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#### ■ Keywords

Organic production, broiler strain, bone mineral density, bone mineral content.

## Edible Giblets and Bone Mineral Characteristics of Two Slow-Growing Chicken Genotypes Reared in an Organic System

### ABSTRACT

This study was conducted to compare edible giblets weight, tibial bone mineral density (BMD), and bone mineral content (BMC) of two slow-growing broiler genotypes (Hubbard S757; S757 and Hubbard Grey Barred JA; GB-JA) reared with outdoor access, and to determine the relationship between these variables. Day-old chicks (straight-run) of the genotypes S757 (n=120) and GB-JA (n=120) were housed for 98 days. Each genotype was assigned to six pens of 20 birds each. Birds were reared in indoor floor pens and moving shelters with outdoor access (during daylight hours). Absolute body (BW), heart (HW), spleen (SW), liver (LW), gizzard (GW), and abdominal fat pad (AFW) weights of the genotype S757 and male birds were statistically higher than that of the genotype GB-JA and female birds. Genotype statistically affected relative HW, whereas sex affected relative GW. Although BMD values were not influenced by genotype or sex, S757 birds and males presented statistically higher tibial BMC, lean, lean+BMC, total mass values (g) and area (cm<sup>2</sup>) compared with GB-JA birds and females. BW, HW, SW, LW, GW and AFW were positively correlated with BMC obtained by DXA. In conclusion, the measured traits influenced by genetic strain and sex. The use of the Hubbard S757 genotype in organic production systems with outdoor access is recommend.

### INTRODUCTION

Although environmental factors such as management, feeding, movement, toxins, and infectious diseases can play very important roles in the development of skeletal structure, genetic background also has a fundamental influence (Rath *et al.*, 2000). Modern broiler genotypes have been subjected to intensive selection for body weight gain. This selection has resulted in major changes in the anatomy and physiology of broilers, such as increased fat deposition, metabolic diseases, and abnormal skeletal development, including leg disorders, lameness, twisted legs, tibial dyschondroplasia, and crooked toes (Bradshaw *et al.*, 2002; Decuyper *et al.*, 2003; Oviedo-Rondon *et al.*, 2006; Scahaw, 2000).

Conventional intensive production conditions do not allow sufficient exercise and typically present poor hygiene, resulting in leg weakness and skin infections (Bessei, 2006; Talaty *et al.*, 2009). It has been demonstrated that the bones of fast growing meat-type chickens are often abnormally developed, and present high porosity and much lower density than those of slow-growing broilers (Bennett, 2008; Williams *et al.*, 2004). Chickens living freely in their natural habitats did not appear to have such problems (Newberry, 1995), and present much stronger muscle structures (Balog, 1997; Le Van, 2000; Shields, 2004). The genetic selection for growth rate and the feed conversion ratio has altered the relative growth of organs in modern broiler lines when



compared with heritage lines (Lippens, 2003; Schmidt *et al.*, 2009) and the increasing muscle weight of modern broiler lines is accompanied by a decrease in edible giblets weight (Janiszewska *et al.*, 1998; Plavnik & Hurwitz, 1982). The changes in the weight of edible giblets occur at different rates (Murawska *et al.*, 2011; Tüzün & Aktan, 2012).

Because of bone ash (Onyango *et al.*, 2003) and its high correlation with live scans (Schreiweis *et al.*, 2005), excised bones can also be scanned using DXA to assess bone quality. Bone mineral density (BMD, g/cm<sup>2</sup>) (Kim *et al.*, 2006; Lian *et al.*, 2004; Onyango *et al.*, 2003; Rath *et al.*, 2000; Shim *et al.*, 2012; Watkins and Southern, 1992) and bone mineral content (BMC, g/cm) (Akpe *et al.*, 1987; Almeida *et al.*, 2008; Kim *et al.*, 2006, Onyango *et al.*, 2003) measurements have been used to evaluate the quality and the status of the porous structure of bone matrix. These two parameters are greatly influenced by mineral intake. BMD is a noninvasive method using dual-energy x-ray absorptiometry (DXA) that has also been employed to predict osteoporosis in humans (Bolotin, 2007; Koo, 2000). DXA could also be used to measure BMC in broilers and laying hens (Schreiweis *et al.*, 2005; Shim *et al.*, 2012; Talaty *et al.*, 2010).

