Increasing dietary vitamin D_3 improves the walking ability and welfare status of broiler chickens reared at high stocking densities

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ABSTRACT A study was conducted to evaluate the effects of varying dietary vitamin D_3 and stocking density on growing performance, carcass characteristics, bone biomechanical properties, and welfare responses in Ross (308) broilers. Experimental diets, containing 1, 10, or 20 times the NRC recommended level of vitamin D_3 (200 IU/kg), were formulated with low, medium, or high vitamin D_3 levels for 3 growing phases. Two stocking densities were 10 and 16 birds/m^2. One-day-old hatchlings (1,872 males) were randomly assigned to 6 pens in each treatment. Results showed that high stocking density decreased the feed intake, BW gain ($P < 0.01$), breast muscle yield ($P = 0.010$), and tibial development ($P < 0.01$), whereas increasing feed conversion ratio ($P < 0.001$), and the scores of gait, footpad and hock burn, and abdominal plumage damage ($P < 0.01$), particularly toward the age when birds attained their market size. Increasing dietary vitamin D_3 improved the birds' walking ability and tibial quality ($P < 0.05$), and reduced the development of footpad or hock dermatitis and abdominal plumage damage ($P < 0.01$), some aspects of which were age-dependent and appeared to vary with stocking density. These data indicate that increasing supplemental vitamin D_3 has a favorable effect on walking ability and welfare status of high stocking density birds, but not on performance.

Key words: stocking density, vitamin D_3, walking ability, welfare status, broiler chicken

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INTRODUCTION

Rapid muscle growth in broiler chickens through genetic selection causes imbalance between meat production and skeletal growth, resulting in abnormal skeletal development (Oviedo-Rondon et al., 2006). Leg problems and skeletal disorders, such as lameness, twisted legs, tibial dyschondroplasia, and crooked toes are the common causes of culling and mortality in broiler chickens (Bradshaw et al., 2002; Oviedo-Rondon et al., 2006). These problems are likely to worsen when birds are reared at high stocking densities (**HSD**), as shown by an increase in leg culls (Hall, 2001) and leg deformities (Grashorn and Kutritz, 1991). Leg weakness is painful or decreases walking ability (Bradshaw et al., 2002), and thus constitutes a welfare problem. A long time spent sitting on wet litter may cause breast and leg skin lesions, which further aggravates the deterioration of flock well-being (Bokkers and Koene, 2004; Bessei, 2006). A causal interrelationship among the HSD, low locomotor activity, and leg problems has been discussed for modern broilers in current literature (Arnould and Faure, 2003; Dozier et al., 2005).

One strategy that can be used to prevent leg problems and skeletal disorders is to stimulate bone growth and strength in the early growth period. One potential nutrient or bioactive molecule for achieving this is vitamin D_3. In contrast with all other vitamins, vitamin D_3 is endogenously produced in the skin exposed to UV light, and several studies indicated that UV light is effective in reducing leg problems in chickens (Elliot and Edwards, 1997; Edwards, 2003). Supplying high levels of vitamin D_3 in broiler diets decreased the severity of lameness by reducing tibial dyschondroplasia, Ca rickets, and other leg abnormalities (Whitehead et al., 2004; Atencio et al., 2005; Driver et al., 2006), in particular under harsh environmental conditions, such as heat stress (Nääs et al., 2012). Little is known, however, if the deleterious effect of HSD on the leg abnormalities has a relationship with dietary vitamin D_3. From the foregoing, we hypothesized that supplemental vitamin D_3 would improve the bone quality and welfare status of broiler chickens stocked at high densities.

The primary objective of the present study was to verify the effectiveness of feeding high levels of vitamin

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D_3 in reducing lameness and skeletal lesions for fast-growing broilers reared at high densities. Additionally, live performance, carcass composition, welfare status, and blood parameters related to calcium and phosphate absorption were determined.

