Protein Source and Nutrient Density in the Diets of Male Broilers from 8 to 21 Days of Age: Effects on Tibia Dimension, Breaking Strength, and Mineral Density

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The effects of supplemental protein source, and amino acid (AA) and apparent metabolizable energy (AME) densities in the diets of broilers on their growth performance and intestinal development were reported in companion studies. The effects of protein source, and AA and AME densities on the tibias of male Ross×Ross 708 broiler chicks were investigated in the current study. A completely randomized block design with a 2×2×2 factorial arrangement of treatments was applied (10 blocks, 8 treatments/block, 14 chicks/pen). Diets fed from 8 to 21 days of age (**d**) were formulated to contain two protein sources [high inclusion of distiller's dried grains with solubles diet (**hDDGS**) or high inclusion of meat and bone meal diet (**hMBM**)], two AA densities (moderate or high) and two AME densities (moderate or high). Tibias from 2 chicks/pen at 21 d were sampled. Chicks fed a high AA diet exhibited longer tibias than did chicks fed a moderate AA diet. Chicks fed hDDGS diets with a high AME density exhibited larger tibia circumferences as compared to those fed hMBM diets with a high AME density. Dietary treatment did not affect tibia breaking strength, ash content, or calcium, phosphorus, potassium, zinc, manganese, and magnesium concentrations in the tibia. Because feeding chicks high AME diets increased their BW, the weights of their tibia, and tibial calcium and phosphorus weights relative to BW were decreased. In birds fed high AA diets, the feeding of hMBM diets improved tibia breaking strength relative to BW when compared to the feeding of hDDGS diets. In conclusion, either hDDGS or hMBM diets can be fed to male broilers from 8 to 21 d without affecting mineral deposition in their tibias. In addition, high AME diets may improve broiler BW without affecting tibia strength.

Key words: amino acid, breaking strength, metabolizable energy, mineral density, tibia

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Introduction

High incidences of leg problems are being observed than ever before in commercial broilers (Thorp and Waddinton, 1997; Bessi, 2006). This may be attributed to an emphasis on the genetic selection of muscle tissue in modern broilers. In order to achieve increased production profits, selection has disproportionately increased rates of meat deposition when compared to that of increased bone growth (Julian, 1998; Bizeray *et al.*, 2000). This imbalance has resulted in increased incidences of skeletal abnormalities, such as tibial dyschondroplasia (Siller, 1970), rickets (Lian *et al.*, 1982), and tendon ruptures (Enwemeka, 1989). The incidences of

visible leg disorders in the broiler industry are between 2 to 3% (Oviedo-Rondon *et al.*, 2009). However, greater numbers of birds suffer from sub-clinical bone weakness (Oviedo-Rondon *et al.*, 2009). Those leg abnormalities limit the broiler chicken's access to feed and water, which results in decreased growth performance and meat quality, as well as increased mortality (Riddell and Springer, 1984; Pattison, 1992; Estevez, 2002).

Bone formation is the result of the interaction between bone osteoblast (synthesis) and osteoclast (decomposition) activities (Salgado *et al.*, 2004). Nutritional factors and local tissue stress may influence these processes (Lanyon, 1984; Sharir and Zelzer, 2011). Dietary calcium (**Ca**) (Clunies *et al.*, 1992), phosphorus (**P**) (Long *et al.*, 1984), vitamin D (Hauschka and Reid, 1978), and fluorine (Marie and Hott, 1986) are major nutritional factors that affect bone formation, with dietary levels of Ca being highly associated with its ash content (Sebastian *et al.*, 1996), density (Onyango *et al.*, 2003), and breaking strength (Skinner *et al.*, 1992). Ratios

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of Ca to P from 1.1:1 to 1.4:1 in poultry diets were recommended to maintain optimal meat and bone growth (Edwards and Veltmann, 1983; Qian *et al.*, 1997).

