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ANIMAL SCIENCE

Growth of long bones in European and Japanese quail from the 13th day of incubation to day 35 post-hatch

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Abstract: This study described the growth, morphometric, biomechanical, and chemical properties of the femur, tibiotarsus, and tarsometatarsus of European and Japanese quail. Analyses were performed at 13 and 15 days of incubation, at hatch, and at 4, 7, 10, 14, 21, 28, and 35 days post-hatch (n=6/subspecies/period). Bone specimens were analyzed by cone-beam computed tomography, biomechanical assays, chemical analyses, and histomorphometry. Variables were fitted by the Gompertz function and its derivative or assessed using the analysis of variance. Analysis of the derivative of Gompertz curves showed that the growth behavior of the tarsometatarsal bone was similar between quail subspecies, and the femur and tibiotarsus of European quail increased first in width and then in length, whereas the opposite occurred in Japanese quail. There was an interaction between quail subspecies and days of growth on femoral, tarsometatarsal, and tibiotarsal bone densities. Femoral and tibiotarsal cross-sectional areas were influenced by the interaction of quail subspecies and day of growth. Interaction effects were significant for breaking strength and phosphorus percentage. European and Japanese quail have different femoral and tibiotarsal growth patterns, especially in the first few days after hatching, whereas tarsometatarsal growth is similar between subspecies.

Key words: calcium, *Coturnix*, densitometry, femur, Gompertz, growth.

INTRODUCTION

Quail production has attracted increasing economic interest in Brazil. In 2020, the national quail herd amounted to 16.5 million birds, with an egg production of 295.9 million dozen eggs (IBGE 2020). Two commercial breeds have been used for egg and meat production. Japanese quail (*Coturnix coturnix japonica*) is mostly used for egg production, and European quail (*Coturnix coturnix coturnix*) is used for both meat and egg production (Bertechini 2010). Although there are important phenotypic and zootechnical differences between subspecies, Japanese quail occupy an important position in commercial production, being explored as a meat supply to meet the demands of consumer markets (Oliveira & Escocard 2010). In the Far East and other parts of Asia, Japanese quail are widely used for meat production (Narinc et al. 2010).

European and Japanese quail differ in several traits, such as body weight, time to maturity, body composition, and nutrient deposition rate, all of which can affect growth patterns (Gous et al. 1999). It is known that bone development and maturity may not accompany the general growth of rapidly developing birds, generating excessive physical load that predisposes bones to deformity and fragility (Rath et al. 2000). Bone growth should be synchronous with muscle and adipose tissue development, which are related to body growth in birds (Pizauto Jr 2002).

Several studies examined bone development in broiler chickens by assessing bone structure, composition, and mechanical parameters (Rath et al. 2000, Farquharson & Jefferies 2000, Shim et al. 2012, Yair et al. 2012). Other investigations focused on relationships between growth patterns of the long bones tibiotarsus and femur (Applegate & Lilburn 2002), ossification processes, and associations between calcium, phosphorus, and other minerals in bones (Han et al. 2015). In Japanese quail, there are descriptions of embryonic development (Ainsworth et al. 2010, Nakane & Tsudzuki 1999) and growth of the tibiotarsus and femur (Ahmed & Soliman 2013). However, there is a lack of studies comparing bone growth between European and Japanese quail, which are intended for slaughter at 35 days.

European and Japanese quail have different growth curves; for reliable comparison, growth should be analyzed under optimal (non-limiting) conditions (Fitzhugh Jr & Taylor 1976). The Gompertz equation is the most commonly used model to describe growth in birds, as it provides a better fit to the data than other nonlinear models and can be used for drawing physiological inferences (Duan-Yai et al. 1999, Freitas 2005). The comparison between the two subspecies is relevant because most of the studies carried out consider only the Japanese quail and only a few studies with European quail are published.

In poultry production research, it is usual to analyze the tibiotarsus when the objective is to describe the effect of diet ingredients on the bone, and in some cases, both the femur and the tibiotarsus are cited (Applegate & Lilburn 2002, Barreiro et al. 2009, Jendral et al. 2008, Osman et al. 2009, Regmi et al. 2016, Shim et al. 2012, Stanquevis et al. 2015). Also, a lot of problems are associated with those bones with economic and welfare issues like dyschondroplasia, epiphysiolysis, femoral head necrosis, osteomyelitis, and bacterial chondronecrosis, especially in broiler breeders and in laying hens the cage layer fatigue or osteoporosis (Rath et al. 2000, Rath & Durairaj 2022). In this work, the authors analyzed the femur, tibiotarsus and tarsometatarsus. The tarsometatarsus plays several essential roles in the overall function and adaptation of birds, and it is studied in different species for evolutionary aspects (Casinos & Cubo 2001).

In this research, the hypothesis that European and Japanese quail will have different growth patterns for long hindlimb bones was tested. In this sense, the growth of the femur, tibiotarsus, and tarsometatarsus of European and Japanese quail were describe and characterized. Bones were analyzed with morphometric, biomechanical, chemical, and histological methods from the end of incubation to day 35 post-hatch.

MATERIALS AND METHODS

This research was approved by the Animal Ethics Committee of the State University of Maringá, Paraná, Brazil (protocol No. 1237250914) and the experiment was conducted at the poultry farm of the State University of Maringá.

Animals and housing conditions

Fertile eggs from European quail (*Coturnix coturnix coturnix*) (n=200) and Japanese quail (*Coturnix coturnix japonica*) (n=200) were incubated to produce embryos and quails. Eggs were selected by mean weight ± 5% (European

11.80 g, and Japanese 9.79 g) and quality (form, uncracked, etc), and incubated in an automatic vertical incubator (Petersime®, model Labo 13, capacity of 3,978 quail eggs) at 60% relative humidity and 37.6 °C with open ventilation. After 348 h of incubation, the eggs were transferred to a hatcher (Petersime®, model Labo 9) at 70% relative humidity and 37.0 °C.

After hatching, quails were housed in conventional conditions according to the species density, light, diet, and temperature. Unsexed chicks were randomly housed in groups of 50 birds per pen in six pens measuring 2.80 × 1.40 m each (3 pens for European quail, n=150, and 3 pens for Japanese quail, n=150). Only part of these quails was used in the bone´s analysis. The diet was based on corn and soybean meal and was formulated to meet the nutrient requirements of quail during the starter and grower phases, according to Silva & Costa (2009). The starter diet (1-21 days) had 25% crude protein, 2,900 kcal/kg metabolizable energy, 0.85% calcium, and 0.32 available phosphorus. While the grower diet (22-35 days) had 21% crude protein, 3,050 kcal/kg metabolizable energy, 0.75% calcium, and 0.30 available phosphorus. The composition of the ingredients was based on the Brazilian poultry and swine tables (Rostagno et al. 2017). Infrared lamps were placed inside each pen to provide heating for the chicks during their first 15 days of life. Chick behavior (crowding or dispersing) and thermohydrometers were used to control the temperature, and the distance between lamps and birds was adjusted accordingly. The temperature was initially set at 36 °C and reduced over the next few days until room temperature was achieved (25 °C), when lamps were turned off. All the quails used in the experiment were intended for meat production. Animal handling and management conditions were similar among pens. The light program was set at 23:1 (Light:Dark) hours (Shanaway 1994).

