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Influence of Stocking Density on Bone Development in Family Chickens Reared up to 18 Weeks of Age Under Intensive System

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Abstract: This study investigated the influence of stocking density on bone development of family chickens up to 18 weeks of age. A total of 232 unsexed day-old family chicks were used in a completely randomized design. Birds were randomly assigned to four stocking densities, i.e., D1 (10 birds/m^2), D2 (13 birds/m^2), D3 (16 birds/m^2) and D4 (19 birds/m^2) in the first phase (0-6 weeks). Each treatment was replicated four times. Two birds were slaughtered at 6, 12 and 18 weeks of age from each replicate to evaluate bone length, bone width, bone weight and bone chemical composition (ash weight, Ca, P and Mg). In the second phase (7 to 12 weeks) the stocking densities were 8 birds/m^2 (D1), 11 birds/m^2 (D2), 14 birds/m^2 (D3) and 17 birds/m^2 and 6 birds/m^2 (D1), 9 birds/m^2 (D2), 12 birds/m^2 (D3) and 15 birds/m^2 (D4) in the final phase (13 to 18 weeks). General Linear Model (GLM) procedure of Statistical Analysis System was used to estimate the differences between treatment means for different stocking densities. Stocking density in all the three phases did not have a significant ($p>0.05$) effect on bone dimensions and chemical composition. It is therefore concluded that stocking density had no influence on bone development of family chickens raised up to 18 weeks of age under intensive system probably due to slaughtering that occurred at six weekly intervals. It appeared that family chickens could be raised at a density of 15 birds/m^2 in winter without any detrimental effect on bone development and related parameters. Further studies should be done using identical densities throughout the research period to avoid disturbing the control which will make blocking by age possible.

Key words: Bone development, family chickens, intensive system, stocking density

INTRODUCTION

Stocking density is a housing variable that can affect chickens' development. Bone development is part of animal growth and the growth of the skeleton determines the size and proportions of the body (Yakubu *et al*., 2010; Buijs *et al*., 2012). Bone is a dynamic tissue that is influenced by physiological, nutritional and physical factors such as mechanical stress and physical activities (Rath *et al*., 2000). The deposition of bone is regulated primarily by parathyroid hormone, which is secreted in response to low serum calcium levels (Klein and Enders, 2010).

Several studies have been conducted to study the effect of stocking density on broiler production. According to Hall (2001), increased stocking density can negatively influence skeletal development of broilers, as shown by an increase in leg culls, which may be due to a decrease in activity as density increases. Skrbic *et al*. (2009) observed that providing more floor space per chicken influenced the level of physical activity, development and firmness of the skeleton, especially legs. The author noted that physical activity of broilers influenced cross section of the cortex and as a result improved their mechanical characteristics by better supply with blood of epiphysis of long bones and sufficient mineralization. In another study, Skrbic *et al*. (2011) observed that more physical activity of broilers in lower stocking density improved the parameters of tibia quality.

According to Sonaiya and Swan (2005), the term "family poultry" is defined as small-scale poultry rearing by households using family labour and locally available feed resources. Family poultry production systems of tropical regions are mainly based on family chickens found in nearly all villages and households in rural areas (Gueye, 1998). Generally, feeding, health control and housing are inadequate in family chicken rearing.

There is little information on how stocking density influences bone development in family chickens. The absence of stocking density standards for family chickens forces farmers to rely on personal experience in determining the space allowances and this may affect their productivity. Therefore, a study was undertaken to investigate the influence of stocking density on bone development of family chickens reared up to 18 weeks of age under intensive system.

MATERIALS AND METHODS

Study site: The experiment was carried out at the Guinea Fowl Unit of the Botswana College of Agriculture (BCA), Sebele for a period of 18 weeks. The site is at an

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altitude of 994 m above sea level and the coordinates are latitude 24°33' S and longitude 24°54' E (Aganga and Omphile, 2000). The experiment ran from April to August 2013. During the study period, environmental temperature averaged 21°C and ranged from 5 to 21°C.

