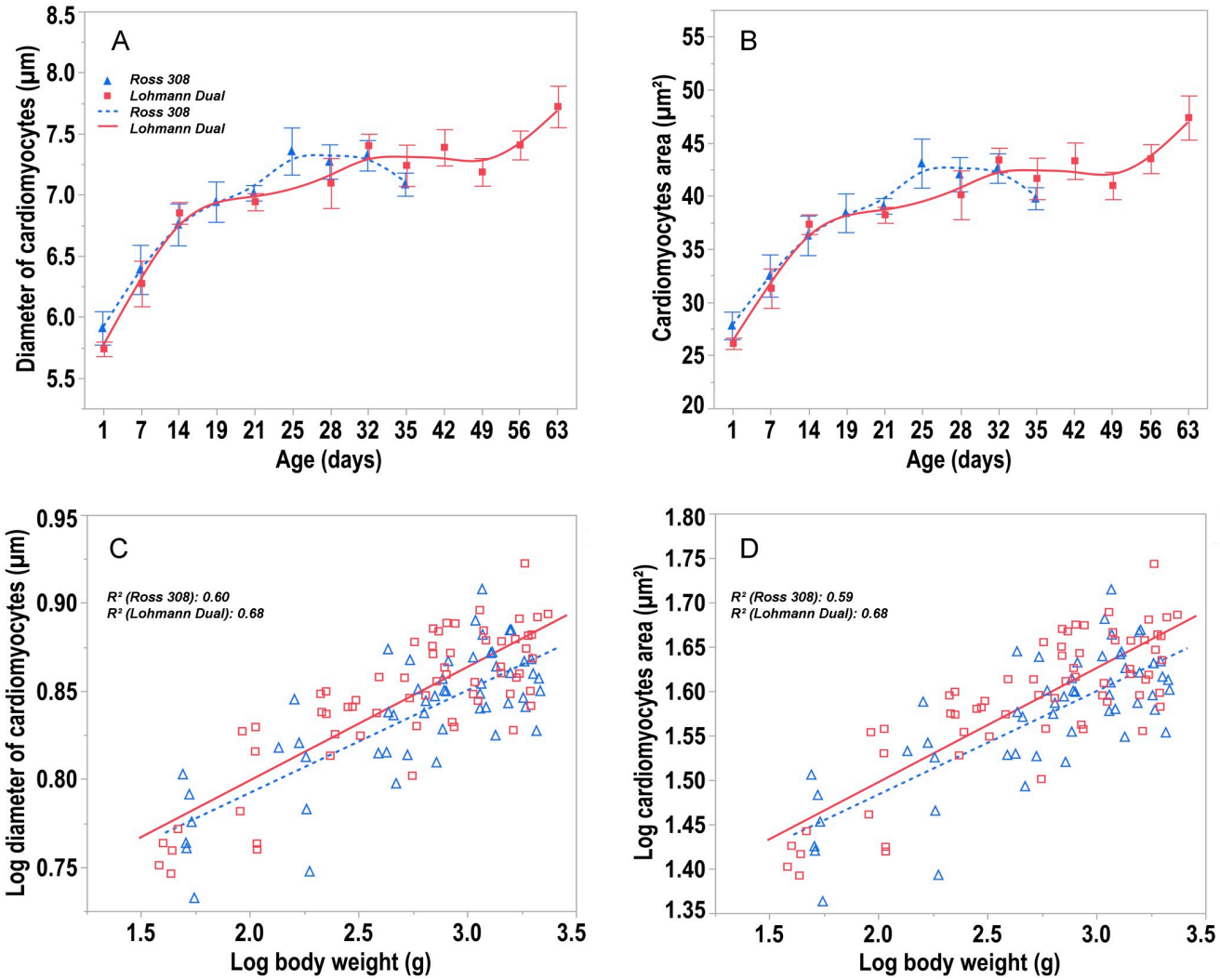

Fig 7. Trendlines of the changes in diameter and cross sectional area of cardiomyocytes versus day post hatching. (A) diameter of cardiomyocytes and (B) cross sectional area of cardiomyocytes versus day post hatching for Ross and LD chicken lines. Bars refer to mean ± standard error of the mean of the chicken samples at each time interval. (C) allometric plot: log transformed diameter of cardiomyocytes and (D) cross sectional area of cardiomyocytes versus log of body weight post hatching for Ross and LD chicken lines. Symbols represent each individual value for each chicken line.

https://doi.org/10.1371/journal.pone.0214158.g007

than did Ross chickens (U-test, $p < 0.05$) (Table 3). The intercapillary distance in the myocardium of LD chickens was shorter than in Ross chickens only at d 35 (U-test, $p = 0.045$). At all other ages there were no differences between both lines (Table 3). The number of cardiomyocytes supplied by one capillary decreased over the time of the study in both chicken lines. The number of cardiomyocytes per capillary did not differ between both chicken lines of the same age, except at 32 days. At this age, one blood capillary supplied 2.36 cardiomyocytes in LD chickens and 3.29 in Ross chickens (U-test, $p = 0.013$). This number decreased from 3.9 to 2.4 in LD chickens and from 3.7 to 3.2 in Ross chickens over the time line of the study (Table 3).

Microscopic examination of the aorta

Aortic wall thickness. The aortic wall thickness increased with age in both lines. The aortic wall thickness showed no differences between LD chickens and Ross chickens at hatching

Table 3. Ventricular capillary and cardiomyocyte measurements over time of LD and Ross chicken lines.

Age (days)	Line (n)	Number of capillary profiles per mm^2	Area occupied by capillaries %	Cardiomyocyte per capillary	Intercapillary distance (μm)
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
1	Ross (6)	3123.44 ± 83.85	4 ± 0	3.73 ± 0.23	10.38 ± 0.32
	LD (6)	3734.42 ± 178.18	7 ± 0	3.91 ± 0.25	11.18 ± 0.52
7	Ross (6)	3857.02 ± 161.73	7 ± 0	3.19 ± 0.21	10.76 ± 0.54
	LD (6)	3955.02 ± 140.06	7 ± 0	3.61 ± 0.4	10.64 ± 0.76
14	Ross (6)	3546.02 ± 182.18	7 ± 0.01	2.52 ± 0.21	10.52 ± 0.71
	LD (6)	4069.02 ± 194.27	9 ± 0.01	3.04 ± 0.32	10.21 ± 0.34
19	Ross (6)	3926.42 ± 111.97	9 ± 0.01	3.04 ± 0.24	9.87 ± 0.54
21	Ross (6)	3884.42 ± 240.99	8 ± 0.01	2.6 ± 0.17	10.93 ± 0.42
	LD (6)	3736.82 ± 166.22	9 ± 0.01	2.43 ± 0.13	10.58 ± 0.57
25	Ross (6)	3968.02 ± 226.02	9 ± 0.01	2.7 ± 0.15	11.17 ± 0.36
28	Ross (6)	3406.81 ± 122.68	8 ± 0.01	2.68 ± 0.08	10.44 ± 0.44
	LD (6)	3722.02 ± 110.93	8 ± 0	2.71 ± 0.17	9.04 ± 0.72
32	Ross (6)	3231.01 ± 160.69	7 ± 0	3.29 ± 0.19	9.92 ± 0.68
	LD (6)	3749.02 ± 111.57	9 ± 0.01	2.36 ± 0.2	9.17 ± 0.37
35	Ross (6)	3860.02 ± 368.08	6 ± 0	2.46 ± 0.11	10.4 ± 0.47
	LD (6)	3563.02 ± 168.18	8 ± 0	2.89 ± 0.21	9.13 ± 0.41
42	LD (6)	3651.02 ± 150.29	10 ± 0	2.48 ± 0.14	8.76 ± 0.2
49	LD (6)	3783.02 ± 109.91	9 ± 0.01	2.3 ± 0.1	8.9 ± 0.48
56	LD (6)	3479.01 ± 112.65	9 ± 0	2.45 ± 0.12	9.13 ± 0.31
63	LD (6)	3605.02 ± 119.03	10 ± 0.01	2.33 ± 0.1	10.46 ± 0.61

LD: Lohmann Dual; Line: genetic line; n: animal number; Ross: Ross 308; SEM: standard error of the mean.

https://doi.org/10.1371/journal.pone.0214158.t003

and at d 7. Thereafter, the aortic wall in LD chickens was significantly thinner than in Ross chickens in all age matched groups (Table 4).

Layers of aortic wall. Analysis of the individual aortic wall layers indicated no difference in the thickness of the tunica intima and adventitia between the two chicken lines in all age matched groups. However, the thickness of the tunica media from d 14 to d 32 was lower in LD chickens than in Ross chickens (U-test $p \leq 0.018$) (Table 4). Furthermore, the intima: media ratio was greater in LD than in Ross birds between d 14 and d 32 (U-test $p \leq 0.018$). The overall ratio of the thicknesses of the aortic layers (intima:media:adventitia) was 1:256:4.8 for LD and 1:302:4.9 for Ross (Table 4).

Aortic lumen radius. The radius of the aortic lumen increased continuously with age in both chicken lines. LD chickens had a lower aortic lumen radius than Ross chickens on d 28 and d 35 (U-test, $p \leq 0.05$) (Table 5). However, when both chicken lines had the same BW, the radius of the aortic lumen was 7.2% greater in LD chickens than in the Ross chickens, $p < 0.001$, $R^2 = 0.85$.

Aortic lumen diameter/aortic wall thickness ratio. The ratio of aortic lumen diameter/ aortic wall thickness increased continuously with age in both chicken lines and was greater in LD chickens than in Ross chickens at d 21 (U-test, $p = 0.025$). There were no significant differences between the chicken lines in all other age groups (Table 5).

Aortic lumen area. The cross sectional area of the aortic lumen increased with age in both chicken lines. There was no difference between both chicken lines in the age matched groups, except on d 28, when the aorta of LD chickens had a smaller lumen than that of Ross chickens (U-test, $p = 0.011$). According to the regression analysis, the chicken line as well as the body weight had an effect on the aortic lumen. On average, the aorta of LD chickens had a

Table 4. Aortic wall measurements over time of LD and Ross chicken lines.

Age (days)	Line (n)	Wall thickness of aorta (mm)	Layers of aortic wall			Intima:media:adventitia ratio	
			Int thickness (μm)	Med thickness (μm)	Adv thickness (μm)	Med:Int	Adv:Int
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
1	Ross (6)	0.47 ± 0.01	2.14 ± 0.05	456 ± 12.77	12.98 ± 0.25	212.9 ± 3.25	6.1 ± 0.1
	LD (6)	0.43 ± 0.06	2.29 ± 0.05	440.48 ± 61.87	12.99 ± 0.07	191.12 ± 24.8	5.7 ± 0.13
7	Ross (6)	0.6 ± 0.05	2.48 ± 0.08	623.55 ± 45.65	13.06 ± 0.1	250.1 ± 13.94	5.3 ± 0.18
	LD (6)	0.59 ± 0.04	2.42 ± 0.07	571.03 ± 39.86	13.01 ± 0.07	236.6 ± 16.83	5.35 ± 0.15
14	Ross (6)	0.8 ± 0.02	2.57 ± 0.04	781.28 ± 20.56	13.19 ± 0.1	303.8 ± 5.83	5.14 ± 0.11
	LD (6)	0.63 ± 0.02	2.68 ± 0.06	611.55 ± 23.88	13.23 ± 0.05	228.83 ± 7.6	5 ± 0.11
19	Ross (6)	0.87 ± 0.05	2.75 ± 0.07	852.02 ± 47.65	13.26 ± 0.06	312.2 ± 22.33	4.84 ± 0.12
21	Ross (6)	0.92 ± 0.04	2.71 ± 0.05	904.69 ± 37.91	13.15 ± 0.07	344.5 ± 10.3	4.83 ± 0.09
	LD (6)	0.69 ± 0.03	2.82 ± 0.08	673.86 ± 43.65	13.20 ± 0.11	239.46 ± 16,83	4.7 ± 0.14
25	Ross (6)	0.95 ± 0.05	2.85 ± 0.05	934.58 ± 55.56	13.39 ± 0.11	322.31 ± 20.81	4.7 ± 0.11
28	Ross (6)	0.99 ± 0.04	2.81 ± 0.03	970.28 ± 39.92	13.19 ± 0.1	344.56 ± 11.56	4.7 ± 0.07
	LD (6)	0.77 ± 0.05	2.71 ± 0.04	752.6 ± 49.48	13.44 ± 0.12	277.52 ± 16.7	5 ± 0.05
32	Ross (6)	0.94 ± 0.04	2.79 ± 0.06	927.25 ± 37.45	13.42 ± 0.07	331.58 ± 9.7	4.82 ± 0.11
	LD (6)	0.73 ± 0.03	2.77 ± 0.02	737.60 ± 28.36	13.33 ± 0.12	266.3 ± 10.4	4.81 ± 0.04
35	Ross (6)	1.01 ± 0.03	2.88 ± 0.06	969.49 ± 33.6	13.2 ± 0.05	336.6 ± 19	4.6 ± 0.1
	LD (6)	0.75 ±0.01	2.89 ± 0.1	737.02 ± 13.57	13.46 ± 0.1	256.18 ± 9.62	4.7 ± 0.16
42	LD (6)	0.88 ± 0.03	3.12 ± 0.05	867.48 ± 28.81	13.67 ± 0.07	279.1 ± 12.76	4.4 ± 0.1
49	LD (6)	0.87 ± 0.02	3.17 ± 0.07	855.24 ± 3	13.94 ± 0.08	271.13 ± 14.74	4.4 ± 0.12
56	LD (6)	0.93 ± 0.04	3.18 ± 0.04	915.73 ± 46.83	13.96 ± 0.12	288.6 ± 14.3	4.4 ± 0.1
63	LD (6)	0.9 ± 0.03	3.16 ± 0.01	878.22 ± 34.37	13.98 ± 0.07	278.1 ± 10.8	4.43 ± 0.02

Adv: tunica adventitia; Int: tunica intima; LD: Lohmann Dual; Line: genetic line; Med: tunica media; n: animal number; Ross: Ross 308; SEM: standard error of the mean.

https://doi.org/10.1371/journal.pone.0214158.t004

14% greater luminal cross sectional area than that of Ross chickens of the same body weight, $p < 0.001$, $R^2 = 0.84$. (Table 5).