Dietary ingredients varying in mineral composition can have different effects on bone quality. Animal source ingredients, such as the meat and bone meal (MBM), contain high levels of available P (Jeng *et al.*, 2006) as well as other minerals. Hendriks *et al.* (2002) reported that the mean ash content of 94 MBM samples tested was 28.4%. In comparison, plant source ingredients contain a high percentage of phytate P, which cannot be utilized by poultry. In contrast to that of MBM, the ash content of distiller's dried grains with solubles (DDGS) is only 4.5% (Kim *et al.*, 2008). Therefore, different inclusion rates of the 2 protein sources in broiler diets may affect mineral absorption and utilization differently.

Even though dietary energy and protein levels have no notable effect on tibia breaking strength (Venalainen *et al.*, 2006), high nutrient densities may improve chicken growth and weight gain (Corzo *et al.*, 2004; Kidd *et al.*, 2004; Zhai *et al.*, 2013), thereby resulting in higher load weight capacities of their leg bones. Absolute bone breaking strength is positively related to body size (Leterrier and Nys, 1992; Shim *et al.*, 2012a, b), however, relative breaking strength, which is expressed as breaking strength per unit of BW, may decrease with increased body mass (Chen *et al.*, 2006; Shim *et al.*, 2012b). Furthermore, high nutrient density diets may affect relative leg bone quality and the walking ability of growing birds (Sorensen *et al.*, 1999; Su *et al.*, 1999; Kestin *et al.*, 2001). In a companion study, the feeding of high apparent metabolizable energy (AME) density diets to broiler chicks increased their BW gain (Wang *et al.*, 2014). In the current study, the effects of amino acid (AA) density, AME density, protein source, and their interactions on tibia breaking strength, dimension, and mineral density were determined.

Materials and Methods

Birds and Diets

A total of 1,120 male Ross × Ross 708 broiler chicks were randomly placed in 80 floor pens with 14 birds/pen in a broiler house with a controlled environment. The pens were grouped into 10 blocks that were evenly distributed throughout the broiler house, and each block served as one replicate unit. Detailed descriptions of the water, feed, light program, and pen facility management procedures were provided in a companion study by Wang *et al.* (2014). All birds were fed a common starter diet from 1 to 7 days of age (d) and experimental diets from 8 to 21 d. The 8 treatment diets, in a 2 × 2 × 2 factorial arrangement, were randomly assigned to 8 pens within each block. Each diet contained either a high inclusion of DDGS (hDDGS) or a high inclusion of MBM (hMBM), moderate or high AA densities, and moderate or high AME densities. The specific ingredients and nutrient composition of experimental diets are provided by Wang *et al.* (2014) (Table 1). All bird handling, management, and sampling procedures were approved by the

Institutional Animal Care and Use Committee of Mississippi State University.

Bone Morphology

Two chicks per pen were randomly selected, tagged, weighed, and euthanized using CO₂ at 21 d. Tibia bones were excised from the legs of each bird, were cleaned of adhering tissue and cartilage, and were weighed. Tibia lengths were measured from the proximal to the distal end of each bone, and circumferences were measured at their mid-points. Tibia volume was estimated by using circumference and length data ($\text{Volume} = \text{length} \times \text{circumference}^2 / 4\pi$). Tibia densities were calculated by dividing the dry tibia weight by the tibia estimated volume. All the tibiae were stored at −20°C for subsequent tests.

Bone Breaking Strength and Ash and Moisture Contents

Left tibias were equilibrated to room temperature before testing breaking strength. Breaking strength tests were carried out by a three-point-bending Instron 5544 System. All the broken pieces from each sample were wrapped with aluminum foil, placed on an aluminum tray, and dried in an oven at 105°C for 24 h. Dry bone weights and subsequent moisture weights were recorded. Percentage of tibia moisture was determined using the difference of fresh and dry bone weight. After allowing bones to reach room temperature in a desiccator, the bones were weighed, ashed in a muffle furnace at 600°C for 16 h, and weighed. Ash samples were stored in whirl-pack bags.

