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Tibia properties in broilers raised on their own commercial diets with different growth rates and sex during a 10-week rearing period

Hilal Çapar Akyüz[1,2] | Esin Ebru Onbaşılar[1] | Sakine Yalçın[3]

[1]Department of Animal Husbandry, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey

[2]The Graduate School of Health Sciences, Ankara University, Ankara, Turkey

[3]Department of Animal Nutrition & Nutritional Diseases, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey

Correspondence
Esin Ebru Onbaşılar, Department of Animal Husbandry, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey.
Email: onbasilar@ankara.edu.tr

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Abstract

Background: Genetic selection applied to broilers results in fast growth and an increase in meat yield. However, this situation causes welfare problems in broilers.

Objectives: The aim of this study is to determine the weekly changes in the tibia characteristics in broilers raised on their own commercial diets.

Methods: In the study, 168 (84 female and 84 male) slow-growing (Hubbard-Isa Red JA) and 168 (84 female and 84 male) fast-growing (Ross-308) day-old broiler chicks were used. Six broilers from each genotype and sex group were weighed weekly and slaughtered to determine the tibia properties during the 10-week fattening period.

Results: Fast-growing broilers had higher tibia weight and longer length, diaphysis diameter and medullary canal diameter than those of slow-growing broilers at the same age. In fast-growing genotypes, the cortical index was low only in the 2nd week, and Robusticity and Seedor indices were observed to be better throughout the whole fattening. The ash content of the total tibia in the fast-growing broilers was higher in all of the examined weeks except the 4th week and the 9th week of fattening than that in the slow-growing broilers. Although the amount of ash per unit body weight in the 1st week of fattening was higher in fast-growing broilers, this situation reversed after the 4th week. The level of all minerals examined in total tibia weight is high in fast-growing broilers, and they differed according to the fattening period.

Conclusions: When comparing tibia characteristics of two different genotypes fed their own commercial diets, the tibia structure was found to be stronger in fast-growing broilers compared to other genotypes at the same age, but slow-growing broilers were more prominent in terms of body weight. It was observed that the mineral density was higher in male broilers, except in the 1st week.

KEYWORDS
broiler, genotype, mechanic properties, mineral content, morphology, sex, tibia

1 | INTRODUCTION

Genetic selection applied to broilers resulted in fast growth, an increase in meat yield, and an earlier slaughter age. Fast-growing broilers are commonly reared nowadays. However, genetic selection applied in the direction of fast growth caused unbalanced body structure in broilers (Caplen et al., 2012). One of the important problems, which is directly linked to their fast growth, is the skeletal problem. Broiler welfare deteriorates due to musculoskeletal disorders, including decreased ability to walk, associated pain, increased risk of injury, reduced access to food and water and an inability to perform natural behaviours (Hartcher & Lum, 2020).

Tibia, the main long bones in broiler legs, carry the bird's body weight (Evaris et al., 2019). The tibia belongs to the most mineralized group of bones, and therefore it is often used as an indicator of overall skeletal mineralization, so when the bone quality is measured, the tibia is typically used (Kraus et al., 2022). Bone strength is determined not only by the volume of bone tissue and the bone measurements but also by the mineralization of the bone. Bone breaking strength and various morphometric measurements, including Robusticity, Seedor and tibiotarsal indexes, density, ash and mineral content, have been used as indicators of bone health (Onbaşılar et al., 2016; Shim et al., 2012). Mineralization affects bone strength, which allows the skeleton to withstand gravity and increased body weight. Although factors such as nutrition, management and diseases play a role in the development of leg problems, growth rate seems to be the main factor (Knowles et al., 2008).

Consumer concern for animal welfare has resulted in the development of a market segment using broilers with slower growth rates. Slow-growing genotypes take longer to reach slaughter weight. When the studies were examined, it was observed that the differences in limited characteristics between the ages of 42 and 70 days of fast- and slow-growing genotypes were examined, whereas there was no information about weekly changes in bone characteristics in broilers reared in intensive conditions. However, determining the weekly changes in bone according to sex will help to understand at what stage the genotype makes a difference and in which period the possible negative effects begin. The weekly differences that will occur in the bone during rearing will help to take the necessary measurements to prevent bone disorders. Therefore, this study was conducted to determine the weekly differences in tibial properties of fast- and slow-growing broilers fed according to their own commercial diets during 10 weeks of rearing.

2 | MATERIALS AND METHODS

2.1 | Experimental design and animals

In the study, 168 (84 female and 84 male) slow-growing (Hubbard-Isa Red JA) and 168 (84 female and 84 male) fast-growing (Ross-308) day-old broiler chicks were used. In the selection of slow-growing and fast-growing genotypes, one of the hybrids commonly used in fattening was chosen. Slow- and fast-growing female and male chicks were put in the litter pens (90 × 80 cm) with 14 chicks each. Each genotype and sex group consisted of six replicates. Each floor pen had wood shavings as litter material. The average temperature was 33°C at chick placement in the first week, and it was reduced by 3°C/week until 24°C was achieved. After the 23L:1D cycle was applied on the first day, the 16L:8D cycle was implemented throughout the experiment. Ad libitum feed and water were provided. In the present study, the diets were formulated to meet or exceed the nutrient requirements for the fast-growing genotype for 6 weeks. The nutrient and energy levels of the diets of Hubbard Red AJ were formulated to be among the experiments (Akyar & Yeter, 2021; Balevi et al., 2019; Cömert et al., 2016; İpek et al., 2009) for 10 weeks. After 6 weeks, Ross 308 was fed the same diet as that of the Hubbard Red AJ for 4 weeks in order to avoid high energy. The ingredients and chemical composition of the diets are shown in Table 1. The nutritional analysis of the diets was made according to Association of Official Analytical Chemists (AOAC) (2000) and the metabolizable energy values of the diets were calculated as described by Carpenter and Clegg (1956). Calcium and phosphorus levels were determined using ICP-OES (Varian Vista MPX CCD Simultaneous ICP-OES). The study lasted 10 weeks (Akyüz et al., 2022).

2.2 | Evaluation of tibia properties

Every week until the end of the experiment, one broiler from a pen (six broilers for each genotype and sex group) was weighed and slaughtered to determine the tibia properties. The surrounding soft tissue of the tibia was removed. The weight and length of the tibia were measured. The relative tibia weight was calculated by proportioning the tibia weight to the slaughter weight. For cortical index calculation, the tibiae were cut at their midpoints. The tibia diaphysis diameter, the thickness of the medial wall, and the lateral wall were measured using a digital caliper. Medullar canal diameter, cortical index, Robusticity index and Seedor index were calculated by the following equations (Mohammed et al., 2021).

Medullary canal diameter (mm) = diameter at the diaphysis − thicknesses of the tibia (the distance from the medial and lateral walls).

Cortical index (%) = {(diaphysis diameter − medullary canal diameter)/diaphysis diameter} × 100.

Robusticity index (mm/g) = bone length/cube root of bone weight.

Seedor index (g/mm) = tibia weight/tibial length.

After bone measurements, tibia samples were defatted in chloroform and methanol (2:1) for 72 h (Çalık et al., 2019). Subsequently, tibia samples were dried for 12 h at 105°C and ashed overnight at 600°C to determine dry matter and ash percentage, respectively. Tibia ash

TABLE 1 Ingredients and chemical composition of the diets of fast and slow growing broilers according to the periods.