MATERIALS AND METHODS

Birds and Management

One-day-old Ross (308) broiler hatchlings, 1,872 males, were used. Chickens were reared in an environmentally controlled room with 36 floor pens (2 m × 2 m). Rice hulls (5 cm in depth) served as litter. The brooding temperature was originally maintained at 34°C (50% RH), and then decreased by 2°C per week to 26°C (45% RH) and was maintained thereafter. The lighting schedule consisted of 22 h of light for the first day, 19 h from d 2 to 3, 16 h from d 4 to 6, 14 h from d 7 to 12, 12 h from d 13 to 22, 15 h from d 23 to 30, 16 h from d 31 to 36, and 17 h from d 37 to 42. Glass windows of the chicken room were completely covered with opaque plastic sheets, and all fluorescent fixtures in the room were covered with plastic sleeves (Arm-a-Lite Thermoplastic Processes, Sterling, NJ) to eliminate UV light exposure and any possible de novo vitamin D_3 synthesis by the chickens (Edwards et al., 1994; Elliot and Edwards, 1997; Mitchell et al., 1997; Edwards, 2003). The whole experimental period was divided into 3 phases: starter (1 to 21 d), grower (22 to 35 d), and finisher (36 to 42 d). Each pen was equipped with tube feeders and nipple water drinkers. Chickens had free access to mash feed and water throughout. All procedures used in this study were approved by the Animal Care Committee of Shandong Agricultural University (P. R. China) and were carried out in accordance with the guidelines for experimental animals of the Ministry of Science and Technology (P. R. China).

Experimental Design

The experimental treatments were arranged in a 2 × 3 factorial completely randomized design, with 2 levels of stocking density (10, low stocking density, **LSD**, and 16 chickens/m^2, HSD, with 40 and 64 birds/pen, respectively) and 3 levels of dietary vitamin D_3 supplementation. Six replicates of each combination treatment were used, with birds maintained under experimental conditions from 1 to 42 d of age.

The NRC nutrient values for ingredients (NRC, 1994) were used to formulate a corn-soybean meal basal diet that contained soybean oil as the fat source. A basal D_3-deficient diet (Table 1) was supplemented with vitamin D_3 (3 levels) with the first one containing the NRC (1994) recommended level (200 IU/kg) and the other 2 containing 10 and 20 times the NRC recommended vitamin D_3 level. Crystalline vitamin D_3 (Sigma Chemical Co., St. Louis, MO.) was dissolved in 10 mL of ethanol then further diluted with 190 mL of propylene glycol to produce a stock solution of 1.25 mg/mL. This was then added to the basal diet at 5, 50, and 100 μg of D_3/kg of diet, respectively, to create low (200 IU/kg of diet, **LVD**), medium (2,000 IU/kg, **MVD**), and high (4,000 IU/kg, **HVD**) vitamin D_3 treatments.

Data Collection

Growth Performance. At the end of each phase, feed intake (**FI**) and BW gain were recorded on a per-pen basis, and feed conversion ratio (**FCR**) was calculated.

At 42 d of age, after a 12-h overnight fast, 3 randomly selected birds from each pen were killed by CO_2 stunning. The abdominal fat, breast (both the pectoralis major and minor), and deboned thigh muscles were harvested and weighed individually, then expressed as percentages of BW.

Walking Ability, Footpad and Hock Burns, and Abdominal Plumage Damage. At the end of each period, all chickens in each pen were examined for walking ability, footpad and hock burns, and condition of the abdominal plumage. Gait scoring was based on a modification of the method of Garner et al. (2002). Birds were subjectively assigned values of 0 (normal gait), 1 (impaired movement), or 2 (reluctant step). Normal was defined as no impairment of walking ability. Birds could walk at least 1.5 m with a balanced gait. Impaired was defined as an obvious impairment of walking. Birds could walk at least 1.5 m but showed an unbalanced gait. Reluctant was defined as having severe impairment of walking ability causing unwillingness to stand and walk.

Footpad and hock burns were scored on a subjective 4-point scale following Bilgili et al. (2006): 0 (no lesion), 1 (mild lesion, less than 0.75 cm in diameter), 2 (greater lesion, less than 1.5 cm), or 3 (severe lesion, more than 1.5 cm). The condition of plumage in the abdominal area was assessed with a modified method of Tauson (1984). An integer score of 0 to 3 was used, where 0 indicated no damage, 1 meant mild damage (less than 1/3 of total area), 2 was greater damage (more than 1/2) with mild breast blisters, and 3 corresponded to severe damage (more than 2/3) with greater breast blisters.

Plasma and Tibial Quality Variables. At 21, 35, and 42 d of age, 3 randomly selected birds from each pen were blood sampled (wing vein, heparinized syringe) then killed by CO_2 stunning. Plasma, obtained by centrifugation at 400 × g for 10 min at 4°C, was stored at −20°C. The left and right tibias from individual birds were excised, sealed in plastic bags, and stored at −20°C for further analysis.