Bone Minerals

Bone ash samples were pulverized into powder form, digested in nitric acid, filtered, diluted, and analyzed by inductively coupled plasma emission spectroscopy (Bou *et al.*, 2004; Chen and Chen, 2004; Venalainen *et al.*, 2006; Tu *et al.*, 2009). Concentrations of Ca, P, potassium (K), zinc (Zn), manganese (Mn), and magnesium (Mg) in the bone ash were determined. Mineral weights were expressed as percentages of dry bone, fresh bone, and whole body weights. Mineral densities were calculated by dividing the mineral weight by the tibia estimated volume.

Statistical Analysis

A complete randomized block design was used with block serving as a unit of replication, and with all treatments being equally represented in each block. The eight treatments were arranged in a 2 × 2 × 2 factorial design structure. All parameters were analyzed using SAS version 9.2 (SAS Institute, 2010). A three-way ANOVA using the PROC MIXED procedure was used to determine the significance of responses to the protein source, AA, and AME densities, and their interactions. The protein source, AA and AME densities were designated as fixed effects and block as a random effect. Linear correlations between breaking strengths and bone traits (Ca, P, ash contents in fresh bone, bone weights, and moistures) and BW at 21 d were analyzed using the PROC CORR procedure. Global effects, differences among least squares means, and correlations were considered to be significant at $P \leq 0.05$.

Table 1. Feed ingredient composition and nutrient contents from 8 to 21 d of age¹

Item	hDDGS ²	hMBM	hDDGS	hMBM	hDDGS	hMBM	hDDGS	hMBM
	×M AA	×M AA	×H AA	×H AA	×M AA	×M AA	×H AA	×H AA
	×M AME	×M AME	×M AME	×M AME	×H AME	×H AME	×H AME	×H AME
Ingredient, %								
Corn	60.48	63.10	53.93	59.34	57.73	64.52	51.16	57.94
Soybean meal	27.93	25.16	33.46	30.23	28.39	24.92	33.93	30.46
DDGS	6.00	2.00	6.00	2.00	6.00	2.00	6.00	2.00
MBM	2.00	6.00	2.00	6.00	2.00	6.00	2.00	6.00
Poultry fat	0.620	0.000	1.660	0.000	2.940	0.750	3.97	1.80
Dicalcium phosphorus	0.620	0.000	0.590	0.000	0.633	0.000	0.600	0.000
Calcium carbonate	1.100	0.530	1.090	0.510	1.097	0.530	1.090	0.510
Salt	0.350	0.330	0.330	0.330	0.346	0.346	0.341	0.330
L-Lysine hydrochloride	0.322	0.342	0.308	0.336	0.315	0.350	0.300	0.332
Premix	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
DL-Methionine	0.163	0.174	0.189	0.196	0.165	0.173	0.190	0.197
Sand	0.000	1.934	0.000	0.634	0.000	0.000	0.000	0.000
L-Threonine	0.096	0.104	0.097	0.107	0.094	0.105	0.096	0.110
Monteban	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Ronozyme P ³	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020
Nutrient contents								
ME (kcal/kg)	2,998	2,998	2,998	2,998	3,100	3,100	3,100	3,100
CP, %	20.80	20.80	22.88	22.88	20.80	20.80	22.88	22.88
Crude fiber, %	3.11	2.87	3.09	2.92	3.05	2.91	3.02	2.86
Crude fat, %	3.48	3.05	4.34	2.97	5.68	3.85	6.55	4.71
Ca, %	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82
None-phytate P, %	0.34	0.40	0.34	0.41	0.34	0.40	0.34	0.41
Na, %	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21
Digestible Lysine, %	1.17	1.17	1.29	1.29	1.17	1.17	1.29	1.29
Digestible Methionine, %	0.46	0.46	0.50	0.50	0.46	0.46	0.50	0.50
Digestible TSAA, %	0.73	0.73	0.80	0.80	0.73	0.73	0.80	0.80
Digestible Threonine, %	0.76	0.76	0.84	0.84	0.76	0.76	0.84	0.84

¹ Feed ingredient composition and nutrient contents from 8 to 21 d of age table is cited from Wang *et al.* (2014).