	FGB		SGB		FGB and SGB
Ingredients (kg/t)	0–21 days	22–42 days	0–21 days	22–42 days	43–70 days
Corn	539.00	544.00	546.74	569.71	548.74
Corn gluten	22.00	–	28.00	–	–
DDGS	–	–	40.00	40.00	50.00
Rice bran	–	–	30.00	40.00	50.00
Wheat feed flour	–	50.00	–	–	–
Chickpea	–	20.00	25.00	30.00	50.00
Fullfat soya	107.00	83.50	–	34.00	97.00
Soyabean meal	293.00	228.00	227.00	196.00	91.00
Sunflower seed meal	–	40.00	40.00	50.00	75.00
Canola seed meal	–	–	20.00	–	–
Monocalcium phosphate	8.75	6.83	7.10	6.40	4.50
Limestone	15.80	13.83	16.70	15.80	12.60
Sodium sulphate	1.47	1.47	0.58	0.57	0.88
Salt	2.66	2.60	2.79	2.82	2.18
Soya oil	–	–	5.00	5.00	7.50
Methionine	3.17	2.73	2.47	2.54	2.72
Lysine	3.72	4.02	5.59	4.22	5.20
Threonine	1.23	0.92	0.83	0.84	1.18
Choline	0.50	0.50	0.60	0.50	0.40
Vitamin mineral premix[a]	1.00	1.00	1.00	1.00	1.00
Xylanase complex enzyme[b]	0.05	0.05	0.05	0.05	0.05
Phytase enzyme[c]	0.05	0.05	0.05	0.05	0.05
Chemical composition					
Dry matter, %	87.8	89.7	88.1	88.2	88.2
Crude protein, %	23.9	21.6	21.0	19.2	18.1
Ether extract, %	5.2	4.3	4.1	4.2	6.2
Crude fibre, %	2.7	3.2	3.1	3.1	3.4
Crude ash, %	5.5	4.9	5.5	5.1	5.0
Calcium, %	1.08	1.00	1.02	0.97	0.82
Total phosphorus, %	0.78	0.73	0.74	0.70	0.68
Metabolisable energy[cd], kcal/kg	3056	3183	2940	2990	3080

Abbreviations: DDGS, distillers dried grains with solubles; FGB, fast growing broiler; SGB, slow growing broiler.
[a]Vitamin mineral premix (1 kg): 12,000,000 IU vitamin A, 5,000,000 IU vitamin D3, 65 g vitamin E, 3 g vitamin K3, 3 g vitamin B1, 7 g vitamin B2, 15 g kalsiyum D pantothenate, 4 g Vitamin B6, 20 g vitamin B12, 60 g niacin, 2 g folic acid, 250 mg biotin, 25 g Fe, 16 g Cu, 120 g Mn, 110 g Zn, 1.25 g I, 300 mg Se.
[b]Hostazym X: endo-1,4-β xylanase (min 30,000 EPU/g), cellulase, hemicellulase, α-amylase, protease.
[c]OptiPhos 250 OTU: 6-phytase.
[c]Calculated as described by Carpenter and Clegg (1956).

samples were crushed manually, weighed (approximately 300 mg), and mixed with 8 mL HCl and 2 mL nitric acid. Afterwards, digested tibia ash samples were diluted, and concentrations of calcium, phosphorus, magnesium, potassium, copper, iron, manganese and zinc were determined by inductively coupled plasma mass spectrometry (Agilent Technologies 7700X Series). The ash and mineral levels in the tibia for each unit of body weight were also determined.

2.3 | Statistical analysis

Statistical analyses were performed using the IBM SPSS software version 22 (SPSS Inc.). Two-way analysis of variance was employed separately for each time period to assess the effects of genotype and sex on all examined tibia traits (Verma, 2013). Differences in examined tibia traits were tested for age, genotype and sex, as well as the sex × genotype interaction using the Generalized Estimating Equations with an identity model. Wald chi-square and *p*-values were calculated.

3 | RESULTS AND DISCUSSION

Fast-growing broilers had tibias having a higher weight and longer length, diaphysis diameter and medullary canal diameter than slow-growing broilers at the same age ($p < 0.05$, Tables 2 and 3). The rapid increase in tibia weight in fast-growing broilers decreased after the 8th week of fattening. The bone may be more porous after this week, and therefore no significant reduction in medullary canal diameter was seen in this study. For this reason, fractures can occur very easily and can cause chronic pain and lameness. This situation was also observed in slow-growing female broilers from the 9th week of fattening. Although the ratio of tibia weight to slaughter weight was higher in fast-growing broilers in the first 2 weeks ($p < 0.05$) and it started to decrease rapidly after the 2nd week in fast-growing female broilers and in the 3rd week in the fast-growing male broilers. This difference between genotypes was reversed until the end of the fattening ($p < 0.05$). This shows that the increase in tibia weight of fast-growing broilers could not keep up with the increase in body weight from the 2nd week. In fast-growing broilers, superiority in terms of tibia length continued until the 9th week of fattening ($p < 0.05$). It was observed that diaphysis diameter and medullary canal diameter in fast-growing broilers from the 2nd week of the fattening were larger than those of slow-growing broilers, and this situation continued throughout the 10 weeks of the fattening period ($p < 0.05$). Similarly, Shim et al. (2012) reported that broilers with slow growth rates had lower tibia weight and shorter length compared to broilers with fast growth rates. González-Cerón et al. (2015) showed that genetic factors determining faster growth also led to heavier, longer and wider leg bones in broilers. However, some researchers (Güz et al., 2022; van der Eijk et al., 2022) showed that slow-growing broilers have better tibia morphological characteristics compared to fast-growing broilers at the same body weight. This discrepancy among studies is likely explained by sampling at different ages and body weights.

When the effect of sex on tibia weight was examined, it was determined that the tibia weight in males was higher than that of females at the 3rd week and after the 5th week of the fattening ($p < 0.05$). Although tibia length was longer in females at the 1st week of fattening ($p < 0.01$), the situation reversed from the 6th week of fattening and became longer in males ($p < 0.05$). Similarly, Faveri et al. (2019) reported a significant sex effect ($p < 0.001$) for tibia weight, length and diameter with mean values of 7.16 g, 92.78 mm and 6.88 mm (for males), respectively, and 5.36 g, 88.06 mm and 6.07 mm (for females) in broilers slaughtered at 42 days of age, respectively. The fact that heavier bone weights in male broilers than females may be due to high feed consumption of males.

The cortical index indicates mineralization of bone; therefore, a higher cortical index showed a higher tibia mineralization level and good bone quality (Ziaie et al., 2011). According to the results, the significant difference between the two genotypes was detected only in the 2nd week of the rearing period ($p < 0.05$, Table 3), and it shows that there is higher mineralization in slow-growing broilers during this week. As the diaphyseal diameter and medullary canal are larger in fast-growing broilers, the bone quality of slow-growing broilers is higher than that of fast-growing broilers at this age. The cortical index decreases with age. This decrease was very rapid between the 3rd and 5th weeks of fattening in both genotypes. Similarly, Mabelebele et al. (2017) found that the cortical index values of the slow-growing pure breed were higher than those of the fast-growing commercial hybrids. The sex effect was not significant for the cortical index of the tibia during the 10 weeks of fattening.