Experimental design and bone sample collection

Growth was monitored from day 13 of incubation to day 35 post-hatch. The femur, tibiotarsus, and tarsometatarsus bones were analyzed at 13 days (312 h) and 15 days (360 h) of incubation, at hatch (420 h), and at 4, 7, 10, 14, 21, 28, and 35-days post-hatch. Six eggs or chicks per subspecies were analyzed per growth period (10 periods × 6 birds × 2 subspecies, totaling 60 European and 60 Japanese quail).

In the incubation groups, eggs were broken and the embryos euthanized by cervical dislocation. At hatch and in groups post-hatch, chicks were anesthetized intraperitoneally with sodium thiopental (10 mg/kg body weight) + lidocaine (10 mg/kg body weight) and euthanized by cervical dislocation. All embryos and chicks were weighed before euthanasia. In groups from 4 to 35 days post-hatch, samples were obtained from 2 quails from each pen to avoid bias.

Bones were dissected from adhered muscle tissues and weighed on an analytical balance. The right bones were wrapped in gauze soaked with saline solution (0.9%) and kept frozen (−18 °C) until used for morphometric, biomechanical, and chemical analyses. For histological analysis, only the tibiotarsus was used, and the left bone was fixed in a 10% paraformaldehyde solution.

Seedor index

Bones were unfrozen, measured with a pachymeter at their greatest length, and then used for computed tomography. Data were used to determine the Seedor index by dividing the result of the weight of the bone by its length. Seedor Index = Weight (mg) / Length (mm).

Computed tomography morphometric analysis

Bones were placed on a flat plate (10 × 10 cm) for computed tomography. Images were acquired using an i-CAT Next Generation® system (Imaging

Sciences International, Hatfield, PA, USA) at 14-bit resolution. Volumes were reconstructed using an isometric voxel of 0.125 mm with an 8 × 8 cm field of view, a tube voltage of 120 kVp, and a tube current of 3–8 mA. Scan images were stored on the laboratory computer and imported into Dolphin Imaging & Management Solutions® 3D software version 11.8 (Dolphin Imaging, Chatsworth, CA, USA) in Digital Imaging and Communications in Medicine (DICOM) format for analysis of bone volume (mm^3), mineral density (Hounsfield units, HU), length (mm), diaphyseal diameter (mm), and diaphyseal thickness (mm). A cross section of the tomography images was used to obtain diaphyseal diameter (mm), and diaphyseal thickness (mm) variables. Specifically, for diaphyseal thickness, four measurements were obtained in four opposite directions around the circumference of the diaphysis, and the results are presented as arithmetic means.

Biomechanical analyses

Biochemical analysis was performed on the same bones. Bone resistance was assessed on right femur and tibiotarsus specimens after 10 days post-hatch and on tarsometatarsus specimens after 14 days post-hatch. Bones were held in place by the epiphyseal region without any grippers in the central region. The anteroposterior position was used for analysis to prevent bones from moving at the time of breakage. Three-point strength tests were performed on a universal testing machine (DL3000 EMIC), with results expressed in newtons (N). Force was applied to the diaphyseal region, always at the same point for all bones. The crosshead speed was 10 mm/s, and the amount of force applied was measured immediately after rupture. The load was set at 200 kgf for all samples. The distance between grippers varied according to bone type and bird age. After bone rupture, the cross-sectional area (elliptical cross-section) of the diaphyseal region was measured according to Turner & Burr (1993), and the cross-sectional area was obtained.

Chemical composition

Mineral matter (%), calcium (Ca), phosphorus (P), and magnesium (Mg) contents were determined on the same bone specimens. Samples were oven-dried at 55 °C for 72 hours and weighed on a precision scale. Then, samples were oven-dried at 105 °C for 24 h, weighed, and calcined at 600 °C in a muffle furnace for 6 h. After cooling, samples were weighed again for determination of ash content (dry matter basis). The resulting ash samples were treated according to the method described by Silva & Queiroz (2006) to obtain a mineral solution. P contents were quantified by a colorimetric method (Silva & Queiroz 2006), whereas Ca and Mg contents were determined by flame spectrophotometry.

Histological analysis

Tibiotarsus samples fixed in paraformaldehyde were decalcified with a solution containing formic acid and sodium citrate to avoid tissue hydrolysis or intumescence. Bones were cut vertically, and the proximal epiphysis, together with part of the diaphysis, was embedded in paraffin. The resulting blocks were cut into 10 µm slices using a microtome, and sections were stained with 2.5% Alcian Blue. Photographs were captured with a digital camera (Moticam 5 MP) coupled to a microscope at 4× magnification. Images were analyzed using Motic Image Plus software version 2.0 to measure the thickness of the epiphyseal plate. Growth plate thickness was measured according to the method proposed by Reich et al. (2005).

Statistical analysis

The data were subjected to statistical analysis using SAS software (SAS 2001). Growth curves were constructed from the bone estimates of different quail subspecies by using the Gompertz equation, according to Fialho (1999), as described below (Eq. 1):

$$W = A\exp^{-\exp^{-B(t-C)}} \quad (1)$$

where *W* is the weight estimate (g) at age *t*, *A* is the asymptotic weight (g) when *t* tends to infinity (interpreted as adult weight), *B* is the relative growth at the inflection point (g/day·g) or the maturity rate, *C* is the age at the inflection point (days) or the time at which the growth rate is maximum (*t* = age (in days), and exp = 2.718281828459.

Growth rates (g/day) were calculated from the derivative of Eq. (1), as suggested by Fialho (1999) (Eq. 2):

$$\frac{dM}{dt} = AB\exp^{-B(t-C)-\exp^{-B(t-C)}} \quad (2)$$

Models were fitted to experimental data for bird weight (g), bone weight (G), bone length (mm), Seedor index (mg/mm), diaphyseal volume (mm^3), diaphyseal diameter (mm), diaphyseal thickness (mm), and ash content (%). Eight growth models (M_1–M_8) were tested to analyze differences in growth curve parameters between European and Japanese quail. The first model (M_1) was developed without adjustments for curve parameters. M_2, M_3, and M_4 differed only in one parameter. M_2 differed in parameter *C*, M_3 differed in parameter *B*, and M_4 differed in parameter *A*. Models M_5, M_6, and M_7 differed in two parameters: *A* and *B*, *A* and *C*, and *B* and *C*, respectively. In M_8, all parameters were the same. The best-fitting model was chosen on the basis of the likelihood ratio test with chi-square approximation, as proposed by Regazzi & Silva (2004). Growth curve parameters were estimated using modified Gauss-Newton methods with the NLIN procedure of SAS (SAS Institute Inc., Cary, NC, USA).

An analysis of variance was performed at the 5% significance level for variables not adjusted to Gompertz curves. Differences between quail subspecies (European and Japanese quail), time (days), and interaction were tested by generalized linear models at the 5% significance level in regression analysis.

For statistical analysis of variables assessed during the incubation period, the day of hatching was considered day 17, and day 35 post-hatch was considered day 52. For all other variables, the day of hatching was considered day 0. In all analyses and variables, each animal was considered the experimental unit.

RESULTS

The growth patterns of the long bones (femur, tibiotarsus, and tarsometatarsus) of European and Japanese quail were described from the 13th day of incubation to day 35 post-hatch. During this period, birds were monitored for body weight, bone weight, bone length, Seedor index, diaphyseal volume, diaphyseal diameter, diaphyseal thickness, breaking strength, cross-sectional area, and bone density. Metaphyseal thickness and ash, Ca, P, and Mg contents in the tibiotarsus were also monitored.

