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Use of Vitamin D to Reduce Lameness in Broilers Reared in Harsh Environments

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ABSTRACT

In tropical poultry-producing countries, poultry houses usually have little environmental control. This study investigated the effect of dietary vitamin D on the incidence of leg abnormalities of a fast-growing broiler strain reared under harsh conditions. In this study, 300 one-day-old male broilers were distributed in two treatments with three replicates of 50 birds each. One group was fed a placebo and the other group was fed 25-hydroxycholecalciferol (25-OH-D3) in a soluble form. The environmental variables were weekly recorded during until birds were 49 days old. Birds were weekly gait scored, and their walking speed and vertical force while walking were assessed. Post-mortem examination was performed and skeletal lesions were assessed. Control birds presented more leg problems than those that ingested 25-OH-D3. These results suggest that dietary vitamin D is effective in decreasing the severity of lameness by reducing tibial dyschondroplasia and other leg abnormalities.

INTRODUCTION

It has been reported that the low locomotor activity of broilers coupled to their high initial growth rate of broilers results in leg problems and gait abnormalities. Long times spent sitting on wet litter may cause breast and leg skin lesions, which may contribute for the deterioration of the flock well-being (McGeown *et al.*, 1999; Vestergaard & Sanotra, 1999; Weeks *et al.*, 2000; Bokkers & Koene, 2004; Bessei, 2006). A causal interrelationship between the rapid growth broiler strains, low locomotor activity and leg problems has been discussed in current literature (Julian, 1998; Vestergaard & Sanotra, 1999; Kestin *et al.*, 2001; Sanotra *et al.*, 2001; Bokkers & Koene, 2003). However, the influence of environmental and management-related factors, such as heat stress, light intensity, age, stocking density and diet on the incidence of leg abnormalities in broiler flocks has also been reported (Edwards Jr., 2000; Edwards Jr., 2003; Dawkins *et al.*, 2004; Bessei, 2006).

The most common skeletal problems that cause leg weakness in broilers are tibial dyschondroplasia, chronic painful lameness, chondrodystrophy or angular bone deformities, valgus-varus deformities, spondylolisthesis, rickets, femoral head necrosis, curled toes, and ruptured gastrocnemius tendon (Angel, 2007). Lameness in broilers with poor gait scores significantly improved after treatment with analgesic and anti-inflammatory drugs, which has led to the assumption that the leg problems in broilers are painful (McGeown *et al.*, 1999; Danbury *et al.*, 2000; Weary *et al.*, 2006; Nääs *et al.*, 2009). Several attempts have been made to determine the broilers' locomotor deficiencies (Dawkins *et al.*, 2004) and to increase the walking ability of the birds (Corr *et al.*, 2007).

Among the studies to reduce lameness in broilers, some are related to the use of feed additives (Rath *et al.*, 1998), and some to the



addition of vitamin D in the diet (Edwards Jr., 2000; Whitehead *et al.*, 2004; Waldenstedt, 2006; Leeson, 2007). In contrast with all other vitamins, vitamin D 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 (Ameenuddina *et al.*, 1985; Edwards Jr. *et al.*, 1994; Elliot & Edwards Jr., 1997; Edwards Jr., 2003). Edwards Jr. (2000) found that broilers fed diets deficient in vitamins, minerals, and amino acids are more susceptible to leg problems.

The objective of this study was to verify the effectiveness feeding a solution of 25-hydroxycholecalciferol (25-OH-D₃) in reducing lameness in fast-growing broilers reared under harsh environmental conditions.

MATERIALS AND METHODS

The facilities and experimental procedures reported herein were approved by the Institutional Animal Care and Use Committee (CEEA 1664-1) of the State University of the Campinas.