The Robusticity index shows the bone fracture strength. If the Robusticity index is high, bone density is low, and therefore the bone becomes fragile (Javid et al., 2022). In our study, the tibia Robusticity index was higher in the slow-growing broilers during the completely fattening period ($p < 0.001$, Table 4). Tibia length and weight are taken into account when calculating the Robusticity index. This indicates that the increase in tibia length is greater than the increase in tibia weight in slow-growing broilers. In addition, in fast-growing broilers, tibia weight and length are more proportional, indicating that the bone is stronger. The bones of fast-growing broilers are stronger than those of slow-growing broilers; however, they are not strong enough to carry the increased body weight. The Robusticity index increases with age, and this will cause the long tibia to have difficulty carrying the weight due to the increase in body weight. According to the Robusticity index results, it is seen that the tibia is stronger in males in the 5th week and after the 7th week of fattening ($p < 0.05$). Mabelebele et al. (2017) reported that there was no significant difference in the Robusticity index between male and female broilers. The difference between the studies may be due to the fact that the studies were conducted with a single and different age group.

The Seedor index, known as the ratio of bone weight to bone length, is an indicator of bone density (Javid et al., 2022). The Seedor index of the tibia was high in fast-growing broilers during all weeks of fattening ($p < 0.001$). The Seedor index was found to be higher in male broilers than that of female broilers at the 3rd week and after the 5th week of fattening ($p < 0.01$). The Seedor index started to decrease after the 8th week in the fast-growing broilers and after the 9th week in the slow-growing broilers. If bone length grows faster than its weight, the Seedor index decreases.

In our study, ash content in total tibia weight of fast-growing broilers was higher for all of the examined weeks except the 4th and 9th weeks of fattening than that in the slow-growing broilers ($p < 0.01$, Table 5). It was seen that this finding was compatible with the Robusticity index value obtained in our study results. The results show that ash contents are optimal. Excessive mineralization could potentially create its own welfare issues by producing an unnecessarily rigid bone (Lewis et al., 2009). Tibia ash content increases with age in both genotypes up to the 9th week of age. This result indicates that the tibia grows up to this age. Han et al. (2015) showed that the ash content of the tibia quadratically increased with age, and the highest ash content was observed at 28 days of age. However, Barreiro et al. (2011) reported that the ash content of the tibia increased from 8 to 22 days of age and

TABLE 2 The effect of genotype and sex on weight and length of tibia.

		Age (week)									
Genotype	**Sex**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Tibia weight (g)											
FG	Female	2.07	4.45	7.00	11.78	15.45	19.84	24.12	28.04	25.58	23.52
	Male	1.86	4.99	9.31	10.97	19.04	23.67	30.35	36.34	36.95	37.83
SG	Female	1.21	2.53	4.36	7.24	10.76	13.91	15.24	15.50	20.66	16.62
	Male	1.11	2.81	5.12	7.08	12.03	16.53	19.70	24.28	27.57	28.64
FG		1.97	4.72	8.15	11.38	17.24	21.76	27.23	32.19	31.26	30.67
SG		1.16	2.67	4.74	7.16	11.39	15.22	17.47	19.89	24.11	22.63
	Female	1.64	3.49	5.68	9.51	13.11	16.88	19.68	21.77	23.12	20.07
	Male	1.49	3.90	7.21	9.03	15.53	20.10	25.02	30.31	32.26	33.24
Pool SEM		0.04	0.13	0.18	0.25	0.27	0.49	0.42	0.49	0.64	0.49
P											
Genotype		***	***	***	***	***	***	***	***	***	***
Sex		–	–	***	–	***	**	***	***	***	***
GenotypeXSex		–	–	*	–	*	–	–	–	–	–
Tibia weight (% slaughter weight)											
FG	Female	0.99	0.96	0.73	0.79	0.73	0.70	0.66	0.70	0.60	0.50
	Male	1.00	0.96	0.92	0.68	0.81	0.75	0.73	0.76	0.79	0.77
SG	Female	0.88	0.84	0.77	0.88	0.91	0.90	0.76	0.70	0.79	0.62
	Male	0.83	0.87	0.92	0.81	0.93	0.93	0.89	0.91	0.97	0.83
FG		1.00	0.96	0.83	0.74	0.77	0.73	0.69	0.73	0.69	0.63
SG		0.86	0.86	0.84	0.85	0.92	0.91	0.82	0.81	0.88	0.72
	Female	0.93	0.90	0.75	0.83	0.82	0.80	0.71	0.70	0.69	0.56
	Male	0.92	0.92	0.92	0.75	0.87	0.84	0.81	0.84	0.88	0.80
Pool SEM		0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02
P											
Genotype		**	*	–	*	***	***	***	*	***	*
Sex		–	–	***	–	–	–	**	***	***	***
GenotypeXSex		–	–	–	–	–	–	–	*	–	–
Tibia length (mm)											
FG	Female	45.70	58.81	70.41	87.50	98.61	108.22	116.97	124.50	124.84	126.86
	Male	43.90	59.93	74.84	84.51	101.48	112.22	122.27	131.78	137.20	137.92
SG	Female	43.24	55.35	68.73	81.32	93.65	104.02	113.75	118.18	128.71	122.70
	Male	41.08	55.59	68.74	80.37	94.94	108.01	115.79	129.88	137.07	140.56
FG		44.80	59.37	72.63	86.00	100.05	110.22	119.62	128.14	131.02	132.39
SG		42.16	55.47	68.73	80.84	94.30	106.01	114.77	124.03	132.89	131.63
	Female	44.47	57.08	69.57	84.41	96.13	106.12	115.36	121.34	126.77	124.78
	Male	42.49	57.76	71.79	82.44	98.21	110.12	119.03	130.83	137.13	139.24
Pool SEM	0.34	0.49	0.77	0.53	0.66	0.72	0.85	0.95	1.14	1.04	
P											
Genotype		**	**	*	***	***	**	*	*	–	–
Sex		**	–	–	–	–	*	*	***	***	***
GenotypeXSex		–	–	–	–	–	–	–	–	–	–

Note: –: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.
Abbreviations: FG, fast growing; SG, slow growing; SEM, standard error of mean.

TABLE 3 The effect of genotype and sex on diaphysis diameter, medullary canal diameter and cortical index of tibia.