Comparative research among different slow-growing broiler strains may be used to establish physiological and morphological adaptations in response to distinct selective pressures. The purpose of this study was to compare the BMD, BMC, and giblet weights of two slow-growing broiler genotypes selected for organic production.

## **MATERIAL AND METHODS**

Two hundred and forty male and female Hubbard S757 (S757) and Grey Barred JA (GB-JA) were equally divided between strains were used. Although both these strains present slow growth rates to be used in organic production systems, Hubbard S757 (S757) and Grey Barred JA (GB-JA) were selected to achieve market weight at 84 and 70 days of age respectively. In the study, one-d-old chicks were weighed, identified by wing band, and randomly allocated to two treatments (genotypes: Hubbard S757 or Hubbard Grey Barred JA) with six replicates of 20 birds each. The experiment was approved by the Ethics Committee of the University of Cumhuriyet in Sivas, Turkey (20.06.2011/50).

Twelve portable shelters (1.5 x 1.5 m), each housing 20 birds per replicate at a stocking density of 10 birds/m<sup>2</sup> placed in each of the 100-m<sup>2</sup> grazing areas. The research was carried out according to the principles and

implementation of regulation on organic agriculture (OFL, 2010) published by the Republic of Turkey, Ministry of Food, Agriculture and Livestock. During the first 14 days, chicks were housed in the portable shelters, and offered feed and water *ad libitum*. After this period chicks were allowed to access outdoors and to graze freely. All feed and water were provided between 07:00-19:00 for all chicks during the entire experimental period.

Birds were fed with a three-phase organic diet: a starter diet (197 g crude protein (CP)/kg and 13.00 MJ of metabolizable energy (ME) per kg of diet) from 0 to 28 days, a grower diet (201 g/kg CP and 12.72 MJ of ME per kg of diet) from 29 to 81 days, and a finisher diet (180 g/kg CP and 12.91 MJ of ME per kg of diet) from 82 to 98 days (Table 1).

Only natural day length lighting was provided for chickens from first days to slaughter age. Ceramic heaters, which are sources of far infrared rays and do not emit light, were used for heating.

All birds used in the experiment were managed following the recommendations of Sirri *et al.* (2011). Birds were fed certified organic feedstuffs, and provided with a cultivated poultry pasture, consisting of equal amounts of *Lotus corniculatus* and *Bromus inermis*.

At the end of the 98-day trial period, bone properties were assessed in 24 males and 24 females whose body weights (BW) were close to the pen average (Eleroğlu *et al.*, 2014a). These 48 birds were fasted for 10 h (with free access to water), weighed, and slaughtered by severing the throat and major blood vessels of the neck in the local processing plant producing organic chicken products (Eleroğlu *et al.*, 2014b). After slaughter, hot carcass weight was determined in a semi-analytic digital scale with 0.001-g precision, and then the left tibial bone, heart, spleen, liver, gizzard, and abdominal fat pad were removed and weighed to determine absolute weight and relative weight as a percentage of live body weight. Bone mineral content (BMC), bone mineral density (BMD), lean, lean+BMC, total mass and mass area of the left tibial bone were determined using a total-body dual energy X-ray absorptiometry DXA scanner (QDR 4500W Acclaim, Hologic, USA) at the Nuclear Medicine Department of the Medicine Faculty at Cumhuriyet University.

Data were analyzed using the General Linear Model (GLM) procedure. The effects of genotype, sex, and their interactions on the absolute and relative weights of the body (BW), heart (HW), spleen (SW), liver (LW), gizzard (GW), and abdominal fat (AFW), as well as



**Table 1** – Ingredients and calculated nutritional composition of the experimental diets

Feedstuffs, %	Age (days)			Nutritional composition (g/kg)			
	0–28 days	29–81 days	82–98 days				
Barley	3.45	4.50	4.50	ME (MJ/kg)	13.00	12.72	12.91
Vegetable oil	4.36	5.00	5.00	Dry matter	899.00	903.00	901.00
Wheat bran	5.00	5.00	5.00	Crude protein	197.00	201.00	180.00
White wheat	12.40	4.00	4.00	Crude ash	4.70	5.90	4.80
Rye	3.00	4.00	4.00	Lysine	10.80	10.60	8.50
Corn	40.00	20.00	20.00	Met + Cys.	6.60	6.70	5.90
Triticale	-	22.00	32.00	Threonine	7.30	7.20	6.20
Oats	2.10	5.00	-	Calcium	10.00	11.60	9.00
Fish meal	7.30	5.00	-	Phosphorus	7.70	6.00	5.90
Soybean meal	20.00	22.00	22.00	Sodium	1.90	1.80	1.50
Dicalcium phosphate	1.10	2.10	2.10	Tryptophan	2.40	2.60	2.50
Limestone	0.74	0.80	0.80	Linoleic acid	31.9	32.1	31.3
Salt	0.30	0.30	0.30				
Vitamin-mineral premix*	0.25	0.30	0.30				