Plasma calcium and phosphorus levels were determined from 4-mL aliquots of ready samples using standard colorimetric methods. Commercial kits (Kone Commercial Kit, Kone Instruments Corp., Espoo, Finland) was used for measurement of Ca by means of a flame atomic absorption spectrophotometer (Shimadzu AA-680, Shimadzu Corporation, Tokyo, Japan) and de-

Table 1. Ingredients and nutrient composition of experimental diets (air-dry basis)

Item	Starter (1 to 21 d)	Grower (22 to 35 d)	Finisher (36 to 42 d)
Ingredient, %			
Corn	57.64	61.37	66.42
Flour (special grade 2)	3.00	5.00	5.00
Soybean oil	1.49	2.21	2.59
Soybean meal (46% CP)	26.7	16.3	9.10
Peanut meal (46% CP)	3.00	4.00	5.00
Cottonseed meal (40% CP)	2.00	3.00	4.00
Corn protein powder (60% CP)	2.00	4.00	4.00
Salt	0.31	0.36	0.36
Limestone	1.47	1.36	1.25
Calcium phosphate	1.63	1.47	1.28
Choline chloride (60%)	0.10	0.08	0.06
L-Lys-H_2SO_4	0.23	0.43	0.53
DL-Met	0.14	0.11	0.09
L-Thr	0.04	0.06	0.07
Vitamin premix[1]	0.05	0.05	0.05
Mineral premix[2]	0.20	0.20	0.20
Total	100	100	100
Nutrient composition			
CP, %	21.0	19.0	17.0
ME, kcal/kg	2,950	3,075	3,150
Ca, %	1.00	0.90	0.80
Total P, %	0.65	0.60	0.55
Available P, %	0.40	0.36	0.32
Total Lys, %	1.12	1.00	0.90
Digestible Lys, %	1.00	0.90	0.80
Total Met, %	0.58	0.48	0.39
Digestible Met, %	0.54	0.45	0.36
TSAA, %	0.93	0.80	0.69
Digestible TSAA, %	0.82	0.71	0.60
Total Thr, %	0.85	0.74	0.64
Digestible Thr, %	0.73	0.63	0.54
Total Trp, %	0.24	0.21	0.18
Digestible Trp, %	0.20	0.18	0.15

[1]Vitamin premix provided the following per kilogram of diet: retinyl acetate, 9,000 IU; vitamin E, 11.0 IU; vitamin K, 1.00 mg; thiamine, 1.20 mg; riboflavin, 5.80 mg; niacin, 66.0 mg; pantothenic acid, 10.0 mg; pyridoxine, 2.60 mg; biotin, 0.10 mg; folic acid, 0.70 mg; vitamin B_{12}, 0.012 mg.

[2]Mineral premix provided the following per kilogram of diet: manganese, 100 mg; zinc, 75.0 mg; iron, 80.0 mg; iodine, 0.65 mg; copper, 80.0 mg; selenium, 0.35 mg.

termination of P by an autoanalyzer (ALCYON-300, Autoanalyser, Abbott Park, IL) using the ammonium molybdate procedure.

For determination of tibial weight, fat was first removed from the left tibias by a 36-h Soxhlet extraction in ethyl alcohol, followed by a 36-h extraction with di-

Table 2. Model and ANOVA results for growth performance of broiler chickens[1]

		Feed intake, g/d		BW gain, g/d		FCR, feed (g)/gain (g)	
Source of variation	df[2]	*F*	*P*	*F*	*P*	*F*	*P*
Between-subjects							
Density[3]	1	9.47	0.004	13.3	0.001	60.6	<0.001
D_3[4]	2	0.17	NS	0.29	NS	5.14	0.012
Density × D_3	2	0.01	NS	0.02	NS	0.63	NS
Error	30						
Within-subjects							
Age[5]	2	2,171	<0.001	1,449	<0.001	4,330	<0.001
Density × age	2	15.8	<0.001	43.3	<0.001	40.0	<0.001
D_3 × age	4	1.04	NS	3.32	0.016	3.85	0.008
Density × D_3 × age	4	0.22	NS	0.27	NS	0.54	NS
Error	60						
Total	107						

[1]FCR, feed conversion ratio; NS, $P > 0.05$.

[2]Degrees of freedom were the same for all variables.

[3]Density effect (high and low stocking densities).

[4]Vitamin D_3 effect (high, medium, and low vitamin D_3 diets).

[5]Age effect (starter, grower, and finisher periods).