Long bone growth rates were modeled using Gompertz equations. Models were fitted to experimental data for body weight, bone weight, bone length, Seedor Index, diaphyseal volume, diaphyseal diameter, diaphyseal thickness, and ash content. However, the Gompertz function did not provide a good fit to bone density, breaking strength, cross-sectional area, metaphyseal thickness, Ca content, P content, or Mg content, which were therefore subjected to ANOVA.

Table I presents the results and models used for the analysis of values at maturity, growth rate, and period of maximum growth rate in European and Japanese quail. Body weight and bone weight (femur, tibiotarsus, and tarsometatarsus) data were best fitted by M_7, according to which

Table I. Femoral, tibiotarsal, and tarsometatarsal parameters estimated for European and Japanese quail by Gompertz equations from the 13th day of incubation to 35 days **post-hatch.**

Parameter	Model	W_m		B		t^*	
		European quail	**Japanese quail**	**European quail**	**Japanese quail**	**European quail**	**Japanese quail**
Body weight (g)	M_7	275	156	0.07	0.07	35.59 (18.59)[1]	35.59 (18.59)[1]
Femur							
Weight (g)	M_7	0.91	0.55	0.07	0.07	34.77 (17.77)	34.77 (17.77)
Length (mm)	M_2	55.60	50.95	0.05	0.04	25.76 (8.76)	25.76 (8.76)
Seedor index	M_7	17.97	12.84	0.08	0.08	26.77 (9.77)	26.77 (9.77)
Volume (mm^3)	M_7	933	604	0.07	0.07	31.98 (14.98)	31.98 (14.98)
Diaphyseal diameter (mm)	M_7	2.96	2.59	0.07	0.07	22.83 (5.83)	22.83 (5.83)
Diaphyseal thickness (mm)	M_4	0.55	0.55	0.07	0.09	15.56	20.09 (3.09)
Tibiotarsus							
Weight (g)	M_7	0.98	0.57	0.08	0.08	31.44 (14.44)	31.44 (14.44)
Length (mm)	M_3	68.27	56.54	0.05	0.05	24.46 (7.46)	21.59 (5.59)
Seedor index	M_7	17.79	11.48	0.09	0.09	25.47 (8.47)	25.47 (8.47)
Volume (mm^3)	M_7	947	645	0.11	0.11	27.65 (9.65)	27.65 (9.65)
Diaphyseal diameter (mm)	M_7	3.15	2.80	0.05	0.05	25.28 (8.28)	25.28 (8.28)
Diaphyseal thickness (mm)	M_5	0.73	0.73	0.06	0.06	20.42 (3.42)	24.16 (7.16)
Ash (%) (dry matter basis)	M_5	44.86	44.86	0.19	0.19	14.10	12.17
Tarsometatarsus							
Weight (g)	M_7	0.47	0.30	0.08	0.08	29.42 (12.42)	29.42 (12.42)
Length (mm)	M_7	36.64	32.41	0.06	0.06	18.59 (1.59)	18.59 (1.59)
Seedor index	M_7	14.21	10.53	0.08	0.08	23.66 (6.66)	23.66 (6.66)
Volume (mm^3)	M_7	525	375	0.08	0.08	26.69 (9.69)	26.69 (9.69)
Diaphyseal diameter (mm)	M_7	2.23	1.98	0.09	0.09	18.09 (1.09)	18.09 (1.09)
Diaphyseal thickness (mm)	M_7	0.64	0.58	0.07	0.07	18.46 (1.46)	18.46 (1.46)

W_m, values at maturity; B, growth rate at maturity; t^*, time to maximum growth rate. [1] Inside parentheses are the days post-hatch.

European and Japanese quail differed only at maturity. Comparison between long bone weights revealed that the tarsometatarsus had the earliest development, whereas the femur had the slowest development. Growth rate patterns for bone weight were similar for the three bones evaluated.

There were significant differences in longitudinal bone growth. Three different models were used to describe growth patterns. M_2 provided the best fit to the femoral data. The model showed that, in addition to having different weights at maturity, European quail had a higher growth rate for femoral length than Japanese quail. For tibiotarsal length data, the best-fitting model was M_3. The model showed that Japanese quail were precocious compared with European quail. Although the maturity values of European quail were higher than those of Japanese quail, both subspecies had similar growth rates. However, the time to reach the maximum growth rate was shorter in Japanese quail. Tarsometatarsal growth values, analyzed using M_7, differed between subspecies only at maturity. A comparison of growth rates for bone weight between the three bones indicated that the tarsometatarsus was the earliest to develop.

In the current study, body weight increased 43.12-fold in European quail and 28.77-fold in Japanese quail from the 13th day of incubation to 35 days post-hatch. In European quail, the weights of the femur, tibiotarsus, and tarsometatarsus increased by 48.85, 44.50, and 25.81 times, respectively, compared with the initial weight. In Japanese quail, femoral, tibiotarsal, and tarsometatarsal weights increased 28.64-, 29.81-, and 15.43-fold, respectively, compared with the initial weight. Tibiotarsal and femoral growth rates were similar to body growth rates. The tarsometatarsus, however, had the lowest increase in weight. Tibiotarsus and femur were found to be closely related to body growth. The length of the femur, tibiotarsus, and tarsometatarsus increased 4.79, 4.48, and 3.70 times, respectively, in European quail and 4.08, 3.93, and 3.78 times, respectively, in Japanese quail.

The Seedor index did not differ in growth rate for the three bones analyzed, which only differed in maturity values. M_7 provided the best fit to the Seedor index data. The Seedor index is calculated as the ratio of bone weight to bone length, serving as a good indicator of bone density. Although differences in growth rate for bone length were observed, they were not sufficient to impact growth curves for the Seedor index.

Bone volume measurements taken using Dolphin® software showed that the femur, tibiotarsus, and tarsometatarsus exhibited similar growth behavior. European quail had a higher bone volume at maturity than Japanese quail. Growth curves were fitted by M_7.

Diaphyseal diameter was also described by model M_7. European and Japanese quail differed in diaphyseal diameter at maturity. The tarsometatarsus showed precocious development and a higher growth rate than the femur and tibiotarsus.

Growth patterns for diaphyseal thickness differed between European and Japanese quail. Femoral bone data were best fitted by M_4, which indicated that both quail subspecies reached similar values at maturity but differed in growth rate and time to maximum rate. Although European quail had lower growth rates than Japanese quail, they were more precocious, undergoing maximum growth during the incubation period. Tibiotarsal data were fitted by model M_5. Quail subspecies differed only in time to maximum growth rate. There were no differences in maturity values or growth rates, suggesting that, in European quail, the tibiotarsus increases first in cortical thickness and then in length. The opposite behavior was observed in Japanese quail.

Data for ash content in the tibiotarsus bone were fitted by model M_5. There were differences between subspecies only in time to maximum growth rate, suggesting differences in bone mineralization. It was also found that bone calcification occurred earlier in Japanese quail.

The derivative of Gompertz equations (Fialho 1999) was applied to body weight, Seedor index, diaphyseal volume, diaphyseal length, diaphyseal diameter, diaphyseal thickness, and tibiotarsal ash content, and the growth rate of these variables (expressed in grams, millimeters, cubic millimeters, or percentage points per day) was calculated according to the age of European and Japanese quail. Growth rates increased with bird age until they reached a maximum value and then started to decrease. These data are graphically represented in Figures 1 and 2.