Genotype	Sex	Age (week)									
		1	2	3	4	5	6	7	8	9	10
Tibia diaphysis diameter (mm)											
FG	Female	2.06	4.10	5.64	6.64	7.27	8.50	9.30	8.97	8.95	8.67
	Male	2.79	4.57	6.45	7.69	7.79	8.59	9.92	10.17	10.33	10.79
SG	Female	2.62	3.51	4.42	5.50	6.33	6.83	6.84	6.78	7.80	7.31
	Male	2.62	3.75	4.78	5.24	6.11	7.03	7.77	8.33	8.51	9.15
FG		2.42	4.34	6.04	7.16	7.53	8.55	9.61	9.57	9.64	9.73
SG		2.62	3.63	4.60	5.37	6.22	6.93	7.31	7.55	8.16	8.23
	Female	2.34	3.80	5.03	6.07	6.80	7.66	8.07	7.87	8.38	7.99
	Male	2.70	4.16	5.61	6.46	6.95	7.81	8.84	9.25	9.42	9.97
Pool SEM		0.09	0.07	0.10	0.09	0.13	0.14	0.13	0.20	0.09	0.15
P											
Genotype		–	***	***	***	***	***	***	***	***	***
Sex		*	*	**	–	–	–	**	**	***	***
GenotypeXSex		*	–	–	**	–	–	–	–	–	–
Tibia medullary canal diameter (mm)											
FG	Female	1.20	2.37	3.29	4.14	4.75	5.59	6.24	6.47	6.57	6.41
	Male	1.58	2.59	3.70	4.41	5.09	5.79	6.92	7.39	7.97	8.36
SG	Female	1.42	1.91	2.50	3.44	3.91	4.49	4.54	4.78	5.88	5.61
	Male	1.38	2.00	2.58	2.97	3.64	4.52	5.23	5.59	6.32	7.05
FG		1.39	2.48	3.49	4.27	4.92	5.69	6.58	6.93	7.27	7.39
SG		1.40	1.95	2.54	3.21	3.77	4.50	4.88	5.19	6.10	6.33
	Female	1.31	2.14	2.89	3.79	4.33	5.04	5.39	5.62	6.22	6.01
	Male	1.48	2.29	3.14	3.69	4.36	5.15	6.08	6.49	7.14	7.70
Pool SEM		0.06	0.04	0.10	0.14	0.11	0.14	0.13	0.19	0.11	0.16
P											
Genotype		–	***	***	**	***	***	***	***	***	**
Sex		–	–	–	–	–	–	*	*	***	***
GenotypeXSex		–	–	–	–	–	–	–	–	*	–
Tibia cortical index (%)											
FG	Female	41.72	41.77	41.69	38.40	34.62	34.34	32.52	28.14	26.64	25.94
	Male	43.28	43.45	42.82	42.79	34.98	32.73	30.36	27.39	22.91	22.59
SG	Female	45.85	45.57	43.92	37.59	37.99	34.01	33.61	29.68	24.68	23.08
	Male	47.35	46.74	46.21	43.27	40.25	35.45	32.53	32.57	25.76	23.18
FG		42.50	42.61	42.25	40.59	34.80	33.53	31.44	27.77	24.78	24.27
SG		46.60	46.15	45.06	40.43	39.12	34.73	33.07	31.12	25.22	23.13
	Female	43.78	43.67	42.80	38.00	36.31	34.17	33.06	28.91	25.66	24.51
	Male	45.32	45.09	44.51	43.03	37.62	34.09	31.44	29.98	24.34	22.89
Pool SEM		1.26	0.85	1.28	1.29	1.18	1.37	1.39	1.43	0.88	1.10
P											
Genotype		–	*	–	–	–	–	–	–	–	–
Sex		–	–	–	–	–	–	–	–	–	–
GenotypeXSex		–	–	–	–	–	–	–	–	–	–

Note: –: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.
Abbreviations: FG, fast growing; SG, slow growing; SEM, standard error of mean.

TABLE 4 The effect of genotype and sex on Robusticity index and Seedor index of tibia.

		Age (week)									
Genotype	**Sex**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Robusticity index (mm/g)											
FG	Female	3588	3587	3707	3854	3963	4003	4053	4111	4239	4430
	Male	3582	3519	3565	3808	3802	3914	3921	3981	4126	4109
SG	Female	4068	4064	4214	4209	4252	4346	4594	4747	4701	4819
	Male	3993	3964	3991	4214	4148	4253	4299	4491	4540	4602
FG		3585	3553	3636	3831	3883	3959	3987	4046	4183	4269
SG		4030	4014	4102	4211	4200	4299	4446	4619	4620	4710
	Female	3828	3825	3960	4031	4108	4175	4323	4429	4470	4624
	Male	3787	3741	3778	4011	3975	4083	4110	4236	4333	4355
Pool SEM	37.61	26.95	48.24	31.27	22.92	28.31	24.31	38.46	23.94	21.41	
P											
Genotype		***	***	***	***	***	***	***	***	***	***
Sex		–	–	–	–	**	–	***	*	*	***
GenotypeXSex		–	–	–	–	–	–	–	–	–	–
Seedor index (g/mm)											
FG	Female	0.05	0.08	0.10	0.13	0.16	0.18	0.21	0.23	0.21	0.19
	Male	0.04	0.08	0.12	0.13	0.19	0.21	0.25	0.28	0.27	0.27
SG	Female	0.03	0.05	0.06	0.09	0.12	0.13	0.13	0.13	0.16	0.14
	Male	0.03	0.05	0.07	0.09	0.13	0.15	0.17	0.19	0.20	0.20
FG		0.04	0.08	0.11	0.13	0.17	0.20	0.23	0.25	0.24	0.23
SG		0.03	0.05	0.07	0.09	0.12	0.14	0.15	0.16	0.18	0.17
	Female	0.04	0.06	0.08	0.11	0.14	0.16	0.17	0.18	0.18	0.16
	Male	0.04	0.07	0.10	0.11	0.16	0.18	0.21	0.23	0.24	0.24
Pool SEM		0.001	0.002	0.003	0.003	0.002	0.004	0.003	0.004	0.004	0.003
P											
Genotype		***	***	***	***	***	***	***	***	***	***
Sex		–	–	**	–	***	**	***	***	***	***
GenotypeXSex		–	–	–	–	*	–	–	–	–	–

Note: –: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.
Abbreviations: FG, fast growing; SG, slow growing; SEM, standard error of mean.

decreased at 42 days of age. Grupioni et al. (2015) showed that selecting for breaking strength and rigidity will result in high mineral content and, consequently, broilers will be more resistant to bone disorders.

In general, there is an inverse relationship between the growth rate of broilers and the mineralization process (Brickett et al., 2007). Although the amount of ash per unit body weight in the 1st week of the fattening was higher in fast-growing broilers ($p < 0.05$, Table 6), it was seen that the amount of ash in the tibia did not increase in parallel with the body weight gain after this week. In general, there is a decrease in ash content per unit body weight after the 9th week of fattening in all genotypes. Tablante et al. (2003) showed that males had a significantly lower percentage of bone ash (52.0% ± 0.22%) compared to females (52.9% ± 0.23%). However, in our study, the ash content in the total tibia weight was found to be high in the males from the 5th week due to the bone weight ($p < 0.01$). The amount of ash according to per unit body weight of females was superior in the 1st week ($p < 0.05$, Table 6) and this situation was reversed from the 7th week of fattening ($p < 0.01$).

Sanchez-Rodriguez et al. (2019) reported that fast-growing broilers always have lower mineralization, resulting in poorer bone quality. The levels of all minerals examined in total tibia weight were found to be higher in fast-growing broilers ($p < 0.001$, Tables 5, 7 and 8) but the amount of minerals in the tibia per unit body weight (Tables 6, 9 and 10) differed according to the fattening period in the present study. The amount of Ca in the tibia per unit body weight was found to be higher in those with rapid growth in the 1st week ($p < 0.05$). In slow growing, the amount of Ca started to increase after the 1st week, and the difference between the genotypes became insignificant in the 2nd week ($p > 0.05$). A rapid decrease was observed after the 3rd week in the fast-growing female and after the 4th week in the male. This decrease occurred in

TABLE 5 The effect of genotype and sex on ash, Ca and P levels in total tibia weight.

