Femur and tibia development in meat-type chickens with different growth potential for 56 days of rearing period

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ABSTRACT We studied the changes in morphological, geometric, densitometric, and mechanical parameters of the femur and tibia during 56 D of rearing chickens with different growth rates. Ten femur and tibia were collected from fast-growing chickens (**FG**) and 2 types of medium-growing chickens (**MGH** and **MGGP**) immediately after hatching (0 D) and on 7, 14, 21, 35, 42, 49, and 56 D of life. The bone parameters of chickens across all genetic groups were found to be similar on 0 D, with exceptions of lower percentage contribution of bone weight (**BW**) in FG chickens ($P < 0.05$), lower total bone volume in MGGP chickens ($P < 0.05$), and lower maximum elastic strength in MGH chickens ($P < 0.05$). The bones developed in FG chickens were longer and wider; however, an increase in bone mineral density (**BMD**) between 42 and 49 D was not observed. The BMD value in FG chickens on 56 D was comparable to that in MGH chickens ($P = 0.089$) and significantly lower than that in MGGP chickens ($P = 0.021$). Mean relative wall thickness, despite longer and thicker bones in FG chickens, was comparable and often lower than that of MGH and MGGP chickens. In conclusion, the results showed that medium-growing chickens could be reared for up to 56 D without the risk of any growth impairment due to problems associated with deterioration of pelvic limb bone quality.

Key words: chicken, growth, bone development, femur, tibia

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INTRODUCTION

Growth rate of chickens is the basic criterion considered in breeding flocks when selecting for meat yield traits. This is due to the huge economic importance and ease of controlling the growth rate. Growth is a dynamic process that leads to an increase in total bone weight (**BW**) and individual tissues (Siegel and Dunnington, 1987). Therefore, the adverse effects during breeding depend on factors such as selection criteria, the number of features controlled at the same time, and the knowledge of correlation between them. Targeted selection of chickens with regard to growth potential traits gives rise to disorders such as difficulties in reproduction (Siegel and Dunnington, 1987; Hocking, 1993), cardiovascular system physiology (Olkowski, 2007), locomotor system functioning (Zhao et al., 2014), excessive body fatness (Tůmová and Teimouri, 2010), impaired immunity (Cheema et al., 2003), and consequently reduced welfare (Knowles et al., 2008). These effects are due to one-sided selection focused on increasing meat yield and improving feed conversion ratio (**FCR**) (Paxton et al., 2014).

Impaired bone development limits the growth of poultry, thereby contributing to increased mortality and losses due to poor classification of carcasses in slaughterhouses, thus concurrently becoming 1 of the 4 main causes that reduces the economics of production (Manning et al., 2007; Damaziak et al., 2014; Gocsik et al., 2014). To address the increasing incidence of tibial chondrodysplasia (**TD**) and femoral head necrosis (**FHN**), X-ray examinations assessing bone pathology and evaluating gait were included in routine selection programs (Kapell et al., 2012). These methods are expected to improve the degree of mineralization and mechanical endurance of broiler pelvic limb bones in the future, while further selection is done using the conventions method of assessing increase in BW, thereby decreasing the rearing period and improving the FCR. However, compensating for the skeletal pathologies that have already occurred in commercial fast-growing

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broilers is difficult and requires many subsequent generations. To date, studies evaluating the quality of skeletal system in meat-type poultry were focused majorly on the final stage of production cycle, which usually is no longer than 42 D of a chicken's life (Aguado et al., 2015; Mosleh et al., 2017). Throughout the production cycle, the focus on bone development in poultry is very less (Charuta and Cooper, 2012; Van Wyhe et al., 2012, 2014, Robison et al., 2015, Stover et al., 2017). Therefore, in the present study, we analyzed the period from hatching to 56 D to show the differences in bone development depending on the growth rate of the chickens. Currently, the consumer demand for less-intensive animal production processes has increased; therefore, a longer rearing period is required in case of poultry. We believe that the results of this study can provide an answer regarding which of the analyzed genetic groups of chickens in terms of bone quality should be maintained during a prolonged rearing period. We assume that slow growth rate is favorable for proper development of long bones and leads to better mineralization and durability as compared with the bones in FG chickens.

Therefore, the aim of this study is to analyze the bone characteristics of meat-type chickens with different growth rates during their growth and development.

MATERIALS AND METHODS

All procedures were performed according to the guidelines for the care and use of research animals and were approved by the Third Local Ethics Committee on Animal Experimentation in Warsaw (SGGW Warsaw) (resolution number 29/2010, Warsaw, PL).

Chickens, Breeding, and Bone Sample Collection

In this study, fast-growing Cobb 500 (FG) chickens and 2 medium-growing chickens MGH (HubbardJA957) and MGGP (only males) were raised up to 56 D of age. MGGP chickens consisted of the second generation of the experimental line created from crossing Polish native Greenleg Partridge hens and commercial parental line heavy type rooster (Cobb 500). A total of 1,080 chickens were reared, 360 of each genotype. Ten replications consisting of 36 chickens kept in the same pen, with an area of 3 m^2, were formed within each genotype. Each pen was equipped with 2 bell drinkers and 2 feeders. Sawdust mixed with straw was used as litter. Only the following ingredients were used in the ad libitum feeding: corn-wheat-soybean diet in a 4-stage system: 0 to 14 D, starter: 12.52 MJ of energy and 220 g crude protein (**CP**)/kg; 15 to 35 D, grower 1: 12.76 MJ of energy and 208 g CP/kg; 36 to 49 D, grower 2: 13.20 MJ of energy and 193 g CP/kg; 50 to 56 D, finisher: 13.47 MJ of energy and 185 g CP/kg. The light program was adapted to commercial conditions, i.e., on day 0 to 5, the light was switched on for 24 h, and on day 6 to 56, the light: dark cycle was 18L:6D.

The BW of individual chickens was determined (±1.0 g) at the time of insertion (0 D) and on 7, 14, 21, 35, 49, and 56 D of life. In each of these growth stages, 10 chickens from each genotype were randomly selected for slaughter. The chickens were slaughtered by decapitation after electrical stunning. After bleeding, femur and tibia bones were collected from the right leg of each chicken. After removal of all soft tissue residues, bone mass (±0.1 g), percentage contribution of femur weight to BW (**PCF**), and percentage contribution of tibia weight to BW (**PCT**) were determined. The length (mm) and width (mm) at mid-length of the bones were then measured with a caliper. After the measurements, all bones were wrapped in cheesecloth, frozen in plastic bags, and systematically transferred for further analysis.

Dual Energy X-ray Absorptiometry

All bones were scanned using the dual energy X-ray absorptiometry (**DEXA**) method. Bone mineral density (**BMD**; g/cm^2) and bone mineral content (**BMC**; g) were determined for whole bone samples. Scanning and measuring procedures of the femur and tibia were performed using Norland XR-46 apparatus (resolution 1.0 × 1.0 mm) and Research Scan software (Norland, Fort Atkinson). All bones were placed on their dorsal surface and scanned in an anterior-posterior direction (Krupski et al., 2018). Low mineral density of bone samples obtained from chickens after the hatching (0 D) limited densitometric measurements to the DEXA method.

Quantitative Computed Tomography

Volumetric bone mineral density (g/cm^3) of the trabecular (**Td**—trabecular bone mineral density) and cortical bone (**Cd**—cortical bone mineral density) of the femur and tibia was determined using the quantitative computed tomography (**QCT**) method and Somatom Emotion scanner supplied with Somaris/5 VB10B software (Siemens, Erlangen, Germany). Whole bone samples were scanned using 1-mm thick (0 to 28 D of life) and 2-mm thick (35 to 56 D of life) cross-sectional sequential scans. The femur Td measurement was performed on a single cross-sectional scan in the distal epiphysis. The measurement of tibia Td was performed in the distal epiphysis. The measurement of Cd was performed on a cross-sectional scan placed at 50% of bone length (midshaft) for both the femur and tibia. Cortical bone area (**CBA**, mm^2) was measured automatically at the midshaft of the femur and tibia. Volume evaluation package (Siemens, Erlangen, Germany) was used to determine the total bone volume (**Bvol**; cm^3) and mean volumetric bone mineral density (**MvBMD**; g/cm^3) of each bone. For Bvol and MvBMD measurements of the femur and tibia from chickens between day 0 and 7, the

volume of interest (**VOI**) was limited by minimum and maximum densities of the investigated bones at −300 and 3,071 Hounsfield units (**HU**), respectively. For the Bvol and MvBMD measurements of the bones from 14 to 56 day-old chickens, the VOI was limited by the minimum and maximum densities of the investigated bones at 0 and 3,071 HU, respectively.

Geometrical properties of the femur and tibia were determined on the basis of measurements of horizontal and vertical diameters (both external and internal) of the mid-diaphyseal cross-section of the bone obtained from computed tomography multiplanar reconstructions. The values of cross-sectional area (**A**), second moment of inertia (**Ix**), mean relative wall thickness (**MRWT**), and cortical index (**CI**) were calculated (Brodzki et al., 2004; Tatara et al., 2005).

Analysis of Bone Mechanical Properties

Mechanical properties of the femur and tibia were determined using a 3-point bending test and an Instron 3367 apparatus (Instron, Canton, MA) integrated with a computer. The relationship between loading force of the evaluated bone and the resulting displacement was recorded. The values of maximum elastic strength (Wy) and ultimate strength (Wf) were determined. The distance between bone supports was set at 40% of the total bone length, and the measuring head loaded bone samples at the midshaft with a constant speed of 50 mm/min (Krupski et al., 2018).