Experimental design: A completely randomized design (CRD) with four treatments was used in the experiment. Each treatment was replicated four times. The four treatment levels were D1 (10 birds/m^2), D2 (13 birds/m^2), D3 (16 birds/m^2) and D4 (19 birds/m^2) in the first phase (i.e., 0 to 6 weeks). The experimental birds were distributed randomly among the four stocking densities. Two birds were slaughtered at 6, 12 and 18 weeks of age from each replicate. In the second phase (i.e., 7 to 12 weeks) the stocking densities were 8 birds/m^2 (D1), 11 birds/m^2 (D2), 14 birds/m^2 (D3) and 17 birds/m^2 and 6 birds/m^2 (D1), 9 birds/m^2 (D2), 12 birds/m^2 (D3) and 15 birds/m^2 (D4) in the final phase (i.e., 13 to 18 weeks).

Animal management: A total of 232 unsexed day-old family chicks were obtained from a farmer in Gaborone north and reared in a deep litter system. Initial body weights of the birds were determined by weighing 10% of the birds prior to allocation to four stocking densities. Birds were individually identified using wing bands. Chicks were housed under deep litter management system in an open-sided shed. The size of each pen was one metre squared (m^2). All pens were bedded with wood-shavings and equipped with one tube feeder and a 10 L waterer. Birds were raised under artificial light for the first two weeks to acclimatize birds to the experimental diets prior to collection of data and thereafter natural light throughout the study period. Feeds and water were provided *ad libitum* throughout the study period. Birds in each replicate were group fed. Chickens in each pen were individually weighed on a weekly basis.

Experimental diets: Birds were fed a commercial broiler starter crumbled diet up to 6 weeks of age, pelleted broiler grower diet from 7 to 12 weeks and pelleted broiler finisher diet from 13 to 18 weeks. Commercial broiler diets were purchased from some retail shops in Gaborone.

Data collection: At 6, 12 and 18 weeks of age, two birds from each replicate were sacrificed to determine bone dimensions and chemical composition. After slaughter carcasses were placed in plastic bags, identified and chilled to 0°C overnight in a cold room and bones removed 24 h post mortem. The right and left tibiae and the right humerus from each of the birds were removed and defleshed without boiling. Thereafter, bones were individually weighed using an electronic balance with a precision of 0.001 g, (Sartorius AG Germany, TE 313S model) and their widths and lengths determined using an electronic calliper with an accuracy of 0.001 cm, (Starrett® 798 B 12"/300 mm model). The left tibiae were used for bone chemical composition analysis (ash, Ca, P, Mg) and the right tibiae for bone dimensions. Bone samples were oven-dried in porcelain crucibles at 105°C for 48 h and weighed (Liu *et al*., 2003). Thereafter, bone samples were ashed in a muffle furnace at 550°C for 8 h. Approximately 1 g of ash samples was dissolved in 10 mL of 3M hydrochloric acid and boiled for 10 min. The samples were then allowed to cool and filtered into a 100 mL volumetric flask. Thereafter, the volume was topped up to 100 mL with distilled water and later analyzed for minerals according to AOAC (1996).

Statistical analysis: General Linear Model (GLM) Procedures of Statistical Analysis System (SAS Institute, 2009) version 9.2.1 was used to analyze the data according to the following statistical model:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

where, Y_{ij}: Response variables (bone width, bone length, bone weight and bone chemical composition). μ: General mean effect. τ_i: i^{th} stocking densities effects on family chickens' growth. Where, I = 1, 2, 3, 4. Where 1 = 10 birds/m^2, 2 = 13 birds/m^2, 3 = 16 birds/m^2, 19 birds/m^2. ε_{ij} = random error.

Least significant difference comparisons were made between treatment means using paired t-test and statistical significance was established at $p \leq 0.05$.