By analyzing the growth rate of European and Japanese quail in relation to body weight, it was observed that both subspecies had the highest growth rates between days 14 and 21; however, the rate of weight gain (g/day) of European quail was almost twice as high as that of Japanese quail.

The rate of femoral weight gain was highest at 21 days of age in European and Japanese quail, decreasing thereafter. From 21 to 35 days of age, femoral weight gain decreased by 39.13% in European quail and 35.71% in Japanese quail. The tibiotarsal growth rate was highest at 14 days of age in both quail subspecies, decreasing thereafter. From 14 to 35 days, tibiotarsal weight gain decreased by 61.29% in European quail and 61.11% in Japanese quail. Similarly, the highest rate of tarsometatarsal weight gain was observed at 14 days of age in both subspecies, decreasing thereafter. The growth rate decreased by 55.55% and 66.66% in European and Japanese quail, respectively, from 14 to 35 days of age.

The rate of bone longitudinal growth differed between European and Japanese quail for the tibiotarsus bone only. Femoral longitudinal growth decreased by 40% and 20% in European and Japanese quail, respectively, up to 35 days of age. Reductions in tibiotarsal longitudinal

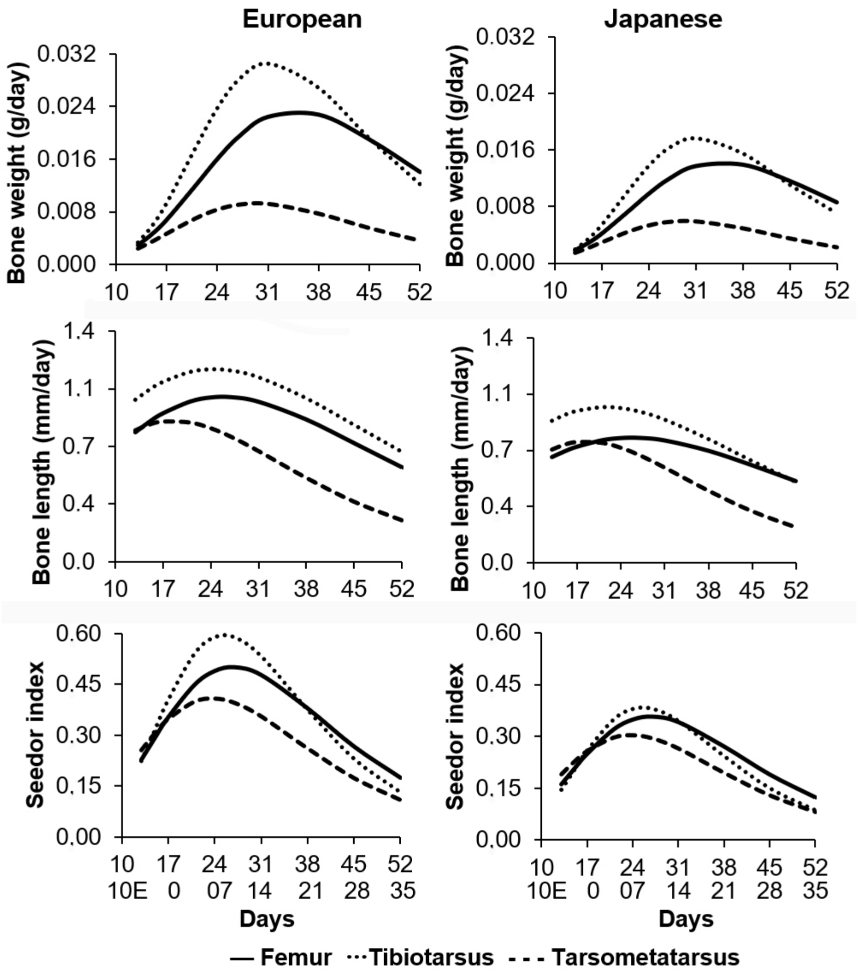


Figure 1. Growth rates of bone weight, bone length, Seedor index of femur, tibiotarsus, and tarsometatarsus in European and Japanese quail. Curves were constructed from the growth estimates shown in Table I using the equations described by Fialho (1999).

growth were 42% in European quail and 48% in Japanese quail. The reduction in tarsometatarsal growth rate was the same in European and Japanese quail, estimated at about 70%.

The Seedor index of the femur and tibiotarsus was highest at 10 days of age, decreasing by 65.2% and 77%, respectively, up to 35 days of age in both quail subspecies. For the tarsometatarsus, the Seedor index was highest at 7 days of age, decreasing by 73% up to 35 days of age in European and Japanese quail.

Femoral volume reached the maximum growth rate at 14 days in both quail subspecies, decreasing by 50.40% up to 35 days of age. In the tibiotarsus, the reduction in volume growth rate was 80%. Tarsometatarsal volume decreased by 70.90% from 10 to 35 days of age in both subspecies.

The highest growth rate of femoral diaphyseal diameter was observed on day 5 in both subspecies. The rate decreased by 68% in European quail and 69% in Japanese quail up to