Statistical Analysis

During the weekly control of BW, the arithmetic mean for all the genetic groups of chickens was calculated. The obtained data were checked for normality, and birds whose BW deviated from the group were eliminated. The chickens for slaughter were randomly selected from the remaining ones. In this study, the mean values of BW were presented only for birds from which bones were collected, i.e., for 10 chickens of each genotype and each of the analyzed developmental stages. Average BW for all chickens was presented and analyzed previously (Michalczuk et al., 2016). All examined variables were compared between chickens of different genetic groups within the same slaughter stage and within the genetic group between different weeks of rearing by 1-way analysis of variance (**ANOVA**) and multiple comparison post-hoc Duncan test. Moreover, 2-way ANOVA was performed. The first factor was the genetic group of chickens with 3 levels (FG, MGH, MGGP), and the second factor was the weekly age of the flock with 7 or 8 levels, depending on the group of variables. An effect of both factors and the interaction between them was evaluated. Two-way ANOVA was conducted using the following model:

$$y_{ijk} = m + a_i + b_j + (ab)_{ij} + e_{ijk}$$

where y is response variable, m is general mean of the variable, a_i is the main effect of the genotype, b_j is the main effect of age, $(ab)_{ij}$ is the interaction of genotype and age, and e_{ijk} is the random error.

All calculations were performed using Statistica 10.0 software (Statistica, 2011) and SPSS 23 (IBM, 2015). The significance level was set at 0.05 ($P < 0.05$).

RESULTS

Effect of Chicken Age and Genotype on Bone Morphological Properties

Results of morphological parameters of chicken bones are presented in Table 1 for the femur and in Table 2 for the tibia. Age, genotype, and their interaction affected bone morphological parameters. On 0 D, FG chickens had the heaviest weight and highest Bvol, but the lowest PCF and PCT ($P < 0.05$). The weight of femur and tibia significantly increased with the age of the chickens ($P < 0.05$). The heaviest BW was observed in FG chickens. The bones in MGGP chickens were usually of the lowest weight, but the difference between the weight of bones of MGH and MMGP chickens was insignificant on 42 and 56 D for tibia ($P > 0.05$). The difference between the BW in MGH and MMGP chickens decreased especially for the femur from 42 D. On 56 D, the weight of the femur in MGGP chickens was significantly higher than that of the femur in MGH chickens ($P = 0.012$), whereas in the case of tibia, the weight difference was not confirmed at that time ($P = 0.063$).

Percentage contribution of femur weight to BW in FG chickens ranged from 0.38% on 0 D to 0.50% on 56 D of life, and in the case of PCT from 0.53 to 0.71%. For MGH chickens, PCF ranged from 0.39% on 0 D to 0.58% on 56 D, while PCT ranged from 0.58 to 0.83% and was the highest for both bones of all groups of chickens (Tables 1 and 2). Percentage contribution of femur weight to BW in MGGP chickens was the lowest on 7 D (0.40%) and highest on 42 D (0.52%). Compared with other groups of chickens, PCF in MGGP chickens was significantly lower from 21 D than that in MGH chickens and comparable or higher than that in FG chickens. Exceptionally, significantly lower PCF in MGGP chickens was observed on 56 D than that observed in FG chickens ($P = 0.025$). The highest PCT in MGGP chickens was observed on 42 D (0.79%) and the lowest on 0 and 7 D (0.62%). Compared with FG chickens, PCT in MGGP chickens was higher on 0, 14, 35, and 42 D and comparable at other growth stages. Compared to MGH chickens, the PCT in MGGP chickens was usually at a similar level with the exception of higher PCT on 0 D ($P = 0.020$) and lower on 7 and 49 D ($P = 0.010$ and $P = 0.014$, respectively).

The length and width of the femur and tibia gradually increased each week. The chicken genotype had no effect on the length of both bones on 0 D. From

Table 1. Means and ±SD for femur bone morphological properties in 3 chicken genotypes.

		Morphological properties of femur						
Age (wk)	Genotype[1]	Body weight (g)	Weight of bone (g)	PCF[2] (%)	Length (mm)	Width (mm)	CBA[3] (mm^2)	Bvol[4] (cm^3)
Hatching (0 D)	FG	46.6 ± 1.3^{a,Z}	0.18 ± 0.01^{a,Z}	0.38 ± 0.02^{b,Z}	22.8 ± 0.63^{a,Z}	1.94 ± 0.10^{a,Z}	nd	0.14 ± 0.01^{a,Z}
	MGH	41.5 ± 1.2^{b,Z}	0.16 ± 0.01^{b,Z}	0.39 ± 0.02^{b,Z}	22.4 ± 0.35^{a,Z}	1.77 ± 0.10^{b,Z}	nd	0.11 ± 0.03^{b,Z}
	MGGP	37.5 ± 1.3^{c,Z}	0.16 ± 0.01^{b,Z}	0.42 ± 0.03^{a,Z}	22.3 ± 0.57^{a,Z}	1.75 ± 0.11^{b,Z}	nd	0.10 ± 0.02^{b,Z}
7 D	FG	151.5 ± 2.5^{a,Y}	0.70 ± 0.03^{a,Y}	0.46 ± 0.02^{a,X}	30.9 ± 0.56^{a,Y}	2.94 ± 0.18^{a,Y}	5.17 ± 0.41^{a,Z}	0.50 ± 0.09^{a,Z,Y}
	MGH	124.1 ± 2.0^{b,Y}	0.54 ± 0.03^{b,Y}	0.43 ± 0.02^{b,Y}	29.6 ± 0.24^{b,Y}	2.58 ± 0.12^{b,Y}	4.50 ± 0.55^{a,b,Z}	0.43 ± 0.07^{a,Z}
	MGGP	110.9 ± 3.0^{c,Y}	0.44 ± 0.02^{c,Z,Y}	0.40 ± 0.02^{c,Z}	28.3 ± 0.54^{c,Y}	2.55 ± 0.12^{b,Y}	4.33 ± 0.82^{b,Z}	0.34 ± 0.04^{b,Z}
14 D	FG	347.4 ± 8.1^{a,X}	1.50 ± 0.08^{a,X}	0.43 ± 0.02^{b,Y}	40.6 ± 0.58^{a,X}	4.29 ± 0.20^{a,X}	12.33 ± 1.63^{a,Y}	0.80 ± 0.18^{a,Y}
	MGH	264.4 ± 5.9^{b,X}	1.28 ± 0.07^{b,X}	0.48 ± 0.02^{a,X}	38.4 ± 0.84^{b,X}	3.84 ± 0.17^{b,X}	11.33 ± 1.37^{a,b,Y}	0.73 ± 0.07^{a,Z}
	MGGP	233.2 ± 6.3^{c,X}	1.15 ± 0.04^{c,Y}	0.49 ± 0.02^{a,X,Y}	37.6 ± 0.54^{c,X}	3.87 ± 0.18^{b,X}	9.83 ± 1.17^{b,Y}	0.57 ± 0.08^{b,Z}
21 D	FG	722.2 ± 23.2^{a,W}	3.37 ± 0.13^{a,W}	0.47 ± 0.02^{b,X}	49.8 ± 0.48^{a,W}	5.85 ± 0.28^{a,W}	18.50 ± 1.22^{c,X}	2.15 ± 0.14^{a,X}
	MGH	536.1 ± 12.3^{b,W}	2.60 ± 0.13^{b,W}	0.57 ± 0.08^{a,W}	47.9 ± 0.89^{b,W}	5.02 ± 0.27^{b,W}	14.83 ± 1.47^{b,X}	1.62 ± 0.18^{b,Y}
	MGGP	416.9 ± 10.0^{c,W}	1.99 ± 0.08^{c,X}	0.48 ± 0.02^{b,Y}	44.7 ± 1.03^{c,W}	4.71 ± 0.26^{c,W}	12.33 ± 1.37^{a,X}	1.17 ± 0.09^{c,Y}
35 D	FG	1670.9 ± 40.7^{a,V}	7.47 ± 0.38^{a,V}	0.45 ± 0.02^{c,X,Y}	65.3 ± 4.14^{a,V}	6.77 ± 0.07^{a,V}	27.00 ± 5.48^{a,W}	4.64 ± 0.64^{a,W}
	MGH	1066.9 ± 17.9^{b,V}	5.57 ± 0.27^{b,V}	0.52 ± 0.02^{a,V}	63.8 ± 1.86^{a,V}	6.48 ± 0.09^{b,V}	22.00 ± 2.68^{b,W}	3.33 ± 0.20^{b,X}
	MGGP	1020.0 ± 16.3^{c,V}	4.91 ± 0.26^{c,W}	0.48 ± 0.03^{b,Y}	63.2 ± 1.86^{a,V}	5.85 ± 0.09^{c,V}	20.00 ± 2.28^{c,W}	2.90 ± 0.28^{c,X}
42 D	FG	2066.5 ± 69.6^{a,U}	10.47 ± 0.27^{a,U}	0.51 ± 0.02^{b,W}	69.9 ± 1.31^{a,U}	7.73 ± 0.28^{a,U}	26.67 ± 1.37^{a,W}	6.03 ± 0.52^{a,V}
	MGH	1350.7 ± 71.7^{b,U}	7.68 ± 0.33^{b,U}	0.57 ± 0.03^{a,V}	68.3 ± 0.87^{b,U}	7.38 ± 0.21^{b,U}	23.67 ± 3.67^{b,W}	4.43 ± 0.73^{b,W}
	MGGP	1306.7 ± 46.4^{b,U}	6.86 ± 0.26^{c,V}	0.52 ± 0.02^{b,X}	66.2 ± 0.54^{c,U}	7.23 ± 0.32^{b,U}	23.17 ± 1.47^{b,V}	4.13 ± 0.33^{b,W}
49 D	FG	2554.5 ± 22.7^{b,T}	11.91 ± 0.58a^{c,T}	0.47 ± 0.02^{c,X}	75.4 ± 0.80^{a,T}	8.98 ± 0.40^{a,T}	27.00 ± 3.03^{a,W}	6.21 ± 0.82^{a,V}
	MGH	1734.3 ± 37.1^{b,T}	9.72 ± 0.58^{b,T}	0.56 ± 0.03^{a,V}	73.1 ± 3.35^{b,T}	8.29 ± 0.31^{b,T}	23.00 ± 2.45^{b,W}	6.12 ± 0.95^{a,V}
	MGGP	1637.8 ± 26.6^{c,T}	8.25 ± 0.50^{c,U}	0.50 ± 0.03^{b,X,Y}	71.1 ± 1.27^{c,T}	7.58 ± 0.56^{c,T}	22.67 ± 1.86^{b,V}	4.86 ± 0.52^{b,V}
56 D	FG	3035.5 ± 137.0^{a,S}	15.26 ± 0.87^{a,S}	0.50 ± 0.05^{b,W}	80.4 ± 1.41^{a,S}	10.64 ± 1.07^{a,S}	30.83 ± 2.48^{a,V}	9.53 ± 0.57^{a,U}
	MGH	2239.5 ± 52.4^{c,S}	12.93 ± 0.74^{b,S}	0.58 ± 0.03^{a,V}	79.8 ± 3.28^{a,S}	9.21 ± 0.38^{b,S}	24.00 ± 1.26^{b,W}	7.64 ± 1.27^{b,U}
	MGGP	2375.2 ± 101.2^{b,S}	10.11 ± 2.86^{c,T}	0.43 ± 0.01^{c,Z}	78.5 ± 1.53^{a,S}	9.67 ± 0.79^{b,S}	25.83 ± 2.79^{b,U}	6.26 ± 1.22^{c,U}
Effect of age		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Effect of genotype		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Effect of age × genotype		<0.001	<0.001	<0.001	<0.001	<0.001	0.016	<0.001

Means within a column, for a specific birds age, without a common small letter ($^{a, b, c}$) in superscript differ significantly between groups and without a common capital letter ($^{Z, Y, X,U, V, W,S,T}$) in superscript differ significantly between ages ($P < 0.05$).