Elasticity of aorta. The area occupied by elastic fibers in the tunica media decreased steadily with age in both chicken lines. It was greater in the LD chickens' aorta than in the Ross chickens' aorta by 13.3% at d 21 and by 50% at d 35 (U-test, $p < 0.05$). The number of elastic lamellae did not change with age in both lines. The number of elastic lamellae in the aortic tunica media ranged from 19.2 to 22.2 in LD chickens and from 19.6 to 22.5 in Ross chickens. There were no significant differences between LD chickens and Ross chickens (Fig 8A). Nevertheless, the number of elastic lamellae per 1 mm of the aortic wall thickness was higher in LD chickens than in Ross chickens from d 14 onward (U-test, $p < 0.05$) (Fig 8B).

Discussion

Our findings confirm other studies that, at all ages, the body weight of Ross chickens was significantly higher than that of LD chickens. In the present investigation the BW at slaughter reached by LD chickens was lower than that of broiler lines such as Ross 308 and Ross MP3 [4,15]. However, at 35 days of age the BW attained by LD males in our study was twice the body weight (791 g) of similar aged Leghorn layer chickens (394 g) reported in a comparable study [16]. At 63 days, the slaughtered LD males in the present experiment had a BW of 2012 g, making them heavier than the Lohmann Brown layer males reported by Habig et al, (2016),

Table 5. Radius of aortic lumen, ratio of aortic lumen diameter/aortic wall thickness and cross sectional area of aortic lumen. All versus day post hatching.

Age (days)	Line (n)	Radius of aortic lumen (mm)	Aortic lumen diameter/aortic wall thickness ratio	Cross sectional area of aortic lumen (mm^2)
		Mean ± SEM	Mean ± SEM	Mean ± SEM
1	Ross (6)	0.25 ± 0.03	1.09 ± 0.14	0.2 ± 0.04
	LD (6)	0.35 ± 0.04	1.72 ± 0.21	0.4 ± 0.11
7	Ross (6)	0.48 ± 0.05	1.63 ± 0.22	0.78 ± 0.14
	LD (6)	0.48 ± 0.07	1.65 ± 0.22	0.87 ± 0.25
14	Ross (6)	0.85 ± 0.0.3	2.15 ± 0.12	2.25 ± 0.21
	LD (6)	0.80 ± 0.03	2.6 ± 0.18	2.12 ± 0.16
19	Ross (6)	1.06 ± 0.07	2.49 ± 0.22	3.6 ± 0.5
21	Ross (6)	1.06 ± 0.07	2.3 ± 0.1	3.7 ± 0.5
	LD (6)	1 ± 0.05	2.98 ± 0.25	3.16 ± 0.3
25	Ross (6)	1.35 ± 0.08	2.86 ± 0.15	5.9 ± 0.7
28	Ross (6)	1.45 ± 0.02	2.96 ± 0.15	6.8 ± 0.24
	LD (6)	1.23 ± 0.4	3.27 ± 0.19	4.74 ± 0.33
32	Ross (6)	1.36 ± 0.08	2.94 ± 0.27	6.1 ± 0.7
	LD (6)	1.32 ± 0.09	3.16 ± 0.19	5.53 ± 0.9
35	Ross (6)	1.62 ± 0.06	3.25 ± 0.2	8.3 ± 0.5
	LD (6)	1.36 ± 0.07	3.62 ± 0.21	6.21 ± 0.7
42	LD (6)	1.53 ± 0.04	3.48 ± 0.2	7.25 ± 0.34
49	LD (6)	1.38 ± 0.04	3.2 ± 0.05	5.82 ± 0.32
56	LD (6)	1.5 ± 0.05	3.23 ± 0.11	7.1 ± 0.4
63	LD (6)	1.52 ± 0.06	3.41 ± 0.14	7.36 ± 0.76

LD: Lohmann Dual; Line: genetic line; n: animal number; Ross: Ross 308; SEM: standard error of the mean.

https://doi.org/10.1371/journal.pone.0214158.t005

that at the same age weighed just 1233 g [15]. The slaughter weight of contemporary broiler chickens at 5 weeks of age exceeds 2.2 kg [6], whereas in commercial layer hens killed when 73 weeks old, the average body weight of spent hens is 1.9 kg [17]. Due to the large differences in the body growth traits of intensively genetically selected broiler breeds to that of layer breeds,

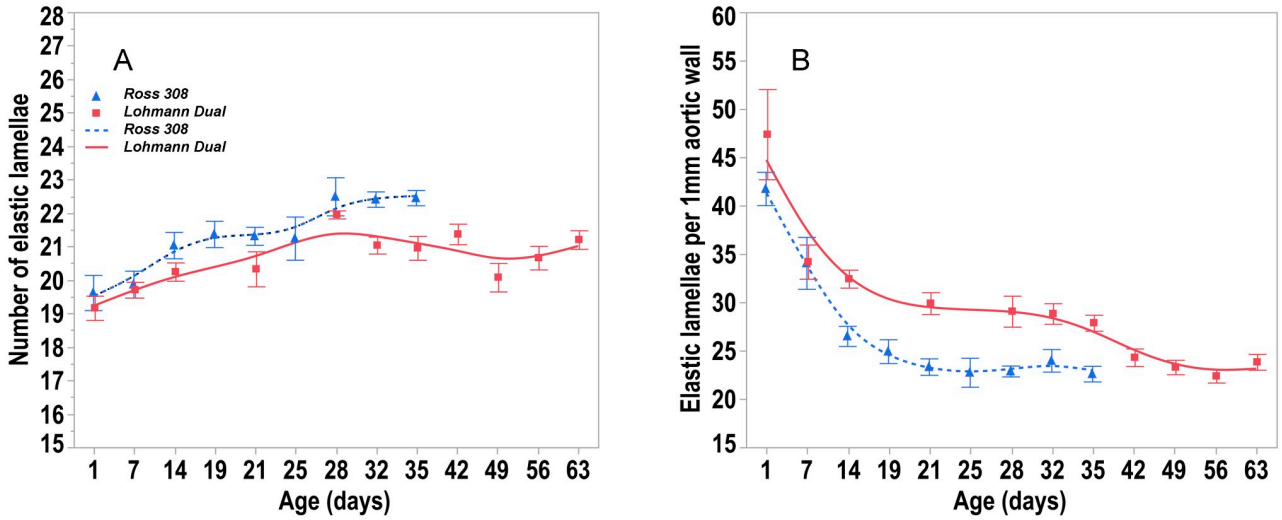

Fig 8. Trendlines of the changes in number of elastic lamellae versus day post hatching. (A) number of elastic lamellae and (B) number of elastic lamellae per 1mm aortic wall versus days post hatching for Ross and LD chicken lines. Bars refer to mean ± standard error of the mean of the chicken samples at each time interval.

https://doi.org/10.1371/journal.pone.0214158.g008

it does not appear possible to breed dual-purpose male chickens to have comparable meat production performance levels to those of the specialized lines.

Our observations highlight that, there were no differences in the growth patterns of the heart of both lines. Although the absolute heart mass of LD chickens was lower than that of Ross chickens over the entire observation period, the relative heart mass in both lines was similar and decreased continuously with age. Our results are in agreement with the findings of similar comparative studies [14,18,19] that reported that the relative heart mass of poultry diminishes with age. However, in these studies the relative heart mass of unselected chicken lines was greater than that of modern meat-type chicken lines. Schmidt et al. (2009) in a comparative study of heart growth in a modern broiler line (Ross 708) to that of the UIUC heritage chickens, where no selection had occurred for over 65 years, found no difference in the relative heart mass between these two lines until 14 days post hatching [8]. They found that after day 14 the relative heart mass of the broiler line became significantly less than that of the heritage line. This drop in the relative heart mass in the broiler lines is, most probably, due to their much larger somatic muscle mass. Presumably, the reduced relative heart mass in the broiler lines could result in diminished cardiac capacity compromising their rapid somatic muscle growth and ultimately the welfare of individual birds [20,21].

Regression analysis of our data showed no difference between the two lines in terms of the allometric growth of the ventricular walls. Nevertheless, the relative thickness of left and right ventricular walls was greater in LD than in Ross chickens. This could be due to the positive correlation between ventricular wall thicknesses and BW growth in LD chickens, whereas in Ross chickens the development of the ventricular wall thickness lags behind BW growth. Many studies report similar findings in slow-growing; chicken [16] and turkey breeds [14] which have relatively thicker ventricular walls than those of fast-growing breeds.

Cardiomyocytes are the individual functional units of cardiac muscle, providing the contractile power of the heart. Domestic chickens, ducks and pheasants all have cardiomyocyte diameters ranging from 3.5 to 6.3 μm [22–24]. In our study the diameter and the cross-sectional area of cardiomyocytes were 7.7 μm and 47.4 μm^2, respectively, in LD birds compared with 7.4 μm and 39.8 μm^2, respectively in Ross birds. However, Li et al. (1997) who isolated the cardiomyocytes enzymatically from layer chickens' hearts recorded the cardiomyocyte diameter to be 8.7 μm and a cross-sectional area of 62.3 μm^2.

When LD and Ross chickens reach the same BW, the cardiomyocytes of LD chickens were about 1.2% larger in diameter and about 2.3% larger in the cross sectional area than those of Ross chickens. Nevertheless both lines had similar ventricular wall thicknesses.

It has been shown, that at the time of hatching, cardiac myocytes cease dividing, but do increase in size as the birds age [25]. Anatskaya et al. (2002) demonstrated in 31 different avian species that the increase in the ventricular wall thickness as the birds age was due to an increase of the size of the cardiomyocytes [26]. In the present study, where the ventricular walls of both genetic lines remained similar in age matched pairs, the number of cardiomyocytes were similar, but those of the LD chickens were larger. This suggests that LD chickens have lower volumes of ventricular wall connective tissue than the Ross chickens.

The number and size of the capillary profiles supplying the cardiomyocytes as well as the diffusion distances between capillaries are important descriptive measures of the oxygen and nutrient supply of the myocardium. In the present study although the LD chickens' heart had less mass than the broilers' heart in all age groups, more of the heart area measured was occupied by capillaries from day 28 onward, at which time the area occupied by capillaries in Ross heart decreased rapidly. Similar to our findings, wild-type turkeys had a higher capillary density compared to highly selected meat-type turkeys [14]. Our observations confirm that capillary density is negatively correlated with total heart weight, i.e. larger hearts of fast growing

genetically selected modern broilers have a lower capillary density which could be a contributor to hypoxia [27].

Michel et al. (1972) reported a relationship of one capillary to four cardiomyocytes in domestic chickens [22]. In our study although the number and the intercapillary distance of blood capillary profiles did not change significantly over time, the number of cardiomyocytes supplied by one capillary decreased from 3.9 to 2.4 in LD chickens and from 3.7 to 3.2 in Ross chickens over the time line of the study. This is probably due to the increase in cardiomyocyte size over time, whilst the number of capillary profiles per unit area remain constant [27].