Table 3. Growth performance of broiler chickens (mean ± SEM) according to stocking density or dietary vitamin D_3 across age[1]

	Stocking density			Vitamin D_3			
Variable	HSD	LSD	SEM	HVD	MVD	LVD	SEM
Feed intake, g/d							
Starter	58.1^{z}	59.0^{z}	0.91				
Grower	$145^{b,y}$	$155^{a,y}$	3.78				
Finisher	$183^{b,x}$	$207^{a,x}$	6.54				
BW gain, g/d							
Starter	40.4^{y}	41.3^{z}	0.88	41.0^{y}	40.7^{y}	41.0^{z}	1.27
Grower	$75.0^{b,x}$	$81.2^{a,y}$	2.62	77.8^{x}	79.0^{x}	77.5^{y}	4.29
Finisher	$73.0^{b,x}$	$89.2^{a,x}$	2.77	78.0^{x}	81.4^{x}	84.0^{x}	5.83
Feed conversion ratio, feed (g)/gain (g)							
Starter	1.44^{z}	1.43^{z}	0.01	1.44^{z}	1.43^{z}	1.43^{z}	0.01
Grower	1.94^{y}	1.92^{y}	0.02	1.94^{y}	1.90^{y}	1.94^{y}	0.04
Finisher	$2.50^{a,x}$	$2.33^{b,x}$	0.02	$2.46^{a,x}$	$2.41^{ab,x}$	$2.38^{b,x}$	0.05

[a,b]Means without a common superscript within a row differ significantly ($P < 0.05$).

[x–z]Means without a common superscript within a column differ significantly ($P < 0.05$).

[1]HSD and LSD: high and low stocking densities; HVD, MVD, and LVD: high, medium, and low vitamin D_3 diets.

ethylether, and then dried at 100°C for 24 h. Tibial ash content was determined by ashing the bones in a muffle furnace for 18 h at 600°C. Calcium was determined by atomic absorption in GBC equipment (932AA, Analitica, Sao Paulo, Brazil), whereas phosphorus was measured spectrophotometrically (B-395, Micronal, Sao Paulo, Brazil).

The right tibia was defleshed by hand, and the patella was removed. The breaking strength of the fresh bone was determined with an All-Digital Electronic Universal Testing Machine (Shenzhen Hengen Instrument Co. Ltd., Shenzhen, China) fitted with a 3-point-bend rig with a load cell capacity of 50 kg, a crosshead speed of 10 mm/min, and a span over which the bone was set of 40 mm.

Statistical Analysis

Data were analyzed using the Statistical Analysis Software (version 8e, SAS Institute Inc., Cary, NC), and bird data were averaged by pen. Prior to analysis, all data were examined for normality by examination of histograms and normal distribution plots (UNIVARIATE procedure).

Mean values per pen and per period for FI, BW gain, FCR, walking ability, footpad and hock burn scores, abdominal plumage condition, tibial quality indices, and plasma measurements were calculated before analysis, and the pen was considered the experimental unit. Data were analyzed using the MIXED procedure of SAS, and the time effect was also evaluated, performing a repeated measurements analysis. Fixed effects were stocking density, vitamin D_3, and their interactions. Gait and lesion scores were square root transformed before analysis to satisfy the ANOVA requirements for normality and homogeneity of variance. Models were rerun using raw data to obtain least squares means for presentation, but P-values were calculated using transformed data.

For the remaining variables (namely, final BW, and processing yield), a 2-way ANOVA model was used to estimate the main effects of stocking density and vitamin D_3, as well as their interactions on a per-pen basis. Comparisons between means used Duncan's tests, and differences were considered significant at $P < 0.05$.

RESULTS

Production Performance

As stocking density increased, both FI and BW gain were decreased, whereas FCR was increased (Table 2). Overall, FCR was significantly higher with the HVD diet than with the LVD treatment (Table 2). A significant interaction between stocking density and age was observed for growth performance of broiler chickens

Table 4. Final BW and carcass characteristics of broiler chickens (mean ± SEM) according to stocking density (STD) and dietary vitamin D_3 (VD)[1]

	High stocking density			Low stocking density			P-value			
Variable[2]	HVD	MVD	LVD	HVD	MVD	LVD	STD	VD	STD × VD	SEM
Final BW, g	2,357	2,401	2,378	2,575	2,607	2,626	0.006	NS	NS	228
Breast muscle, %	18.5	18.0	18.0	19.0	18.9	18.5	0.010	NS	NS	0.65
Thigh muscle, %	15.6	15.4	15.6	15.8	15.7	15.5	NS	NS	NS	0.33
Abdominal fat, %	2.21	2.19	2.20	2.22	2.29	2.22	NS	NS	NS	0.15

[1]HVD, MVD, and LVD: high, medium, and low vitamin D_3 diets; NS: $P > 0.05$.