² hDDGS=high inclusion of distiller's dried grains with soluble, hMBM=high inclusion of meat and bone meal, M stands for moderate, and H stands for high.

³ Ronozyme P=Ronozyme Phytase.

Results

Tibias were 0.12 cm longer in chicks fed high AA diets than those fed moderate AA diets (6.97 cm and 6.85 cm, respectively; $P=0.046$, Table 2). Tibias from chicks fed hDDGS diets were larger in circumference (0.12 cm) than those fed hMBM diets with high AME levels (2.49 cm and 2.37 cm, respectively; $P=0.016$). Tibias were heavier ($P=0.017$) from chicks fed hDDGS diets with high AA levels (6.03 g) than those from chicks fed hDDGS diets with moderate AA levels (5.62 g), hMBM diets with high AA levels (5.53 g), or hMBM diets with moderate AA levels (5.70 g).

No significant dietary treatment effects were observed for the breaking strength (Table 3), ash content, tibia density, or mineral concentrations (Ca, P, K, Mg, Mn, and Zn) of the dry bone, fresh bone, or whole body samples (data not shown). However, a high AME diet increased broiler chicken BW at 21 d by 27 g (794 g and 821 g, respectively; $P=0.038$; Table 3), which resulted in decreased relative weights of the tibia,

and decreased relative Ca and P weights, all as percentages of total BW ($P=0.012$, 0.024, and 0.014, respectively). When chicks were fed a high AA level diet, the inclusion of high MBM levels in their diets increased the breaking strength of their tibias relative to BW (22.81 kgf/kg and 20.25 kgf/kg, respectively; $P=0.043$; Table 3). Tibia and Ca densities were not affected by dietary treatment, however P density was lower ($P=0.008$) in chicks fed an hMBM diet with a high AA level and a high AME level (3.86 mg/cm³), when compared to those fed an hMBM diet with a moderate AA level and a high AME level (4.67 mg/cm³), or an hDDGS diet with a moderate AA level and a high AME level (4.60 mg/cm³).

Tibia breaking strength was positively correlated with the percentages of Ca, P, and ash in fresh bone samples, and with fresh bone, dry bone, and whole body weights, whereas tibia breaking strength was negatively correlated with tibia moisture (Table 4). Relative breaking strengths of tibiae were also positively correlated with the percentages of Ca, P, and ash in fresh bone samples.

Table 2. Effect of dietary protein source and nutrient density on chicken tibia dimension and fresh weight at 21 days of age^{1,2}