[1]FG—fast-growing Cobb500; MGH—medium-growing HubbardJA957; MGGP—medium-growing experimental line.

[2,3,4]Percentage contribution of femur weight to BW (only one—right bone); CBA = cortical bone area; Bvol = total bone volume.

[4]Bvol in 0 and 7-day-old groups was measured using VOI limitation between –300 and 3,071 HU, while in the older groups the VOI limitation was between 0 and 3,071 HU.

Table 2. Means and ±SD for tibia bone morphological properties in 3 chicken genotypes.

Age (wk)	Genotype[1]	Morphological properties of tibia					
		Weight of bone (g)	PCF[2] (%)	Length (mm)	Width (mm)	CBA[3] (mm^2)	Bvol[4] (cm^3)
Hatching (0 D)	FG	$0.25 \pm 0.02^{a,Z}$	$0.53 \pm 0.05^{c,Z}$	$31.1 \pm 0.61^{a,Z}$	$1.66 \pm 0.14^{a,Z}$	nd	$0.20 \pm 0.02^{a,Z}$
	MGH	$0.24 \pm 0.02^{a,b,Z}$	$0.58 \pm 0.03^{b,Z}$	$31.3 \pm 1.10^{a,Z}$	$1.69 \pm 0.12^{a,Z}$	nd	$0.16 \pm 0.04^{a,b,Z}$
	MGGP	$0.23 \pm 0.01^{b,Z}$	$0.62 \pm 0.02^{a,Z}$	$31.3 \pm 0.88^{a,Z}$	$1.60 \pm 0.16^{a,Z}$	nd	$0.12 \pm 0.05^{b,Z}$
7 D	FG	$0.95 \pm 0.05^{a,Y}$	$0.63 \pm 0.04^{a,b,Y}$	$41.5 \pm 1.13^{a,Y}$	$2.51 \pm 0.22^{a,Y}$	$5.50 \pm 0.55^{a,Z}$	$0.70 \pm 0.10^{a,Z}$
	MGH	$0.81 \pm 0.05^{b,Y}$	$0.65 \pm 0.04^{a,Y}$	$41.1 \pm 0.58^{a,Y}$	$2.51 \pm 0.18^{a,Y}$	$6.00 \pm 0.89^{a,Z}$	$0.66 \pm 0.09^{a,Z}$
	MGGP	$0.68 \pm 0.02^{c,Z,Y}$	$0.62 \pm 0.02^{b,Z}$	$39.9 \pm 0.70^{b,Y}$	$2.38 \pm 0.18^{a,Y}$	$5.33 \pm 0.52^{a,Z}$	$0.54 \pm 0.04^{b,Z}$
14 D	FG	$2.23 \pm 0.11^{a,X}$	$0.64 \pm 0.02^{b,Y}$	$54.1 \pm 1.33^{a,X}$	$3.05 \pm 0.10^{a,X}$	$9.83 \pm 0.98^{a,Y}$	$1.23 \pm 0.18^{a,Z}$
	MGH	$1.85 \pm 0.14^{b,X}$	$0.70 \pm 0.05^{a,X,W}$	$53.3 \pm 1.53^{a,X}$	$3.00 \pm 0.11^{a,X}$	$9.00 \pm 0.63^{a,Y}$	$1.13 \pm 0.15^{a,Z}$
	MGGP	$1.62 \pm 0.04^{c,Y}$	$0.69 \pm 0.03^{a,Y,X}$	$51.6 \pm 1.30^{b,X}$	$2.75 \pm 0.16^{b,X}$	$7.67 \pm 0.82^{b,Y}$	$0.82 \pm 0.08^{b,Z,Y}$
21 D	FG	$4.54 \pm 0.17^{a,W}$	$0.63 \pm 0.01^{b,Y}$	$66.3 \pm 1.16^{a,W}$	$5.28 \pm 0.26^{a,W}$	$15.33 \pm 0.82^{a,X}$	$3.02 \pm 0.19^{a,Y}$
	MGH	$3.62 \pm 0.28^{b,W}$	$0.67 \pm 0.05^{a,Y,X}$	$64.5 \pm 1.01^{b,W}$	$5.04 \pm 0.83^{a,b,W}$	$13.67 \pm 1.21^{b,X}$	$2.38 \pm 0.32^{b,Y}$
	MGGP	$2.72 \pm 0.17^{c,X}$	$0.65 \pm 0.05^{a,b,Z,Y}$	$62.0 \pm 1.22^{c,W}$	$4.69 \pm 0.25^{b,W}$	$10.17 \pm 0.98^{c,X}$	$1.72 \pm 0.12^{c,Y}$
35 D	FG	$10.74 \pm 0.83^{a,V}$	$0.64 \pm 0.05^{b,Y}$	$89.7 \pm 2.37^{a,V}$	$6.96 \pm 0.15^{a,V}$	$23.83 \pm 3.92^{a,W}$	$7.44 \pm 0.38^{a,X}$
	MGH	$7.78 \pm 0.33^{b,V}$	$0.73 \pm 0.03^{a,W,V}$	$86.6 \pm 2.82^{b,V}$	$7.05 \pm 0.21^{a,V}$	$17.50 \pm 3.62^{b,W}$	$4.95 \pm 0.25^{b,X}$
	MGGP	$7.18 \pm 0.32^{c,W}$	$0.70 \pm 0.03^{a,Y,X}$	$83.8 \pm 2.98^{c,V}$	$6.76 \pm 0.31^{b,V}$	$17.33 \pm 2.34^{b,W}$	$4.43 \pm 0.36^{c,X}$
42 D	FG	$14.68 \pm 1.25^{a,U}$	$0.71 \pm 0.07^{b,X}$	$99.9 \pm 2.74^{a,U}$	$7.59 \pm 0.37^{a,U}$	$25.50 \pm 2.74^{a,W}$	$8.75 \pm 0.34^{a,W}$
	MGH	$10.14 \pm 0.64^{b,U}$	$0.75 \pm 0.06^{a,b,V}$	$94.3 \pm 6.58^{b,U}$	$7.22 \pm 0.22^{b,V}$	$21.67 \pm 2.94^{b,V}$	$6.07 \pm 1.36^{b,W}$
	MGGP	$10.32 \pm 0.49^{b,V}$	$0.79 \pm 0.05^{a,W}$	$93.9 \pm 2.54^{b,U}$	$7.55 \pm 0.29^{a,U}$	$20.00 \pm 0.89^{b,V}$	$5.25 \pm 1.10^{b,X}$
49 D	FG	$17.52 \pm 0.55^{a,T}$	$0.69 \pm 0.02^{b,X}$	$109.9 \pm 3.06^{a,T}$	$8.28 \pm 0.27^{a,T}$	$24.50 \pm 2.43^{a,W}$	$9.68 \pm 1.16^{a,W}$
	MGH	$13.81 \pm 0.83^{b,T}$	$0.80 \pm 0.05^{a,U}$	$104.9 \pm 3.60^{b,T}$	$7.92 \pm 0.30^{b,U}$	$22.50 \pm 1.05^{a,V}$	$8.33 \pm 1.67^{a,b,V}$
	MGGP	$11.69 \pm 0.70^{c,U}$	$0.71 \pm 0.05^{b,X}$	$104.5 \pm 2.56^{b,T}$	$7.99 \pm 0.17^{b,T}$	$19.33 \pm 1.75^{b,V}$	$7.22 \pm 0.84^{b,W}$
56 D	FG	$21.65 \pm 1.84^{a,S}$	$0.71 \pm 0.08^{b,X}$	$114.1 \pm 3.23^{a,S}$	$9.21 \pm 0.27^{b,S}$	$29.50 \pm 2.59^{a,V}$	$14.44 \pm 2.09^{a,V}$
	MGH	$18.55 \pm 1.05^{b,S}$	$0.83 \pm 0.05^{a,U}$	$117.1 \pm 3.24^{a,S}$	$8.19 \pm 0.23^{c,T}$	$23.00 \pm 2.90^{b,V}$	$11.71 \pm 1.04^{a,U}$
	MGGP	$17.33 \pm 4.00^{b,T}$	$0.73 \pm 0.18^{a,b,X}$	$107.7 \pm 4.83^{b,S}$	$9.63 \pm 0.35^{a,S}$	$23.17 \pm 1.17^{b,X}$	$9.82 \pm 1.96^{b,V}$
Effect of age		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Effect of genotype		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Effect of age × genotype		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means within a column, for a specific birds age, without a common small letter ($^{a, b, c}$) in superscript differ significantly between groups and without a common capital letter ($^{Z, Y, X,U,V,W,S,T}$) in superscript differ significantly between ages ($P < 0.05$).

[1]FG—fast-growing Cobb500; MGH—medium-growing HubbardJA957; MGGP—medium-growing experimental line.