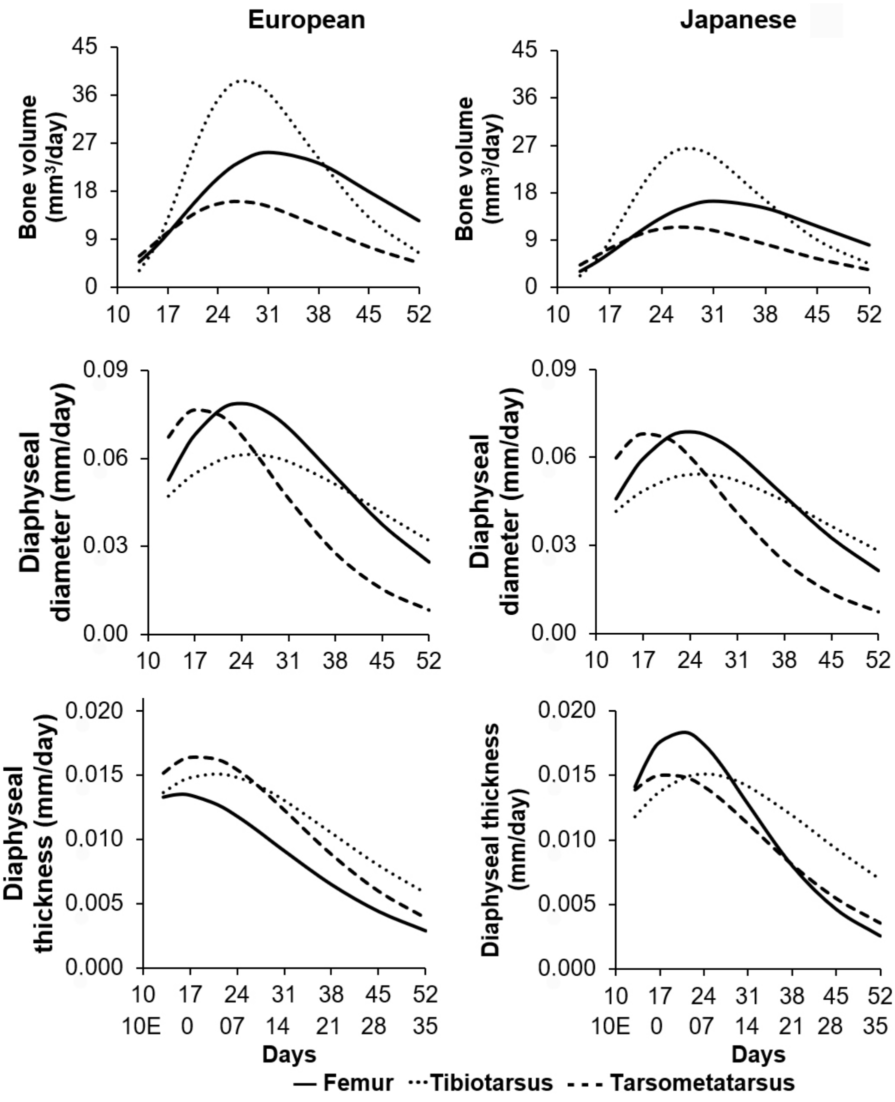


Figure 2. Growth rates of bone volume, diaphyseal diameter, and thickness of femur, tibiotarsus, and tarsometatarsus in European and Japanese quail. Curves were constructed from the growth estimates shown in Table I using the equations described by Fialho (1999).

35 days of age. Tibiotarsal diaphyseal diameter growth was highest at 8 days of age in both quail subspecies, decreasing by 47% and 48% in European and Japanese quail, respectively, by the end of the evaluation period.

Femoral diaphyseal thickness was highest at 15 days of incubation in European quail and 3 days post-hatch in Japanese quail. Reductions in the growth rate of femoral diaphyseal thickness were 78.57% and 83.33% in European and Japanese quail, respectively. Tibiotarsal thickness growth was highest at 3 days of age in European quail and 7 days of age in Japanese quail, decreasing by 60% and 53%, respectively, at 35 days of age.

In European quail, as compared with Japanese quail, diaphyseal thickness increased before longitudinal growth of the bone. That is, the maximum rate of bone remodeling associated with diaphyseal thickness occurred before the maximum rate of longitudinal growth. The opposite was observed in Japanese quail, in which the maximum longitudinal growth rate was achieved 3 days before the maximum diaphyseal thickness growth rate.

Tarsometatarsal diaphyseal thickness decreased by 89.16% in European quail and 89.10% in Japanese quail from the day of maximum growth rate to 35 days of age.

Ash content was highest in the last days of embryonic development. The maximum deposition rate was observed on day 15. From day 21 onward, ash deposition rate decreased by 97% in European and Japanese quail. In other words, from this day on, ash deposition in bones was virtually nonexistent.

Regression analysis (Tables II and III) revealed interaction effects ($p < 0.05$) between quail subspecies and day of growth on femoral, tibiotarsal, and tarsometatarsal densities, as assessed by the Hounsfield scale. The cubic model showed that there were differences in bone mineralization between European and Japanese quail of the same age for the three bones evaluated. European quail showed increased mineralization from 7 days onward in the femur and tibiotarsus and from 4 days onward in the tarsometatarsus (Figure 3).

Femoral, tibiotarsal, and tarsometatarsal resistance were influenced by the main effect of day of growth ($p < 0.05$). A linear effect was exerted on femoral and tibiotarsal resistance; that is, resistance increased over time. Tarsometatarsal resistance had a quadratic relationship with time, also increasing with age (Table III). Quail subspecies and day of growth exerted significant interaction effects on the cross-sectional area of the femur and tibiotarsus ($p < 0.05$) (Table III). Bone cross-sectional areas increased with time (Figure 4). Femoral cross-sectional area increased 1.96-fold in European quail and 1.94-fold in Japanese quail from 10 to 35 days of age.

Epiphyseal plate thickness in the tibiotarsus was influenced by the main effect of day of growth ($p < 0.05$), showing that tibiotarsal length varied over time in both European and Japanese quail (Figure 5). The thickness increase along days and the reduction of the epiphyseal plate thickness is clear after 24 days. At 35 days the thickness is reduced by more than 50%, characterizing the start of the ossification process in the long bones.

Results of mineral contend are described on Table IV. An interaction effect was exerted on tibiotarsal P content ($p < 0.05$) (Figure 6). A cubic effect of day of growth was observed on tibiotarsal Mg content ($p < 0.05$). The Mg percentage was highest on day 15 of embryonic development and in the first day's post-hatch, decreasing thereafter. This behavior was observed in European and Japanese quail. No significant differences in tibiotarsal Ca content were observed between European and Japanese quail.

Table II. Mean bone density of the femur, tibiotarsus, and tarsometatarsus in European and Japanese quail from the 13th day of incubation (13e) to 35 days post-hatch.

Bone density, Hounsfield scale units				
Day		**Fem**	**Tib**	**Tmt**
European quail				
13e	(13)*	−803	−787	−696
15e	(15)	−704	−707	−672
hatch	(17)	−663	−651	−597
04	(21)	−567	−535	−515
07	(24)	−567	−412	−440
10	(27)	−425	−362	−384
14	(31)	−200	−223	−276
21	(38)	−240	−267	−254
28	(45)	−164	−148	−197
35	(52)	−119	−85	−123
Japanese quail				
13e	(13)*	−811	−807	−833
15e	(15)	−624	−686	−627
hatch	(17)	−653	−636	−597
04	(21)	−555	−545	−522
07	(24)	−427	−432	−420
10	(27)	−315	−348	−386
14	(31)	−317	−320	−378
21	(38)	−324	−331	−377
28	(45)	−268	−239	−278
35	(52)	−218	−193	−275
CV (%)		−12.61	−11.15	−12.29
p-value				
Quail		0.847	0.363	0.500
Day		0.003	<0.0001	0.006
Quail × Day		0.002[1]	0.0007[2]	<0.0001[3]
Regression equation				R^2
Fem	[1]Eur: $y = -1709.264 + 90.8478x - 1.8993x^2 + 0.0142x^3$ Jap: $y = -1625.092 + 86.8806x - 1.893x^2 + 0.0142x^3$			0.93
Tib	[2]Eur: $y = -1771.2855 + 100.1889x - 2.2779x^2 + 0.0187x^3$ Jap: $y = -1714.2003 + 97.101x - 2.2779x^2 + 0.01873x^3$			0.95
Tmt	[3]Eur: $y = -1446.8261 + 72.9746x - 1.5723x^2 + 0.01264x^3$ Jap: $y = -1371.3113 + 68.8089x - 1.5723x^2 + 0.01264x^3$			0.91

*** Days inside parentheses were used for regression analysis. Fem: femur; Tib: tibiotarsus; Tmt: tarsometatarsus, CV: coefficient of variation.**

Table III. Mean breaking strength and cross-sectional area of the femur, tibiotarsus, and tarsometatarsus in European and Japanese quail from 10 to 35 days post-hatch.