Protein source	Treatment		Length (cm)	Circumference (cm)	Estimated volume ³ (cm ³)	Fresh weight (g)
	AA level	AME level				
hDDGS			6.90	2.44	3.26	5.83
hMBM			6.91	2.42	3.21	5.61
SEM			0.043	0.025	0.082	0.089
	High		6.97 ^a	2.44	3.33	5.78
	Moderate		6.85 ^b	2.42	3.14	5.66
	SEM		0.044	0.025	0.082	0.093
		High	6.90	2.43	3.25	5.77
		Moderate	6.92	2.43	3.22	5.67
		SEM	0.044	0.026	0.082	0.093
hDDGS	High		7.00	2.46	3.43	6.03 ^a
hDDGS	Moderate		6.81	2.43	3.09	5.62 ^b
hMBM	High		6.93	2.41	3.24	5.53 ^b
hMBM	Moderate		6.90	2.42	3.19	5.70 ^b
SEM			0.056	0.035	0.116	0.125
hDDGS		High	6.85	2.49 ^a	3.32	5.87
hDDGS		Moderate	6.95	2.40 ^{ab}	3.20	5.78
hMBM		High	6.94	2.37 ^b	3.18	5.66
hMBM		Moderate	6.89	2.46 ^{ab}	3.24	5.56
SEM			0.056	0.035	0.116	0.125
	High	High	7.00	2.41	3.31	5.91
	High	Moderate	6.93	2.46	3.35	5.64
	Moderate	High	6.79	2.45	3.19	5.62
	Moderate	Moderate	6.91	2.40	3.09	5.70
	SEM		0.058	0.035	0.116	0.132
hDDGS	High	High	6.99	2.47	3.43	6.18
hDDGS	High	Moderate	7.00	2.45	3.41	5.88
hDDGS	Moderate	High	6.72	2.50	3.20	5.56
hDDGS	Moderate	Moderate	6.89	2.35	2.98	5.68
hMBM	High	High	7.01	2.35	3.18	5.65
hMBM	High	Moderate	6.85	2.48	3.29	5.41
hMBM	Moderate	High	6.86	2.40	3.18	5.68
hMBM	Moderate	Moderate	6.93	2.45	3.20	5.71
SEM			0.067	0.049	0.163	0.177
Source of variation (<i>P</i> -value)						
Protein source			0.802	0.417	0.689	0.077
AA			0.046	0.731	0.104	0.369
AME			0.665	0.932	0.808	0.468
Protein source × AA			0.125	0.504	0.214	0.017
Protein source × AME			0.187	0.016	0.445	0.958
AA × AME			0.087	0.128	0.526	0.201
Protein source × AA × AME			0.681	0.731	0.802	0.743

^{a-b} Means in a column for a given main effect or interaction not sharing a common superscript are different ($P \leq 0.05$).

¹ Observed means were calculated from 10 replicate values using the pen as the experimental unit.

² Two supplemented protein sources:

hDDGS=high inclusion of distiller's dried grains with soluble, hDDGS diets contained 6% DDGS and 2% MBM; hMBM=high inclusion of meat and bone meal, hMBM diets contained 2% DDGS and 6% MBM.

Moderate AA diets contained 1.17, 0.46, 0.73, and 0.76% of digestible lysine, methionine, TSAA, and threonine, respectively.

High AA diets contained 1.29, 0.50, 0.80, and 0.84% of digestible lysine, methionine, TSAA, and threonine, respectively.

Moderate AME=2,998 kcal/kg.

High AME=3,100 kcal/kg.

³ Estimated volume=length × circumference²/4[π].

Table 3. Effects of dietary protein source and nutrient density on BW, relative weight and density of tibia and minerals, and relative breaking strength at 21 days of age^{1,2}