Table 5. Model and ANOVA results for walking ability and welfare status of broiler chickens[1]

		Gait score		Hock burn score			Footpad burn score		Abdominal plumage score	
Source of variation	df[2]	*F*	*P*	*F*	*P*	df[2]	*F*	*P*	*F*	*P*
Between-subjects										
Density[3]	1	36.6	<0.001	173	<0.001	1	192	<0.001	110	<0.001
D_3[4]	2	4.80	0.016	5.44	0.010	2	6.23	0.006	6.32	0.005
Density × D_3	2	6.97	0.003	8.64	0.001	2	7.80	0.002	4.94	0.014
Error	30					30				
Within-subjects										
Age[5]	1	171	<0.001	49.0	<0.001	2	334	<0.001	386	<0.001
Density × age	1	2.59	NS	12.9	0.001	2	111	<0.001	146	<0.001
D_3 × age	2	0.35	NS	0.70	NS	4	2.51	0.051	11.3	<0.001
Density × D_3 × age	2	8.63	0.001	0.41	NS	4	5.92	<0.001	8.13	<0.001
Error	30					60				
Total	71					107				

[1]NS, $P > 0.05$.

[2]Degrees of freedom were the same for both variables.

[3]Density effect (high and low stocking densities).

[4]Vitamin D_3 effect (high, medium, and low vitamin D_3 diets).

[5]Age effect (5 and 6 wk for gait and hock burn scores, and 3, 5, and 6 wk for footpad burn and abdominal plumage scores).

(Table 2). In the grower or finisher period, HSD decreased FI and BW gain, while increasing FCR (Table 3). The effect of vitamin D_3 on BW gain and FCR was clearly age dependent (Table 2). Body weight gain increased with age, and peaked in the grower period for HVD and MVD treatments, and in the finisher period for the LVD (Table 3). The FCR was progressively higher with elevated vitamin D_3 in the finisher period, but not in the starter and grower periods when 3 diets seemed to be equal (Table 3).

Final BW and carcass composition were hardly affected by stocking density, supplemental vitamin D_3, and their interactions, except for final BW and breast muscle yield being decreased by HSD (Table 4).

Gait, Footpad and Hock Burns, and Abdominal Plumage Condition

In contrast to footpad burns and abdominal plumage damage, hock burn and gait problems were not observed at 3 wk of age. They were all influenced by stocking density, vitamin D_3, and their interactions (Table 5). In the case of stocking density, these scores were higher with HSD treatment. With respect to vitamin D_3, they were reduced by the HVD diet. When stocking density and vitamin D_3 were considered together, increasing dietary vitamin D_3 improved the walking ability and welfare status of broiler chickens in the HSD, but not LSD treatment (Tables 6 and 7). The effect of stocking

Table 6. Walking ability and welfare status of broiler chickens (mean ± SEM) according to stocking density and dietary vitamin D_3 across age[1]

	High stocking density			Low stocking density			
Variable	HVD	MVD	LVD	HVD	MVD	LVD	SEM
Gait score[2]							
5 wk	0.44^{b}	$0.65^{a,y}$	$0.77^{a,y}$	$0.31^{bc,y}$	$0.25^{c,y}$	$0.33^{bc,y}$	0.13
6 wk	0.50^{b}	$0.84^{a,x}$	$0.96^{a,x}$	$0.56^{b,x}$	$0.39^{b,x}$	$0.50^{b,x}$	0.17
Footpad burn score[3]							
3 wk	0.32^{z}	0.35^{z}	0.33^{z}	0.28^{z}	0.31^{y}	0.33^{y}	0.07
5 wk	$1.09^{b,y}$	$1.44^{a,y}$	$1.56^{a,y}$	$0.62^{c,y}$	$0.59^{c,x}$	$0.66^{c,x}$	0.15
6 wk	$1.45^{b,x}$	$1.96^{a,x}$	$2.19^{a,x}$	$0.80^{c,x}$	$0.64^{c,x}$	$0.69^{c,x}$	0.29
Abdominal plumage score[4]							
3 wk	0.41^{z}	0.39^{z}	0.36^{z}	0.41^{z}	0.39^{z}	0.40^{z}	0.08
5 wk	$1.01^{b,y}$	$1.17^{b,y}$	$1.48^{a,y}$	$0.54^{c,y}$	$0.51^{c,y}$	$0.58^{c,y}$	0.19
6 wk	$1.19^{c,x}$	$1.60^{b,x}$	$1.97^{a,x}$	$0.70^{d,x}$	$0.66^{d,x}$	$0.75^{d,x}$	0.23

[a–d]Means without a common superscript within a row differ significantly ($P < 0.05$).