Genotype	Sex	Age (week) 1	2	3	4	5	6	7	8	9	10
Ash (in total tibia weight)											
FG	Female	0.25	0.88	1.87	1.89	2.51	3.68	3.61	4.66	4.90	3.85
	Male	0.18	1.14	2.05	1.86	3.08	4.24	5.31	5.10	6.10	6.06
SG	Female	0.13	0.62	0.94	1.60	1.84	2.66	2.82	2.91	3.62	2.87
	Male	0.11	0.72	1.43	1.47	2.10	2.94	3.43	4.43	5.78	4.82
FG		0.22	1.01	1.96	1.87	2.80	3.96	4.46	4.88	5.50	4.96
SG		0.12	0.67	1.19	1.54	1.97	2.80	3.13	3.67	4.70	3.84
	Female	0.19	0.75	1.41	1.75	2.17	3.17	3.22	3.79	4.26	3.36
	Male	0.15	0.93	1.74	1.66	2.59	3.59	4.37	4.76	5.94	5.44
Pool SEM		0.01	0.05	0.12	0.09	0.08	0.14	0.08	0.14	0.26	0.15
P											
Genotype		***	**	**	–	***	**	***	***	–	**
Sex		**	–	–	–	**	–	***	**	**	***
GenotypeXSex		–	–	–	–	–	–	**	–	–	–
Ca (g in total tibia weight)											
FG	Female	0.29	0.64	1.08	1.96	2.67	3.50	4.33	5.08	4.66	4.17
	Male	0.25	0.71	1.41	1.78	3.22	4.10	5.29	6.41	6.63	6.70
SG	Female	0.17	0.38	0.69	1.25	1.91	2.49	2.76	2.84	3.81	3.02
	Male	0.15	0.42	0.80	1.21	2.12	2.91	3.52	4.36	5.08	5.18
FG		0.27	0.68	1.25	1.87	2.95	3.80	4.81	5.75	5.65	5.43
SG		0.16	0.40	0.74	1.23	2.01	2.70	3.14	3.60	4.44	4.10
	Female	0.23	0.51	0.89	1.61	2.29	2.99	3.54	3.96	4.24	3.60
	Male	0.20	0.56	1.11	1.49	2.67	3.50	4.41	5.38	5.85	5.94
Pool SEM		0.01	0.02	0.03	0.04	0.05	0.08	0.09	0.10	0.11	0.10
P											
Genotype		***	***	***	***	***	***	***	***	***	***
Sex		–	–	**	–	**	**	***	***	***	***
GenotypeXSex		–	–	–	–	–	–	–	–	–	–
P (g in total tibia weight)											
FG	Female	0.13	0.28	0.47	0.87	1.17	1.55	1.97	2.21	2.00	1.72
	Male	0.11	0.31	0.61	0.78	1.36	1.79	2.31	2.79	2.82	2.87
SG	Female	0.07	0.17	0.31	0.57	0.87	1.16	1.26	1.26	1.65	1.26
	Male	0.07	0.18	0.36	0.53	0.95	1.30	1.58	1.94	2.18	2.23
FG		0.12	0.29	0.54	0.82	1.26	1.67	2.14	2.50	2.41	2.30
SG		0.07	0.17	0.34	0.55	0.91	1.23	1.42	1.60	1.91	1.74
	Female	0.10	0.22	0.39	0.72	1.02	1.36	1.62	1.74	1.82	1.49
	Male	0.09	0.24	0.49	0.65	1.15	1.54	1.95	2.36	2.50	2.55
Pool SEM		0.01	0.01	0.01	0.02	0.02	0.04	0.03	0.04	0.05	0.04
P											
Genotype		***	***	***	***	***	***	***	***	***	***
Sex		*	–	**	–	**	*	***	***	***	***
GenotypeXSex		–	–	–	–	–	–	–	–	–	–

Note: –: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.
Abbreviations: FG, fast growing; SG, slow growing; SEM, standard error of mean.

TABLE 6 The effect of genotype and sex on ash, Ca and P levels per unit body weight.

		Age (week)									
Genotype	Sex	1	2	3	4	5	6	7	8	9	10
Ash × 10^{-6} (per unit body weight)											
FG	Female	1195	1905	1938	1263	1188	1296	985	1164	1145	811
	Male	977	2176	2005	1162	1313	1344	1273	1063	1299	1235
SG	Female	966	2030	1674	1943	1553	1724	1412	1319	1381	1072
	Male	817	2218	2550	1693	1620	1639	1546	1660	2017	1389
FG		1086	2041	1972	1212	1250	1320	1129	1114	1222	1023
SG		892	2124	2112	1818	1587	1681	1479	1489	1699	1231
	Female	1081	1967	1806	1603	1370	1510	1199	1242	1263	942
	Male	897	2197	2278	1427	1467	1491	1410	1361	1658	1312
Pool SEM		35.03	95.62	123.18	80.48	38.36	52.83	35.82	40.98	74.02	42.83
P											
Genotype		*	–	–	**	***	**	***	***	**	*
Sex		*	–	–	–	–	–	**	–	*	***
GenotypeXSex		–	–	–	–	–	–	–	*	–	–
Ca (µg per unit body weight)											
FG	Female	1362	1390	1131	1309	1264	1238	1180	1269	1095	884
	Male	1350	1357	1401	1110	1380	1302	1271	1344	1411	1362
SG	Female	1232	1277	1214	1526	1609	1613	1374	1289	1455	1126
	Male	1139	1296	1435	1390	1640	1629	1581	1634	1777	1496
FG		1356	1373	1266	1209	1322	1270	1225	1306	1253	1123
SG		1186	1287	1324	1458	1624	1621	1477	1461	1616	1311
	Female	1297	1334	1172	1417	1436	1426	1277	1279	1275	1005
	Male	1244	1326	1418	1250	1510	1466	1426	1489	1594	1429
Pool SEM		34.57	31.51	26.75	36.21	27.06	28.43	30.04	30.68	35.01	31.69
P											
Genotype		*	–	–	**	***	***	***	*	***	**
Sex		–	–	***	*	–	–	*	**	***	***
GenotypeXSex		–	–	–	–	–	–	–	*	–	–
P (µg per unit body weight)											
FG	Female	605	607	495	578	554	549	538	553	470	365
	Male	577	590	607	483	581	567	554	585	600	584
SG	Female	539	548	554	689	736	752	626	570	630	469
	Male	496	560	643	606	735	728	712	728	761	643
FG		591	599	551	531	568	558	546	569	535	475
SG		517	554	598	648	736	740	669	649	696	556
	Female	572	578	524	634	645	650	582	562	550	417
	Male	536	575	625	545	658	648	633	656	681	614
Pool SEM		13.29	12.33	11.26	16.35	12.09	16.06	11.04	13.81	14.72	13.57
P											
Genotype		*	–	*	**	***	***	***	**	***	**
Sex		–	–	***	*	–	–	–	**	***	***
GenotypeXSex		–	–	–	–	–	–	–	*	–	–

Note: –: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Abbreviations: FG, fast growing; SG, slow growing; SEM, standard error of mean.

TABLE 7 The effect of genotype and sex on Mg, K and Zn levels in total tibia weight.