Generally, birds have a higher blood pressures than mammals of comparable body weights due to the relatively larger volumes of their heart chambers and greater stroke volumes resulting in larger cardiac outputs than found in mammals [28]. Furthermore, the increase in blood pressure in chickens appears to be age and gender dependent. In males is higher than in females [29]. Girard (1973) reported that one-day-old chicks have a systolic / diastolic blood pressure of 60.2 / 37.2 mm Hg and a heart rate of 156 beats/min. By 3–4 weeks these had risen to 114.6 / 72.9 mm Hg, 345 beats/min and at 5–6 weeks to 130.9 / 95.3 mm Hg and 376 beats/min [30]. The aorta acts as an elastic reservoir, storing blood transiently during systolic ejection, and providing an even flow to the periphery during diastole. Here the elastic recoil of the artery wall converts the pulsatile output of the heart into a smooth flow in the peripheral circulation [31].

Laplace's law describes the tension in the walls of arteries. This geometric law applied to a tube explains that for a given internal fluid pressure, the wall tension will be proportional to the radius of the vessel [Wall tension = Transmural pressure × vessel radius / wall thickness] [32]. The application of this law to large arteries, that have comparable blood pressures, is that the larger the vessel radius, the greater the wall tension required to withstand a given internal fluid pressure. In the present study, the aortic wall thickness in birds of both LD and Ross lines increased with age as the vascular system adapted to higher blood pressures. When both lines had the same body weight, they had the same thickness of the aortic wall, but the luminal radius of aorta was 7.2% greater in LD birds than in the Ross birds. In accordance with Laplace's law, when the transmural pressure is similar in both lines, the LD aorta has a greater wall tension than the Ross chickens. Consequently, the LD birds must have a stronger aortic wall to withstand the wall tension than that found in the Ross aorta. However, the mechanical properties of the arterial wall depend not only on the arterial wall thickness but also on the micro-architecture and composition of the arterial wall [33]. The vast majority of the aortic wall is made up of layers of smooth muscle embedded in elastin fibers, alternating with layers of collagen [31]. Elastin is a protein with rubber-like properties arranged as a network of highly extensible fibers in the elastic artery wall [24]. In the present study the proportion of the elastic fibers in the tunica media of the aorta decreased consistently with age in both lines confirming Ruiz-Feria et al findings [34]. Sans and Moragas (1993) suggested that the decrease in elastin concentration was, in part, due to the increase of other components such as collagen, while the total elastin content did not change [35]. Another study of the thoracic aorta of chickens demonstrated that the percentage of elastic fibers increased from the 1st to the 30th day of age and then decreased from this point on until the age of 36 months [36]. The elastic fibers in the tunica media are arranged in concentric fenestrated layers, the lamellae. Our findings show that the number of elastic lamellae in both chicken lines did not change with age. Similarly, Wagenseil and Mecham (2009) reported that the number of elastic lamellae does not change after birth in the aorta of vertebrates such as rats, rabbits and pigs [37]. Other studies have reported between 25 and 30 elastic lamellae in the aortic tunica media of adult chickens [38] and swans [39]. Each elastic lamella alternates with a physically connected concentric ring of smooth muscle cells to form lamellar units. A single lamellar unit has elastic properties

allowing it to work within and to withstand a set range of mechanical load, that is best described by the parameter 'tension' [40]. There is a direct relationship between the tension and the number of elastic lamellae units, i.e. the number of lamellar units increases as arterial wall tension increases [41]. Faury (2001) suggested, that the tension per lamella is constant across species [40]. It has been estimated that each individual elastic lamella withstands in the range of 1–3 Nm^{-1} (Newton × $Meter^{-1}$) tension in mammalian species [33]. Our results showed that the aorta of LD birds had a proportionally larger number of elastic lamellae and greater percentage of elastic fibers giving the aortic wall of LD chickens the ability to withstand greater wall tension.

Conclusion

Our study indicates that the heart of both LD and Ross chicken lines has similar ventricular walls geometry. Furthermore, the blood capillary density was greater in LD's heart than that in Ross's heart. The micro-architecture of aorta, specifically the number of elastic lamellae and the percentage of elastic fibers are greater in LD chickens than in Ross chickens. Therefore, LD chickens have better aortic mechanical properties than those of Ross chickens, suggesting that the LD' aorta has a greater ability to adapt to changes of blood pressure, than that of the Ross chickens.

Supporting information

S1 Table. Number of elastic lamellae in the aortic wall, lamellae number per 1mm aortic wall and percentage area occupied by elastic fiber bundles of both chicken lines. All versus day post hatching.
(DOCX)

Acknowledgments

We would like to thank Karin Briest-Forch and Barbara Drewes for their help in the collection and histological preparation of the samples. Also, we thank Johanna Leber for assistance with sample collection.

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3 Basic morphometry, microcomputed tomography and mechanical evaluation of the tibiotarsal bone of a dual-purpose and a broiler chicken line

Published in:

PLOS ONE, March 11, 2020
Public Library of Science

Please find the original article via the following digital object identifier:
https://doi.org/10.1371/journal.pone.0230070

PLOS ONE

RESEARCH ARTICLE

Basic morphometry, microcomputed tomography and mechanical evaluation of the tibiotarsal bone of a dual-purpose and a broiler chicken line

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Citation: Harash G, Richardson KC, Alshamy Z, Hünigen H, Hafez HM, Plendl J, et al. (2020) Basic morphometry, microcomputed tomography and mechanical evaluation of the tibiotarsal bone of a dual-purpose and a broiler chicken line. PLoS ONE 15(3): e0230070. https://doi.org/10.1371/journal.pone.0230070

Editor: David Fyhrie, University of California Davis, UNITED STATES

Received: August 8, 2019

Accepted: February 20, 2020

Published: March 11, 2020

Data Availability Statement: All relevant data are within the manuscript.

Funding: JP and HMH received the award (grant number 28RZ372049). The project is supported by funds of the German Government's Special Purpose Fund held at Landwirtschaftliche Rentenbank: https://www.rentenbank.de/. We acknowledge support by the German Research Foundation and the Open Access Publication Fund of the Freie Universität Berlin: www.fu-berlin.de.

Abstract

Continuous loading of the skeleton by the body's weight is an important factor in establishing and maintaining bone morphology, architecture and strength. However, in fast-growing chickens the appendicular skeleton growth is suboptimal making these chickens predisposed to skeletal mineralization disorders and fractures. This study compared the macro- and microstructure as well as the mechanical properties of the tibiotarsus of a novel dual-purpose, Lohmann Dual (LD) and a highly developed broiler, Ross (Ross 308) chicken line. Eighty one-day-old male chicks of each line were grown until their body weight (BW) reached 2000g. Starting at the day of hatching, six birds of each line were sampled weekly. The weight, length and width of the tibiotarsus were measured and its mechanical properties (rigidity, M-Max and the M-fracture) were evaluated using the three-point bending test. Additionally, the mineral density of both, trabecular and cortical bone, the bone volume fraction, the trabecular number, thickness and separation plus cortical thickness of both chicken lines were analyzed using microcomputed tomography. The growth of the tibiotarsus in both chicken lines followed a similar pattern. At the same age, the lighter LD chickens had shorter, thinner and lighter tibiotarsi than those of Ross chickens. However, the LD chickens had a similar cortical thickness, bone volume fraction and similar mineral density of both trabecular and cortical bone to that of Ross chickens. Furthermore, the tibiotarsus of LD chickens was longer, heavier and wider than those of Ross chickens of the same BW. In addition the rigidity of the LD tibiotarsus was greater than that of Ross chickens. This suggests that the tibiotarsus of LD chickens had more bending resistance than those of Ross chickens of the same BW. Consequently, fattening LD chickens to the marketable weight should not affect their leg skeleton stability.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Abbreviations: BV/TV, bone volume fraction; BW, body weight; Ct.Ar, Cortical bone area; Ct.Ar/Tt.Ar, Cortical area fraction; Ct.BMDv, Volumetric bone mineral density in the cortical bone; Ct.Th, Cortical thickness; d, day; LD, Lohmann Dual; M-fracture, fracture bending moment; M-Max, maximum bending moment; Med.Ar, Medullary section area; N, newton; ROI, region of interest; Ross, Ross 308; Tb.BMDv, Volumetric bone mineral density in the trabecular bone; Tb.N, Trabecular number; Tb.Sp, Trabecular separation; Tb.Th, Trabecular thickness; Tt.Ar, Total cross-sectional area; μCT, microcomputed tomography.

Introduction

Worldwide, male chickens from layer genetic lines are killed immediately after hatching due to their inability to lay eggs and their slow muscle growth [1]. Farming dual-purpose chickens is an alternative to the culling of males. Here females are used to produce eggs and the males are grown for meat production. This approach is exemplified by the new commercial dual-purpose chicken, the Lohmann Dual (LD), that has been developed by crossing meat and layer chicken lines [2]. Earlier comparative studies show that LD chickens have a better myocardial capillary supply and better aortic mechanical properties compared to the highly specialized broiler, Ross 308, chickens [3]. Nevertheless, LD chickens have a slower growth rate compared to Ross 308 chickens that appears to be associated with the LD chickens having a smaller intestinal absorptive surface area [4].

A recent comparative study between two different slow-growing broiler genotypes showed that the tibiotarsus traits influenced by genetic strain [5]. In earlier studies on rapidly growing meat-producing poultry the tibiotarsal bone was found to be the most affected bone in clinical and subclinical leg problems as tibial dyschondroplasia and rickets [6–8]. Additionally, the tibiotarsus was shown to have significantly higher mechanical and geometrical parameter values as well as higher mineralization than did other bones of the pelvic limb [9, 10]. Consequently, the tibiotarsal bone has greater mechanical resistance to deformation and as such is the most appropriate of the leg bones for research. Although the LD chicken line is grown commercially there is little information on their principal weight bearing bones notably the long bones of the pelvic limb. Therefore, studies on the ultrastructure and bending properties of the LD tibiotarsus are important so that production protocols can be adopted to lessen potential skeletal disorders and thus ensure that their welfare is optimised.

As postulated in Wolff's Law, during development and aging, bone architecture adapts to withstand the extremes of functional load-bearing [11, 12]. However, this adaptive process appears to be suboptimal in chickens that have been selected for rapid and high growth rates [13]. Thus in rapidly growing broiler lines the tibiotarsal bones are strongly loaded by the body weight of the birds and are more prone to mineralization disorders and fractures [14]. Moreover, market age chickens often suffer from lameness and bone deformities that can cause bone fracture during capture and transportation [15]. A probable reason for the poor pelvic limb bone health in broiler chickens is a reduction in bone quality. The tibiotarsus in broiler chickens is less mineralized and less dense than found in slow-growing genetic lines [13]. Several studies have shown that an increased load on the bone increases bone mass [16, 17].

Previous studies assert that the bone quality of slow-growing broilers is better compared to that of the fast-growing ones and that the faster growing chickens are disadvantaged by their heavier body weight, i.e. the cortical bone of fast-growing broilers is highly porous and poorly mineralized. They report that the association between tibiotarsal fracture strength and growth rate was negative [13, 18–21].

An evaluation of the quality and integrity of the tibiotarsus includes the examination of morphological variables such as bone mass and length [22] as well as details of the microstructure properties of both trabecular and cortical bones including bone mineral density, plus the assessment of mechanical properties such as bone fracture strength and stiffness [20, 21, 23, 24]. Tibiotarsal bone strength is influenced by numerous properties including shape, size, mass, structure, and composition [25–27]. Furthermore, bone strength is correlated positively with trabecular properties [trabecular number, thickness and separation] [28, 29], bone mineral density and bone weight [30, 31].

The aim of this study was to investigate the macro- and microstructural as well as the mechanical properties of the tibiotarsal bone of a dual-purpose chicken line (Lohmann Dual,

LD) and a modern broiler chicken line (Ross 308) throughout the period from time of hatching until they reach their market body weight.

Material and methods

Animals and husbandry

The tibiotarsal bones of male chicks from two different lines, a dual-purpose line (Lohmann Dual, LD) and a broiler line (Ross 308) were used for this study. The same chickens had previously been used to investigate the gastrointestinal tract [4] as well as the heart and aorta [3]. Animals and husbandry conditions were described in detail in the above studies [3, 4]. Briefly, the chickens were reared under similar husbandry conditions until they reached a body weight (BW) of 2000 g, i. e. 35 days for Ross chickens and 63 days for LD chickens. The study was approved by the Animal Welfare Committee "Landesamt für Gesundheit und Soziales", Berlin, Germany, ID: 0236/15.