[x–z]Means without a common superscript within a column differ significantly ($P < 0.05$).

[1]HVD, MVD and LVD: high, medium, and low vitamin D_3 diets.

[2]Values represent scoring the gait as 0 (normal gait), 1 (impaired movement), or 2 (reluctant step).

[3]Values represent scoring the footpad as 0 (no lesion), 1 (mild lesion, less than 0.75 cm in diameter), 2 (greater lesion, less than 1.5 cm), or 3 (severe lesion, more than 1.5 cm).

[4]Values represent scoring the abdominal plumage as 0 (no damage), 1 (mild damage, less than one-third of total area), 2 (greater damage, more than one-half), or 3 (severe damage, more than two-thirds).

Table 7. Hock burn scores of broiler chickens (mean ± SEM) according to stocking density across age or in interaction with dietary vitamin D_3[1]

Item	Hock burn score[2]		
	High stocking density	Low stocking density	SEM
Age, wk			
5	1.46[a,y]	0.50[b]	0.10
6	1.78[a,x]	0.60[b]	0.12
Vitamin D_3			
HVD	1.21[a,y]	0.61[b]	0.11
MVD	1.75[a,x]	0.49[b]	0.13
LVD	1.91[a,x]	0.56[b]	0.10

[a,b]Means without a common superscript within a row differ significantly ($P < 0.05$).

[x,y]Means without a common superscript within a column differ significantly ($P < 0.05$).

[1]HVD, MVD, and LVD: high, medium, and low vitamin D_3 diets.

[2]Values represent scoring the hock as 0 (no lesion), 1 (mild lesion, less than 0.75 cm in diameter), 2 (greater lesion, less than 1.5 cm), or 3 (severe lesion, more than 1.5 cm).

density was related to age for all lesion scores (Table 5). The differences in footpad burn and abdominal plumage scores between stocking densities reached statistical significance from 5 wk on (Table 6). Meanwhile, the hock burn score increased with age in HSD birds, but not in LSD group (Table 7). The interaction between vitamin D_3 and age was slight for footpad burn scores and was significant for abdominal plumage scores (Table 5), whose differences among vitamin D_3 were not observed until 5 wk of age. There was an interaction of stocking density, vitamin D_3, and age on almost all the scores, except those of hock burn (Table 5). The gait score of only HSD birds in the HVD treatment did not increase with age (Table 6). The differences in footpad burn and abdominal plumage scores among vitamin D_3 were significant only in HSD birds at 5 and 6 wk of age (Table 6).

Tibial Mineral Composition and Breaking Strength, and Plasma Variables

High stocking density had no influence on tibial Ca, but significantly decreased tibial ash, P, and breaking strength (Table 8). As supplemental vitamin D_3 increased, these indices were elevated (Tables 8 and 9). The interaction between stocking density and age was slight for tibial Ca and breaking strength, and was significant for percentages of ash and P (Table 8). They were decreased by HSD at 5 and 6 wk of age (Table 10). There was an interaction of vitamin D_3 and age on tibial breaking strength (Table 8), which was progressively higher with increasing vitamin D_3 at 5 or 6 wk (Table 10).

Plasma concentrations of P tended to be affected by stocking density, and the interaction between vitamin D_3 and age (Table 8). With increasing vitamin D_3, plasma Ca and P were elevated (Tables 8 and 9).

Table 8. Model and ANOVA results for tibial development and blood responses of broiler chickens[1]

Source of variation	df[2]	Tibial development								Blood parameter			
		Ash, %		Ca, %		P, %		Breaking strength, N		Ca, mg/dL		P, mg/dL	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Between-subjects													
Density[3]	1	12.7	0.001	2.06	NS	7.53	0.010	8.26	0.007	1.99	NS	3.09	0.089
D_3[4]	2	5.57	0.009	6.36	0.005	5.41	0.010	16.5	<0.001	8.18	0.002	4.81	0.016
Density × D_3	2	0.44	NS	0.22	NS	0.08	NS	0.73	NS	0.04	NS	0.03	NS
Error	30												
Within-subjects													
Age[5]	2	550	<0.001	380	<0.001	1,644	<0.001	4,002	<0.001	970	<0.001	356	<0.001
Density × age	2	9.64	<0.001	3.04	0.055	13.9	<0.001	3.12	0.051	1.78	NS	0.13	NS
D_3 × age	4	0.49	NS	0.32	NS	1.07	NS	5.53	0.001	1.15	NS	2.17	0.083
Density × D_3 × age	4	0.45	NS	0.29	NS	0.25	NS	0.86	NS	0.16	NS	0.86	NS
Error	60												
Total	107												

[1]NS, $P > 0.05$.