[2,3,4]Percentage contribution of tibia weight to BW (only one—right bone); CBA = cortical bone area; Bvol = total bone volume.

[4]Bvol in 0 and 7-day-old groups was measured using VOI limitation between –300 and 3,071 HU, while in the older groups the VOI limitation was between 0 and 3,071 HU.

7 to 21 D and on 49 D, the highest length of the femur was observed in FG chickens, followed by the MGH group and the lowest for the MGGP group. On 35 and 56 D, femur lengths reached similar values for all chickens (Table 1). For the tibia, similar length was observed for FG and MGH chickens on 7 and 14 D ($P = 0.080$ and $P = 0.060$, respectively), whereas MGGP chickens had significantly lower value of bone length in this period ($P < 0.05$). From 21 to 56 D, the tibia in FG chickens was the longest, whereas for MGH and MGGP chickens, no differences were confirmed from 42 D (Table 2). The width of the femur and tibia on 0 D and the width of the tibia on 7 D were comparable for chickens in all groups ($P > 0.05$). From 7 D to the end of the rearing period, FG chickens had the widest femur, whereas the comparison of width in this bone between MGH and MGGP chickens showed differences only on 21, 35, and 49 D, when MGH chickens had a wider bone (Table 1). The width of the tibia in FG chickens was comparable to that in MGH chickens on 14, 21, and 35 D ($P > 0.05$).

A significant increase in CBA of the femur and tibia in FG chickens was observed without exception up to 35 D, while it remained at a constant level from 35 to 49 D and increased in the last week (Tables 1 and 2). CBA of the femur and tibia in MGH chickens increased significantly without exception up to 56 D. For MGGP chickens, an absence of CBA increase in both the femur and tibia was observed between 42 and 49 D. The FG chicken group showed mainly the highest CBA values of the femur except on 7 and 14 D. From 21 D, the CBA values of the femur in MGH and MGGP chickens were at similar levels. A similar trend was observed for the tibia (Table 2).

Total bone volume of both bones of all chicken groups increased systematically. For both femur and tibia, the highest Bvol on 0, 21, 35, 42, and 49 D was found for FG chickens. In addition, the Bvol of the femur in FG chickens was also higher than that in MGGP and MGH chickens on 56 D (Table 1). On 7 and 14 D, the Bvol of the femur and tibia in FG and MGH chickens reached similar values ($P = 0.059$ and $P = 0.060$, respectively) and were higher than that in MGGP chickens ($P = 0.020$). Total bone volume of the femur and tibia in MGGP chickens was always lower than that in MGH chickens, with the exception of 49 D (Tables 1 and 2).

Table 3. Means and ±SD for femur bone geometrical properties in 3 chicken genotypes.

Age (wk)	Genotype[1]	Geometrical properties of femur[2]			
		A (mm^2)	Ix (mm^4)	MRWT	CI
7 D	FG	$4.19 \pm 0.33^{b,Z}$	$2.96 \pm 0.55^{a,Z}$	$1.48 \pm 0.06^{a,W}$	$59.68 \pm 1.03^{a,V}$
	MGH	$4.97 \pm 0.46^{a,Z}$	$2.03 \pm 0.30^{b,Z}$	$1.44 \pm 0.11^{a,W}$	$58.90 \pm 1.85^{a,V}$
	MGGP	$3.85 \pm 0.35^{b,Z}$	$1.76 \pm 0.25^{b,Z}$	$1.59 \pm 0.23^{a,X}$	$61.02 \pm 3.31^{a,V}$
14 D	FG	$14.39 \pm 2.93^{a,Y}$	$21.60 \pm 7.66^{b,Z}$	$1.83 \pm 0.31^{b,V}$	$64.03 \pm 4.43^{b,U}$
	MGH	$13.73 \pm 1.16^{a,b,Y}$	$17.47 \pm 2.33^{a,b,Z}$	$2.24 \pm 0.25^{a,V}$	$68.91 \pm 2.40^{a,U}$
	MGGP	$11.27 \pm 2.17^{b,Y}$	$13.29 \pm 4.73^{b,Z}$	$1.79 \pm 0.22^{b,W}$	$63.61 \pm 2.72^{b,V}$
21 D	FG	$19.86 \pm 2.89^{a,X}$	$50.25 \pm 12.88^{a,Z}$	$1.17 \pm 0.14^{a,X}$	$53.55 \pm 3.05^{a,W}$
	MGH	$16.44 \pm 2.12^{b,Y}$	$37.75 \pm 9.49^{b,Z}$	$0.96 \pm 0.14^{b,X}$	$48.57 \pm 3.45^{b,W}$
	MGGP	$13.73 \pm 1.02^{c,X}$	$25.31 \pm 2.23^{c,Z}$	$1.09 \pm 0.16^{a,b,Y}$	$51.84 \pm 3.63^{a,b,W}$
35 D	FG	$23.92 \pm 3.04^{a,W}$	$144.35 \pm 51.13^{a,Y}$	$0.57 \pm 0.05^{a,Y}$	$35.99 \pm 2.20^{a,X}$
	MGH	$19.60 \pm 1.61^{b,X}$	$94.99 \pm 27.11^{b,Y}$	$0.60 \pm 0.11^{a,Y}$	$37.37 \pm 4.04^{a,X}$
	MGGP	$16.80 \pm 0.79^{c,W}$	$67.13 \pm 8.03^{b,Y}$	$0.62 \pm 0.11^{a,Z}$	$37.86 \pm 4.04^{a,X}$
42 D	FG	$27.97 \pm 5.38^{a,V}$	$206.88 \pm 36.90^{a,X}$	$0.51 \pm 0.05^{a,Z,Y}$	$33.46 \pm 2.38^{a,Y,X}$
	MGH	$22.76 \pm 2.33^{a,X}$	$140.60 \pm 40.06^{b,X}$	$0.48 \pm 0.06^{a,Z,Y}$	$32.23 \pm 2.64^{a,Y}$
	MGGP	$19.87 \pm 2.24^{b,V}$	$129.95 \pm 21.67^{b,X}$	$0.54 \pm 0.07^{a,Z}$	$34.90 \pm 2.98^{a,Y,X}$
49 D	FG	$31.88 \pm 2.59^{a,V}$	$247.87 \pm 57.06^{a,X}$	$0.46 \pm 0.08^{a,Z,Y}$	$31.83 \pm 3.59^{a,Y}$
	MGH	$25.56 \pm 4.28^{b,W}$	$189.67 \pm 64.20^{a,b,W}$	$0.41 \pm 0.06^{a,Z}$	$27.52 \pm 2.14^{b,Z}$
	MGGP	$25.33 \pm 1.78^{b,V}$	$159.86 \pm 16.24^{b,X}$	$0.47 \pm 0.03^{a,Z}$	$31.73 \pm 1.21^{a,Z,Y}$
56 D	FG	$34.18 \pm 1.97^{a,V}$	$349.96 \pm 82.35^{a,W}$	$0.37 \pm 0.05^{b,Z}$	$25.78 \pm 2.84^{b,Z}$
	MGH	$29.10 \pm 3.88^{b,W}$	$248.42 \pm 42.82^{b,V}$	$0.38 \pm 0.01^{b,Z}$	$27.76 \pm 0.55^{b,Z}$
	MGGP	$26.89 \pm 1.34^{b,U}$	$274.79 \pm 79.41^{a,b,W}$	$0.44 \pm 0.05^{a,Z}$	$30.74 \pm 2.49^{a,Z}$
Effect of age		<0.001	<0.001	<0.001	0.001
Effect of genotype		<0.001	<0.001	0.753	0.056
Effect of age × genotype		0.021	0.016	<0.001	0.001

Means within a column, for a specific birds age, without a common small letter ($^{a, b, c}$) in superscript differ significantly between groups and without a common capital letter ($^{Z, Y, X, U, V, W, S, T}$) in superscript differ significantly between ages ($P < 0.05$).

[1]FG—fast-growing Cobb500; MGH—medium-growing HubbardJA957; MGGP—medium-growing experimental line.

[2]A = cross-sectional area; Ix = second moment of inertia; MRWT = mean relative wall thickness; CI = cortical index.

Effect of Chicken Age and Genotype on Bone Geometrical Properties

The results of geometric parameters of chicken bones are presented in Tables 3 and 4 for the femur and tibia, respectively. All bone geometric parameters depended on the age of the chickens. The genotype of chickens was important for cross-sectional area and second moment of inertia ($P < 0.05$) but had no effect on MRWT and CI for both bones ($P > 0.05$). A significant age × genotype interaction was confirmed for femur MRWT ($P < 0.001$) and only Ix of tibia ($P < 0.001$).

Cross-sectional area in the femur increased with the age of chickens. With the exception of 21 D for MGH chickens and 56 D for FG and MGH chickens, the value of A in the tibia increased significantly initially between 7 and 14 D, and subsequently, there was no increase on 21 D for FG and MGGP chickens. The next significant increase in this parameter for FG and MGGP chickens occurred only on 35 and 56 D. On 7 D, the A of the femur in MGH chickens was significantly higher than that in FG and MGGP chickens ($P < 0.05$). In the case of tibia, the lowest A value was confirmed for MGGP chickens ($P < 0.05$), whereas A value for tibia of FG and MGGP chickens was similar ($P > 0.05$). Later, the A value of the femur and tibia in FG chickens was mainly the highest on 14 D for both bones and on 42 D for the femur only. On 21, 49, and 56 D, the A value of tibia in FG chickens was comparable to that in MGH chickens. The lowest A value was observed for the bones of MGGP chickens; however, the differences between MGGP and MGH chickens were insignificant on 14, 49, and 56 D for the femur and on 14, 35, 42 (Table 3), and 49 D for the tibia (Table 4). Only on 56 D, the value of A of the tibia in MGGP chickens was significantly higher than that in MGH chickens ($P = 0.011$).