Explanation: \* - Each kg of vitamin-mineral premix contained: vitamin A, 4,400,000 IU; vitamin D<sub>3</sub>, 1,600,000 IU; vitamin E, 20,000 mg; vitamin K<sub>3</sub>, 1,600 mg; vitamin B<sub>1</sub>, 1,200 mg; vitamin B<sub>2</sub>, 3,200 mg; vitamin B<sub>3</sub>, 20,000 mg; vitamin B<sub>5</sub>, 6,000 mg; vitamin B<sub>6</sub>, 1,600 mg; vitamin B<sub>9</sub>, 800 mg; vitamin B<sub>12</sub>, 8 mg; biotin, 80 mg; antioxidant, 50,000 mg; Cu, 6,000 mg; Fe, 20,000 mg; Mn, 48,000 mg; Se, 80 mg; Zn, 40,000 mg; Co, 80 mg; I, 500 mg.

on BMD and BMC were evaluated using multifactor analysis of variance. The percentage data were converted to arcsines prior to analysis. The relationship between bone mineral characteristics and edible giblets were determined using one-tailed Pearson correlation procedure (SPSS Inc. 2010, Release 16.0). Treatment effects were considered significant at  $p < 0.05$ . Data were expressed as mean values with pooled standard errors (standard errors of the mean, SEM).

## RESULTS

The results of the effects of genotype and sex on edible giblets, abdominal fat, and bone characteristics are given in Table 2 and 3, respectively.

Body weight at slaughter (BW) of the two genotypes investigated under the conditions of the present experiment was accurately measured, and was different from each other. Before slaughter, genotype and sex significantly influenced BW ( $p < 0.01$ ). As shown in Table 2, S757 males were heavier (2847 g) than GB-JA males (2128 g) and S757 females (2194 g) were heavier than GB-JA females (1634 g). Males of both evaluated strains were heavier than females ( $p < 0.01$ ). There were significant differences between males and females of the same genotypes ( $p < 0.01$ ). S757 males showed higher BW (2847 g) than females (2194 g), whereas the calculated BW of males and females of the GB-JA genotype were 2128 g and 1634 g, respectively.

**Table 2** – Absolute and relative weights of edible giblets and abdominal fat of male and female broilers of two different slow-growing genotypes.

	GB-JA <sup>1</sup>		S757 <sup>1</sup>		SEM <sup>2</sup>	p value	
	Female	Male	Female	Male		G <sup>3</sup>	S <sup>4</sup>
<b>Absolute weight (g)</b>							
Body	1634	2128	2194	2847	65.31	**	**
Heart	7.13	9.57	8.59	11.91	0.31	**	**
Spleen	2.07	2.40	3.17	3.74	0.13	**	*
Liver	24.55	31.35	31.73	38.71	0.9	**	**
Gizzard	32.14	37.16	41.37	46.42	1.04	**	**
Abdominal fat pad	27.75	38.44	39.14	50.34	2.25	**	**
<b>Relative weight (g/100 g body weight)</b>							
Heart	0.44	0.45	0.39	0.42	0.008	**	NS
Spleen	0.13	0.11	0.15	0.13	0.005	NS	NS
Liver	1.51	1.47	1.45	1.36	0.024	NS	NS
Gizzard	1.97	1.75	1.90	1.63	0.042	NS	**
Abdominal fat pad	1.70	1.79	1.78	1.78	0.087	NS	NS

Explanations: <sup>1</sup> - GB-JA = Hubbard Grey Barred JA; S757 = Hubbard S757; <sup>2</sup> - Standard error of the mean, G<sup>3</sup> - Genotype; S<sup>4</sup> - Sex; \* -  $p < 0.05$ ; \*\* -  $p < 0.01$ ; NS - Not significant ( $p > 0.05$ )