Sample collection

Six birds from each line were sampled randomly on days 1, 7, 14, 19, 21, 25, 28, 32 and 35 for Ross chickens and on days 1, 7, 14, 21, 28, 32, 35, 42, 49, 56 and 63 for LD chickens.

On sampling day, the live body weight (BW) of each bird was determined to an accuracy of 0.1 g using a mechanical scale (Sartorius, Göttingen, Germany).

The tibiotarsal bones of both left and right legs were excised and cleaned of surrounding muscles and soft tissues and separated from the fibula. Weight (g), relative to body weight (g/100 g BW), length (cm) [between the ends of proximal and distal epiphyses], relative length (cm/100 g BW), mass per unit of length (g/cm) and width (cm) [at the calculated midpoint, i.e. 50% of length] of the left and right tibiotarsal bones were measured and the average values for both bones was calculated. The bones were sealed individually in plastic bags and stored at -20˚C until required for further analysis when they were warmed up to 20˚C before mechanical testing and microcomputed tomography (μCT) analysis.

Mechanical properties

The mechanical properties of the right tibiotarsal bones were determined by the three-point bending test, using a Zwick testing machine (Zwick/Roell Z010, Ulm, Germany). The cranial face of each tibiotarsus was placed horizontally down on two support holders and submitted to a mid-shaft vertical force (Fig 1A). A span length of 25 mm was used for the tibiotarsi of birds aged from day 1 to day 21 and 50 mm for the bones of older birds. The vertical force testing speed was 0.1 $mm.s^{-1}$ until fracture. The bending force [N] and the displacement (deflection) [mm] were recorded using a TestXpert II software (TestXpert II, Zwick, Ulm, Germany) at a sample rate of 100 Hz. The loading force ranged from 40 N to 1 kN (Fig 1A–1C).

The resulting load-displacement curve was analyzed using a customized Matlab script (The MathWorks, Inc. USA). The following parameters were determined (Fig 1D):

- Stiffness (N/mm): the linear slope of the elastic part of the curve.
- Maximum load (N): the highest load reached.
- Fracture load (N): the load where the bone ultimately failed.

These measurements were normalized for the different span widths according to the equations described previously [32]:

- Rigidity (Nmm^2) = stiffness (N/mm) × $(\text{span length})^3$ / 48

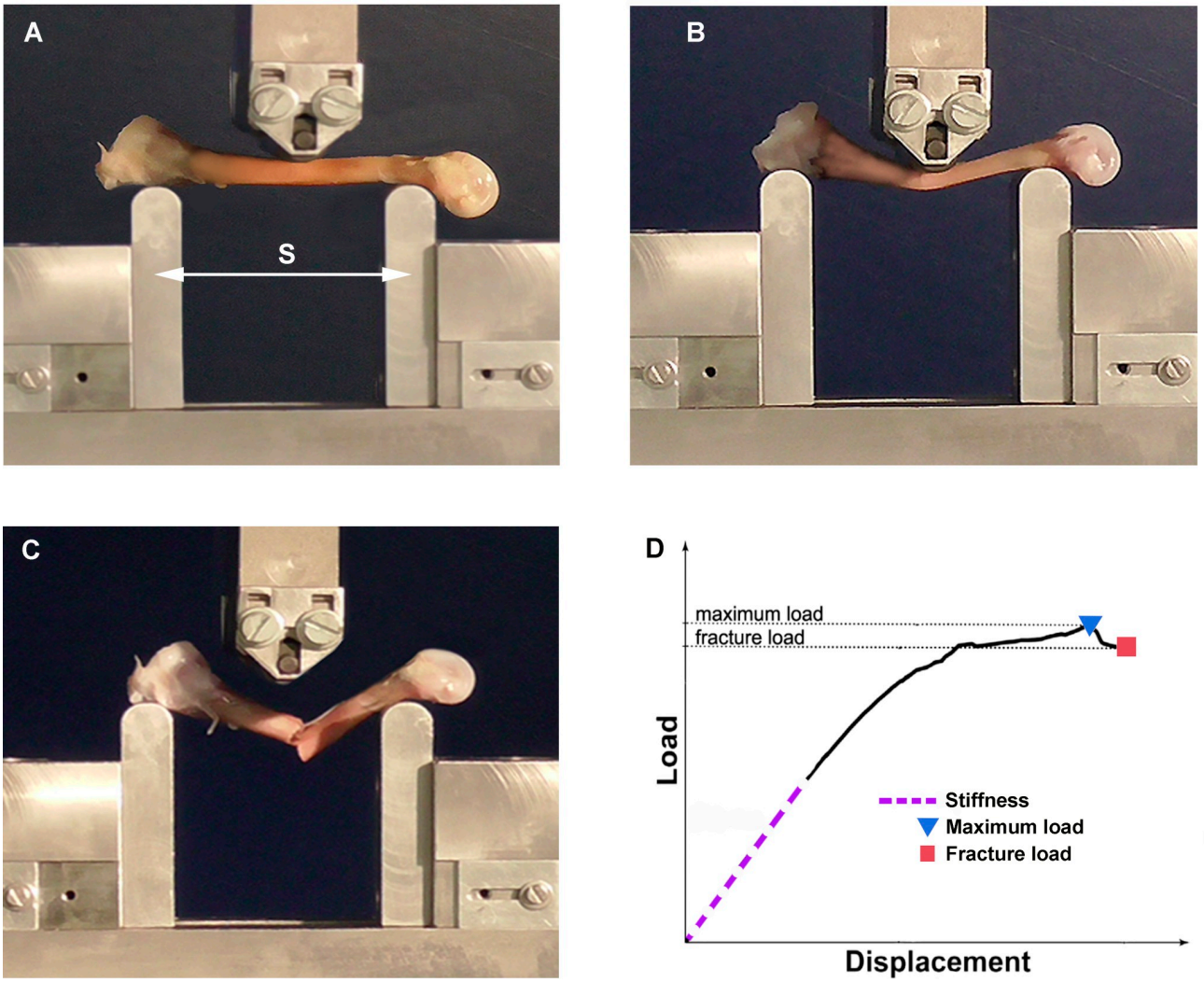

Fig 1. Three-point bending test. (A) Zwick testing machine with the tibiotarsus placed horizontally cranial face down on two support holders and submitted to a vertical force from above. Here (S): span length between two support holders. Zwick testing machine showing (B) maximum load (before the fracture) and (C) fracture load (bone fracture). (D) Load-displacement curve illustrating the estimated mechanical properties.

https://doi.org/10.1371/journal.pone.0230070.g001

- Maximum bending moment (Nmm) = maximum load (N) × span length / 4
- Fracture bending moment (Nmm) = fracture load (N) × span length / 4

Then these parameters were calculated per unit of BW.

Microcomputed tomography imaging (μCT)

The μCT analysis was carried out on the left tibiotarsal bones for Ross birds at the ages of 1, 7, 21 and 35 days and for LD birds at the age of 1, 7, 21, 35 and 63 days. The tibiotarsi were scanned using a high-resolution microcomputed tomography (μCT) scanner (VivaCT40, Scanco Medical, Brüttisellen, Switzerland; nominal isotropic image resolution 21 μm, 70 kVp, 114 μA, 381 ms integration time).

Microcomputed tomography analysis was performed on trabecular and cortical regions of interest selected using ImageJ software (ImageJ 1.52h, Wayne Riband, National Institutes of

Health, USA) according to the methodologies reported by Castrillón et al [33]. Trabecular bone samples [trabecular ROI(region of interest)] were taken from 3% below the distal-most point of the proximal growth plate and extended distally for 7.5% of the tibiotarsus length. Cortical bone samples (cortical ROI) were obtained from the diaphyseal region from 15% distal to the proximal growth plate and extended distally 3% of the tibiotarsus length [33] (Fig 2A–2C).

To segment the bone from marrow and soft tissue, firstly the colour threshold was measured manually for each bird using the gray scale. Thereafter, the average of the colour threshold values of each individual age group were calculated. Then, the software generated the estimated parameters based on the previously calculated threshold.

The following parameters were measured for the trabecular ROI:

- Volumetric bone mineral density (Tb.BMDv; mg/cm^3): Mass of mineralized bone per total volume in the trabecular ROI.

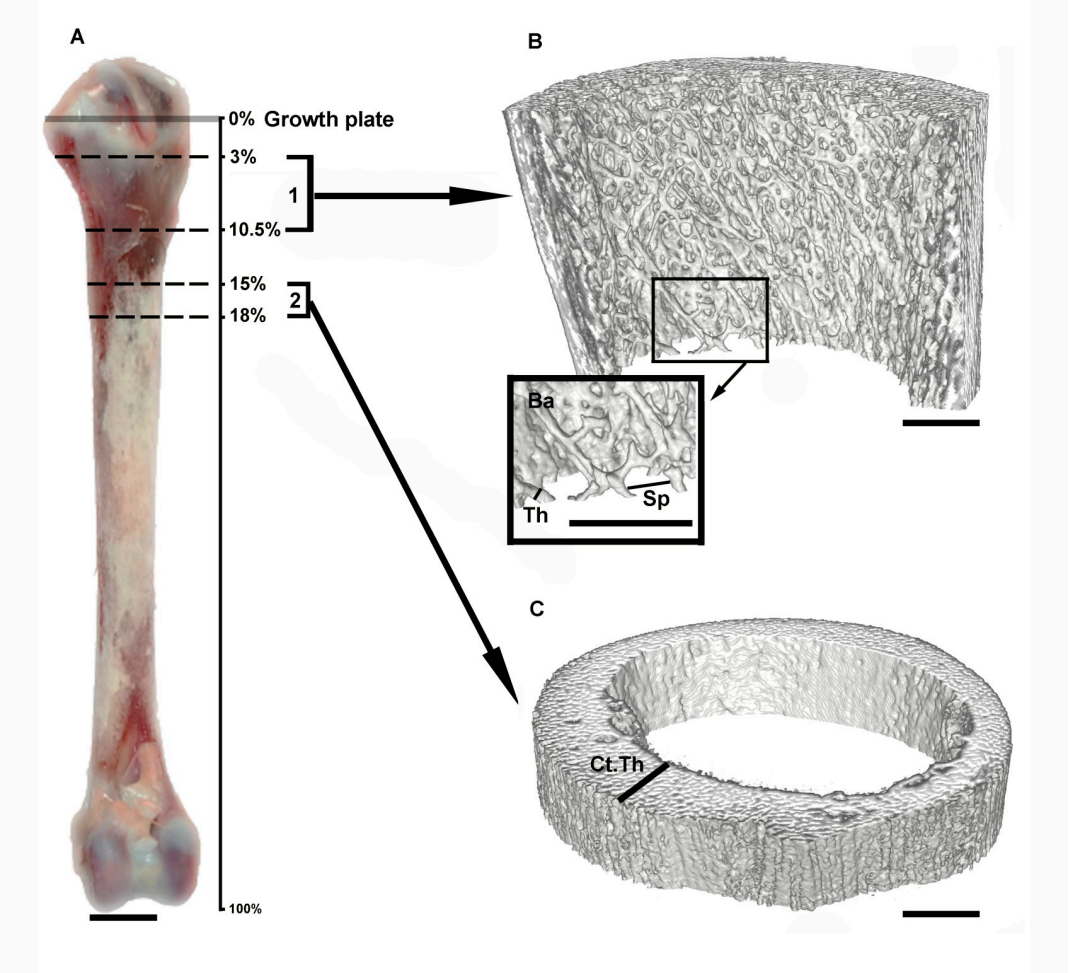

Fig 2. Measurement of the trabecular and cortical bone properties using μCT analysis. (A) Tibiotarsal bone of LD chicken at day 63: (1) the trabecular sample taken from 3% below the distal-most point of the growth plate and extended distally for 7.5% of the tibiotarsus length. (2) The cortical region of interest selected from the diaphyseal region from 15% distal to the proximal growth plate and extending distally 3% of the tibiotarsus length. (B and Ba) Trabecular bone. (C) Cortical bone. Ct. Th: cortical thickness, Th: trabecular thickness and Sp: trabecular separation. Bar = 1 cm for A, and 1 mm for B, Ba and C.