Genotype	Sex	Age (week) 1	2	3	4	5	6	7	8	9	10
Mg (mg in total tibia weight)											
FG	Female	6.9	15.8	25.0	44.4	62.5	80.7	105.1	120.2	105.2	97.2
	Male	6.1	16.7	33.6	40.0	74.2	96.3	125.6	150.8	148.6	153.8
SG	Female	4.2	9.4	16.6	29.4	45.9	63.0	68.8	67.7	84.9	68.6
	Male	3.7	10.3	19.3	28.7	51.2	72.0	86.2	99.5	113.7	119.0
FG		6.5	16.3	29.3	42.2	68.3	88.5	115.4	135.5	127.4	125.5
SG		3.9	9.9	17.9	29.0	48.6	67.5	77.5	83.6	99.3	93.8
	Female	5.6	12.6	20.8	36.9	54.2	71.8	87.0	93.9	95.0	82.9
	Male	4.9	13.5	26.4	34.3	62.7	84.1	105.9	125.1	131.7	136.4
Pool SEM		0.17	0.44	0.66	1.06	1.30	2.28	1.95	2.32	2.42	2.12
P											
Genotype		***	***	***	***	***	***	***	***	***	***
Sex		*	–	***	–	**	*	***	***	***	***
GenotypeXSex		–	–	*	–	–	–	–	–	–	–
K (mg in total tibia weight)											
FG	Female	11.2	20.6	30.2	44.0	52.0	59.2	69.1	69.5	58.4	51.0
	Male	9.7	22.8	40.2	40.6	62.3	70.2	82.9	84.3	82.9	80.1
SG	Female	6.3	13.0	20.5	27.5	36.7	43.3	44.9	41.0	50.2	37.3
	Male	5.3	13.8	22.5	25.6	39.8	51.0	56.2	64.9	63.9	61.2
FG		10.5	21.7	35.2	42.3	57.2	64.7	76.0	76.9	70.7	65.5
SG		5.8	13.4	21.5	26.5	38.3	47.1	50.6	53.0	57.1	49.3
	Female	8.8	16.8	25.3	35.7	44.4	51.3	57.0	55.2	54.3	44.2
	Male	7.5	18.3	31.4	33.1	51.1	60.6	69.5	74.6	73.4	70.6
Pool SEM		0.21	0.66	0.90	1.16	1.30	1.86	1.44	1.60	1.54	1.12
P											
Genotype		***	***	***	***	***	***	***	***	***	***
Sex		*	–	**	–	*	*	***	***	***	***
GenotypeXSex		–	–	*	–	–	–	–	–	–	–
Zn (μg in total tibia weight)											
FG	Female	827	1804	2689	4312	4867	6103	7301	7233	6592	6195
	Male	674	2000	3143	3663	5886	7291	8971	8653	9184	9315
SG	Female	489	1062	1638	2584	3756	4729	4581	4678	6190	4537
	Male	447	1167	1922	2505	4055	5266	5761	7039	8009	7445
FG		750	1902	2916	3987	5377	6697	8136	7943	7888	7755
SG		468	1115	1780	2544	3905	4997	5171	5858	7099	5991
	Female	658	1433	2163	3448	4312	5416	5941	5955	6391	5366
	Male	561	1584	2532	3084	4970	6279	7366	7846	8597	8380
Pool SEM		15.6	53.1	71.8	99.9	99.5	167.4	151.2	209.2	197.0	151.0
P											
Genotype		***	***	***	***	***	***	***	***	***	***
Sex		**	–	*	–	**	*	***	***	***	***
GenotypeXSex		–	–	–	–	–	–	–	–	–	–

Note: –: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.
Abbreviations: FG, fast growing; SG, slow growing; SEM, standard error of mean.

TABLE 8 The effect of genotype and sex on Fe, Mn and Cu levels in total tibia weight.

		Age (week)									
Genotype	Sex	1	2	3	4	5	6	7	8	9	10
Fe (µg in total tibia weight)											
FG	Female	283	832	1336	1784	1793	2236	2639	2918	2310	1725
	Male	235	880	1614	1596	2182	2571	3287	3203	2981	2544
SG	Female	192	506	1000	1043	1284	1675	1756	1573	1926	1317
	Male	156	494	1011	986	1354	1769	2233	2282	1919	2073
FG		259	856	1475	1690	1987	2404	2963	3060	2646	2135
SG		174	500	1005	1014	1319	1722	1995	1927	1922	1695
	Female	238	669	1168	1413	1538	1956	2198	2245	2118	1521
	Male	195	687	1312	1291	1768	2170	2760	2742	2450	2309
Pool SEM		5.60	25.57	35.40	45.86	36.85	53.63	54.38	71.22	48.69	42.16
P											
Genotype		***	***	***	***	***	***	***	***	***	***
Sex		**	–	–	–	**	–	***	**	**	***
GenotypeXSex		–	–	–	–	*	–	–	–	**	–
Mn (µg in total tibia weight)											
FG	Female	20.27	46.21	70.91	99.01	121.83	158.43	194.41	227.85	205.25	180.71
	Male	18.14	50.32	89.23	92.55	148.72	187.11	240.74	294.90	269.46	290.25
SG	Female	12.89	25.73	39.26	64.91	94.06	109.77	120.66	131.79	164.56	131.43
	Male	10.90	27.83	45.58	58.28	105.31	126.23	152.67	191.28	217.39	207.02
FG		19.21	48.27	80.07	95.78	135.28	172.77	217.57	261.38	237.36	235.48
SG		11.89	26.78	42.42	61.60	99.68	118.00	136.67	161.54	190.98	169.23
	Female	16.58	35.97	55.09	81.96	107.95	134.10	157.54	179.82	184.91	156.07
	Male	14.52	39.08	67.40	75.42	127.01	156.67	196.71	243.09	243.43	248.63
Pool SEM		0.45	1.29	1.77	2.09	2.25	3.58	3.43	3.72	4.49	3.66
P											
Genotype		***	***	***	***	***	***	***	***	***	***
Sex		*	–	**	–	***	**	***	***	***	***
GenotypeXSex		–	–	–	–	–	–	–	–	–	*
Cu (µg in total tibia weight)											
FG	Female	11.66	24.22	21.62	45.83	68.06	98.39	117.45	129.80	125.48	107.23
	Male	9.18	25.39	23.06	42.15	75.76	116.27	142.62	171.25	183.29	172.49
SG	Female	6.77	12.07	12.74	28.20	47.35	64.48	68.40	72.49	105.49	72.34
	Male	5.84	12.66	15.48	26.59	53.54	76.47	84.22	107.94	138.92	123.01
FG		10.42	24.81	22.34	43.99	71.91	107.33	130.03	150.53	154.38	139.86
SG		6.30	12.37	14.12	27.39	50.44	70.48	76.31	90.22	122.21	97.68
	Female	9.22	18.15	17.18	37.01	57.71	81.44	92.93	101.15	115.48	89.78
	Male	7.51	19.02	19.27	34.37	64.65	96.37	113.42	139.60	161.11	147.75
Pool SEM		0.21	0.68	0.62	1.12	1.27	2.08	2.13	2.47	3.17	2.29
P											
Genotype		***	***	***	***	***	***	***	***	***	***
Sex		**	–	–	–	*	**	***	***	***	***
GenotypeXSex		–	–	–	–	–	–	–	–	–	–

Note: –: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Abbreviations: FG, fast growing; SG, slow growing; SEM, standard error of mean.

TABLE 9 The effect of genotype and sex on Mg, K and Zn levels per unit body weight.