Birds and husbandry

In this study, 300 one-day-old Cobb® 500 male chicks with $47 \text{ g} \pm 0.014$ average initial weight were equally distributed into six small-scale broiler houses (3.2 x 1.4 m) located in an open area. Birds were obtained from a commercial hatchery located near the experimental area. Houses were East-West oriented, with open sides and closed walls at the ends. Lateral openings were covered with yellow polypropylene ($170 \mu \text{ mm}^{-1}$) curtains that could be wrapped up when required. The concrete floor was covered with 50-mm deep wood shavings. Litter was regularly replaced to maintain its proper conditions during the experiment. There was a brooder in one corner of each house to provide supplemental heat during the first weeks. Each house was equipped with a tube feeder and a bell drinker. Fresh water was supplied from a plastic water recipient located at the south end of each house. The feeding program consisted of a starter diet (ME = 3,125 kcal kg⁻¹, 22% CP) fed for the first two weeks; a grower diet (ME = 3,125 kcal kg⁻¹, 20% CP) fed from 15 to 42 d of age, and a finisher diet (ME = 3,150 kcal kg⁻¹, 20% CP) fed until birds were 49 days old. Feed and water were offered *ad libitum*. Maximum flock density was 30 kg m⁻² during the study.

Treatments

Birds were divided in two groups: group A (control) was fed a placebo solution (0.2 kg 1000 L⁻¹), and group

B a 25-hydroxycholecalciferol (25-OH-D₃) solution (0.2 kg 1000 L⁻¹, equivalent to 0.069 kg ton⁻¹). Each group included three replicates of 50 birds each, which were reared in distinct houses. The vitamin D supplied to group B birds was diluted in drinking water. Both groups were reared under the conditions described above and were exposed to natural ventilation and weather conditions (temperature and relative humidity) as presented during the summer of 2008/09 in Campinas, Brazil (47°03' W, 22°54' S, 854 m altitude). The local season average dry bulb temperature was 27.5°C with 83% relative humidity and SE prevailing wind.

Experimental procedure

Five birds were randomly selected from each house daily, and their weights were recorded. Feed and water intakes were daily recorded by weighing the offered amount and the residues remaining both in the feeder and drinker. Ambient temperature, relative humidity, wind velocity, and light intensity inside the houses were continuously recorded using a data logger placed in the center of the house at a height of 0.8 m. Environmental data of the surrounding area were also recorded.

Fifteen broilers were randomly removed from groups A and B (five from each house) at 28, 35, 42, and 49 days of age for locomotion evaluation. Birds were weighed and identified with a color marker according to the house they were reared, and they were kept in a waiting area. Each bird was scored using the subjective gait scoring system (GS) suggested by Dawkins (2004) to evaluate broilers' locomotor ability. Scores were given by observing 10 consecutive steps of the bird, according to the following scale: 0 for the birds that walked 10 steps normally; 1 for the birds that walked 10 steps with some difficulty, showing unstable walking; and 2 for the birds that could not walk more than 4 steps, sitting afterwards. GS was evaluated in individual birds by a trained observer.

Broilers were then stimulated to walk on a force measurement platform system (FMPS) that consisted of a 0.01 m wide pressure-sensitive mat with piezoelectric crystal sensing elements inside. The MatScan® pressure measurement system (Tekscan, Inc, South Boston, MA, USA) was used to determine the resulting force distribution as a percentage of the body weight (Nääs *et al.*, 2010). The pressure mat was connected to a computer where data were recorded, saved, and processed using a software program (MatScan® – Clinical foot version 5.72). This allowed the resulting force distribution image to be visualized and data to



be processed and analyzed (Nääs *et al.*, 2009). The mat was calibrated with the average weight of the birds in each house as a function of age group. The time birds spent crossing the FMPS was also recorded by the software, which was used to determine their speed of walking.

Video recording was performed while the birds walked across the FMPS using two digital video cameras (Sony® HDR –SR5, 30 FPS). The first camera was placed at a distance of approximately 1.0 m to the side of the plane of motion, and the second camera was placed over the middle of the mat, attached to a 1.3-m high pole. Both cameras were aligned on their vertical and horizontal axes, using a 90° angle from the plane of motion to record the walking alignment of the broilers, which was analyzed by dividing the top images of the mat into three virtual lanes of approximately 0.30 m each, consisting of a central lane and the borders. When a bird walked on the mat consistently in the same lane, it was considered to have proper walking alignment. The walking alignment assessment was done by watching the recorded videos.

Post-mortem examination

Immediately after the walking observation was completed, all birds were euthanized by cervical dislocation, and submitted to *post-mortem* examination to determine the presence or absence of physical leg abnormalities (LA), including tibial dyschondroplasia (TD), valgus-varus deformities (VVD), angular bone deformities (ABD), spondylolisthesis (S), femoral degenerative joint lesion (FHNR, right leg and FNHL, left leg), curled toes (CT), and ruptured gastrocnemius tendon (RGT).