RESULTS AND DISCUSSION

Bone dimensions: Stocking density had no significant ($p>0.05$) influence on tibia and humerus length of family chickens (Tables 2 to 4). However, the longest tibia (78.41 mm) and humerus length (55.52 mm) was found in the stocking densities of 19 bird/m^2 at week 6, 14 bird/m^2 (131.33 and 85.61 mm) at week 12 and 9 bird/m^2 (149.67 and 93.14 mm) at week 18, respectively. These

Table 1: Chemical composition of experimental diets fed to family chickens from 0 to 18 weeks of age

Chemical composition	Broiler starter crumbles (0-6 wks)	Broiler grower pellets (7-12 wks)	Broiler finisher pellets (13-18 wks)
	Feed type and age of birds		
	Amount in g/kg		
Protein (min)	200.0	180.0	160
Moisture (max)	120.0	120.0	120.0
Fibre (max)	50.0	60.0	70.0
Calcium (min)	8.0	7.0	6.0
Calcium (max)	12.0	12.0	12.0
Fat (min)	25.0	25.0	25.0
Phosphorus (min)	6.0	5.5	5.0
Total lysine (min)	12.0	10.0	9.0

Source: OPTI Feeds Botswana (Pty) Ltd, 2014

Table 2: Least square means for bone dimensions of family chickens at 6 weeks of age reared under intensive system

Treatment	Tibia			Humerus		
Bird density/m^2	Length (mm)	Width (mm)	Weight (g)	Length (mm)	Width (mm)	Weight (g)
10	77.22[a]	5.25[a]	4.63[a]	55.07[a]	5.56[a]	2.50[a]
13	75.71[a]	4.80[a]	4.31[a]	54.02[a]	5.10[a]	2.63[a]
16	78.02[a]	5.10[a]	4.50[a]	54.91[a]	5.40[a]	2.31[a]
19	78.41[a]	4.86[a]	4.25[a]	55.52[a]	5.30[a]	2.06[a]
SE	1.74	0.21	0.48	1.13	0.15	0.21
p-value	0.7103	0.4103	0.9425	0.8211	0.2377	0.2865
CV	4.5045	8.2050	21.8815	4.1286	5.6659	17.3232

Means within the same column within a parameter not having similar superscripts are significantly different ($p<0.05$)
SE: Standard Error, CV: Coefficient of variation

Table 3: Least square means for bone dimensions of family chickens at 12 weeks of age reared under intensive system

Treatments	Tibia			Humerus		
Bird density/m^2	Length (mm)	Width (mm)	Weight (g)	Length (mm)	Width (mm)	Weight (g)
8	125.82[a]	7.88[a]	13.81[a]	83.54[a]	7.96[a]	5.69[a]
11	126.03[a]	7.89[a]	13.69[a]	81.18[a]	7.88[a]	5.75[a]
14	131.33[a]	8.41[a]	14.75[a]	85.61[a]	8.25[a]	6.44[a]
17	127.79[a]	8.46[a]	15.06[a]	83.48[a]	8.21[a]	6.63[a]
SE	2.16	0.31	0.88	1.58	0.13	0.45
p-value	0.2939	0.3983	0.6258	0.3141	0.1876	0.3773
CV	3.3858	7.5774	12.2578	3.7764	3.3032	14.6456

Means within the same column within a parameter not having similar superscripts are significantly different ($p<0.05$)
SE: Standard Error, CV: Coefficient of variation

Table 4: Least square means for bone dimensions of family chickens at 18 weeks of age reared under intensive system

Treatments	Tibia			Humerus		
Bird density/m^2	Length (mm)	Width (mm)	Weight (g)	Length (mm)	Width (mm)	Weight (g)
6	143.30[a]	9.39[a]	18.31[a]	90.05[a]	9.40[a]	8.38[a]
9	149.67[a]	10.11[a]	20.69[a]	93.14[a]	9.61[a]	8.75[a]
12	143.28[a]	9.68[a]	19.19[a]	88.91[a]	9.44[a]	8.44[a]
15	145.07[a]	9.53[a]	18.81[a]	88.93[a]	9.01[a]	8.44[a]
SE	2.39	0.38	1.11	1.34	0.21	0.49
p-value	0.2421	0.5895	0.4925	0.1376	0.2876	0.9478
CV	3.2834	7.9160	11.5248	2.9597	4.5447	11.5950