**Table 3** – Bone characteristics of male and female broilers of two different slow-growing genotypes

Genotype (G) <sup>1</sup>	Sex (S)	BMC (g)	Lean (g)	Lean+BMC (g)	Total mass (g)	Mass Area (cm <sup>2</sup> )	BMD (g/cm <sup>2</sup> )
GB-JA	F	2.37	55.78	58.15	57.10	9.96	0.237
	M	4.55	86.75	91.30	89.38	16.52	0.269
S757	F	3.57	81.65	85.22	84.31	14.45	0.256
	M	5.88	109.45	115.35	113.78	21.18	0.269
SEM <sup>2</sup>		0.29	3.54	3.78	3.76	0.76	0.009
Main effect of							
G <sup>3</sup>		**	**	**	**	**	NS
S <sup>4</sup>		**	**	**	**	**	NS

Explanations: <sup>1</sup> - GB-JA = Hubbard Grey Barred JA; S757 = Hubbard S757; <sup>2</sup> - Standard error of the mean; G<sup>3</sup> - Genotype; S<sup>4</sup> - Sex; \* -  $p < 0.05$ ; \*\* -  $p < 0.01$ , NS - Not significant ( $p > 0.05$ )

Absolute HW, SW, LW, GW and AFW (g) were influenced by genotype, with S757 birds presenting higher values than GB-JA birds ( $p < 0.01$ ). On the other hand, no differences in HW, SW, LW, GW, and AFW relative to body weight (g/100 g body weight) were detected between genotypes, except for HW, which was higher ( $p < 0.01$ ) in GB-JA (0.45 g) than in S757 (0.41 g) birds.

The absolute HW (11.91 g), SW (3.74 g), LW (38.71 g), GW (46.42 g) and AFW (50.34 g) of S757 males were higher ( $p < 0.01$ ) than absolute HW (9.57 g), SW (2.40 g), LW (31.75 g), GW (37.16 g) and AFW (38.44 g) of GB-JA males. Similarly, absolute HW (8.59 g), SW (3.17 g), LW (31.73 g), GW (41.37 g) and AFW (39.14 g) values of S757 females were higher compared with GB-JA females, which presented HW (7.13 g), SW (2.07 g), LW (24.55 g), GW (32.14 g) and AFW (27.75 g) ( $p < 0.01$ ,  $p < 0.05$ ). Neither genotype nor sex influenced ( $p > 0.05$ ) relative SW, LW and AFW (g/100 g BW). However, relative HW ( $p < 0.01$ ) was significantly higher in S757 birds compared with GB-JA birds, independently of sex, and males presented higher relative GW than females ( $p < 0.01$ ), independently of genotype.

A strong effect of genotype and sex was observed on BMC, Lean, Lean+BMC, total mass and mass area ( $p < 0.01$ ). In contrast, genotype and gender effects on BMD were not significant ( $p > 0.05$ ).

As shown in Table 3, the average BMC (4.72 g), Lean (95.55 g), Lean+BMC (100.28 g), total mass (99.05 g) and mass area (17.82 cm<sup>2</sup>) values of S757 birds were higher ( $p < 0.01$ ) than the average BMC (3.46 g), Lean (71.27 g), Lean+BMC (74.73 g), Total mass (73.24 g) and Mass area (13.24 cm<sup>2</sup>) values of GB-JA genotype. On the other hand similar differences were observed between sex, the average BMC (5.51 g), Lean (98.1 g), Lean+BMC (103.33 g), Total mass (101.58 g) and Mass area (18.85 cm<sup>2</sup>) values of male were higher than the average BMC (2.97 g), Lean (68.72 g), Lean+BMC (71.69 g), Total mass (70.71 g) and Mass area (12.21 cm<sup>2</sup>) values of female ( $p < 0.01$ ).

Table 4 shows the correlations between BW, giblets, abdominal fat, BMC, and BMD values. HW, SW, LW, GW and AFW were significantly correlated with BW ( $r = 0.839, 0.711, 0.826, 0.668$  and  $0.526$ , respectively,  $p < 0.01$ , Table 4). A high correlation of 0.839 ( $p < 0.01$ ) was especially noted between HW and BW, whereas the correlation of AFW between HW was low ( $r = 0.307$ ;  $p < 0.05$ ). In addition, BMD, BW, HW, SW, LW and AFW were highly significantly correlated with BMC ( $r = 0.687, 0.543, 0.415, 0.396, 0.462$  and  $0.434$ , respectively,  $p < 0.01$ ) and the correlation GW with BMC was low ( $r = 0.319$ ;  $p < 0.05$ ).