The second moment of inertia remained unchanged for all groups of chickens up to 21 D for femur. Similarly, no significant changes in Ix were found to 21 D in tibia for FG and MGGP chickens, whereas Ix tibia of MGH chickens increased significantly by 19.82 mm^4 from 7 to 21 D ($P < 0.05$). An exception was a significant increase in Ix for the tibia in MGH chickens on 21 D. A significant increase in Ix was observed for all groups of chickens on 35 D for both bones, on 49 D for the femur, and on 42 and 49 D for the tibia exclusively in MGH chickens. On 49 D, only bones in MGH chickens showed a significant increase in Ix value. On 56 D compared to 49 D, an increase in Ix was recorded for both bones in all groups of chickens (Tables 3 and 4). On 7 D and from 21 to 42 D for the femur (Table 3) and on 14 D and from 35 to 56 D for the tibia (Table 4), the highest Ix values were confirmed for FG chickens when compared with MGH and MGGP chickens. On 14 and 49 D for

Table 4. Means and ±SD for tibia bone geometrical properties in 3 chicken genotypes.

Age (wk)	Genotype[1]	Geometrical properties of tibia[2]			
		A (mm^2)	Ix (mm^4)	MRWT	CI
7 D	FG	5.62 ± 0.48^{a,Z}	3.47 ± 0.51^{a,b,Z}	1.55 ± 0.11^{a,b,W}	60.70 ± 1.64^{a,b,W}
	MGH	5.76 ± 0.34^{a,Z}	3.83 ± 0.66^{a,Z}	1.40 ± 0.15^{b,X}	58.19 ± 2.74^{b,W}
	MGGP	5.11 ± 0.26^{b,Z}	2.88 ± 0.20^{b,Z}	1.61 ± 0.20^{a,V}	61.42 ± 2.84^{a,V}
14 D	FG	11.94 ± 3.27^{b,Y}	15.13 ± 6.50^{b,Z}	1.79 ± 0.62^{a,W}	62.56 ± 8.12^{a,W}
	MGH	9.80 ± 1.45^{a,b,Y}	9.87 ± 2.95^{a,b,Z,Y}	1.68 ± 0.54^{a,X}	61.45 ± 6.76^{a,W}
	MGGP	8.24 ± 0.65^{a,Y}	8.06 ± 1.99^{a,Z}	1.32 ± 0.15^{a,W}	56.46 ± 2.63^{a,W}
21 D	FG	15.16 ± 0.66^{a,Y}	28.77 ± 2.12^{a,Z}	1.08 ± 0.08^{a,X}	51.59 ± 2.01^{a,X}
	MGH	13.54 ± 2.61^{a,X}	23.65 ± 9.29^{a,Y}	0.99 ± 0.15^{a,Y}	49.29 ± 4.06^{a,X}
	MGGP	10.03 ± 0.99^{b,Y}	12.74 ± 2.89^{b,Z}	1.06 ± 0.10^{a,X}	51.23 ± 2.51^{a,X}
35 D	FG	27.59 ± 4.56^{a,X}	95.22 ± 32.54^{a,Y}	0.78 ± 0.06^{a,Y,X}	43.24 ± 1.79^{a,Y}
	MGH	20.26 ± 3.09^{b,W}	58.41 ± 20.75^{b,X}	0.71 ± 0.08^{a,Z,Y}	41.47 ± 2.71^{a,Y}
	MGGP	18.56 ± 2.34^{b,X}	51.63 ± 11.57^{b,Y}	0.72 ± 0.09^{a,Y}	41.73 ± 3.10^{a,Y}
42 D	FG	27.99 ± 2.64^{a,X}	117.32 ± 14.03^{a,Y}	0.65 ± 0.08^{a,Z,Y}	39.40 ± 3.40^{a,Y}
	MGH	22.06 ± 3.04^{b,W,V}	79.68 ± 20.66^{b,W}	0.56 ± 0.13^{a,Z}	35.29 ± 5.42^{a,Z}
	MGGP	21.26 ± 1.76^{b,X}	72.35 ± 12.05^{b,Y}	0.61 ± 0.04^{a,Z,Y}	37.73 ± 1.37^{a,Z}
49 D	FG	27.47 ± 4.21^{a,X}	136.14 ± 34.36^{a,Y}	0.50 ± 0.07^{b,Z,Y}	32.80 ± 3.48^{b,Z}
	MGH	24.42 ± 3.11^{a,b,V}	112.37 ± 8.84^{a,V}	0.52 ± 0.11^{a,b,Z}	36.11 ± 4.35^{a,b,Z,Y}
	MGGP	21.20 ± 0.88^{b,X}	69.33 ± 8.91^{b,Y}	0.60 ± 0.04^{a,Z,Y}	37.36 ± 1.61^{a,Z}
56 D	FG	35.84 ± 6.82^{a,W}	278.14 ± 86.90^{a,X}	0.45 ± 0.10^{a,Z}	32.42 ± 5.08^{a,Z}
	MGH	29.73 ± 3.96^{a,U}	152.29 ± 24.07^{b,U}	0.54 ± 0.18^{a,Z}	33.58 ± 6.89^{a,Z}
	MGGP	30.39 ± 5.64^{a,W}	163.93 ± 42.47^{b,X}	0.53 ± 0.10^{a,Z}	34.47 ± 4.76^{a,Z}
Effect of age		<0.001	<0.001	<0.001	<0.001
Effect of genotype		<0.001	<0.001	0.411	0.494
Effect of age × genotype		0.061	<0.001	0.062	0.151

Means within a column, for a specific birds age, without a common small letter ($^{a, b, c}$) in superscript differ significantly between groups and without a common capital letter ($^{Z, Y, X, U, V, W, S, T}$) in superscript differ significantly between ages ($P < 0.05$).

[1]FG—fast-growing Cobb500; MGH—medium-growing HubbardJA957; MGGP—medium-growing experimental line.

[2]A = cross-sectional area; Ix = second moment of inertia; MRWT = mean relative wall thickness; CI = cortical index.

the femur (Table 3) and on 7 and 21 D for the tibia (Table 4), Ix in FG chickens was comparable to that in MGH chickens and significantly ($P < 0.05$) higher than that in MGGP chickens. The second moment of inertia of the femur and tibia in MGGP chickens was most often similar for this parameter in MGH chickens, except for significantly lower values on 21 D for the femur ($P = 0.026$) and on 7 and 21 D for the tibia ($P = 0.016$ and $P = 0.025$, respectively).

Mean relative wall thickness of the femur increased between 7 and 14 D for all groups of chickens, while CI values of the femur increased in the same period only for FG and MGH chickens. A significant decrease in MWRT and CI values of the femur was observed on 21 and 35 D when compared with that on 7 and 14 D for all groups of chickens. From 42 to 56 D, the MRWT values of the femur did not change, while femur CI values decreased further, with a decrease in the CI of MGH chickens on 42 and 49 D, and for FG and MGGP chickens in 56 D. For the tibia, contrary to the femur, no increase in MRWT and CI was observed (Table 4). A significant decrease in MRWT values was noted for MGGP chickens from 14 to 35 D and CI from 14 to 42 D. A significant decrease in MRWT value in FG and MGH chickens in the tibia occurred only on 21 D, and no changes were observed up to 56 D. A decrease in CI values of the tibia in FG and MGH chickens was observed on 21 and 35 D, on 49 D only for FG chickens, and on 42 D for MGH chickens (Table 4).

Effect of Chicken Age and Genotype on Bone Densitometric Properties

The results of evaluation of densitometric parameters are presented in Tables 5 and 6 for the femur and tibia, respectively. All the densitometric parameters were dependent on the age of the chickens ($P < 0.001$). Chicken genotype affected BMC, MvBMD, and Cd of both bones and Td of the femur ($P < 0.001$). Significant age × genotype interactions were confirmed for Td, BMD, and BMC of the femur and for Td, BMC, and Cd of the tibia ($P < 0.001$).

An increase in femoral Td was found on 14 D for FG and MGH chickens, on 21 D for MGH and MGGP chickens, and on 49 D for chickens from all genetic groups (Table 5). On 56 D, Td of the femur in FG, MGH, and MGGP chickens decreased significantly when compared with the values on 42 and 49 D ($P < 0.05$) (Table 5). For the tibia, an increase in Td was observed for all groups of chickens only on 14 D and for the MGGP group on 21 D. From 21 to 56 D, Td of the tibia in FG and MGGP chickens remained unchanged. A decrease in Td value of the tibia in MGGP chickens was observed from 49 to 56 D (Table 6).

Table 5. Means and ±SD for femur bone densitometric properties in 3 chicken genotypes.