Treatment			Relative weight ³						Density (mg/cm ³)		
Protein source	AA level	AME level	BW (g)	Tibia (mg/g)	Ca (μg/g)	P (μg/g)	BS ⁴ (kgf)	RBS ⁵ (kgf/kg)	Tibia	Ca	P
hDDGS			808	2.79	31.5	15.7	17.72	21.31	74.1	9.04	4.46
hMBM			807	2.85	31.7	15.8	17.80	22.28	73.2	8.46	4.22
SEM			8.1	0.047	0.08	0.04	0.456	0.538	2.58	0.301	0.138
	High		805	2.87	32.3	16.0	17.70	21.52	71.8	8.45	4.21
	Moderate		811	2.78	30.9	15.4	17.82	22.06	75.4	9.05	4.48
	SEM		7.8	0.046	0.09	0.04	0.456	0.538	2.76	0.296	0.131
		High	821 ^a	2.75 ^b	30.4 ^b	15.1 ^b	17.92	21.68	73.8	8.83	4.39
		Moderate	794 ^b	2.90 ^a	32.8 ^a	16.3 ^a	17.60	22.91	73.4	8.68	4.30
		SEM	8.4	0.047	0.08	0.04	0.489	0.520	2.65	0.305	0.139
hDDGS	High		805	2.87	32.1	15.9	17.20	20.25 ^b	72.3	8.77	4.32
hDDGS	Moderate		811	2.71	30.9	15.4	18.23	22.38 ^{ab}	75.8	9.30	4.60
hMBM	High		804	2.87	32.5	16.2	18.20	22.81 ^a	71.4	8.13	4.10
hMBM	Moderate		810	2.84	30.8	15.4	17.41	21.75 ^{ab}	75.1	8.80	4.35
SEM			10.6	0.060	0.11	0.05	0.620	0.760	3.44	0.400	0.172
hDDGS		High	815	2.72	30.5	15.1	18.35	21.76	72.6	9.21	4.55
hDDGS		Moderate	801	2.87	32.5	16.2	17.09	20.87	75.5	8.86	4.38
hMBM		High	827	2.78	30.3	15.1	17.49	21.60	75.0	8.44	4.23
hMBM		Moderate	788	2.93	33.1	16.5	18.12	22.96	71.4	8.49	4.22
SEM			11.1	0.062	0.11	0.05	0.540	0.736	3.37	0.410	0.183
	High	High	821	2.78	30.6	15.2	17.38	20.80	73.5	8.34	4.14
	High	Moderate	789	2.96	33.9	16.9	18.03	22.26	70.2	8.56	4.27
	Moderate	High	822	2.72	30.2	15.1	18.47	22.55	74.2	9.31	4.64
	Moderate	Moderate	800	2.84	31.6	15.7	17.18	21.57	76.7	8.79	4.32
	SEM		11.1	0.060	0.11	0.05	0.645	0.736	3.5	0.541	0.223
hDDGS	High	High	808	2.79	31.1	15.3	17.23	20.09	74.7	9.06	4.43 ^{ab}
hDDGS	High	Moderate	802	2.95	33.1	16.5	17.18	20.41	69.9	8.48	4.21 ^{ab}
hDDGS	Moderate	High	822	2.64	30.0	15.0	19.48	23.42	70.5	9.37	4.67 ^a
hDDGS	Moderate	Moderate	800	2.78	31.9	15.9	16.98	21.32	81.1	9.24	4.54 ^{ab}
hMBM	High	High	833	2.77	30.2	15.0	17.53	21.52	72.2	7.62	3.86 ^b
hMBM	High	Moderate	775	2.97	34.8	17.4	18.87	24.11	70.5	8.64	4.34 ^{ab}
hMBM	Moderate	High	821	2.79	30.3	15.2	17.46	21.68	77.9	9.25	4.60 ^a
hMBM	Moderate	Moderate	800	2.89	31.3	15.6	17.37	21.81	72.2	8.35	4.10 ^{ab}
SEM			15.0	0.081	0.14	0.07	0.877	1.040	4.54	0.540	0.223
Source of variation (<i>P</i> -value)											
Protein source			0.954	0.271	0.890	0.805	0.885	0.212	0.780	0.183	0.208
AA			0.558	0.081	0.259	0.293	0.842	0.489	0.345	0.136	0.117
AME			0.038	0.012	0.024	0.014	0.647	0.742	0.903	0.903	0.635
Protein source×AA			0.993	0.231	0.761	0.594	0.134	0.043	0.977	0.977	0.910
Protein source×AME			0.254	0.958	0.676	0.757	0.118	0.122	0.299	0.299	0.624
AA×AME			0.616	0.553	0.241	0.167	0.110	0.095	0.333	0.333	0.099
Protein source×AA×AME			0.152	0.683	0.255	0.264	0.671	0.992	0.108	0.270	0.008

^{a-b} Means in a column for a given main effect or interaction not sharing a common superscript are different ($P \leq 0.05$).