[2]Degrees of freedom were the same for all variables.

[3]Density effect (high and low stocking densities).

[4]Vitamin D_3 effect (high, medium, and low vitamin D_3 diets).

[5]Age effect (3, 5, and 6 wk).

DISCUSSION

The present study has investigated the interactive effect of stocking density and dietary vitamin D_3 supplementation on the growth and welfare of broilers of different growing periods. The findings indicate the importance of considering both density and nutritional factors when making production decisions.

Production Performance

In accordance with previous studies (Sørensen et al., 2000; Moreira et al., 2006; Buijs et al., 2009; Ventura et al., 2010; Sun et al., 2011), BW gain, FCR, and breast muscle yield were all adversely affected by the HSD treatment, indicating detrimental effects on growth and slaughter quality. The disadvantage of HSD appeared to relate to age. In the finisher period, the suppressed BW gain and FCR was consistent with the findings by Dozier et al. (2005), who found that the harm of HSD to cumulative BW was caused mainly between 35 and 49 d of age. It was speculated that some of the negative effects of HSD on live performance might stem from difficulty accessing feeders (Sørensen et al., 2000). In the present study, aggravated gait problems occurred simultaneously with decreased FI in HSD broilers from 35 to 42 d of age, suggesting that leg weakness around marketing age may contribute to the decreased live performance of HSD birds.

To evaluate the effect of HSD on the vitamin D_3 requirement, 3 experimental diets differing in vitamin D_3 concentrations were used here. As in previous findings (Edwards, 2002; Shirley et al., 2003), FCR was increased by the higher vitamin D_3 level, especially in the finisher phase. The effect of vitamin D_3 on BW gain was age dependent; the increase of BW gain from grower to finisher period was of lower magnitude in HVD and MVD chickens than in LVD chickens. The results suggest that feeding a large dose of vitamin D_3 for a long time may compromise broiler performance. This should have nothing to do with the toxicity resulting from excess vitamin D_3, because broiler chickens could tolerate as much as 200 times the requirement level within an exposure time of no more than 60 d (NRC, 1987). A P-adequate diet used in the present study is the likely explanation. In fact, vitamin D_3 improves the growth of broilers by increasing phytate phosphorus utilization, so its positive effect on performance is exerted almost exclusively in P-deficient diets (Biehl and Baker, 1997; Edwards, 2002).

Although HSD reduced growth and body composition variables, there was no significant interaction between this factor and level of dietary vitamin D_3, indicating that increasing dietary vitamin D_3 cannot alleviate the negative effect of HSD on live performance. The relationship between stocking density and nutritional factors, such as dietary energy and Lys levels, has been reported in a few studies (Berri et al., 2008; Sun et al., 2011). They found that the nutritional requirement for optimum growth was hardly altered by the stocking density.

Welfare Status

As in previous work (Sørensen et al., 2000; Moreira et al., 2006; Buijs et al., 2009; Ventura et al., 2010; Sun et al., 2011), the present study found that footpad and hock lesions, and abdominal feather damage increased with stocking density in particularly growing chickens, which may be ascribed to the higher litter moisture and less activity with age in HSD conditions (Dozier et al., 2006). As hypothesized, higher level of dietary vitamin D_3 decreased these scores, especially near marketing age, indicating a special age-related nutritional requirement exists for birds' well-being. In the present study, stocking density had significant interactions with vitamin D_3 on these scores. They were progressively lower with elevated vitamin D_3 level in HSD birds, but not in LSD chickens, indicating that increasing dietary vitamin D_3 improves the welfare of broilers stocked at high densities. The smaller differences in footpad burn and abdominal plumage scores among the vitamin D_3 levels were only significant for HSD birds at 5 or 6 wk, suggesting that the favorable welfare response of HSD birds to increasing vitamin D_3 might be broadly applicable to older chickens.