		Densitometric properties of femur[2]				
Age (wk)	Genotype[1]	Td (g/cm^2)	BMD (g/cm^2)	BMC (g)	MvBMD[3] (g/cm^3)	Cd (g/cm^3)
Hatching (0 D)	FG	nd	nd	nd	$0.93 \pm 0.02^{a,Z}$	nd
	MGH	nd	nd	nd	$0.92 \pm 0.02^{a,Z}$	nd
	MGGP	nd	nd	nd	$0.91 \pm 0.01^{a,Z}$	nd
7 D	FG	$1.06 \pm 0.03^{a,Z}$	$0.046 \pm 0.00^{a,Z}$	$0.048 \pm 0.01^{a,Z}$	$1.07 \pm 0.04^{a,b,Y}$	$1.29 \pm 0.09^{a,Z}$
	MGH	$1.07 \pm 0.04^{a,Z}$	$0.040 \pm 0.01^{b,Z}$	$0.024 \pm 0.00^{b,Z}$	$1.07 \pm 0.02^{a,Y}$	$1.28 \pm 0.16^{a,Z}$
	MGGP	$1.04 \pm 0.05^{a,Z}$	$0.039 \pm 0.00^{b,Z}$	$0.017 \pm 0.00^{c,Z}$	$1.04 \pm 0.02^{b,Y}$	$1.15 \pm 0.11^{a,Z}$
14 D	FG	$1.17 \pm 0.04^{a,Y}$	$0.088 \pm 0.00^{a,Y}$	$0.263 \pm 0.06^{a,Y}$	$1.23 \pm 0.04^{a,b,X}$	$1.48 \pm 0.09^{a,b,Y}$
	MGH	$1.18 \pm 0.06^{a,Y,XW}$	$0.081 \pm 0.00^{a,Z,Y}$	$0.208 \pm 0.02^{b,Y}$	$1.26 \pm 0.02^{a,X}$	$1.50 \pm 0.03^{b,Y}$
	MGGP	$1.16 \pm 0.04^{a,Y}$	$0.120 \pm 0.17^{a,Z}$	$0.182 \pm 0.02^{b,Y}$	$1.22 \pm 0.03^{b,X}$	$1.40 \pm 0.09^{a,Y}$
21 D	FG	$1.22 \pm 0.03^{a,X}$	$0.108 \pm 0.01^{a,X}$	$0.576 \pm 0.04^{a,X}$	$1.32 \pm 0.02^{a,W}$	$1.78 \pm 0.10^{a,X}$
	MGH	$1.13 \pm 0.11^{a,Z,YX}$	$0.221 \pm 0.31^{a,X}$	$0.466 \pm 0.02^{b,X}$	$1.35 \pm 0.02^{a,W,V}$	$1.73 \pm 0.07^{a,X}$
	MGGP	$1.18 \pm 0.06^{a,Y}$	$0.085 \pm 0.02^{a,Z}$	$0.351 \pm 0.03^{c,X}$	$1.29 \pm 0.02^{b,W}$	$1.52 \pm 0.06^{b,X}$
35 D	FG	$1.25 \pm 0.04^{a,X}$	$0.145 \pm 0.01^{a,W}$	$1.269 \pm 0.10^{a,W}$	$1.34 \pm 0.02^{a,W}$	$1.91 \pm 0.06^{a,X,W}$
	MGH	$1.18 \pm 0.05^{b,X,YW}$	$0.134 \pm 0.01^{b,Y,X}$	$0.854 \pm 0.38^{b,W}$	$1.36 \pm 0.03^{a,V}$	$1.90 \pm 0.11^{a,W}$
	MGGP	$1.19 \pm 0.04^{b,Y,X}$	$0.125 \pm 0.00^{c,Z}$	$0.917 \pm 0.07^{b,W}$	$1.31 \pm 0.02^{b,W,V}$	$1.67 \pm 0.05^{b,X,W}$
42 D	FG	$1.25 \pm 0.02^{a,X}$	$0.176 \pm 0.01^{a,V}$	$1.850 \pm 0.07^{a,V}$	$1.31 \pm 0.03^{a,b,W}$	$1.86 \pm 0.13^{a,X,W}$
	MGH	$1.24 \pm 0.02^{a,W}$	$0.145 \pm 0.01^{b,Y,X}$	$1.331 \pm 0.11^{b,V}$	$1.33 \pm 0.04^{a,W,V}$	$1.83 \pm 0.22^{a,X,W}$
	MGGP	$1.24 \pm 0.04^{a,X}$	$0.140 \pm 0.01^{b,Z}$	$1.201 \pm 0.08^{c,V}$	$1.29 \pm 0.03^{b,W}$	$1.62 \pm 0.10^{b,X}$
49 D	FG	$1.22 \pm 0.03^{a,X}$	$0.179 \pm 0.01^{a,V}$	$2.385 \pm 0.20^{a,U}$	$1.33 \pm 0.04^{a,W}$	$1.86 \pm 0.10^{a,b,X,W}$
	MGH	$1.20 \pm 0.04^{a,X,W}$	$0.161 \pm 0.01^{b,Y,X}$	$1.722 \pm 0.11^{b,U}$	$1.34 \pm 0.03^{a,W,V}$	$1.94 \pm 0.06^{a,W}$
	MGGP	$1.18 \pm 0.07^{a,Y}$	$0.156 \pm 0.01^{b,Z}$	$1.561 \pm 0.08^{c,U}$	$1.33 \pm 0.05^{a,V}$	$1.78 \pm 0.16^{b,W,V}$
56 D	FG	$1.07 \pm 0.03^{b,Z}$	$0.200 \pm 0.02^{b,U}$	$2.522 \pm 0.26^{a,T}$	$1.33 \pm 0.04^{a,W}$	$1.96 \pm 0.17^{a,W}$
	MGH	$1.13 \pm 0.04^{a,Z,Y}$	$0.177 \pm 0.02^{b,X}$	$2.058 \pm 0.11^{b,T}$	$1.31 \pm 0.05^{a,W}$	$1.94 \pm 0.12^{a,W}$
	MGGP	$1.07 \pm 0.04^{b,Z}$	$0.388 \pm 0.25^{a,Y}$	$1.873 \pm 0.10^{c,T}$	$1.30 \pm 0.02^{a,W,V}$	$1.81 \pm 0.13^{a,V}$
Effect of age		<0.001	<0.001	<0.001	<0.001	<0.001
Effect of genotype		0.033	0.686	<0.001	<0.001	<0.001
Effect of age × genotype		0.036	0.007	<0.001	0.700	0.715

Means within a column, for a specific birds age, without a common small letter ($^{a, b, c}$) in superscript differ significantly between groups and without a common capital letter ($^{Z, Y, X,U, V, W, S, T}$) in superscript differ significantly between ages ($P < 0.05$).

[1]FG—fast-growing Cobb500; MGH—medium-growing HubbardJA957; MGGP—medium-growing experimental line.

[2]Td = volume mineral density of the trabecular bone; BMD = bone mineral density; BMC = bone mineral content; MvBMD = mean volumetric bone mineral density; CD50% = cortical bone mineral density.

[3]MvBMD in 0 and 7-day-old groups was measured using VOI limitation between –300 and 3,071 HU, while in the older groups the VOI limitation was between 0 and 3,071 HU.

Throughout the experiment, Td of the femur was comparable in all groups of chickens, with an exception of higher value in FG chickens on 35 D (Table 5). On the contrary, Td of the tibia from 7 to 21 D was strongly dependent on chicken genotype. On 7 D, Td of the tibia in MGGP chickens was higher than that in the FG group ($P = 0.010$), and on 14 D the value was lower than that in FG and MGH chickens ($P = 0.023$ and $P = 0.018$, respectively). On 21 D, the highest Td values of the tibia were observed in MGH chickens when compared with the other groups ($P < 0.05$). From 35 to 49 D, no differences in the Td value of the tibia were found between all groups, whereas on 56 D, Td of the tibia in MGH chickens was lower than that in MGGP chickens ($P = 0.011$).

BMD of the femur and tibia increased gradually only in FG chickens. A significant increase in BMD in MGH chickens was reported only on 21 D for the femur and on 21 and 35 D for the tibia, while BMD of both these bones in MGGP chickens increased significantly only on 56 D (Table 5 and 6). BMC values of the femur increased gradually across all groups of chickens from 7 to 56 D. A similar trend was observed for BMC of the tibia; however, an increase in the value of this parameter in the MGGP group of chickens started from 35 D (Table 6). An increase in MvBMD for both femur and tibia was also observed across all groups of chickens up to 21 D. The values of MvBMD of the femur from 35 to 56 D did not change significantly in all groups of chickens, as did the values of MvBMD of the tibia for FG and MGGP chickens ($P > 0.05$). For MGH chickens, the values of MvBMD of the tibia increased significantly on 49 D and decreased on 56 D. The highest BMD values of both the bones were observed in FG chickens on 49 D. It is worth noting that this relationship was reversed on 56 D and a very strong increase in BMD in both the femur and tibia of chickens between 49 and 56 D led to the highest values of this parameter for this group of chickens in the last week. For BMC of both the femur and tibia, the highest values were observed for FG chickens. BMC values for MGGP chicken bones were the lowest on 14, 21, and 35 D for the femur (Table 5) and on 14, 35, and 49 D for the tibia (Table 6). These values were comparable to BMC values of MGH chicken bones ($P > 0.05$). From 0 to 56 D, MvBMD of the femur and tibia in FG and MGH chickens did not differ ($P > 0.05$). The MvBMD values of the femur in MGGP chickens were lower than those in MGH

Table 6. Means and ±SD for tibia bone densitometric properties in 3 chicken genotypes.