Data analysis

Initially, the data from prime birds (PBs) at all ages were selected. PBs corresponded to the broilers which GS was zero, walked on the mat with proper alignment, and did not present any skeletal disorders. A comparison was made between PBs and the other tested non-prime birds (NPBs). The effect of treatment on the collected data was initially analyzed using the Principal Components Analysis procedure, which shows the closeness of the vectors representing the studied variables. The variables first tested were treatment, GS, PBs, NPBs, and LA found in the *post-mortem* examination. In the second analysis, the effect of treatment on all identified lesions and leg abnormalities was tested. The Chi-Square test was applied to quantify the effect of treatment on the detected lesions, and odds ratio (OR) was calculated. The effects of treatment and ambient temperature on the gait score, leg abnormalities, and leg lesions were submitted to analysis of variance (GLM procedure). Data were square-root transformed to obtain homogeneity of variances. Performance and walking pressure data were compared using Student's paired t-test. Effects were considered significant when $p < 0.05$ and, in some described cases, when $p < 0.10$. All analyses were performed using a statistical software program (Minitab, 2005).

RESULTS AND DISCUSSION

Ambient temperature and performance data

After the 5th week of rearing, when broilers were close to market age, the mean ambient temperature increased following the increase in the maximum ambient temperatures (Table 1).

Table 1 – Average internal environmental data during rearing (air temperature, relative humidity, air velocity and light intensity) and outside environmental conditions (air temperature, relative humidity, air velocity).

| Rearing period (d) | Treatment | Inside air temp. (°C) | | Out. air temp. (°C) | | Inside air RH (%) | | Outside air RH (%) | | Air velocity (m s ⁻¹) | Light intensity* (lx) | |
|--------------------|-----------|-----------------------|----------|---------------------|------|-------------------|-----------|--------------------|-------|-----------------------------------|-----------------------|----------|
| | | Min | Max | Min | Max | Min | Max | Min | Max | Mean | Min | Max |
| 21 - 28 | A | 23.9±1.6 | 30.0±2.8 | | | 62.6±6.3 | 46.2±14.2 | | | | | |
| | B | 24.4±1.5 | 30.2±2.1 | 24.8 | 30.0 | 70.4±8.0 | 64.0±9.0 | 57.35 | 98.93 | 1.98 | 18.1±1.0 | 38.4±1.9 |
| 29 - 35 | A | 20.8±0.8 | 31.1±2.9 | | | 64.0±8.1 | 43.5±16.0 | | | | | |
| | B | 21.3±0.7 | 31.7±2.7 | 25.1 | 30.2 | 58.2±5.5 | 56.3±11.7 | 57.17 | 95.47 | 1.85 | 20.9±0.8 | 41.6±0.9 |
| 36 - 42 | A | 21.7±1.0 | 32.9±1.6 | | | 65.7±7.9 | 41.1±12.2 | | | | | |
| | B | 22.1±1.9 | 34.2±0.8 | 25.2 | 30.8 | 66.4±5.2 | 58.2±9.7 | 52.04 | 97.67 | 1.93 | 37.7±1.6 | 52.4±2.4 |
| 43 - 49 | A | 24.5±3.6 | 33.8±1.7 | | | 62.7±2.3 | 31.9±1.3 | | | | | |
| | B | 24.4±3.7 | 34.1±1.8 | 25.1 | 31.0 | 64.6±2.5 | 44.4±3.3 | 44.88 | 94.87 | 1.58 | 25.3±1.4 | 47.6±1.6 |

*Natural light regimen during the summer (14L:10D)