	Breaking strength, N			Cross-sectional area, mm^2		
Day	**Fem**	**Tib**	**Tmt**	**Fem**	**Tib**	**Tmt**
European quail						
10	19.22	21.47	-	8.46	6.58	-
14	21.06	25.15	18.76	11.62	8.58	8.00
21	29.24	24.37	29.89	14.92	12.31	9.62
28	32.28	28.61	17.36	15.98	15.54	11.25
35	44.43	36.52	21.80	16.66	15.83	11.06
Japanese quail						
10	16.87	20.68	-	6.88	5.38	-
14	15.75	20.50	12.57	7.79	6.28	5.54
21	20.37	21.33	17.48	10.78	9.07	6.61
28	36.88	27.18	15.35	11.17	11.46	8.94
35	28.67	24.50	10.82	13.38	12.16	8.78
CV (%)	35.30	30.18	35.46	13.45	14.33	14.71
p-value						
Quail	0.075	0.682	0.003	<0.0001	0.001	0.0003[6]
Day	<0.0001[1]	0.001[2]	0.046[3]	0.005	0.005	<0.0001[7]
Quail × Day	0.327	0.167	0.683	0.046[4]	0.034[5]	0.600
Regression equation						R^2
Fem	[1]Eur/Jap: $y = 8.14231 + 0.8465x$					0.43
Tib	[2]Eur: $y = 19.1437 + 0.3762x$; Jap: $y = 14.5623 + 0.3762x$					0.24
Tmt	[3]Eur: $y = 1.3282 + 1.957x - 0.0412x^2$; Jap: $y = -6.5827 + 1.957x - 0.0412x^2$					0.33
Fem	[4]Eur: $y = -5.4256 + 1.8996x - 0.0568x^2 + 0.00058x^3$ Jap: $y = -1.9919 + 1.2306x - 0.0432x^2 + 0.00058x^3$					0.82
Tib	[5]Eur: $y = 6.626 - 0.3612x + 0.0473x^2 - 0.00083x^3$ Jap: $y = 5.7634 - 0.4571x + 0.0473x^2 - 0.00083x^3$					0.93
Tmt	[7]Eur: $y = 6.0435 + 0.1595x$; Jap: $y = 3.5922 + 0.1595x$					0.61

*** Days used for regression analysis of BD. Fem: femur; Tib: tibiotarsus; Tmt: tarsometatarsus, CV: coefficient of variation.**

DISCUSSION

This study aimed to describe the growth of the femur, tibiotarsus, and tarsometatarsus in European and Japanese quail from 13 days of incubation to 35 days post-hatch. The Gompertz growth curve was used as it provided a good fit to the experimental data.

Bone growth is dynamic and structurally modified in response to internal and external stresses stemming from physiological, nutritional, and physical factors (Rath et al. 2000). Structural and anatomical parameters are genetically determined from metabolic properties via bone cells (Banks 1992).

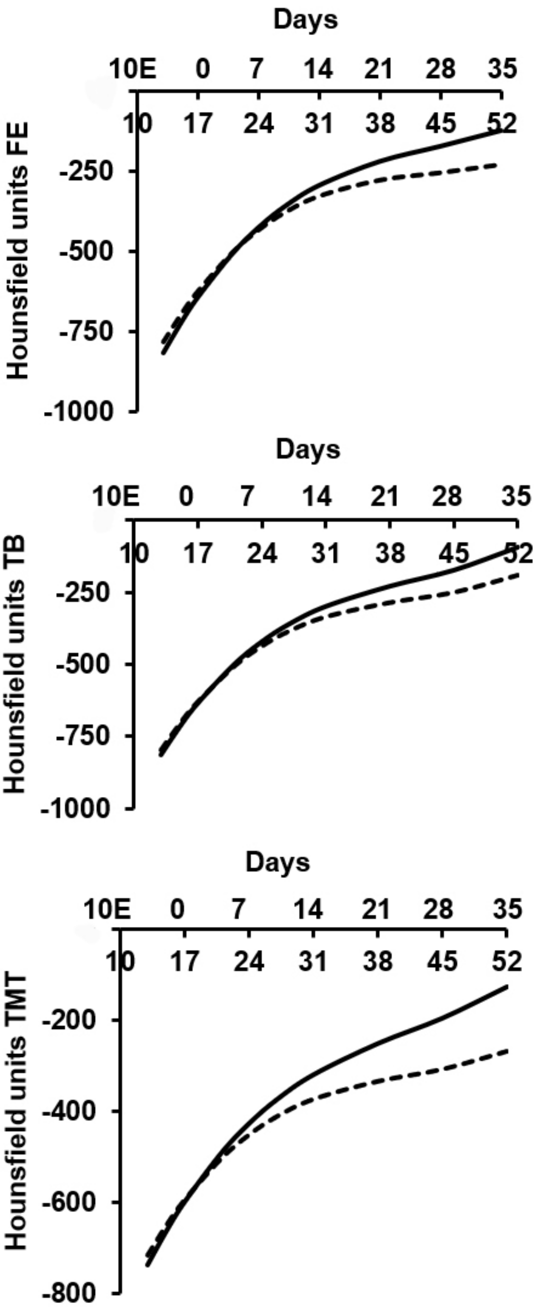


Figure 3. Unfolding interaction of bone density expressed in Hounsfield units of the femur (FE), tibiotarsus (TB), and tarsometatarsus (TMT) in European (¾) and Japanese (---) quail.

Endochondral formation begins on the third day of bird embryonic development, consisting of a hyaline cartilage model that is later replaced by bone tissue (Gartner & Hiatt 2003). In quail embryo development, Nakame & Tsudzuki (1999) described that on the fourth day, it is possible to observe the cartilage model of the tibiotarsus and femur. On the fifth day, the femur reaches a length of 50.67 ± 0.15 mm. Ossification begins on day 7 of embryonic development. The ossification percentage of the femur is 7.3%. Intense calcification of long bones begins on the 8th day of incubation. On day 13, the upper and lower limbs show calcifications

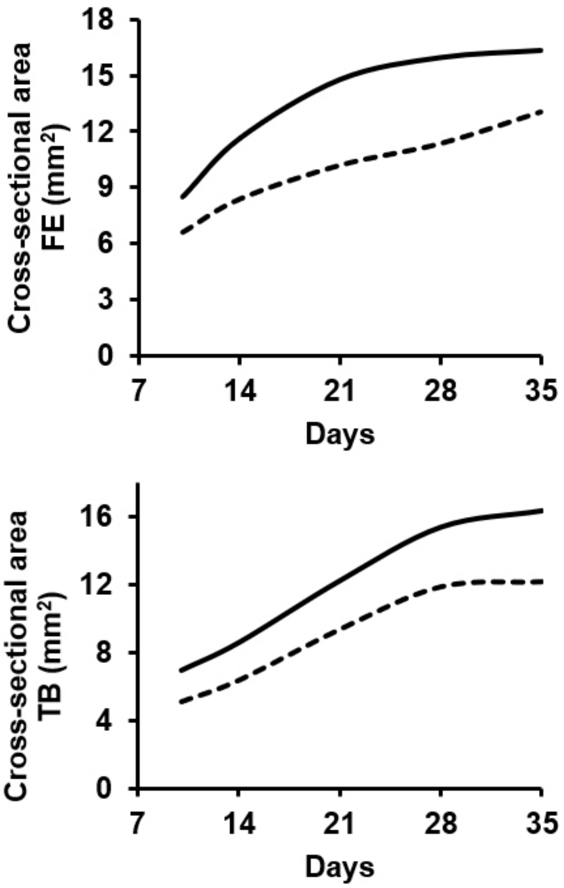


Figure 4. Unfolding interaction of bone density of the cross-sectional area of the femur (FE) and tibiotarsus (TB) in European (¾) and Japanese (---) quail. Curves were constructed from the growth estimates shown in Table 1 using the equations described by Fialho (1999).

of 66.5% and 74.8%, respectively, increasing to 73.7% and 82.8%, respectively, by day 15 of embryonic development. On day 17, the day of hatching, 74.7% of the upper limbs and 86.5% of the lower limbs are calcified.