Age (wk)	Genotype[1]	Densitometric properties of tibia[2]				
		Td (g/cm^2)	BMD (g/cm^2)	BMC (g)	MvBMD[3] (g/cm^3)	Cd (g/cm^3)
Hatching (0 D)	FG	nd	nd	nd	0.94 ± 0.01^{a,Z}	nd
	MGH	nd	nd	nd	0.94 ± 0.02^{a,Z}	nd
	MGGP	nd	nd	nd	0.91 ± 0.02^{b,Z}	nd
7 D	FG	1.05 ± 0.03^{b,Z}	0.073 ± 0.03^{a,Z}	0.049 ± 0.02^{a,Z}	1.09 ± 0.01^{a,Y}	1.22 ± 0.08^{a,Z}
	MGH	1.08 ± 0.03^{a,b,Z}	0.063 ± 0.09^{a,Z}	0.037 ± 0.00^{b,Z}	1.09 ± 0.02^{a,Y}	1.20 ± 0.12^{a,Z}
	MGGP	1.11 ± 0.05^{a,Z}	0.039 ± 0.01^{a,Z}	0.034 ± 0.00^{b,Z}	1.06 ± 0.02^{b,Y}	1.07 ± 0.09^{b,Z}
14 D	FG	1.12 ± 0.02^{a,Y}	0.091 ± 0.01^{a,Y}	0.355 ± 0.03^{a,Y}	1.24 ± 0.04^{a,b,X}	1.60 ± 0.10^{a,Y}
	MGH	1.14 ± 0.02^{a,Y}	0.085 ± 0.00^{b,Z,Y}	0.285 ± 0.03^{b,Y}	1.27 ± 0.03^{a,X}	1.63 ± 0.07^{a,Y}
	MGGP	1.09 ± 0.03^{b,Z}	0.073 ± 0.01^{c,Z}	0.167 ± 0.02^{c,Z}	1.20 ± 0.03^{b,X}	1.40 ± 0.12^{b,Y}
21 D	FG	1.17 ± 0.02^{b,Y,X}	0.112 ± 0.00^{a,X}	0.804 ± 0.03^{a,X}	1.30 ± 0.01^{a,W}	1.90 ± 0.05^{a,X,W}
	MGH	1.24 ± 0.04^{a,X}	0.108 ± 0.00^{b,Y}	0.690 ± 0.03^{b,X}	1.34 ± 0.02^{a,V,W}	1.84 ± 0.13^{a,X,W}
	MGGP	1.17 ± 0.03^{b,Y}	0.094 ± 0.00^{c,Z}	0.466 ± 0.03^{c,Y}	1.26 ± 0.05^{b,W}	1.52 ± 0.14^{b,Y,X}
35 D	FG	1.23 ± 0.15^{a,X,W}	0.155 ± 0.01^{a,W}	1.970 ± 0.15^{a,W}	1.34 ± 0.03^{a,b,W}	2.05 ± 0.08^{a,W}
	MGH	1.22 ± 0.05^{a,X}	0.164 ± 0.08^{a,X}	1.388 ± 0.11^{b,W}	1.35 ± 0.02^{a,U,V}	2.01 ± 0.08^{a,V}
	MGGP	1.20 ± 0.01^{a,Y}	0.124 ± 0.01^{a,Z}	1.307 ± 0.08^{b,X}	1.31 ± 0.02^{b,W,V}	1.77 ± 0.10^{b,W,V}
42 D	FG	1.19 ± 0.02^{a,Y,X}	0.176 ± 0.01^{a,V}	2.681 ± 0.09^{a,V}	1.31 ± 0.02^{a,W}	1.98 ± 0.07^{a,X,W}
	MGH	1.19 ± 0.03^{a,Y,X}	0.149 ± 0.01^{b,X}	1.914 ± 0.07^{b,V}	1.32 ± 0.03^{a,W}	1.81 ± 0.20^{a,b,X}
	MGGP	1.20 ± 0.04^{a,Y}	0.144 ± 0.01^{b,Z}	1.880 ± 0.07^{b,W}	1.29 ± 0.04^{a,W,V}	1.67 ± 0.18^{b,X,W}
49 D	FG	1.33 ± 0.02^{a,V}	0.183 ± 0.01^{a,V}	2.881 ± 0.29^{a,U}	1.31 ± 0.08^{a,W}	1.85 ± 0.33^{a,X}
	MGH	1.33 ± 0.05^{a,W}	0.164 ± 0.00^{b,X}	2.552 ± 0.14^{b,U}	1.37 ± 0.04^{a,U}	2.08 ± 0.16^{a,V}
	MGGP	1.29 ± 0.03^{a,X}	0.156 ± 0.01^{c,Z}	2.249 ± 0.11^{c,V}	1.32 ± 0.04^{a,V}	1.92 ± 0.16^{a,V}
56 D	FG	1.26 ± 0.03^{a,b,W,V}	0.203 ± 0.01^{b,U}	3.825 ± 0.33^{a,T}	1.34 ± 0.03^{a,W}	2.07 ± 0.12^{a,W}
	MGH	1.22 ± 0.05^{b,X}	0.175 ± 0.01^{b,X}	3.277 ± 0.20^{b,T}	1.32 ± 0.03^{a,b,W}	1.99 ± 0.07^{a,W,V}
	MGGP	1.28 ± 0.03^{a,X}	0.288 ± 0.42^{a,Y}	3.215 ± 0.51^{b,U}	1.30 ± 0.02^{b,V}	1.76 ± 0.16^{b,W,V}
Effect of age		<0.001	<0.001	<0.001	<0.001	<0.001
Effect of genotype		0.557	0.607	<0.001	<0.001	<0.001
Effect of age × genotype		0.016	0.233	<0.001	0.156	0.016

Means within a column, for a specific birds age, without a common small letter ($^{a, b, c}$) in superscript differ significantly between groups and without a common capital letter ($^{Z, Y, X,U, V,W, S, T}$) in superscript differ significantly between ages ($P < 0.05$).

[1]FG—fast-growing Cobb500; MGH—medium-growing HubbardJA957; MGGP—medium-growing experimental line.

[2]Td = volume mineral density of the trabecular bone; BMD = bone mineral density; BMC = bone mineral content; MvBMD = mean volumetric bone mineral density; CD50% = cortical bone mineral density.

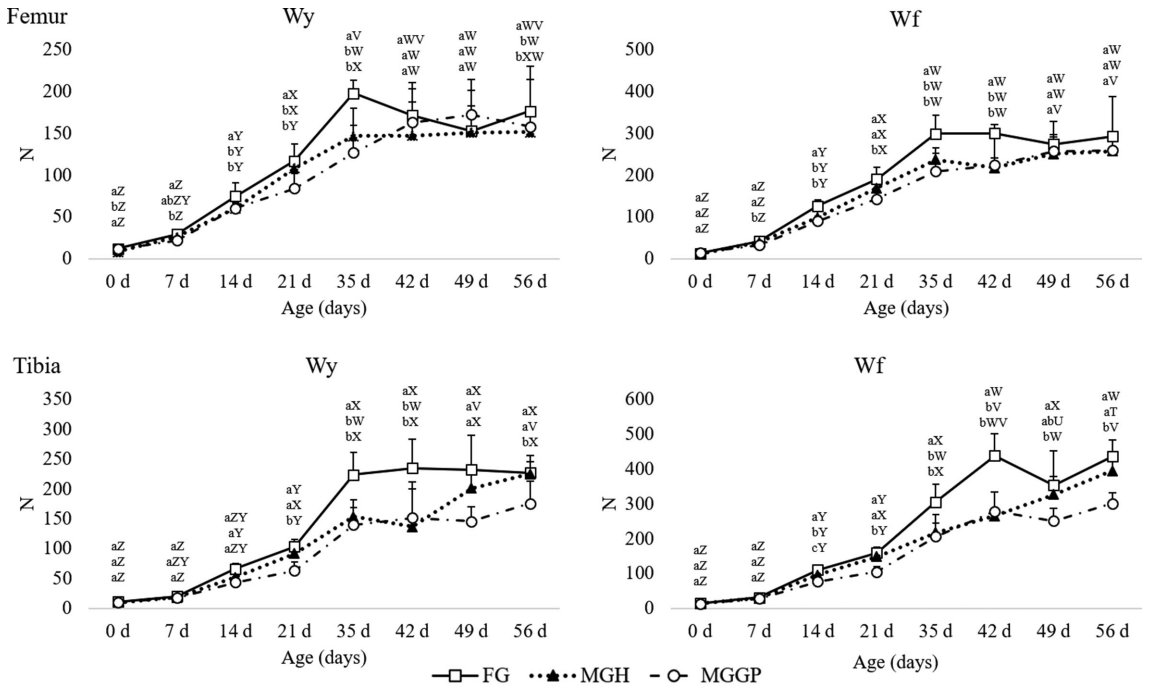
[3]MvBMD in 0 and 7-day-old groups was measured using VOI limitation between –300 and 3,071 HU, while in the older groups the VOI limitation was between 0 and 3,071 HU.

chickens from 14 to 42 D and significantly lower than those in FG chickens on 14, 21, and 35 D. On 0, 49, and 56 D, MvBMD values of the femur across all chicken groups were similar (Table 6). The MvBMD values of the tibia in MGGP chickens were lower than those in MGH chickens from 0 to 35 D, and no differences between these groups of chickens were observed from 42 to 56 D. The MvBMD of the tibia in MGGP chickens was significantly lower than that in FG chickens on 0, 7, 21, and 56 D.

A significant increase in Cd of the femur across all groups of chickens was observed on 14 and 21 D. At the later time points, Cd of femur in FG chickens remained unchanged, while on 35 D, an increase in this parameter in MGH chickens ($P = 0.035$) and on 49 D in MGGP chickens ($P = 0.040$) was observed. For the tibia, Cd in FG chickens increased on 14, 21, and 56 D and in MGGP chickens on 14, 35, and 49 D (Table 6). An increase in Cd of the tibia in MGH chickens was observed from 14 to 35 D, followed by a significant decrease on 42 D and a further increase on 49 D. The value of femoral Cd in all groups of chickens on 7 D showed similar values ($P > 0.05$). On 14 D, higher Cd values of the femur were found in MGH chickens than in MGGP chickens ($P = 0.032$). From 21 to 42 D, Cd of the femur in MGGP chickens was lower than that in FG and MGH chickens ($P < 0.05$). On 49 D, Cd of the femur in MGH chickens was higher than that in MGGP chickens ($P = 0.028$) and comparable to that in the FG group ($P = 0.069$). On 56 D, no significant differences were found for Cd of the femur in all the investigated groups of chickens ($P > 0.05$). From 7 to 35 D and on 56 D, Cd of the tibia in MGGP chickens was lower than that in FG and MGH groups of chickens ($P < 0.05$). On 42 and 56 D, Cd of the tibia in MGGP chickens was lower than that in FG chickens ($P = 0.012$ and $P = 0.028$, respectively).