Table 2 – Summary of performance data

| Rearing period (d) | Treatment | Mean weight (g) | | Mean weekly weight gain (g) | | Mean water consumption (mL bird ⁻¹) | | Weekly mortality (%) |
|--------------------|-----------|-----------------|------|-----------------------------|------|---|-------|----------------------|
| | | Value | SE | Value | SE | Value | SE | |
| 1 - 7 | A | 155.00 | 0.23 | 109.00 | 0.19 | 63.05 | 4.54 | 0.00 |
| | B | 157.00 | 0.22 | 109.00 | 0.18 | 58.19 | 4.26 | 0.68 |
| 8 -14 | A | 533.00 | 0.42 | 378.00 | 0.36 | 127.45 | 6.52 | 0.68 |
| | B | 524.00 | 0.42 | 367.00 | 0.35 | 127.41 | 6.39 | 2.85 |
| 15 -20 | A | 1082.00 | 0.61 | 549.00 | 0.44 | 214.29 | 8.57 | 0.68 |
| | B | 1084.00 | 0.60 | 560.00 | 0.43 | 215.46 | 8.24 | 0.07 |
| 21 - 28 | A | 1867.00 | 0.79 | 784.00 | 0.49 | 334.19 | 10.56 | 8.53 |
| | B | 1831.00 | 0.78 | 747.00 | 0.50 | 327.27 | 10.38 | 7.41 |
| 29 - 35 | A | 2550.00 | 0.94 | 683.00 | 0.51 | 365.56 | 11.20 | 10.57 |
| | B | 2590.00 | 0.92 | 759.00 | 0.48 | 344.15 | 10.51 | 9.84 |
| 36 – 42 | A | 2981.00 | 1.02 | 432.00 | 0.39 | 547.25 | 13.54 | 24.79 |
| | B | 2912.00 | 0.99 | 323.00 | 0.37 | 588.92 | 13.48 | 30.13 |
| 43 - 49 | A | 3414.00 | 1.10 | 433.00 | 0.43 | 650.10 | 14.46 | 37.14 |
| | B | 3312.00 | 1.00 | 399.00 | 0.11 | 743.38 | 15.47 | 39.70 |

SE = standard error

The recorded temperatures were above the thermoneutral zone and lasted approximately five hours (from 13h00 min to 18h00 min), and most birds in both treatments were panting and showed behavioral signals of heat distress. Average weight, weekly weight gain and weekly mortality in both groups were similar ($p > 0.05$). Mortality increased as birds aged. In addition, weekly mortality significantly increased after the 5th week of rearing, while weekly weight gain decreased and water intake increased (Table 2). Average water consumption was not different between the control group and the treated group ($p = 0.09$).

Treatment and presence of lesions

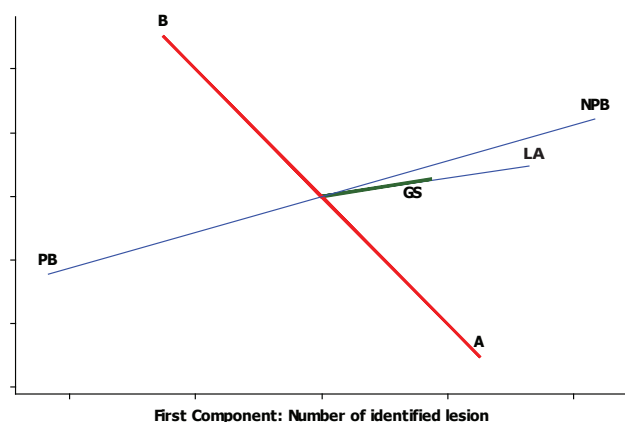


Figure 1 – Principal Components Analysis results and association among total number of lesions, gait score and the number of prime birds related to the treatment.

Data on the detected lesions are summarized in Table 3. The result of the Principal Components Analysis (Figure 1) shows a strong association between group A (control, no treatment) and skeletal lesions and leg abnormalities (LA) that were observed during the *post-mortem* examination. The analysis showed that PBs were associated with the treatment in group B. Increases in the LA, GS and NPB numbers were also associated with the control group.

The Principal Components Analysis applied using all leg abnormalities and lesions detected during the *post-mortem* examination showed that the incidence of tibial dyschondroplasia (TB) both in the right (TBR) and left (TBL) legs, leg alignment (valgus-varus deformities, VVDR, and VVDL), femoral degenerative joint lesions (FHNr and FHNL) and spondylolisthesis (S) were associated with the birds in the control group (Figure 2).

The Pearson's Chi-Square test results showed that the total number of lesions was highly associated with the birds in the control group ($p = 0.024$). The results of further analysis, considering the lesions (TD and VVD) associated with the control group A (Figure 2), showed that birds in the control group had 5.1 times higher probability of presenting tibial dyschondroplasia ($OR = 5.1$; $p = 0.004$) than the birds in the treatment group B. Likewise, the broilers in the control group had 3.8 times higher probability of presenting valgus-varus deformity ($OR = 3.8$; $p = 0.019$).