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- Bone volume fraction (BV/TV; %): Ratio of the segmented bone volume to the total volume of the trabecular ROI.
- Trabecular thickness (Tb.Th; mm) (Fig 2Ba).
- Trabecular number (Tb.N; 1/mm).
- Trabecular separation (Tb.Sp; mm) (Fig 2Ba).

Trabecular bone analysis was performed only from day 7 onward due to the scarcity of trabeculae on day 1, which could have led to unreliable results [28].

For the cortical ROI, the parameters measured included:

- Volumetric bone mineral density (Ct.BMDv; mg/cm^3): Mass of mineralized bone per total volume in the cortical ROI.
- Cortical thickness (Ct.Th; mm) (Fig 2C).
- Cortical bone area (Ct.Ar; mm^2).
- Total cross-sectional area (Tt.Ar; mm^2).
- Cortical area fraction (Ct.Ar/Tt.Ar; %).
- Medullary section area (Med.Ar; mm^2) was calculated as follows:

Med.Ar = Tt.Ar—Ct.Ar

Statistical analysis

Statistical analyses were performed using the statistical package program IBM SPSS Statistics 23 (IBM Corporation, New York, USA). The graphs were generated using the statistical package program JMP® Pro 13 (SAS Institute Inc., Cary, USA). Comparison of the data between the two lines at the same age were performed using the Mann–Whitney U test. One-way analysis of variance (ANOVA) with the post hoc Dunnett's test was performed to evaluate the effect of age on the tibiotarsus parameters. Pearson's correlation coefficient was used to test the relation between mechanical properties (rigidity, M-Max and M-fracture) and the other densitometric and geometric parameters as well as between Tb.BMDv and Ct.BMDv. To explore the effect of chicken line and BW on the tibiotarsus parameters, all data collected was regressed against the chicken line and the BW using the log-log regression model. All statistical analyses were two-sided with significance defined as a p-value of < 0.05.

Results

Morphometric properties

Length (Fig 3A), width and weight of the tibiotarsus increased steadily with age in both lines ($p < 0.05$). From day (d) 1 to d 35 post hatching, the length and the weight of the tibiotarsus of Ross chickens increased at a rate of 0.21 cm/d and 0.49 g/d, respectively, whereas the LD chickens tibiotarsus increased by a rate of 0.17 cm/d and 0.23 g/d over the same period, and from d 1 to 63 the increase was by a rate of 0.15 cm/d and 0.32 g/d. For Ross birds, the highest rate of increase in the bone length (0.23 cm/d) and weight (1 g/d) was between d 28–35, while for LD birds, the highest rate of increase in the bone length (0.2 cm/d) was between d 21–28 and the greatest increase in bone weight (0.52 g/d) was between d 42–49.

The tibiotarsus of LD chickens was significantly shorter, thinner and lighter than those of Ross chickens at all ages between d 1and d 35 post hatching (Table 1). However, when length

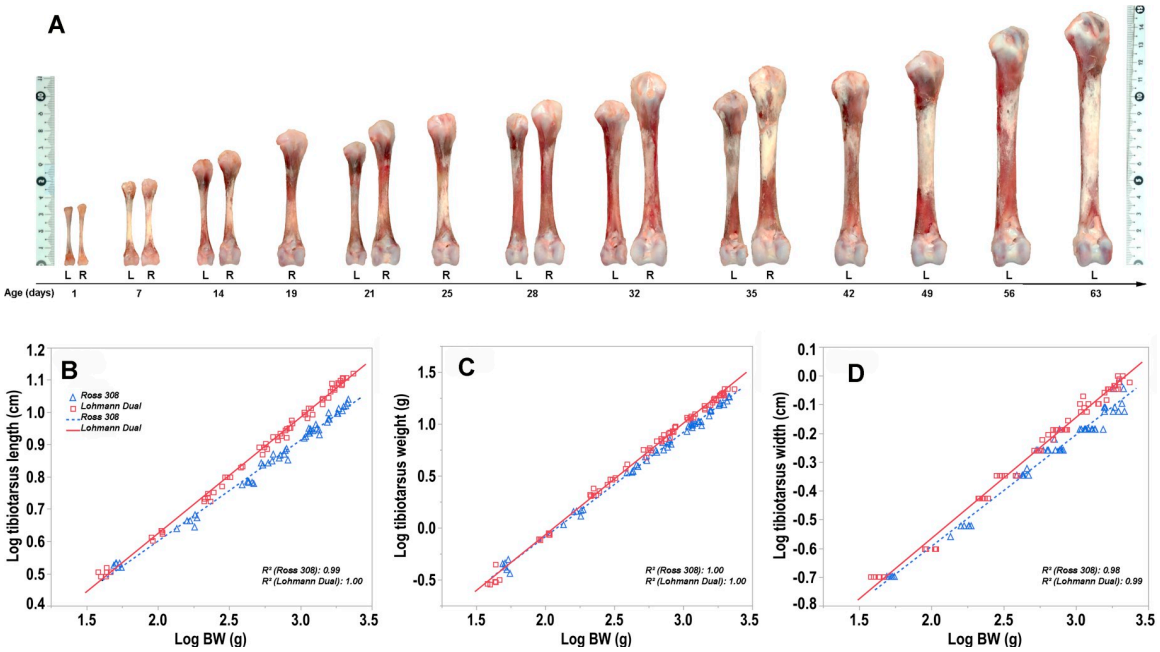

Fig 3. (A) Tibiotarsus length of Ross (R) and LD (L) chicken lines. (B-D) Allometric plots: log transformed length, weight and width of the tiobiotarsus versus log of body weight (BW) post hatching for Ross and LD chicken lines. Symbols represent each individual value for each chicken line.

https://doi.org/10.1371/journal.pone.0230070.g003

Table 1. Body weight, tibiotarsus length, weight and width of LD and Ross chicken lines versus day post hatching.

Age (day)	Line (n)	BW (g)		Tibiotarsus length (cm)		Tibiotarsus weight (g)		Tibiotarsus width (cm)	
		Means	SD	Means	SD	Means	SD	Means	SD
1	Ross (6)	52.26	2.29	3.36	0.05	0.43	0.04	0.20	0.01
	LD (6)	42.45	3.06	3.18	0.08	0.32	0.01	0.20	0.01
7	Ross (6)	169.47	19.41	4.58	0.17	1.36	0.12	0.30	0.01
	LD (6)	101.20	8.12	4.19	0.12	0.84	0.06	0.25	0.01
14	Ross (6)	435.35	28.03	6.04	0.06	3.61	0.20	0.45	0.01
	LD (6)	224.77	13.01	5.45	0.15	2.15	0.12	0.38	0.01
19	Ross (6)	640.73	110.77	7.04	0.17	5.34	0.57	0.55	0.01
21	Ross (6)	746.58	58.32	7.45	0.18	6.35	0.43	0.58	0.04
	LD (6)	329.17	46.05	6.39	0.34	3.22	0.41	0.45	0.01
25	Ross (6)	1191.67	92.54	8.60	0.14	9.63	0.25	0.65	0.01
28	Ross (6)	1221.00	108.99	8.82	0.28	10.00	0.56	0.65	0.01
	LD (6)	575.33	62.55	7.82	0.27	5.75	0.46	0.57	0.03
32	Ross (6)	1677.83	172.74	9.71	0.32	13.71	0.70	0.74	0.05
	LD (6)	754.17	93.82	8.59	0.41	7.71	0.64	0.65	0.03
35	Ross (6)	2013.17	142.70	10.43	0.36	17.02	0.87	0.85	0.08
	LD (6)	791.67	58.41	8.86	0.35	8.21	0.41	0.65	0.01
42	LD (6)	1130.50	59.16	9.98	0.28	11.66	0.27	0.79	0.04
49	LD (6)	1522.50	112.73	11.19	0.46	15.33	1.22	0.83	0.04
56	LD (6)	1817.33	134.21	12.20	0.35	18.60	1.47	0.92	0.03
63	LD (6)	2011.83	182.87	12.64	0.37	20.16	1.06	0.95	0.04

BW: live body weight; LD: Lohmann Dual; Line: genetic line; n: animal number; Ross: Ross 308; SD: standard deviation of the mean.

https://doi.org/10.1371/journal.pone.0230070.t001

and weight of the tibiotarsus were expressed relative to body weight, the tibiotarsus of LD chickens had a greater relative length, width and weight than Ross chickens at all age groups ($p < 0.05$) (Table 1).

The relative tibiotarsus length of both chicken lines decreased significantly over the entire study period for Ross chickens and until day 42 for LD chickens, thereafter the decrease was not significant. The relative tibiotarsus width of both chicken lines decreased until day 28 for both chicken lines, subsequently the relative tibiotarsus width did not change significantly. The relative tibiotarsus weight of both chicken lines did not differ over the study period except between d 7 and d 14 for LD chickens (Fig 4).

Regression analysis showed that the chicken line had an effect on the length, weight and width of the tibiotarsus, where the tibiotarsus of LD chickens had a greater length by 6%, weight by 7% and width by 5.2% than those of Ross chickens of the same BW ($p < 0.001$), adjusted $R^2 = 0.99$, 0.99 and 0.98, respectively (Fig 3B–3D).

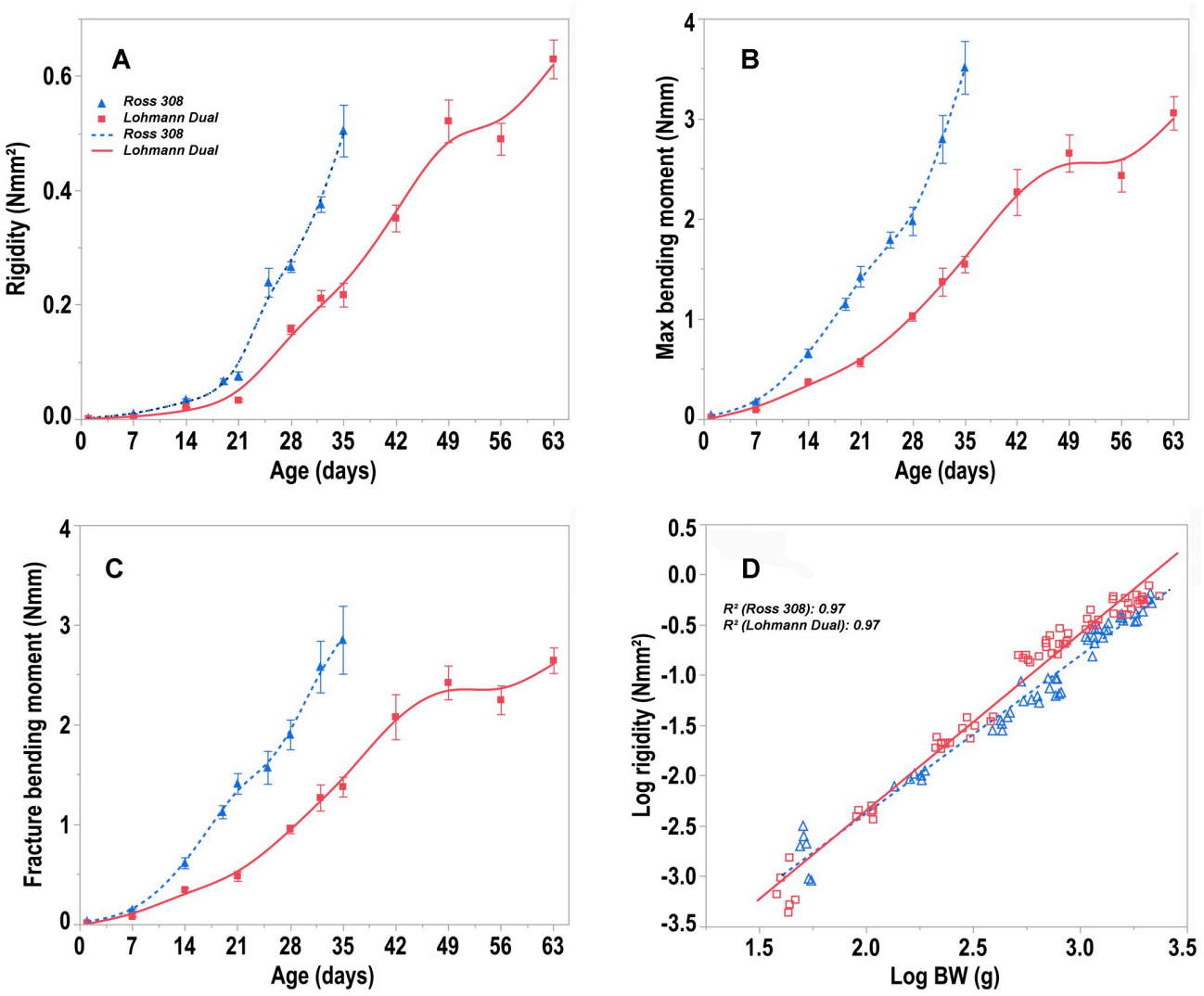

Fig 4. Trendlines of the changes in the mechanical properties of the tibiotarsus versus day post hatching. (A) rigidity, (B) maximum bending moment and (C) fracture bending moment of the tibiotarsus versus day post hatching for Ross and LD chicken lines. Bars refer to mean ± standard error of the mean of the chicken samples at each time interval. (D) allometric plot: log transformed rigidity versus log of body weight (BW) post hatching for Ross and LD chicken lines. Symbols represent each individual value for each chicken line.