Effect of Chicken Age and Genotype on Bone Mechanical Properties

The results of mechanical evaluation of bones are presented in Figure 1. Both Wy and Wf of the femur increased gradually between 14 and 35 D for FG and MGH chickens and between 14 and 42 D for MGGP



Femur Wy
Effect of age <0.001
Effect of genotype = 0.006
Effect of age × genotype = 0.119

Wf
Effect of age <0.001
Effect of genotype <0.001
Effect of age × genotype = 0.078

Tibia Wy
Effect of age <0.001
Effect of genotype <0.001
Effect of age × genotype = 0.009

Wf
Effect of age <0.001
Effect of genotype <0.001
Effect of age × genotype <0.001

Figure 1. Means and ±SD for femur and tibia bone mechanical properties in 3 chicken genotypes. [a, b, c] superscripts differ significantly between groups and without a common capital letter and [Z, Y, X, ..] superscripts differ significantly between ages ($P < 0.05$), where the upper means a group FG, in the middle means a group MGH and at the bottom means a group MGGP. The first alphabetical letter in the series indicates the largest mean. FG–fast growing Cobb500; MGH–medium growing HubbardJA957; MGGP-medium growing experimental line; Wy = maximum elastic strength; Wf = ultimate strength.

chickens. At the later growth stages, the values of Wy and Wf did not change. The only exception was a significant increase in Wf of the femur in MGGP chickens on 49 D. For the tibia, an increase in Wy was noted between 14 and 35 D, which was the only significant change for FG and MGGP chickens. In MGH chickens, an increase in this parameter was observed on 21 and 49 D ($P = 0.030$ and $P = 0.035$, respectively). Changes in Wf values of the tibia during chicken growth were strongly affected by interactions with the genotype. For MGH chickens, a significant increase in Wf of the tibia was observed from 14 D at all time points, except on 42 D. For MGGP chickens, an increase in Wf of the tibia also started between 7 and 14 D; however, no changes were noted on 21 and 42 D. In contrast, Wf of the tibia in FG chickens initially increased on 14, 35, and 42 D and then decreased from 438.50 N on 42 D to 353.67 N on 49 D. A further increase to 436.00 N was then observed on 56 D, reaching the value comparable to that on 42 D. A comparison of the genetic groups of chickens showed that Wy of the femur in MGH chickens on 0 D was lower than that in FG ($P = 0.022$) and MGGP chickens ($P = 0.018$). The maximum elastic strength of the femur in FG chickens was higher than that in MGH and MGGP chickens on 14, 35, and 56 D ($P < 0.05$). Similar results were obtained for Wy of the tibia but only on 14 and 42 D. On 7 and 21 D for the femur and on 7, 21, 35, and 42 D for the tibia, FG chickens also showed higher Wy values than only MGGP chickens ($P < 0.05$). MGH chickens had higher Femur Wy values than MGGP chickens only on 21 D and higher tibia Wy values on 21 and 35 D. On 0 D, the Wf values of both the bones did not differ between all the genetic groups of chickens ($P > 0.05$). The ultimate strength values of the femur and tibia in FG chickens were higher than those in MGGP chickens at all time points, except for 7 D for the tibia and 49 and 56 D for the femur (Figure 1). Compared to Wf in MGH chickens, higher values of this parameter in FG chickens were confirmed on 14 and 42 D for femur and on 14, 35, 42, and

49 D for the tibia ($P < 0.05$). Bones in MGGP chickens compared to those in MGH chickens were characterized by lower Wf values on 7, 21, and 35 D for the femur and on 14, 21, and 56 D for the tibia ($P < 0.05$).

DISCUSSION

The aim of this study was to compare the development of the femur and tibia in chickens with different growth potential. The first important result noted was the differences in chicken bone parameters on 0 D. FG chickens were characterized by the highest Bvol values and MGH chickens by very low maximum elastic strength values for the femur. There is not enough information in the literature on the detailed parameters of broiler bones on 0 D, but based on the obtained results and comparison of the groups, it can be concluded that the bones from FG chickens were the most developed at that time, particularly in comparison with MGGP chicken bones. Above all, femur of FG chickens on 0 D was heaviest and longest, whereas tibia was characterized by highest PCT in comparison with bones of the remaining chicken groups. Moreover, FG chicken bones on 0 D were characterized by relatively high values of mechanical parameters. Although BMD and BMC of bones were not determined on 0 D, the values of MvBMD in this period indicated that physiological bone mineralization process in FG chickens was correct, and MvBMD values of the tibia in these chickens were even higher than that in MGGP chickens. High BMD and BMC values in FG chickens were also observed on 7 D. First, the bones in FG chickens had significantly higher BMC values, which is a measure of the mineral part of bone structure, than those of the bones of the other chicken groups. These results indicate that intensive selection aimed at accelerating the growth rate and increasing meat yield did not result in negative changes in bone development and mineralization during embryogenesis.

It has been confirmed many times before that the cause of bone developmental disorders in broilers is due to the unnatural fast growth rate, especially of the breast and leg muscles. Because of high BW in relation to age, the skeletal load increases disproportionately to the skeletal growth rate and bone mineralization process (Patterson et al., 1986; Rath, et al., 2000; Erdal et al., 2012; de Verdal et al., 2013). Reddish and Lilburn (2004) analyzed the dimensions of chicken leg bones and showed that males of lines A and B, which are used to produce heavy broilers, had shorter bones than pure-bred Barred Rock chickens. In the present study, all dimensions of bones in FG chickens were usually higher than those in the MGH and MGGP groups of chickens. The observation on 35 and 42 D, when broilers are intended for slaughter in commercial breeding, seems to be particularly important. It can be assumed that differences in the results obtained in this study and in the study of Reddish and Lilburn (2004) are a consequence of breeding selection of different genetic chicken material, as some skeletal traits may be breed-specific. We compared the bone dimensions of birds selected and nonselected for rapid growth rate and muscle development, while MGH and MGGP chickens in this experiment were the birds intended for improved meat production. In particular, they originated from the crossbreeding of slow-and fast-growing birds (MGGP) or were subjected to selection for moderate growth potential (Hubbard, 2018). The result of this study confirms the observations of González-Cerón et al. (2015), who showed that genetic factors determining faster growth also lead to heavier, longer, and wider leg bones in broilers. A recent study by Stover et al. (2017) showed that an increase in the size of the bones of the hind legs of heavy turkeys selected for fast growth rates is similar to or only slightly faster than the growth rate of the same bones in completely wild birds; this finding also seems to be interesting. In addition, Stover et al. (2017) observed that because of the need to adjust the longitudinal bone growth rate to the increasing BW, the bone diameter is increased by more radial deposition of the bone tissue-building material. This arrangement of bone tissue contributes to a higher stiffness of the bone, and consequently to a higher strength that can maintain a high BW (Lieberman et al., 2004). Furthermore, Zhong et al. (2012) demonstrated that while modulation of bone geometry is the key factor correlated with BW changes, sufficient mineralization time and matrix maturation is significant for the mechanical competence of bones. This is probably the reason for the highest CBA values for both the femur and tibia as well as A value, especially for femur obtained from FG chicken in this study. However, assistance in supporting relatively large BW through bone diameter expansion and bone system reorganization can be limited due to decreasing MRWT value. In this study, we observed that for FG chickens, despite longer and thicker bones their MRWT was comparable to or thinner than the MRWT in MGH and MGGP chickens.

However, bone quality problems in broilers are not exclusively related to their morphological or geometric features. A majority of researchers attribute locomotory difficulties and high frequency of fractures primarily to the deterioration of densitometric properties (Williams et al., 2000; Talaty et al., 2010). The reduced mechanical strength, i.e., the force needed to crack or to fracture bone, can be attributed to inadequate bone mineralization process in fast-growing birds. In general, an inversely proportional relationship between the growth rate of birds and mineralization process, bone mineral density, and mechanical endurance of bones was confirmed (Corr et al., 2003; Brickett et al., 2007). The main argument was the fact that osteoblasts can only optimally secrete bone tissue components in slow-growing birds (Williams et al., 2004; Brickett et al., 2007). One of the simplest ways to determine bone quality is to use BMC as an indicator as it measures bone minerals in bone tissue structure, and BMD as it is a

mathematical derivative of BMC adjusted to a particular bone area (Licata and Williams, 2014). The current study demonstrated that BMC of both the femur and tibia systematically increased with age of all genetic groups of chickens until 56 D of life, and it was always significantly higher for bones from FG chickens than for bones from the other groups. However, this is not the case with BMD values, which were found to be elevated between 42 and 49 D in FG chicken bones. On 56 D, BMD in bones of FG chickens increased compared to that in the earlier developmental stages; however, it was only comparable to BMD in MGH chickens and significantly lower than that in the MGGP group. An increase in mechanical parameters (Wy and Wf) in FG chicken bones also showed the lack of improvement in BMD during the same time. Consequently, the femur strength in all groups of chickens was equal in this period. For the tibia, identical outcomes occur for FG and MGH chickens, while MGGP chickens show inferior mechanical endurance indices in comparison with the other groups. On the basis of the analyses conducted, it is difficult to explain the reason for the observed changes. However, both the densitometric and mechanical evaluations of bones prove the existence of the critical developmental period for the long pelvic limb bones between 42 and 49 D or even later. The available literature usually lacks information on bone mineral density in broilers over 42 D of age. By comparing the results of this study with the results of other authors who analyzed bone mineral density of the same genetic group of fast-growing chickens (Cobb 500), it can be concluded that densitometric indices of the femur and tibia in FG chickens on 42 D were normal or even improved (Shim et al., 2012; González-Cerón et al., 2015). Presumably, the deterioration of BMD after 42 D was related to nutrition. The use of an appropriate nutrition regimen in chicken flock has been proven to have a major impact on bone mineralization in broilers (McDevitt et al., 2006; Fleming, 2008; Simsek et al., 2011; Świątkiewicz et al., 2011; Favero et al., 2013). As a standard breeding procedure, broilers are maintained up to 35 to 42 D, by changing feed mixtures according to the metabolic needs of birds. The obtained results suggest that the demand in FG chickens for minerals required for bone mineralization may grow consequently with age and should be changed after 42 D; however, this approach cannot be applied to birds with slower growth rate, such as MGGP chickens.

Summarizing the results of this study, it can be concluded that bones in FG chickens are longer, wider, and thicker than those in MGH and MGGP chickens. This clearly indicates that the genetic factors determining an increase in BW may also affect the growth and development of bones in the skeleton. However, the densitometric and mechanical quality of the bones in FG chickens significantly deteriorates between 42 and 49 D of life. The observation of MRWT is also disquieting, indicating that the MRWT of longer and thicker bones in FG chickens is comparable to and often lower than that in MGH and MGGP chickens. This finding is of great importance, as alternatives to intensive poultry production are currently being sought, in which longer than 42 D rearing cycle is a necessity. Analysis of the bone quality indices in MGH and MGGP chickens indicates that both genetic groups can be reared up to 56 D of life without any negative effect on their growth due to problems associated with deterioration of leg bone characteristics. In FG chickens, this can be possible if the factors leading to reduced mineralization and elevated bone strength after 35 D of age are eliminated. Considering the results obtained in the current experiment, further studies on bone mineralization in FG chickens after 42 D are recommended, taking into account nutrition changes after this period as well as factors affecting the developmental expansion of bone wall thickness in pelvic limb bones.

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