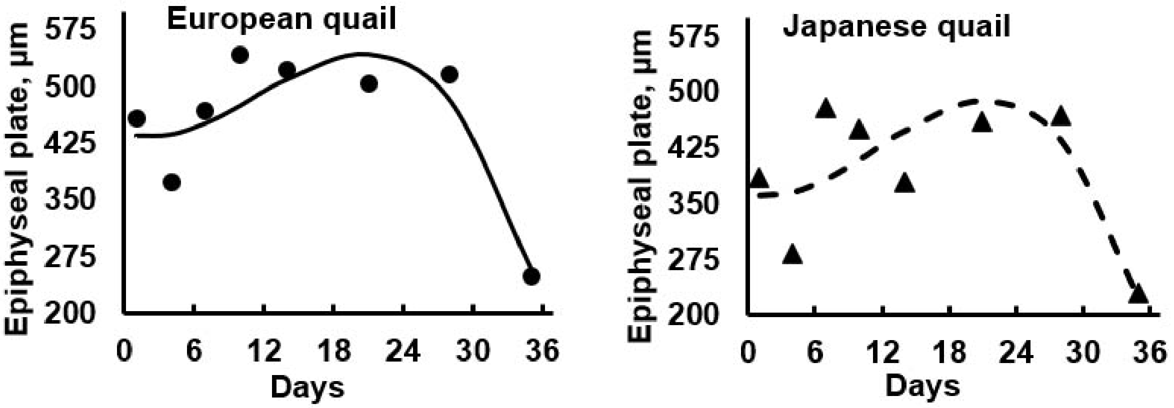
Longitudinal growth of the long bones followed the same patterns in European and Japanese quail. The analysis of the derivative of the Gompertz equation in bones showed that tarsometatarsus had the highest longitudinal and diaphyseal diameter growth rates at 17 days of incubation in both quail subspecies. This finding suggests the occurrence of intense bone deposition in the last few days of incubation.

As shown by analysis of maximum growth rates, the tibiotarsus of European and Japanese quail increased first in length and then in diameter, indicating high osteoclastic activity. The opposite behavior was observed in the femur: the highest growth rates were observed first in relation to diameter, then to length.

In ducks, the tibiotarsus and femur exhibited different responses to changes in diameter. Tibiotarsal length correlated positively with the increase in diameter, resulting in large bones. On the other hand, the femur had a small diameter at the beginning of life, when longitudinal growth was more pronounced. In this case, longitudinal bone growth without proportional changes in diameter may predispose to skeletal problems (Van Wyhe et al. 2014).

In a study conducted with broilers, Applegate & Lilburn (2002) observed differences in the growth patterns of tibiotarsus and femur from hatching onward. The ratio of femoral length to live weight reached its maximum value at 35 days of age, whereas that of the tibiotarsus increased continuously with time. No significant differences in bone width as a function of live weight were observed between the femur and tibiotarsus. The bones exhibited different growth patterns. Given that mineralization in the diaphyseal region was lower in the femur than in the tibiotarsus, the femur might be responsible for skeletal abnormalities of the long bones during the final growth period of broilers.

The epiphyseal plate thickness was analyzed only in the tibiotarsus in this experiment. In this bone the highest thickness value was in periods of low growth rates. On day 35, both longitudinal growth rate and epiphyseal plate thickness values were about half of the maximum values



$y = 438.8517 - 3.6299x + 1.1595x^2 - 0.0335x^3$ $y = 363.2829 - 3.6299x + 1.1595x^2 - 0.0335x^3$

Figure 5. Graphics of regression analysis of the thickness of the epiphyseal plate of the tibiotarsus in European (¾) and Japanese (---) quail from 1 to 35 days. There were effects of quail subspecies (p=0.007) and days (p=0.001). Note the thickness reduction after 28 days characterizing the period of the ossification process.

observed during the experimental period, and probably in few days ahead it will disappear.

The rate of longitudinal bone growth is controlled by biomechanical factors and growth mediators, which interact to regulate the activity of chondrocytes in the growth plate. Chondrocyte proliferation, matrix synthesis and degradation, and changes in chondrocyte size are essential, well-coordinated activities for rapid changes during bone growth (Farquharson & Jefferies 2000). There is synchrony between the speed of mitotic activity in the proliferation zone and the speed of resorption in the ossification zone. When speeds are equal, the growth plate remains the same and the bone becomes longer. When birds reach maturity, the speed of activity decreases, and the ossification zone reaches proliferation and reserve cartilage zones. Reserve cartilage is replaced by a calcified bone, no longer able to grow longitudinally (Gartner & Hiatt 2003). Results observed in the histology of the tibiotarsus reinforce the results in the growth of the three bones analyzed in the quail's subspecies.

Reich et al. (2005) loaded broiler chicks with bags weighing 10% of their body weight and observed a significant reduction in growth plate thickness, leading to a decrease in total length. No changes were observed in the epiphyseal plate growth zone, but loading influenced mineralization and ossification, which accompany growth. These findings indicate a high rate of calcification, which decreases epiphyseal plate thickness.

Ash content in the tibiotarsus reached values similar to those observed at the end of the study period (35 days post-hatch) only at 7 days of age: 46.5% and 45.9% in European and Japanese quail, respectively. Similar values were reported by Osman et al. (2009) in quail. Ash content growth rate, as estimated from the derivative of the Gompertz equation, was

Table IV. **Mineral contents in the tibiotarsus of European and Japanese quail from the 13th day of incubation (15e) to 35 days post-hatch.**

		Tibiotarsus mineral content		
Day		**Ca (%)**	**P (%)**	**Mg (%)**
European quail				
15e	(15)*	14.07	4.46	2.47
hatch	(17)	11.68	4.06	1.49
04	(21)	17.47	6.48	0.95
07	(24)	12.08	5.11	0.53
10	(27)	12.24	4.64	0.48
14	(31)	10.48	4.42	0.41
21	(38)	15.51	5.97	0.30
28	(45)	15.49	5.58	0.29
35	(52)	14.48	5.77	0.30
Japanese quail				
15e	(15)*	15.02	4.70	2.70
hatch	(17)	15.76	5.37	1.79
04	(21)	16.42	7.60	1.17
07	(24)	11.96	5.17	0.45
10	(27)	11.23	4.73	0.52
14	(31)	13.58	4.26	0.43
21	(38)	16.36	4.35	0.29
28	(45)	14.47	4.89	0.29
35	(52)	16.16	5.43	0.30
CV (%)		21.97	24.93	38.81
p-value				
Quail		0.255	0.903	0.751
Day		0.166	0.060	<0.0001[2]
Quail × Day		0.750	0.016[1]	0.209

Percent values are expressed on a dry matter basis.
***Days inside parentheses were used in regression analysis.**
[1] **Eur: $y = -0.4266 + 0.5603x - 0.0183x^2 + 0.00019x^3$; Jap: $y = 1.1647 + 0.5089x - 0.0183x^2 + 0.00019x^3$ ($R^2 = 0.10$) (interaction effect).**
[2] **Eur/Jap: $y = 10.1959 - 0.7899x + 0.0205x^2 - 0.00017x^3$ ($R^2 = 0.83$) (isolated effect).**

3.14% and 2.81% per day in European and Japanese quail, respectively, at 15 days of incubation, decreasing to 1.10% and 0.79% per day, respectively, at 7 days of incubation. From day 15 of incubation to day 21 post-hatch, the rate of ash deposition decreased by 97%. This finding suggests that at 21 days post-hatch, the tibiotarsus is already calcified. From 15 days of incubation to 7 days post-hatch, the ash content of bones increased by 149% in European quail and 62% in Japanese quail. From 7 to 35 days of age, the ash content decreased to 13% and 18% in European and Japanese quail, respectively. Because of this behavior, it was only possible to perform bone resistance analysis at 7 days of age for the femur and tibiotarsus and at 10 days of age for the tarsometatarsus.