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The tibiotarsus mass per unit of length in Ross chickens increased with age over the whole study period and in LD chickens until d 56 ($p < 0.05$). The tibiotarsus mass per unit of length of LD chickens was significantly lower than that of Ross chickens in all comparable age groups between d 1 and d 35 post hatching ($p < 0.05$).

Mechanical properties

Rigidity, M-Max and M-fracture of the tibiotarsus all increased with age. The LD chickens' tibiotarsus had a lower: rigidity, M-Max and M-fracture than those of Ross chickens in all age groups (Fig 4A-4C) ($p < 0.05$). However, there were no differences between both chicken lines, when these parameters were expressed relative to body weight.

Furthermore, at the same BW, the chicken line had an influence on the tibiotarsus rigidity ($p < 0.001$). The tibiotarsus rigidity of LD chickens was greater on average by 1.7% than that of Ross chickens, $R^2 = 0.97$ (Fig 4D). The correlations between the mechanical properties and the other densitometric and geometric parameters are presented in Table 2.

Trabecular bone structural properties

The volumetric bone mineral density (BMDv) increased steadily with age over the entire investigation in the LD birds ($p < 0.05$). However, in Ross birds the tibiotarsus did not change from d 1 to d 21, but increased from d 21 to d 35 ($p < 0.05$). Following day one, there were no differences in the volumetric bone mineral density of trabecular bone between the two lines (Table 3). According to the regression analysis, the chicken line had an influence on the trabecular BMDv ($p < 0.001$). The trabecular BMDv of LD chickens was greater, on average, by 3.2% than that of Ross chickens of the same BW, $R^2 = 0.74$.

The bone volume fraction of the LD tibiotarsus decreased between d 7 and d 21 post hatching ($p < 0.05$). Thereafter, it remained unchanged till the end of the study. From d 7 to d 35, the bone volume fraction of the Ross tibiotarsus decreased ($p < 0.05$). Excluding d 21, no differences in the bone volume fraction of the tibiotarsus between the LD and Ross chickens were observed over the study period (Table 4).

Trabecular number of the tibiotarsus in both chicken lines decreased from d 7 to d 35 ($p < 0.05$). Between d 35 and d 63, the values did not change in the LD tibiotarsus. Trabecular

Table 2. Pearson's correlation coefficient (r) between the mechanical properties and the other densitometric and geometric parameters of LD and Ross chicken lines.

Parameters	Line	BMDv trab	BMDv cortical	Ct.Th (mm)	B.Ar (mm^2)	Med.Ar (mm^2)	Tib.Den (g/cm)	Tib.Lth (cm)	Tib.Wth (cm)	Tib.Wt (g)	BW (g)
BW (g)	Ross	0.61	0.84	0.87	0.94	0.94	0.99	0.98	0.97	0.99	1
	LD	0.95	0.88	0.92	0.96	0.94	0.99	0.99	0.99	0.99	1
Rigidity (Nmm^2)	Ross	0.88	0.59	0.64	0.90	0.94	0.92	0.94	0.86	0.96	0.96
	LD	0.81	0.66	0.82	0.94	0.91	0.95	0.97	0.93	0.97	0.97
M-max (Nmm)	Ross	0.85	0.66	0.74	0.97	0.99	0.96	0.98	0.97	0.97	0.96
	LD	0.89	0.73	0.89	0.98	0.91	0.96	0.95	0.95	0.94	0.95
M-fracture (Nmm)	Ross	0.78	0.65	0.71	0.95	0.95	0.93	0.94	0.91	0.92	0.92
	LD	0.89	0.73	0.90	0.98	0.90	0.96	0.95	0.95	0.94	0.94

All correlations were significant at p-value ≤ 0.01.

B.Ar: cortical bone area; BMDv cortical: volumetric bone mineral density of cortical bone; BMDv trab: volumetric bone mineral density of trabecular bone; BW: body weight; Ct.Th: cortical thickness; LD: Lohmann Dual; Line: genetic line; Med.Ar: medullary section area; M-fracture: fracture bending moment; M-max: maximum bending moment; Ross: Ross 308; Tib.Den: weight (g) per 1 cm of the tibiotarsus; Tib.Lth: tibiotarsus length; Tib.Wth: tibiotarsus width; Tib.Wt: tibiotarsus weight.

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Table 3. Volumetric bone mineral density of trabecular and cortical bone of LD and Ross chicken lines versus day post hatching.

Age (d)	Line (n)	BMDv trabecular (g/cm^3)		BMDv cortical (g/cm^3)	
		Mean	SD	Mean	SD
1	Ross (6)	433.60	23.43	522.59	61.75
	LD (6)	350.10	34.08	535.68	35.18
7	Ross (6)	422.59	17.71	685.27	8.47
	LD (6)	431.56	21.60	718.55	31.51
21	Ross (6)	443.93	41.04	692.41	38.09
	LD (6)	497.87	20.83	703.31	13.21
35	Ross (6)	573.79	29.25	757.72	36.47
	LD (6)	589.61	31.79	781.33	30.00
63	LD (6)	647.02	18.94	811.61	11.57

BMDv cortical: volumetric bone mineral density of cortical bone; BMDv trabecular: volumetric bone mineral density of trabecular bone; LD: Lohmann Dual; Line: genetic line; n: animal number; Ross: Ross 308; SD: standard deviation of the mean.

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thickness of the tibiotarsus in both lines did not alter from d 7 post hatching until the end of the study. Trabecular separation in the LD tibiotarsus increased from d 7 to d 35 ($p < 0.05$). Between d 35 and d 63, the values did not differ. For the Ross tibiotarsus, trabecular separation increased from d 7 to d 35 ($p < 0.05$). There were no differences in trabecular number, trabecular thicknesses and trabecular separation between LD and Ross chickens over the whole study period ($p > 0.05$) (Table 4).

Cortical bone structural properties

The cortical BMDv in both lines increased from d 1 to d 7 ($p < 0.05$), then did not differ between d 7 and d 21. From d 21 to d 35, it increased again ($p < 0.05$). For the LD tibiotarsus, the values did not change from d 35 to d 63. No line differences in the volumetric bone mineral density of cortical bone were found in any age groups (Table 3). According to the regression analysis, the chicken line had an influence on the cortical BMDv ($p < 0.001$). The cortical BMDv of LD chickens was greater, on average, by 3.5% than Ross chickens of the same BW, R^2

Table 4. Trabecular bone properties of LD and Ross chicken lines at different ages.

Age (d)	Line (n)	BV/TV (%)		Tb.Nb (1/mm)		Tb.Th (mm)		Tb.Sp. (mm)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	Ross (6)	26.55	4.47	1.41	0.35	0.15	0.02	0.59	0.15
	LD (6)	28.75	2.11	2.40	0.14	0.15	0.03	0.27	0.03
7	Ross (6)	48.64	6.10	3.35	0.49	0.12	0.01	0.18	0.05
	LD (6)	45.44	12.24	2.93	0.81	0.13	0.03	0.25	0.15
21	Ross (6)	41.47	5.28	1.70	0.57	0.12	0.01	0.54	0.26
	LD (6)	36.13	2.31	1.26	0.16	0.12	0.01	0.69	0.10
35	Ross (6)	28.32	2.16	0.89	0.23	0.13	0.01	1.08	0.37
	LD (6)	30.04	3.95	0.76	0.24	0.13	0.01	1.30	0.44
63	LD (6)	29.97	2.52	0.70	0.18	0.15	0.01	1.34	0.36

BV/TV: bone volume fraction; LD: Lohmann Dual; Line: genetic line; n: animal number; Ross: Ross 308; SD: standard deviation of the mean; Tb.Sp.: trabecular separation; Tb.Nb: trabecular number; Tb.Th: trabecular thickness.

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= 0.71. The correlation between the trabecular BMDv and the cortical BMDv of the tibiotarsus was positive in both chicken lines and greater in LD chickens than in Ross chickens, r = 0.91 and 0.66 for LD and Ross lines, respectively.

The cortical thickness of the tibiotarsus in both chicken lines increased from d 1 to d 35 ($p < 0.05$), then it remained unchanged until d 63 for LD chickens. There were no differences in cortical thickness between LD and Ross chicken lines at the same age over the study (Table 5).

The cortical bone area, total cross-sectional area and medullary section area of both LD and Ross chicken lines increased with age over the study period ($p < 0.05$). The LD tibiotarsus had a lower cortical bone area at all ages, a lower total cross-sectional area on days 21 and 35 and a lower medullary section area from d 7 onwards than those of the Ross tibiotarsus ($p < 0.05$) (Table 5). Between d 1 and d 7 the cortical area fraction of the LD tibiotarsus remained unchanged, thereafter it increased gradually until d 21. From d 21 onwards the cortical area fraction of the LD tibiotarsus did not change. The cortical area fraction of the Ross tibiotarsus remained unchanged over the study period. On days 1 and 7, the LD tibiotarsus had a lower cortical area fraction than those of the Ross tibiotarsus ($p < 0.05$). Thereafter, there were no differences in the cortical area fraction of the tibiotarsus between both chicken lines (Table 5).

Discussion

The choice to examine the tibiotarsal bone in this study was based on earlier research findings in poultry, that birds genetically selected for rapid growth and heavy muscle mass have tibiotarsi that are greatly stressed and are prone to mineralization disorders and fractures [10, 14, 34]. During growth, the skeleton of fast-growing chickens must adapt and modify its morphology and material properties to successfully withstand the effects of their rapidly increasing body weight [35].

These observations validate the results of the present investigation, where the tibiotarsal; length, width and weight of both Ross and LD chicken lines similar strongly correlated with the BW (Table 2), indicating that the growth of the tibiotarsus had a similar pattern of growth in both genetic lines. When both LD and Ross chicken lines had the same age, the tibiotarsus of Ross chickens was longer, thicker and heavier than that of LD chickens. However, when length, width and weight of the tibiotarsus were expressed relative to body weight, the tibiotarsus of Ross chickens was shorter, thinner and lighter than that of LD chickens. This could be

Table 5. Cortical bone properties of LD and Ross chicken lines at different ages.

Age (d)	Line (n)	Ct.Th (mm)		B.Ar (mm^2)		T.Ar (mm^2)		Ct.fraction (%)		Med.Ar (mm^2)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	Ross (6)	0.20	0.02	1.68	0.14	5.24	0.57	0.32	0.03	3.56	0.52
	LD (6)	0.19	0.01	1.37	0.10	5.25	0.49	0.26	0.02	3.88	0.46
7	Ross (6)	0.29	0.04	4.04	0.57	12.54	1.26	0.32	0.04	8.5	1.06
	LD (6)	0.24	0.02	2.66	0.25	9.86	0.51	0.27	0.03	7.2	0.53
21	Ross (6)	0.34	0.05	14.97	1.75	44.25	3.88	0.34	0.02	29.3	2.42
	LD (6)	0.29	0.03	9.82	1.16	29.04	3.45	0.34	0.03	19.2	2.77
35	Ross (6)	0.38	0.04	26.29	1.06	86.53	7.84	0.31	0.02	60.24	7.02
	LD (6)	0.41	0.04	15.91	0.91	53.24	4.56	0.30	0.03	37.34	4.69
63	LD (6)	0.45	0.05	27.45	2.25	90.03	17.85	0.32	0.10	62.6	17.93

B.Ar: cortical bone area; Ct.fraction: cortical area fraction; Ct.Th: cortical thickness; LD: Lohmann Dual; Line: genetic line; Med.Ar: medullary section area; n: animal number; Ross: Ross 308; SD: standard deviation of the mean; T.Ar: total cross-section area.

https://doi.org/10.1371/journal.pone.0230070.t005

due to the metabolic inability to support optimal growth in tibiotarsus length, width and weight at the same rate as muscle growth in fast-growing chickens [19]. In this study the LD chicken line had longer, thicker and heavier tibiotarsi than those of the Ross chicken line at the same BW, as reported previously in findings between unselected and selected chickens for meat production [25].