## DISCUSSION

The obtained absolute and relative HW, SW, LW, GW and AFW results account indicate that bird growth was isometric, and their genotype, management, and feeding program allowed their normal growth and development under the organic rearing conditions (Plavnik & Hurwitz, 1982; Lippens, 2003).

The differences in the relative HW detected between the S757 and GB-JA genotypes may be explained by the selection for different growth rates. The differences in BW led to different relative GW values. Edible internal organ weights relative to hot carcass weight were expected to be higher as the BW at slaughter of broilers reared in organic systems is higher (Eleroglu et al., 2014b). The findings of the current study confirmed this fact.

BMD was not influenced by genotype or sex, on account of this can be said to be similar applied to environmental conditions for both genotype and gender.

Simsek et al. (2009) observed BMC and BMD values of 1.94; 0.12 and 2.01; 0.14 in 42-d-old broilers reared in an enriched and a conventional housing system, respectively. In the present study, BMC values ranged between 2.37 and 5.88, and BMD values between 0.237 and 0.269 in broilers reared in an organic system. The higher BMC and BMD values obtained in



**Table 4** – Correlation coefficients among BW, edible giblets, abdominal fat, BMC and BMD

Items	BMD	BMC	BW	HW	SW	LW	GW
BMD	1						
BMC	0.687**	1					
BW	0.125	0.543**	1				
HW	0.065	0.415**	0.839**	1			
SW	0.136	0.396**	0.711**	0.537**	1		
LW	0.058	0.462**	0.826**	0.767**	0.668**	1	
GW	0.059	0.319*	0.668**	0.620**	0.509**	0.679**	1
AFW	0.145	0.434**	0.526**	0.307*	0.399**	0.484**	0.136

Explanations: \*\* - Correlation is significant at \* $p < 0.05$ ; \*\* -  $p < 0.01$

the present study may be attributed to age at slaughter (98 days), whereas in Simsek *et al.* (2009), broilers were slaughtered with 42 days of age. Rath *et al.* (2000) indicated that BMC and BMD vary according to the age of the skeletal structure, Williams *et al.* (2004) and Bennett (2008) slow-growing genotypes present better skeletal structure compared fast-growing broilers.

In the present study, a positive correlation between BW and edible giblets and abdominal fat was determined. Genetics and environmental factors, such as feeding, housing, and management, influence BW and the relative growth of internal organs (Ramadan *et al.*, 2014; Tavárez *et al.*, 2016), resulting in changes in the coefficients of correlation between BW and organ relative weights. The correlation coefficients were unexpected and possibly related with allometric internal organ growth.

The positive correlation between BW and BMC observed in the present study is in agreement with previous studies (Schreiweis *et al.*, 2004; Salas *et al.*, 2012). In addition, this correlation indirectly affected the correlation of BMC with the internal organs. Bone breaking strength, bone ash concentration, and bone weight are positively correlated with BMD and BMC obtained by DXA in chickens (Fleming *et al.*, 1994; Mazzuco and Hester, 2005; Kim *et al.*, 2006, 2008). There is a strong correlation between BMD and BMC ( $r=0.81$ ;  $p < 0.01$ ) in White Leghorns (Hester *et al.*, 2004) as well in male broilers of commercial lines (Talaty *et al.*, 2010). In addition, relatively larger bones present higher BMC correlation with total bone mineral content and are stronger and BMD and BMC values increase with broiler BW (Schreiweis *et al.*, 2004).

The correlation between BMC and tibia ash content is reported as 86% in commercial broilers (Onyango *et al.*, 2003), and BMC has been described as a better indicator of Ca and P nutrition than BMD (Yan *et al.*, 2005). It is concluded that DXA can be used to assess bone mineralization status and bird welfare as a function of their correlation with BMC and BMD.

## CONCLUSIONS

The results of this study showed the importance of genetic and environmental influences on the bone development of broilers. The positive correlation between BMD values and the weight of internal organs should be further studied.

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