Ahmed & Soliman (2013) showed that the formation and position of the tibiotarsus and femur at 17 days of incubation are very similar to those of bones in adult birds. In mammals, cartilaginous matrix resorption occurs after calcification, which is crucial for breaking strength and the formation of the primary ossification center. In birds, matrix resorption occurs even when calcification is not taking place, reducing the resistance of the bones. Calcification is only high on the last day of incubation and in the first few days post-hatch. According to the authors, only the femur has two ossification centers: the primary is located in the metaphysis region and the secondary in the epiphysis. No secondary ossification centers were observed in the tibiotarsus.

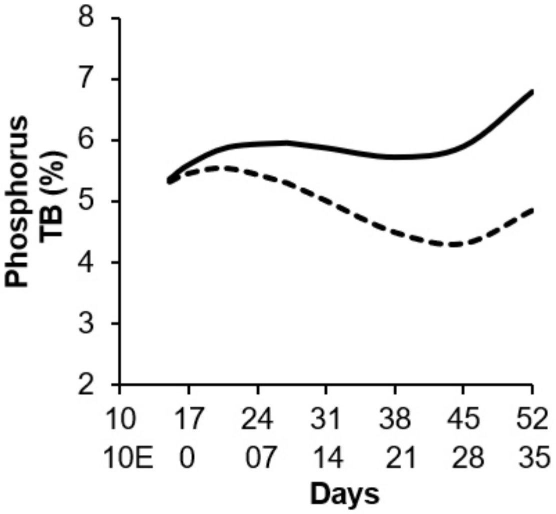


Figure 6. Unfolding interaction of ashes of tibiotarsus content in European (¾) and Japanese (---) quail.

Tarsometatarsus showed the highest bone resistance on day 21 in European and Japanese quail. Tibiotarsus showed greater resistance to fracture at 35 days in European quail and 28 days in Japanese quail. The same behavior was observed for the femur.

Tibiotarsus ash percentage was highest on days 28 (46.68%) and 35 (46.50%) in European quail. These values were similar to those observed by Benites et al. (2020) in the tibiotarsus of European quail at 35 days of age (50.77% ash). In Japanese quail, the highest values were observed on day 28, suggesting that higher breaking resistance is associated with higher ash concentrations. Although the tarsometatarsus and femur have different characteristics, their similar ash contents might indicate similar behavior with regard to fracture. Ca and P are essential elements that play important roles in bone development and mineralization (Underwood & Suttle 1999, Standford 2006). Rath et al. (2000) highlighted that bone ash content is proportional to hardness degree. Thus, the balance between these bone components may contribute to resistance to fracture. Bone mineral density is a measure of bone mineralization directly influenced by ash content.

In this study, the Mg content of tibiotarsus in quail was highest when the ash deposition rate was high. At 15 and 17 days of incubation, calcification was intense. From 7 days post-hatch onward, the ash deposition rate decreased along with Mg percentage. Mg is involved in cell metabolism and bone development, and its actions are closely related to those of Ca and P (Shastak & Rodehutscord 2015). In a study with rats fed Mg-deficient diets, it was observed that osteoblastic activity was impaired. The number of osteoblasts did not differ from the control group; however, markers of osteoblastic bone formation were reduced in Mg-deficient rats, suggesting that osteoblast function is impaired as much as hydroxyapatite formation. In *in vitro* experiments, osteoblasts grown in Mg-deficient environments showed reduced proliferation and differentiation rates. Although it is known that Mg deficiency results in bone mass loss, the mechanisms of such effects are still unknown (Rude et al. 2004).

Stanquevis et al. (2015) reported similar Ca values in European quails to those found in the current study. The authors found that the tibiotarsus of 14-day-old contained 11.77% Ca. Barreiro et al. (2009) assessed the percentages of Ca, P, and Mg in broilers aged 8, 22, and 43 days. The authors identified that mineral contents did not differ between days 22 and 43, with values greater than those observed at 8 days of age.

Femoral, tibiotarsal, and tarsometatarsal density results, as assessed by the Hounsfield scale using cone-beam computed tomography and Dolphin® software, were found to increase with age in European and Japanese quail. The Hounsfield scale represents the relative density of tissues according to a grayscale, in which air has a density of −1000 HU, water has a density of 0 HU, and bone tissue has a density of 1000 HU (Silva et al. 2012). When the bone surface is subjected to stress, an electric potential is created, producing tissue deformation. Stress is important to provide bone tissue with nutrients, acting as a stimulus for bone formation, which could explain the effect of physical activity on bone properties and resistance, as observed in the present study (Turner et al. 1995). Bones are more sensitive to physical loads during growth; thus, outcomes are often characterized by an increase in bone mass via the periosteum and endosteal apposition, with or without changes in mineral density (Regmi et al. 2016).

In a study with rats, Sharir et al. (2011) demonstrated that muscle strength regulates bone morphology, possibly enhancing the load-bearing capacity of the bone structure. The authors concluded that, from embryogenesis to adulthood, the bone adapts to applied loads, shaping the tissue to accommodate changes in animal growth and development. Tarsometatarsal density differed between European and Japanese quail to a greater extent than tibiotarsal and femoral densities. Such a result might be influenced by the high rate of bone remodeling, given that the highest rates of width, diaphyseal thickness, and longitudinal growths occurred 1-day post-hatch.

Bone cross-sectional area is associated with the ability of bones to withstand loads during development, in that the larger the area, the greater the response to applied loads (Yair et al. 2012). Some authors suggested that bones subjected to overload have increased breaking strength because of changes in geometry and the subsequent redistribution of bone mass (Heinonen et al. 2001).

When provided with sufficient space, birds can run, jump, and flap their wings, activities that apply a greater load on bones, increasing breaking strength (Jendral et al. 2008). European quail exhibited a larger bone cross-sectional area than Japanese quail. As animals grow, the geometric shape of bones becomes less circular, with possible differences in cross-sectional areas. There is a strong correlation between bone cross-sectional area and body weight: the heavier the bird, the larger the cross-sectional area (Williams et al. 2004).

Overall, our results demonstrate the differences among forelimb long bone growth in the two subspecies most commonly used on quail farms. The results are important to promote future research in the field of quail production, given that little information on the topic is available in the literature.

CONCLUSIONS

European and Japanese quail have different femoral and tibiotarsal growth patterns, especially in the first few days after hatching, whereas tarsometatarsal growth is similar between subspecies.

Acknowledgments

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