Means within the same column within a parameter not having similar superscripts are significantly different ($p<0.05$)
SE: Standard Error, CV: Coefficient of variation

results are consistent with those obtained by Oliveira *et al.* (2012) who found that the lengths of both tibiae and humeri were not affected by stocking density of 10 and 16 birds/m^2. In disagreement with current results, Buijs *et al.* (2012) found that increased stocking density (15.5, 18.5 and 21.8 birds/m^2) in broilers resulted in shorter tibiae. The variations in the results for the two studies may be due to the differences in genotype. In this study family chickens of slow growing genotype were used compared to broilers of fast growing genotype in the study of Buijs *et al.* (2012). This suggests that as broilers increase in body weight, the tibiae increase in width to support the muscle mass thus forcing them to curve. In this study, the length of tibia increased by 65 and 13.8% between 6 and 12 weeks and 12 and 18 weeks, respectively. On the other hand, humerus length increased by 52 and 8% between 6 and 12 weeks and 12 and 18 weeks, respectively. This indicates that bone development and growth in family chickens were most pronounced during the first 12 weeks, a similar observation was made by Moreki *et al.* (2011) who found that tibia and humerus length increased by 46 and 36% in broiler breeder pullets between 6 and 12 weeks, respectively. According to Ross Breeders (2006) the skeletal size of broiler breeder is fixed at 12 weeks of age. Rath *et al.* (2000) stated that the increment in bone length is correlated with an increase in the content of hydroxylysylpridinoline and lysylpyridinoline, the collagen crosslinks.

No significant influence of stocking density on tibial and humerus width of family chickens could be detected (Tables 2 to 4). However, the greatest tibial and humerus width was found in the stocking densities of 10 bird/m^2 (5.25 and 5.56 mm) at week 6, 17 bird/m^2 and 14 birds/m^2 (8.46 mm for tibia; 8.25 mm for humerus) at week 12 and 9 bird/m^2 (10.11 mm; 9.61 mm) at week 18, respectively. Similarly, Simsek *et al.* (2011) found that the width of tibia was not affected by stocking densities of 22.5, 18.75, 15, 11.25, 7.5 broilers/m^2. Ventura *et al.* (2010) also reported no influence of stocking density (8 birds/m^2, 13 birds/m^2 and 18 birds/m^2) on width of tibia in broilers. In disagreement with the present

Table 5: Least square means for bone chemical composition of family chickens at 6 weeks of age reared under intensive system

Treatments	Variables			
Bird density/m^2	Ash (g)	Ca (%)	P (%)	Mg (%)
10	0.84^a	32.68^a	24.23^a	0.82^a
13	0.79^a	32.83^a	24.25^a	0.83^a
16	0.86^a	32.94^a	24.35^a	0.90^a
19	0.80^a	32.84^a	24.27^a	0.85^a
SE	0.07	0.20	0.21	0.02
p-value	0.8236	0.8363	0.9760	0.7086
CV	16.1889	1.2104	1.6986	3.4858

Means within the same column within a parameter not having similar superscripts are significantly different ($p<0.05$)
SE: Standard Error, CV: Coefficient of variation

Table 6: Least square means for bone chemical composition of family chickens at 12 weeks of age reared under intensive system

Treatments	Variables			
Bird density/m^2	Ash (g)	Ca (%)	P (%)	Mg (%)
8	2.83^a	32.90^a	23.50^a	0.78^a
11	2.89^a	32.90^a	23.65^a	0.78^a
14	3.04^a	33.28^a	23.86^a	0.83^a
17	3.29^a	32.68^a	23.41^a	0.82^a
SE	0.16	0.27	0.21	0.03
p-value	0.2383	0.5097	0.4803	0.3244
CV	10.7187	1.6686	1.7801	6.3518