		Age (week)									
Genotype	**Sex**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Mg (µg per unit body weight)											
FG	Female	33.12	33.96	26.08	29.63	29.55	28.54	28.68	30.02	24.69	20.58
	Male	32.49	32.21	33.26	24.96	31.74	30.59	30.09	31.66	31.90	31.32
SG	Female	30.72	31.37	29.23	35.82	38.76	40.79	34.35	30.66	32.38	25.53
	Male	27.48	31.94	34.57	32.94	39.55	40.28	38.74	37.27	39.73	34.36
FG		32.80	33.09	29.67	27.30	30.64	29.56	29.38	30.84	28.29	25.95
SG		29.10	31.66	31.90	34.38	39.16	40.53	36.55	33.96	36.06	29.95
	Female	31.92	32.66	27.65	32.72	34.15	34.66	31.51	30.34	28.53	23.06
	Male	29.99	32.08	33.92	28.95	35.64	35.43	34.42	34.46	35.82	32.84
Pool SEM		0.94	0.78	0.61	0.94	0.61	0.82	0.70	0.72	0.76	0.73
P											
Genotype		–	–	–	**	***	***	***	*	***	*
Sex		–	–	***	–	–	–	–	**	***	***
GenotypeXSex		–	–	–	–	–	–	–	–	–	–
K (µg per unit body weight)											
FG	Female	53.31	44.53	31.46	29.34	24.58	20.88	18.84	17.35	13.72	10.80
	Male	52.40	43.77	39.79	25.35	26.80	22.32	19.83	17.73	17.58	16.27
SG	Female	45.97	43.14	36.06	33.45	31.04	28.09	22.38	18.58	19.16	13.91
	Male	40.17	42.85	40.37	29.35	30.70	28.44	25.27	24.35	22.35	17.70
FG		52.86	44.15	35.62	27.34	25.69	21.60	19.33	17.54	15.65	13.54
SG		43.07	42.99	38.21	31.40	30.87	28.27	23.83	21.46	20.75	15.81
	Female	49.64	43.83	33.76	31.39	27.81	24.49	20.61	17.97	16.44	12.36
	Male	46.29	43.31	40.08	27.35	28.75	25.38	22.55	21.04	19.97	16.99
Pool SEM		1.17	1.19	0.83	0.92	0.76	0.65	0.46	0.49	0.42	0.38
P											
Genotype		***	–	–	*	**	***	***	**	***	**
Sex		–	–	**	*	–	–	*	**	***	***
GenotypeXSex		–	–	–	–	–	–	–	*	–	–
Zn (µg per unit body weight)											
FG	Female	3.95	3.90	2.81	2.88	2.30	2.16	1.99	1.81	1.55	1.31
	Male	3.63	3.84	3.12	2.28	2.51	2.32	2.15	1.82	1.96	1.90
SG	Female	3.55	3.54	2.88	3.14	3.17	3.06	2.28	2.12	2.36	1.69
	Male	3.37	3.63	3.44	2.88	3.13	2.95	2.59	2.64	2.81	2.15
FG		3.79	3.87	2.97	2.58	2.41	2.24	2.07	1.82	1.76	1.60
SG		3.46	3.58	3.16	3.01	3.15	3.01	2.43	2.38	2.58	1.92
	Female	3.75	3.72	2.85	3.01	2.74	2.61	2.14	1.96	1.95	1.50
	Male	3.50	3.73	3.28	2.58	2.82	2.63	2.37	2.23	2.38	2.02
Pool SEM		0.10	0.09	0.07	0.08	0.04	0.06	0.05	0.07	0.06	0.05
P											
Genotype		–	–	–	*	***	***	**	***	***	**
Sex		–	–	**	*	–	–	*	–	**	***
GenotypeXSex		–	–	–	–	–	–	–	v	–	–

Note: –: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Abbreviations: FG, fast growing; SG, slow growing; SEM, standard error of mean.

TABLE 10 The effect of genotype and sex on Fe, Mn and Cu levels per unit body weight.

		Age (week)									
Genotype	**Sex**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Fe (µg per unit body weight)											
FG	Female	1.35	1.79	1.40	1.19	0.85	0.79	0.72	0.73	0.54	0.37
	Male	1.26	1.69	1.60	1.00	0.93	0.82	0.79	0.67	0.63	0.52
SG	Female	1.40	1.68	1.76	1.28	1.09	1.09	0.87	0.71	0.74	0.49
	Male	1.17	1.54	1.81	1.14	1.05	1.00	1.00	0.86	0.67	0.60
FG		1.31	1.74	1.50	1.09	0.89	0.81	0.76	0.70	0.59	0.44
SG		1.28	1.61	1.79	1.21	1.07	1.04	0.94	0.79	0.70	0.55
	Female	1.37	1.73	1.58	1.23	0.97	0.94	0.80	0.72	0.64	0.43
	Male	1.22	1.61	1.71	1.07	0.99	0.91	0.90	0.76	0.65	0.56
Pool SEM		0.03	0.05	0.04	0.04	0.02	0.03	0.02	0.02	0.02	0.01
P											
Genotype		–	–	**	–	***	***	***	–	**	**
Sex		**	–	–	–	–	–	**	–	–	***
GenotypeXSex		–	–	–	–	–	–	–	*	*	–
Mn (ng per unit body weight)											
FG	Female	96.94	99.69	74.00	66.11	57.65	56.11	52.99	56.87	48.17	38.35
	Male	97.50	96.59	88.42	57.72	63.55	59.48	57.71	61.89	57.37	59.18
SG	Female	93.61	85.69	69.27	79.10	79.53	71.08	60.15	59.71	62.93	49.00
	Male	81.95	86.39	81.75	67.00	81.37	70.68	68.58	71.69	76.16	59.81
FG		97.22	98.14	81.21	61.91	60.60	57.80	55.35	59.38	52.77	48.76
SG		87.78	86.04	75.51	73.05	80.45	70.88	64.36	65.70	69.55	54.41
	Female	95.27	92.69	71.64	72.61	68.59	63.60	56.57	58.29	55.55	43.66
	Male	89.72	91.49	85.08	62.36	72.46	65.08	63.14	66.79	66.77	59.50
Pool SEM		2.46	2.08	1.54	1.79	1.12	1.27	1.14	1.19	1.58	1.41
P											
Genotype		–	**	–	**	***	***	**	*	***	–
Sex		–	–	***	*	–	–	**	**	**	***
GenotypeXSex		–	–	–	–	–	–	–	–	–	–
Cu (ng per unit body weight)											
FG	Female	55.75	52.18	22.60	30.57	32.20	34.87	32.04	32.44	29.43	22.72
	Male	49.31	48.77	22.81	26.36	32.42	37.01	34.15	36.00	39.00	35.19
SG	Female	49.16	40.10	22.57	34.42	40.03	41.80	34.09	32.87	40.21	26.94
	Male	43.86	39.33	27.71	30.56	41.41	42.82	37.83	40.45	48.65	35.52
FG		52.53	50.48	22.70	28.46	32.31	35.94	33.09	34.22	34.22	28.95
SG		46.51	39.72	25.14	32.49	40.72	42.31	35.96	36.66	44.43	31.23
	Female	52.46	46.14	22.59	32.49	36.12	38.33	33.06	32.66	34.82	24.83
	Male	46.58	44.05	25.26	28.46	36.92	39.92	35.99	38.22	43.82	35.35
Pool SEM		1.13	1.09	0.59	0.95	0.66	0.82	0.66	0.78	0.98	0.78
P											
Genotype		*	***	–	*	***	**	*	–	***	–
Sex		*	–	*	*	–	–	*	**	***	***
GenotypeXSex		–	–	*	–	–	–	–	–	–	–

Note: –: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.
Abbreviations: FG, fast growing; SG, slow growing; SEM, standard error of mean.