The increase in the tibiotarsal: cortical thickness, total bone cross section area, cortical area fraction and medullary section area over time followed a similar pattern in both chicken lines. The increase in the total bone cross section area is caused by, in part, periosteal apposition with the production of new osteons at the periosteal surface, whereas the increases in the medullary section area are due to endosteal resorption through increased osteoclastic activity at the endosteal surface [31].

The cortical area fraction remained constant with age in both LD and Ross chicken lines, indicating that total bone and medullary cross section areas in both chicken lines correlated strongly positive with each other. However, total bone width increased more rapidly in Ross chickens than in LD chickens, which is in agreement with previous studies that compared fast with slow-growing chickens [13, 19, 36]. Although the bone width was greater in Ross chickens than in LD chickens of the same age, the cortical thickness was similar in both chicken lines over the study period. This indicates that the medullary section area in Ross chickens is greater than in LD chickens. LeBlanc et al. (1985) who compared fast- and slow-growing turkey genotypes also found that the cortical thickness of the tibiotarsus was similar in both genotypes [8]. In contrast, William et al. (2000) used two distinct genetic lines of Ross birds; a slow-growing chicken line not selected for growth performance since 1972 and a modern fast-growing chicken line selected for rapid growth, efficient food conversion and optimal skeletal quality [19]. They found that fast-growing chickens had a thicker cortical thickness than did slow-growing chickens. They concluded that the greater cortical thickness is an essential element for the optimal dimensions of the tibiotarsus to support the rapid increase in body weight in fast-growing chickens [19].

The proximal metaphysis is preferred for μCT analysis of avian trabecular bone because it contains a large amount of trabecular bone that distributes impact loads applied to the cortex thus contributing appreciably to the mechanical strength of the long bones [37–39]. Trabecular bone analysis was performed only from day 7 onward due to the scarcity of trabeculae on day 1. Yair et al. (2013) were also not able to analyze the trabecular properties in chickens before day 7 of age [28]. They attributed that to the impaired bone development during the perinatal period. It has been supposed that one of the reasons of this "slow-down" phenomenon is the nutrient depletion seen prenatally leading to impaired bone development [40].

The results of this study showed that the tibiotarsus of both chicken lines had a reduction in trabecular numbers over time with unchanged trabecular thickness. Consequently, the trabecular separation increased resulting in a decreased bone volume fraction with age. Similar growth patterns of the trabecular bone have been reported recently in chickens [28], geese [41] and in humans [42]. The tibiotarsal mechanical properties such as rigidity, maximal strength, and fracture strength are indicators of skeletal integrity and associated with differing bone characteristics including both densitometric (cortical and trabecular BMDv) and geometric parameters (bone weight, bone weight per unit of length, bone width, total and cortical bone area) [23, 32, 43]. Our results show that the bone volume fraction decreases with age in both chicken lines, resulted from the reduction in trabecular numbers over time with unchanged trabecular thickness, which could diminish the bone fracture strength. However, cortical and trabecular BMDv inversely increased thus enhancing the bone strength.

Williams et al. (2004) investigated the tibiotarsus growth in a fast-growing chicken line selected for optimal weight gain and skeletal quality. They found that although the morphological

properties of the tibiotarsus in the fast-growing chickens correlate with the rapid weight increase, the tibiotarsus of the fast-growing chickens was less mineralized than that of the slow-growing chickens [13]. Consequently, they hypothesized that the tibiotarsus of the fast-growing chickens would have a lower bone fracture strength. Contrary to this, McDevitt et al. (2006) found that the bone fracture strength of the tibiotarsus of fast-growing chickens was higher than that of slow-growing chickens at the same age [25]. They explained that the tibiotarsus of fast-growing chickens was heavier and had greater bone mineral density than did the tibiotarsus of slow-growing chickens at the same age. McDevitt et al. (2006) measured the actual bone fracture strength using the three-point bending test, whereas Williams et al. (2004) suggested indirectly that the bone would have a lower effective bone fracture strength. We support the conclusion of McDevitt et al. (2006) because the tibiotarsal bone strength is influenced by many factors including bone weight, structure, and composition [25–27]. In the present study, the tibiotarsi of the Ross chickens were twice as strong as those of the LD chickens at the same age. Here the tibiotarsi of Ross chickens had a greater mass per unit of length, greater width and greater cortical cross section area than those of LD chickens at the same age.

Shim et al. (2012) and Rawlinson et al. (2009) reported a negative correlation between tibiotarsal fracture strength and growth rate, where slow-growing chickens had a greater relative bone fracture strength than that of fast-growing chicken [20, 36]. They showed that bone mineral density correlated negatively with growth, i.e. in age-matched birds the fast-growing chickens had a relatively lower bone mineral density than that of the slow-growing chickens. In contrast, our results showed a positive correlation between tibiotarsal fracture strength and growth rate in both chicken lines resulting from the similar relative mechanical properties of both chicken lines. Furthermore, the cortical and trabecular BMDv and the cortical thickness were similar in both chicken lines at the same ag over the study.

This study demonstrated that the tibiotarsal bone of the novel dual-purpose chicken line, LD, had a similar growth pattern to that of the Ross broiler chicken line. Furthermore, at the same BW, the tibiotarsus of LD chickens had a greater rigidity than that of Ross chickens. We suggest that this is due to the superior morphometric properties (weight, width and length) and microarchitecture parameters (cortical and trabecular bone BMDv) of the LD chickens when compared to those of the Ross chickens at the same body weight. These conclusions support the finding that the tibiotarsal bone of the LD chicken line had more bending resistance than did that of Ross chickens. Consequently, growing LD chickens to a similar BW to that of Ross chickens at the time of normal commercial slaughter will not affect their leg skeleton stability.

Acknowledgments

We would like to thank Dag Wulsten, head technician of the biomechanical test laboratory and Mario Thiele, medical-technical assistant at the Julius Wolff Institut, Charité - Universitätsmedizin Berlin, Germany, for their excellent technical support.

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4 Discussion

4.1 The heart

4.1.1 Heart mass and relative heart mass

The present study confirmed the decrease of the relative heart mass in both LD and Ross chicken lines continuously with age. Similarly, many studies reported that the relative heart mass in chickens (Hassanzaden et al., 2005; Tickle et al., 2014) and in turkeys (Al Masri et al., 2017) decreased from hatch to slaughter weight, as a result of the high genetic selection. Comparing LD and Ross chicken lines, the heart mass was greater in the broiler chickens (Ross) than that in the dual-purpose chickens (LD). However, LD chickens had a greater relative heart mass than that of Ross chickens. Other comparative studies were in agreement with these findings: Schmidt et al. (2009) indicated that the relative heart mass of unselected chicken lines was greater than that of modern meat-type chicken lines. Comparable results between unselected and selected turkey lines were found by Al Masri et al (2017). A greater relative heart mass in LD chickens indicates a better cardiac performance as investigated by Schmidt et al. (2009).

4.1.2 Cardiomyocytes

The present study revealed that the ventricular wall thicknesses increased with age in both chicken lines. This increase was due to an increase of the size of the cardiomyocytes as found by Anatskaya et al. (2002) when they studied the heart in different avian species. My results showed that the cardiomyocytes in both chicken lines had a similar pattern growth. The diameter and the cross-sectional area of the cardiomyocytes increased with age and were similar in both LD and Ross chickens at the same age. However, when both chicken lines have the same BW, the myocardium of LD chickens had larger cardiomyocytes in diameter and cross-sectional area than those of Ross chickens. Helms et al. (2010) indicated in a comparative study that myocardium with larger cardiomyocytes, in the physiological state, showed better performance. Consequently, we assume, in term of integrity of the cardiac muscle, that the heart of the LD chickens shows better physiological performance.

4.1.3 Capillary blood supply of the myocardium

When examined at the same age, the cardiomyocytes of the LD chickens were supplied by a greater number of blood capillaries than that of Ross chickens. This means that oxygen and nutrient supply of the cardiomyocytes in LD chickens is better with than that in Ross chicken. Similar to these findings, the heart of wild-type turkeys has a higher capillary density compared

to highly selected meat-type turkeys (Al Masri et al., 2017). These findings confirm a negative correlation between total heart weight and its blood capillary density, as reported by Hudlická (1982).

4.2 The aortic wall

The aorta is the most proximal artery connected directly to the heart and acts both as a conduit and an elastic chamber. The elasticity of the aortic wall converts the heart's pulsatile flow to nearly steady flow in peripheral vessels (Dzialowski and Crossley, 2014). To achieve this goal, the aortic wall has very special mechanical properties.

4.2.1 The aortic wall thickness

In the present study, the aortic wall thickness of both LD and Ross chickens increased with age to withstand the increased blood pressures. Similar results were found in chickens by Ocal et al. (1997). It was reported by Kamimura et al. (1995) that blood pressure in chickens increases with age. Compared to the aortic wall thickness of Ross chickens, LD chickens had a thinner aortic wall at the same age. However, at the same body weight, the thickness of the aortic wall was the same in both chicken lines. This means that both LD and Ross chickens display a similar growth pattern of the aortic wall to withstand the changes of blood pressure.

4.2.2 Elastic fibers and lamellae

The mechanical properties of the arterial wall depend on the micro-architecture and composition of the arterial wall (Wolinsky and Glagov, 1967). My results showed that the proportion of the elastic fibers in the tunica media of the aortic wall decreased with age in both chicken lines. Similar results were found by Ruiz-Feria et al. (2009).

In contrast, the number of elastic lamellae in both chicken lines did not change with age. Similarly, the number of elastic lamellae was found unchanged after birth in the aortic wall of vertebrates such as rats, rabbits and pigs (Wagenseil and Mecham, 2009).

A single lamellar unit has elastic properties to withstand the circumferential tension in the aortic wall (Faury, 2001). There is a direct relationship between the number of elastic lamellae units and the arterial wall tension, i.e. the number of lamellar units increases as tension increases (Dobrin; 1988). My results showed that the aortic wall of the LD chickens had a proportionally larger number of elastic lamellae and greater percentage of elastic fibers than those of the Ross chickens, giving the aortic wall of LD chickens the ability to withstand greater wall tension.

This suggests that the LD chickens have superior aortic mechanical properties than those of Ross chickens, i.e. the aortic wall of the LD chickens has a greater ability to adapt to changes of blood pressure than that of the Ross chickens.

4.3 The tibiotarsus

4.3.1 Morphology of the tibiotarsus

There are strong links between bone dimensions and growth performance. The skeleton of chickens constantly adapts its macro- and microstructure with growth to withstand the increasing body weight (Williams et al., 2004). The results of this study showed a similar strongly correlation between weight, length, width and cross section area of the tibiotarsus and the BW of both Ross and LD chicken lines. This indicates that the growth of the tibiotarsus in both chicken lines followed a similar pattern of growth.

At the same age, Ross chickens had the heavier, longer and thicker tibiotarsi than those of the LD chickens, due to the BW difference between Ross and LD chicken lines. However, the tibiotarsi of Ross chickens were lighter, shorter and thinner than those of the LD chickens at the same BW. This could be due to an inability to produce growth in tibiotarsus weight, length and width at the same rate as muscle growth in the fast-growing chickens (Williams et al., 2000). A similar observation has been reported previously in a comparison between selected and unselected chickens for meat production (McDevitt et al., 2006).

Bones enlarge their circumference in response to increased loads during weight gain (Williams et al., 2004). The total cross section area of the tibiotarsus increased more rapidly in the Ross chickens than in the LD chickens, while the cortical thickness similarly increased in both chicken lines. The growth of the medullary section area, therefore, occurred more rapidly in the Ross chickens.

William et al. (2000), used two distinct genetic lines of Ross birds; a slow-growing chicken line and a modern fast-growing chicken line selected for skeletal health in addition to growth performance. In contrast to my results, they found that the cortical thickness of the fast-growing chickens' tibiotarsus was greater than that of the slow-growing chickens. They concluded that the greater cortical thickness is an essential element for the optimal dimensions of the tibiotarsus to support the rapid increase in body weight in fast-growing chickens.

4.3.2 The effect of macro- and microstructure of the tibiotarsus on bone strength

The mechanical properties of the tibiotarsus such as rigidity, M-max and M-fracture, which are indicators of bone strength, increased with age in both Ross and LD chicken lines. Bone

strength is influenced by several different bone characteristics including geometric parameters (bone weight, bone weight per unit of length, bone width, total and cortical bone area) and densitometric parameters (cortical and trabecular BMD) (González-Cerón et al., 2015; Jepsen et al., 2015; Yair et al., 2015).