Means within the same column within a parameter not having similar superscripts are significantly different $p<0.05$
SE: Standard Error, CV: Coefficient of variation

Table 7: Least square means for bone chemical composition of family chickens at 18 weeks of age reared under intensive system

Treatments	Variables			
Bird density/m^2	Ash (g)	Ca (%)	P (%)	Mg (%)
6	4.66^a	34.19^a	24.52^a	0.76^a
9	5.16^a	34.23^a	24.55^a	0.79^a
12	4.81^a	34.31^a	24.76^a	0.81^a
15	4.72^a	34.25^a	24.57^a	0.79^a
SE	0.27	0.29	0.11	0.02
p-value	0.5812	0.9933	0.4586	0.2904
CV	11.17686	1.6891	0.9188	4.4908

Means within the same column within a parameter not having similar superscripts are significantly different ($p<0.05$)
SE: Standard Error, CV: Coefficient of variation

results, Oliveira *et al.* (2012) observed that the width of humerus in Ross 308 and Hybro PG broilers was affected by stocking density of 10 and 16 birds/m^2 at 42 days of age. The differences in the results of the current study and that of Oliveira *et al.* (2012) may be due to differences in birds' genotype. Several studies have observed that hybrid broilers raised intensively grow rapidly and that as they approach market age and weight, their bodies take up most of the allotted space, leaving no room to perform simple exercises which may lead to a decrease in bone mass of the wings (Lewis *et al.*, 1997; Jones, 2010). In this study, width of tibia increased by 63.1 and 18.6% between 6 and 12 weeks and 12 and 18 weeks, respectively, whereas humerus width increased by 51.2 and 16.0% between 6 and 12 weeks and 12 and 18 weeks, respectively. This suggests that in response to increased body weight, bones of the family chickens increased in bone width.

The weight of tibia and humerus was not significantly ($p>0.05$) influenced by stocking density (Table 2 to 4). In agreement with the current findings on tibia, Buijs *et al.* (2012) found that stocking density had no effect on tibia weight in broilers. Similarly, Oliveira *et al.* (2012) observed that weight of tibia and humerus in broilers was not affected by stocking density at 42 days of age.

Bone chemical composition: Stocking density had no significant ($p>0.05$) effect on chemical composition of bones (Table 5 to 7). The highest bone ash weight was found in the stocking densities of 16 bird/m^2 (0.86 g) at week 6, 17 bird/m^2 (3.29 g) at week 12 and 9 bird/m^2 (5.16 g) at week 18. In agreement with current results, Tablante *et al.* (2003) found that bone ash of broilers was not affected by stocking densities of 10, 15 and 20 birds/m^2. Although the mean weight of ash did not differ significantly among birds reared at different stocking densities bone weight increased with age. Similar observation was made by Moreki *et al.* (2011) who reported a significant increase in bone ash with age in broiler breeders up to 18 weeks of age. In the present study, the highest levels of Ca, P and Mg were observed at 16 birds/m^2 in week 6 (32.94, 24.35 and 0.90%), 14 birds/m^2 in week 12 (33.28, 23.86 and 0.83%) and 12 birds/m^2 in week 18 (34.31, 24.76 and 0.81%), respectively. The levels of Ca, P and Mg decreased from 6 to 12 weeks of age and from 12 to 18 weeks only Ca and P contents increased, whereas Mg content continued to decline. The decline of Ca and P from 6 to 12 weeks of age could be due to the birds increased demand for nutrients for increased muscle mass.

Conclusion: Bone dimensions and bone chemical composition were not influenced by stocking density. Therefore, it can be concluded that stocking density had no influence on bone development probably because of slaughtering that occurred at 6, 12 and 18 weeks of age. Further studies should be done using identical densities throughout the research period to avoid disturbing the control which will make blocking by age possible.

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