¹ Observed means were calculated from 10 replicate values using the pen as the experimental unit.

² Two dietary protein sources:

hDDGS=high inclusion of distiller's dried grains with soluble, hDDGS diets contained 6% DDGS and 2% MBM; hMBM=high inclusion of meat and bone meal, hMBM diets contained 2% DDGS and 6% MBM.

Moderate AA diets contained 1.17, 0.46, 0.73, and 0.76% of digestible lysine, methionine, TSAA, and threonine, respectively.

High AA diets contained 1.29, 0.50, 0.80, and 0.84% of digestible lysine, methionine, TSAA, and threonine, respectively. Moderate AME=2,998 kcal/kg.

High AME=3,100 kcal/kg.

³ Relative weight was defined as mg of bone or mineral in one tibia bone per g of BW. Ca=calcium, P=phosphorus.

⁴ BS=breaking strength.

⁵ RBS=relative breaking strength, which was defined as breaking strength per kg of BW.

Table 4. Pearson correlation coefficients (r) and probability values (in parentheses) between bone breaking strength and bone traits and BW at 21 days of age

	Ca ¹ (%)	P ¹ (%)	Ash ¹ (%)	Moisture (%)	Fresh bone weight (g)	Dry bone weight (g)	BW (kg)
Breaking strength (kgf)	0.253 (0.031)	0.250 (0.033)	0.183 (0.026)	-0.234 (0.001)	0.207 (0.011)	0.280 (0.001)	0.312 (<0.001)
Relative breaking strength (kgf/kg) ²	0.291 (0.014)	0.281 (0.018)	0.667 (0.001)	0.198 (0.086)	0.066 (0.574)	0.218 (0.058)	0.073 (0.534)

¹ Ca=calcium, P=phosphorus. Ca, P, and ash content represented as percentage in fresh bone.

² Relative breaking strength=breaking strength/BW.

Discussion

The results of the current study showed that the tibias of broiler chicks fed a higher AA diet from 8 to 21 d were longer than those fed diets with lower AA levels. It has been reported that longer tibiae in chickens are associated with a larger body size (Leterrier and Nys, 1992). However, in the current study, high AA diets increased tibia length without increasing BW at 21 d. Conversely, high AME diets increased BW at 21 d without increasing tibia size. Therefore, the longer tibiae of chickens that resulted from the feeding of high AA density diets may not necessarily be due to an increase body size, but may be due to a direct effect of AA density on bone growth. Particularly, when chicks were fed hDDGS diets with a higher AA level, their tibias were heavier. Few research is available on the effects of dietary AA density or protein level on bone quality. However, early research in humans has shown that high dietary protein intake can improve bone mineral content (Metz *et al.*, 1993; Kerstetter *et al.*, 2003; Chevalley *et al.*, 2008). This suggests that a high protein intake may be beneficial to bone quality in animals. Furthermore, Bonjour *et al.* (2001) reported that high intake levels of dietary protein up-regulates insulin growth factor I expression in rats, which is a major factor responsible for the longitudinal bone growth. In the current trial, the high AA diets contained higher protein levels (22.88% CP vs 20.80% CP, Wang *et al.*, 2014). The intake of these higher protein levels subsequently may have increased tibial elongation in the chicks.

When chicks were fed an hDDGs diets with high AME levels, their tibias were greater in circumferences than those of chicks fed an hMBM diet. The observed relationship between fiber intake and bone circumference is unclear, nevertheless, it has been reported that moderate fiber intake in animals benefits the intestinal environment, lowers serum LDL-cholesterol, and decreases their mortality (Gomez-Conde *et al.*, 2007; Anderson *et al.*, 2009; Jimenez-Moreno *et al.*, 2009a). In addition, Jimenez-Moreno *et al.* (2009a) reported that moderate dietary fiber levels increased the production of HCl in the proventriculus, which may improve the solubility and absorption of mineral salts. It has been

further reported that dietary fiber inclusion can improve the retention of soluble ash in the gastrointestinal tract (Jimenez-Moreno *et al.*, 2009b). Therefore, the fiber in DDGS may have improved mineral absorption and deposition and thereby increased tibial circumference.