At the same age, my results revealed advanced mechanical properties of the tibiotarsus in Ross chickens than in LD chickens. The tibiotarsus of Ross chickens had greater mass per unit of length, greater width and greater cortical cross section area than those of LD chickens at the same age. In agreement with my results, McDevitt et al. (2006) found that the bone fracture strength of the tibiotarsus of fast-growing chickens was higher than that of slow-growing chickens at the same age and that the tibiotarsus of fast-growing chickens was heavier than that of slow-growing chickens at the same age.

Previous studies (William et al., 2004; Shim et al., 2012) indicate a relatively greater bone mineral density of the slow-growing chickens' tibiotarsus than that of the fast-growing chickens. This results in a greater relative bone fracture strength of the slow-growing chickens than of the fast-growing chickens (Rawlinson et al., 2009; Shim et al., 2012). In addition, William et al. (2000) reported that the tibiotarsus of the slow-growing chickens was more mineralized than that of the fast-growing chickens at the same age. Consequently, they assumed that the tibiotarsus of the fast-growing chickens would have a lower bone fracture strength. In contrast, my results showed that the bone mineral density of both cortical and trabecular bone and the cortical thickness were similar in both chicken lines at the same age. This supports the assumption that similar mechanical properties of the tibiotarsus in both chicken lines exist at the same BW. Moreover, at the same BW, the tibiotarsus of LD chickens had a greater rigidity than that of Ross chickens, suggesting that this is due to the greater morphometric properties (weight, width and length) and microarchitecture parameters (cortical and trabecular bone BMD) of the LD chickens.

5 Summary

Cardiovascular and skeletal diseases of rapid growing chickens are likely to be associated with intensive genetic selection for growth and feed conversion efficiency. The objective of this thesis was to assess the integrity of both cardiovascular and skeletal systems of a new genetic line of dual-purpose chickens (Lohmann Dual, LD) which was bred as an alternative to avoid killing day-old male chicks of egg-laying chickens. It should be investigated whether the LD male chickens are physically able to withstand the intensive fattening conditions. Parameters of LD should be compared to those of a highly selected fast-growing conventional broiler line (Ross 308).

Eighty one-day-old male chicks of each line were housed for 5 weeks (Ross) and 9 weeks (LD). Six birds of each line were sampled weekly and the body weight was recorded. Heart mass, relative heart mass, tibiotarsus weight, tibiotarsus length and tibiotarsus width were measured. Thickness of ventricular walls, cardiomyocyte size and blood capillary density as well as aortic diameter and thickness, number of elastic lamellae and elastic fiber percentage in the aortic wall were histologically determined. Mechanical properties of the tibiotarsus (rigidity, M-Max and the M-fracture) were evaluated using the three-point bending test. Additionally, bone mineral density of both trabecular and cortical bone, bone volume fraction, trabecular number, trabecular thickness and cortical thickness of both chicken lines were analyzed using microcomputed tomography. Results showed physiologcial growth of the heart, the aorta and the leg skeleton in the LD male chickens with increasing BW. Moreover, LD male chickens seem to have more stable circulatory systems compared to conventional broilers. The assessment of the heart and the aorta showed that the heart of the LD male chickens has a larger number of blood capillaries and the aortic wall has proportionally larger numbers of elastic lamellae and more elastic fibers. This means that LD male chickens have a better myocardial capillary supply and superior aortic mechanical properties, indicating better performance characteristics of the heart and the aorta.

At the same body weight of both LD and Ross chicken lines, the mechanical properties and mineral density of both trabecular and cortical bone of the leg skeleton were similar. However, LD male chickens have longer, heavier and wider leg bones than those of Ross male chickens, which results in greater rigidity. This suggests that the leg skeleton of LD chickens had more bending resistance than those of Ross chickens of the same BW.

Overall, these investigations have not lead to indications that fattening the LD male chickens under the described experiment conditions to the marketable weight of 2000 g

will impair the anatomical integrity of the heart, the aorta and the lower leg bone as well as the mechanical properties of the lower leg bone.

6 Zusammenfassung

Anatomische, histologische und morphometrische Vergleichsstudie des Herz-Kreislaufapparates und des Skeletts von Zweinutzungshühnern und Broilern Hähnen

Ca. 5 Millionen männlicher Küken werden jährlich alleine in Deutschland getötet, da sie nicht so gut Muskelfleisch ansetzen, wie das Küken tun, welche als schnell wachsende Masthühnchen gezüchtet wurden. Die bei letzteren auftretenden Herz-Kreislauf- und Skeletterkrankungen stehen vermutlich im Zusammenhang mit der intensiven genetischen Selektion für Wachstum und Futterverwertungseffizienz. Tiere der neuen Zweinutzungslinie Lohmann Dual (LD) sollen nun sowohl in befriedigendem Maße Eier legen (weibliche Tiere) als auch Fleisch ansetzen (männliche Tiere). Um herauszufinden, ob die männlichen Tiere der LD Linie den intensiven Mastbedingungen körperlich gewachsen sind, war das Ziel dieser Arbeit, ihr Herz-Kreislauf- und das Skelett zu untersuchen. Zum Vergleich wurden herkömmliche, schnell wachsende Masthähnchen (Ross 308) ausgewählt.

Insgesamt wurden 80 männliche Ross 308 und 80 männliche LD herangezogen. Die Tiere der Ross–Gruppe wurden bis zur 5. Lebenswoche, die Tiere der LD-Gruppe bis zur 9. Lebenswoche gehalten. Von jeder Gruppe wurden 6 Tiere wöchentlich untersucht. Nachdem das Lebendgewicht der Tiere ermittelt worden war, wurden die Tiere getötet und ihre Organe vermessen. Es wurden die absolute und die relative Herzmasse gewogen, sowie die Wanddicke der Herzventrikel und der Aorta gemessen. Von der Aorta wurde auch der Durchmesser bestimmt. Darüber hinaus wurden das Gewicht, die Länge und die Breite des Tibiotarsus ermittelt. Histologisch wurde am Herz die Größe der Kardiomyozyten, sowie die Kapillardichte des Myokards untersucht. Bei der Aorta wurde die Anzahl der elastischen Lamellen und der Anteil der elastischen Fasern an der Wandstärke der Aorta bestimmt. Die mechanischen Eigenschaften des Tibiotarsus (Biegesteifigkeit, Maximales Biegemoment und Bruchbiegemoment) wurden durch 3-Punkt-Biegeversuch ermittelt. Zusätzlich wurden die Dichte der Mineralien im trabekulären und im kortikalen Knochen analysiert. Es wurden der Knochenvolumenanteil, die Trabekelzahl und die Trabekeldicke mittels hochauflösendem Micro-CT gemessen.

Die Ergebnisse zeigten, dass sich das Herz, die Aorta und der Tibiotarsus von LD-Tieren mit dem Körperwachstum physiologisch entwickelten. Darüber hinaus lassen die Ergebnisse vermuten, dass die LD-Broiler im Vergleich zu den herkömmlichen Ross Broilern ein robusteres Kreislaufsystem entwickeln. Bei der Untersuchung von Herz und Aorta stellte sich nämlich heraus, dass das Herz der LD-Broiler eine höhere Anzahl von Blutkapillaren besitzt und deren Aorta nicht nur eine höhere Anzahl an elastischen Lamellen sondern auch einen höheren Anteil an elastischen Fasern in der Wand der Aorta aufweist. Daraus kann geschlossen werden, dass das Herz der LD-Tiere stärker durchblutet ist, als das der Ross-Tiere und die Aorta der LD-Broiler günstigere elastische Eigenschaften als die Aorta der Ross-

Broiler zeigt, was auf robustere Leistungsmerkmale des Herzens und der Aorta hinweist. Beim gleichen Körpergewicht waren sowohl die mechanischen Eigenschaften als auch die Mineraldichte der trabekulären und kortikalen Knochen des Tibiotarsus der LD- und Ross-Broiler gleich. Allerdings zeigten die LD- im Vergleich zu den Ross-Broilern längere, schwerere und breitere Beinknochen in derselben Gewichtklasse, was auf eine größere Biegesteifigkeit des Beinskeletts bei den LD-Broilern hinweist. Das heißt, dass das Beinskelett der LD-Tiere im Vergleich zu den Ross-Tieren bei gleichem Körpergewicht eine größere Biegefestigkeit hat.

Insgesamt ergaben diese Untersuchungen keine Hinweise darauf, dass die Mast der LD-Hähne zum marktfähigen Körpergewicht von 2000 g und unter den beschriebenen Versuchsbedingungen die anatomische Integrität des Herzens, der Aorta und des Unterschenkelknochens sowie die mechanischen Eigenschaften des Unterschenkelknochens beeinträchtigt.

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8 List of own publications

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9 Danksagung (Acknowledgment)

Allen voran gilt mein Dank meiner Doktormutter Prof. Dr. Johanna Plendl für ihre umfangreiche Unterstützung und ihre fachliche und professionelle Betreuung während der gesamten Dauer der Arbeit und den steten Ansporn. Besonders hervorzuheben ist ihre Hilfe beim Bewerbungsverfahren für mein Stipendium an der Konrad-Adenauer-Stiftung. Auf ihre Hilfe in jeglichen Belangen konnte ich mich stets verlassen. Ich habe viel von ihnen gelernt.

Herzlicher Dank gilt Dr. Salah AL Masri. Bei ihm möchte ich mich ebenfalls für die Betreuung meiner Dissertation bedanken, für seine bereichernden und konstruktiven Anregungen, die freundliche Betreuung und ständige Erreichbarkeit. So ist er mit den Jahren wie ein großer Bruder für mich geworden.

Mein Dank gilt auch Herr Prof Kenneth Richardson für seine geleistete Unterstützung, besonders für die wertvollen fachlichen Anregungen, die entscheidend zum Gelingen dieser Arbeit beigetragen haben.

Ebenso bedanke ich mich bei Herrn Prof. H.M. Hafez für die Unterstützung bei den Probennahmen am Institut für Geflügelkrankheiten und seine wissenschaftlichen Ratschläge. Besonders hervorzuheben ist auch seine Hilfe beim Bewerbungsverfahren für mein Stipendium an der Konrad-Adenauer-Stiftung.

Mein besonderer Dank gilt Frau Dr. Hana Hünigen, die mir jederzeit mit Rat und Tat zur Seite stand und die mir mit ihrem unerschöpflichen Wissen immer weitergeholfen hat.

Des Weiteren möchte ich mich bei meinem Projektkollegen Zaher Alshamy für die nette und vertrauensvolle Zusammenarbeit bedanken, die uns zu einem guten Team gemacht hat.

Einen großen Dank möchte ich allen Mitarbeitenden des Instituts für Veterinär-Anatomie der Freien Universität Berlin aussprechen. Besonders Sindy Schulz für ihre unermüdliche Bereitschaft mir zu helfen. Für die technische Assistenz im Labor und Hilfe bei allen kleinen und großen Fragen möchte ich mich sehr bei Karin Briest-Forch and Barbara Drewes bedanken. Ein großer Dank gilt Dr. Sabine Käßmeyer für ihre Mithilfe.

Ich danke dem Team des Julius Wolff Instituts der Charité Berlin. Vor allem Herrn Dag Wulsten und Herrn Mario Thiele, für die Unterstützung bei der Durchführung und Auswertung der Knochentests.

Herzlicher Dank gilt der Konrad-Adenauer-Stiftung für die finanzielle Förderung.

Eine große Hilfe war mir auch meine Gemeinde „Gemeinde St.Georgios Berlin“ unter der Leitung S.E. Bischof Hanna Haykal. Ich bedanke mich für die große Unterstützung.

Ein großer Dank gilt meine Freunde aus Weinheim besonders Kathrin Schulz für ihre freundliche Unterstützung.

Bei meinen Eltern Bushra und Rami sowie meinen Geschwistern Samer, Housam, Samia, Rama und ihrem Mann Fares bedanke ich mich von ganzem Herzen dafür, dass sie mich stets darin bestärkt meinen eigenen Weg zu gehen.

Nicht zuletzt danke ich ebenfalls von ganzem Herzen meiner Freundin Nataly.

10 Declaration of academic honesty

I hereby confirm that the text at hand is solely my own work. I assure that I only used the cited sources and the thesis has not been submitted in any form for another degree at any university or other institute.

Berlin, 11.08.2020

George Harash

Druck: mbvberlin mensch und buch verlag