Gait scores, a variable used to evaluate walking ability (Garner et al., 2002), correspond well with footpad and hock burns, and abdominal plumage condition in response to varying stocking densities, vitamin D_3 concentrations, and their interactions. Poorer gait scores may be related to a reduction in exercise as seen by the

Table 9. Tibial development and blood responses of broiler chickens (mean ± SEM) according to dietary vitamin D_3

Variable	High vitamin D_3 diet	Medium vitamin D_3 diet	Low vitamin D_3 diet	SEM
Tibial development				
Ash, %	35.4[a]	35.0[ab]	34.1[b]	1.16
Ca, %	12.9[a]	12.7[ab]	12.2[b]	0.47
P, %	5.75[a]	5.59[ab]	5.36[b]	0.34
Blood parameter				
Ca, mg/dL	8.99[a]	8.76[ab]	8.55[b]	0.24
P, mg/dL	5.74[a]	5.57[a]	5.30[b]	0.14

[a,b]Means without a common superscript within a row differ significantly ($P < 0.05$).

Table 10. Tibial development of broiler chickens (mean ± SEM) according to stocking density or dietary vitamin D_3 across age[1]

Age, wk	Tibial ash, %			Tibial P, %			Tibial breaking strength, N			
	HSD	LSD	SEM	HSD	LSD	SEM	HVD	MVD	LVD	SEM
3	30.1^{z}	29.9^{z}	0.68	4.18^{z}	4.16^{z}	0.14	$45.2^{a,z}$	$43.9^{a,z}$	$41.4^{b,z}$	1.85
5	$34.7^{b,y}$	$36.6^{a,y}$	0.35	$5.43^{b,y}$	$5.85^{a,y}$	0.12	$92.4^{a,y}$	$88.9^{b,y}$	$81.6^{c,y}$	2.72
6	$37.9^{b,x}$	$39.8^{a,x}$	0.35	$6.68^{b,x}$	$7.09^{a,x}$	0.10	$114^{a,x}$	$109^{b,x}$	$103^{c,x}$	2.58

[a–c]Means without a common superscript within a row differ significantly ($P < 0.05$).

[x–z]Means without a common superscript within a column differ significantly ($P < 0.05$).

[1]HSD and LSD: high and low stocking densities; HVD, MVD, and LVD: high, medium, and low vitamin D_3 diets.

decline in distance traveled by broilers at high densities (Lewis and Hurnik, 1990; Andrews et al., 1997; Estevez et al., 1997), or may be a result of the rapid decline in litter quality associated with high densities, which is known to affect leg health (Ekstrand, 1993; Wang et al., 1998). When the effect of stocking density within each diet was analyzed, it was observed that gait scores gradually diminished as vitamin D_3 increased in the HSD. Thus, they were equal for both stocking densities when the HVD diet was fed. These findings may corroborate the tendency of increasing dietary vitamin D_3 for HSD broilers, which improves walking ability, decreasing contact of the foot, hock and keel with the litter, thus reducing the development of footpad and hock dermatitis, and abdominal plumage damage.

Tibial Quality

As in previous findings (Buijs et al., 2012), tibial mineral composition and breaking strength were decreased by HSD treatment at 5 or 6 wk, indicating a negative effect of increased stocking density on some aspects of bone quality. To reduce incidence of skeletal disorders, increasing supplemental vitamin D_3 may be a solution. In line with a previous study (Kim et al., 2011), the present study showed that high levels of vitamin D_3 increased tibial mineral deposition and shear strength. With the support of the elevated plasma Ca and P concentrations, the result suggests that the addition of vitamin D_3 facilitates calcium and phosphate absorption, enhancing tibial development. Similarly, Shirley et al. (2003) reported that chicks fed a vitamin D_3-adequate diet (40 μg of D_3/kg of diet) had greater plasma Ca concentrations and a higher percentage of tibial ash than in chicks fed a diet deficient in vitamin D_3 (5 μg of D_3/kg of diet). Although a higher stocking density reduced tibial quality, there were very few significant interactions between this factor and level of dietary vitamin D_3. Additional regression analyses were performed (data not shown) to test the linearity of the dietary vitamin D_3 response and if it was different under LSD or HSD; there no significant differences between the density groups, suggesting similar responses. These data indicate that the tibial quality seems to be unrelated to improved walking ability and welfare status of HSD birds fed the vitamin D_3-adequate diet.

In conclusion, the present study showed that HSD decreased both growth and welfare of broilers, particularly toward the end of the rearing period. Supplemental vitamin D_3, at almost 10 to 20 times NRC (1994) recommended levels, improved the birds' walking ability and tibial quality, and reduced the development of footpad or hock dermatitis and abdominal plumage damage, some aspects of which were age-dependent and appeared to vary with stocking density. The result indicates that increasing dietary vitamin D_3 alleviates the negative effect of HSD on well-being, but not on performance.

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