TABLE 11 Differences in variables by age, genotype, sex and genotypeXSex interaction using generalized estimating equations.

	Wald chi-square/P			
Traits	Age	Genotype	Sex	GenotypeXSex
Tibia weight	741.1/***	309.6/***	159.3/***	3.4/–
Tibia weight (% slaughter weight)	89.7/***	26.1/***	36.8/***	0.0/–
Tibia length	15,308.7/***	28.2/***	46.7/***	0.0/–
Tibia diaphysis diameter	3373.2/***	174.3/***	45.9/***	3.0/–
Tibia medullary canal diameter	1672.3/***	123.1/***	25.3/***	4.3/*
Tibia cortical index	540.7/***	11.0/**	1.5/–	2.7/–
Robusticity index	834.2/***	369.6/***	36.8/***	0.5/–
Seedor index	964.9/***	418.4/***	124.3/***	2.5/–
Ash (in total tibia weight)	1230.6/***	89.0/***	70.4/***	0.0/–
Ca (g in total tibia weight)	1119.6/***	796.1/***	428.5/***	5.8/*
P (g in total tibia weight)	1062.1/***	523.0/***	286.8/***	3.0/–
Mg (g in total tibia weight)	1057.2/***	507.5/***	273.6/***	3.9/*
K (g in total tibia weight)	877.8/***	438.9/***	165.9/***	3.4/–
Zn (g in total tibia weight)	1310.8/***	286.1/***	140.7/***	0.8/–
Fe (g in total tibia weight)	712.6/***	709.4/***	126.8/***	7.0/**
Mn (g in total tibia weight)	1379.2/***	845.7/***	328.7/***	10.7/**
Cu (g in total tibia weight)	1017.2/***	620.1/***	250.3/***	6.2/*
Ash (per unit body weight)	269.1/***	43.5/***	13.3/***	1.8/–
Ca (per unit body weight)	69.7/***	78.0/***	43.0/***	0.1/–
P (per unit body weight)	66.5/***	107.4/***	31.6/***	0.3/–
Mg (per unit body weight)	74.8/***	106.6/***	35.4/***	0.0/–
K (per unit body weight)	1172.0/***	22.5/***	7.4/**	0.5/–
Zn (per unit body weight)	792.0/***	66.2/***	9.1/**	1.1/–
Fe (per unit body weight)	1532.9/***	46.2/***	0.1/–	1.8/–
Mn (per unit body weight)	585.4/***	26.1/***	16.9/***	0.6/–
Cu (per unit body weight)	880.7/***	15.5/***	13.9/***	0.7/–

Note: –: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

the slow-growing female at the 3rd week and in the slow-growing male at the 4th week. The rapid increase in body weight during these weeks indicates that the tibia weight and the amount of Ca in the tibia could not reach this speed. Then, in parallel with the increase in bone weight, the amount of Ca per unit body weight also increased. However, after the 8th week in the fast-growing female and the 9th week in the fast-growing male, the amount of Ca in the tibia started to decrease again rapidly. Ca content per unit body weight was found to be high in the males at certain weeks; this difference was found to be significant at the 3rd and 4th weeks and from the 7th week of fattening ($p < 0.05$).

Bone quality is defined not only by the Ca content but also by the P content, which is in close relationship with Ca and necessary for bone structure (Kraus et al., 2022). In terms of the amount of P per unit body weight, it was found to be higher in those who developed rapidly at the age of 1 week ($p < 0.05$). The amount of P per unit body weight was found to be higher in male broilers at 3 weeks of age and after the 7 weeks of age. The difference between genotypes in terms of Mg and Zn levels per unit body weight started to be seen from the 4th week ($p < 0.05$), and it was observed that the Mg and Zn levels per unit body weight were very low in fast-growing broilers. The change in Mg level with age was similar to that of the P level. Krunt et al. (2021) stated that Mg in the tibia and femur bones of rabbits was an effective mineral in bone fracture resistance. The potassium amount per unit body weight was high in fast-growing broilers at week 1 ($p < 0.001$) and this situation was reversed from the week 4 ($p < 0.05$). The amount of K per unit body weight was found to be higher in male broilers, and this difference was found to be significant at the 3rd week and from the 7th week of fattening ($p < 0.05$). Fe, Mn and Cu levels per body weight in slow-growing broilers were observed to be higher in some weeks during the 10-week fattening period. Bone abnormalities may develop with Mn deficiency (Spears, 2019). It was observed that there was a genotype-sex interaction in certain weeks of the present study. This interaction was due to the large difference between males and females in fast-growing broilers.

The generalized estimating equations revealed that the age and genotype were determined significant ($p < 0.01$) for all examined parameters over time (Table 11). However, when the effect of sex was examined overall, it was determined that only the change in tibia cortical index and Fe (per unit body weight) were not significant ($p > 0.05$). According to generalized estimating equations, genotype-sex interaction was detected in tibia medullary canal diameter and Ca, Fe, Mn and Cu (g, in total tibia weight).

The diets used for hybrid fattening in the commercial are adjusted in accordance with the standards set by the NRC and differ in slow and fast-growing ones. In the study, the diets used in the commercial husbandry were given to the slow and fast-growing broilers. Thus, it was ensured to determine the situation in the commercial husbandry in terms of bone development for both genotypes. Fast growing broilers are fed for 42 days, and slow-growing broilers are fed for up to 70 days in commercial rearing. In this study, both genotypes were fed for up to 70 days. Thus, fast growing broilers fed for 42 days were adapted to commercial production and compared with the other genotype in terms of bone development until the end of 70 days. As a conclusion, although tibia weight, Seedor index, ash and mineral levels in total tibia weight were higher and Robusticity index was lower in fast-growing broilers, the amount of minerals did not increase compared to the body weight except in the 1st week. In fact, it has been observed that the tibia structure is strong in fast-growing broilers, but fast-growing broilers come to the fore when compared to body weight. In general, there is a decrease in the mineral level in the tibia due to the decrease in tibia weight after the 8th week of the fattening in fast-growing broilers and after the 9th week of the fattening in slow-growing broilers. This situation indicates that a porous structure begins to form in the cortical layer. Skeletal problems may occur easily in broilers during this period due to the increased body weight. It was observed that the mineral density was higher in male broilers, except in the 1st week, and they had higher Seedor index and lower Robusticity index values than those of female broilers. The relationship between the cortical index value and other features was not consistent.

AUTHOR CONTRIBUTIONS

Study conception, design, material preparation, data collection, data analysis and writing of the manuscript were performed by Hilal Çapar Akyüz, Esin Ebru Onbaşılar and Sakine Yalçın. This study was summarized from the PhD thesis of the first author.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and that the appropriate ethical review committee approval has been received. All procedures used in the present study were approved by the Ethic Committee of the Ankara University (2018-6-44).

ORCID

Hilal Çapar Akyüz https://orcid.org/0000-0002-4741-6893
Esin Ebru Onbaşılar https://orcid.org/0000-0002-1321-0280
Sakine Yalçın https://orcid.org/0000-0001-8640-2729

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