Bone breaking strength, ash content, tibia density, and mineral percentages (Ca, P, K, Mg, Mn, and Zn) were not affected by the dietary treatments. The mineral composition and gastrointestinal track retentions of different dietary ingredients may vary (Oso *et al.*, 2011). However, in the current trial, dietary mineral levels were formulated to meet or exceed requirements in all the diets. Because the nutrient levels used met the growth requirements of the chicks, those fed an hMBM diet were provided the same type and amounts of minerals for deposition in their tibias as those fed an hDDGS diet.

It has been shown that the density and mineral content of leg bones of fast growing broilers are lower (Williams *et al.*, 2000, 2004; Yalcin *et al.*, 2001). In the present trial, BW was increased in chicks fed a high AME diet (Table 3). Tibia bone, tibial Ca, and tibial P weights relative to BW were decreased with increasing dietary AME level. These results of the current study are, therefore, consistent with those of the previous studies. Even though bone breaking strength may not be necessarily decreased with increased BW, fast growing birds may have a higher incidence of tibial dyschondroplasia because of a larger loading weight that they must bear (Shim *et al.*, 2012a). The current results also indicate that high energy diets may improve chicken muscle deposition without equally improving bone quality.

In addition, relative breaking strength (breaking strength to BW ratio) was affected by an interaction between dietary AA level and diet type. When fed a high AA diet, chicks fed an hMBM diet exhibited relatively stronger tibias in proportion to whole body weight. The MBM contains higher available phosphorus levels than DDGS. The P levels were formulated so as to be at the same level in all the diets of the current trial. In order to maintain equal P levels among the diets, dicalcium phosphate was added to the hDDGS diets (Wang *et al.*, 2014). It has been reported that the tibial ash content in broiler chickens fed diets with MBM was higher

than those fed diets with dicalcium phosphate (Waldroup and Adams, 1994). Other minerals or ingredients in addition to Ca and P in the MBM may have beneficial effects on bone mineral deposition, with a subsequent strengthening effect on the tibias.

Tibial P density was affected by a 3-way interaction between protein source and nutrient density. Use of the higher AA levels in the diets of chicks fed an hMBM diet with high AME levels decreased tibial P density. In mammals, high protein intake may increase bone mineral loss due to urinary excretion (Orwoll *et al.*, 1990; Dawson-Hughes and Harris, 2002). For birds, high AA intakes may impose a chronic metabolic acid load, which may lead to bone mineral loss (Riond, 2001). The decrease in P density in birds fed high AA level diets in the current trial may be partly due to a loss in P.

Both absolute breaking strength and relative breaking strength were positively correlated with Ca, P, and ash content in the bone, which is in agreement with earlier research (Bell *et al.*, 1941; Crenshaw *et al.*, 1981; Wilson, 1991; Orban *et al.*, 1993; Onyango *et al.*, 2003). It is generally accepted that dietary Ca and P levels affect ash content in bone (Clunies *et al.*, 1992; Viveros *et al.*, 2002; Onyango *et al.*, 2003; Powell *et al.*, 2011), which may subsequently influence bone breaking strength. In the current trial, BW was positively correlated with absolute bone breaking strength, but was not correlated with relative breaking strength (bone breaking strength to BW). Although it is expected that larger broilers should also experience some increase in bone strength, it is apparent that the increase in bone strength does not exhibit a sufficient proportional increase in order to adequately support the weight of modern strain broilers.

In conclusion, either hDDGS or hMBM may be used in male broiler diets from 8 to 21 d without affecting mineral deposition in their tibias. In addition, high AME levels in diets improved broiler BW without affecting tibia strength at 21 d.

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