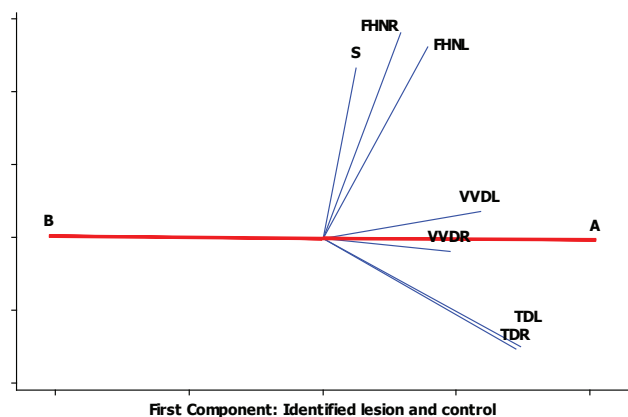


Figure 2 – Principal Components Analysis results and association with the specific lesions observed with the treatment.

When associating total lesions (TLs) with body weight, it was found that the use of dietary vitamin D had a positive effect, reducing the lesions ($p = 0.04$), independently of body weight (Figure 3).

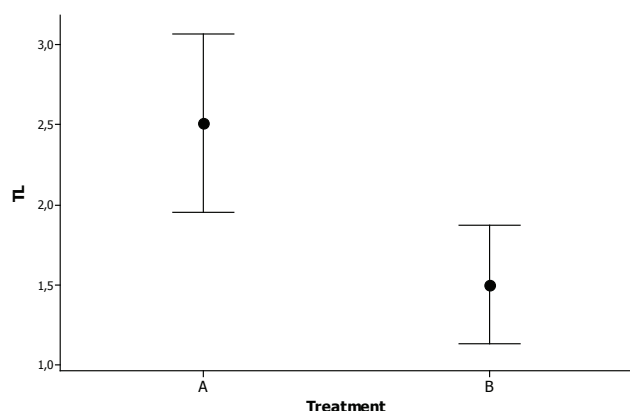


Figure 3 – Box plot of the total number of lesions found in the post-mortem examination and its relation with the treatment.

Locomotor variables (GS, mean walking speed and alignment, and mean vertical pressure)

GS values were not influenced by treatment ($p > 0.05$), but were highly influenced by ambient temperature ($p = 0.01$). For each increase of 4 °C in ambient temperature, there was an increase of approximately 1 unit in the GS values. GS was not different between treatments (Table 3). Mean walking speed of the NPBs was different between treatments on weeks 5 and 7 ($p = 0.04$). The older birds (43 – 49 d) in the control group A walked considerably slower (0.07 m s⁻¹) when compared with the birds in the treatment group B (0.21 m s⁻¹). All birds of the same age group walked at the same speed, independently of their selection as prime or non-prime broilers. Only group B birds presented alignment during walking, and this specific characteristic was used to select prime birds. Mean vertical pressure was significantly different between the right and the left leg at all ages ($p = 0.03$). Between 36 and 42 days of age, the mean vertical pressures exerted by both feet (right and left) on the FMPS by the non-prime birds in treatment group B was not different from that of the prime broilers at the same age ($p > 0.05$); however, they were significantly different from those of the control group ($p = 0.01$). Mean vertical pressure was unbalanced between the right and the left foot at 21 to 28 d and 29 to 35 days of age in birds of the control group and at 43 to 49 days of age in the birds of the treatment group B. Between 36 and 42 days of age, mean vertical pressure of the birds of treatment group B was significantly higher ($p = 0.01$) than that of broilers of the control group.

Table 3 – Summary of locomotor data for prime and non-prime birds.

| Rearing period (d) | Treatment | Gait score | | Mean walking speed NPB (m s ⁻¹) | | Mean walking speed PB (m s ⁻¹) | | Mean vertical pressure of NPB (% BW) | | | | Mean vertical pressure of PB (% BW) | |
|--------------------|-----------|------------|------|---|------|--|------|--------------------------------------|------|--------|------|-------------------------------------|------|
| | | Value | SE | Value | SE | Value | SE | R | SE | L | SE | Value | SE |
| 21 - 28 | A | 0.7 | 0.03 | 0.07* | 0.01 | NA | NA | 92.5B | 17.7 | 98.5A | 14.3 | NA | NA |
| | B | 0.8 | 0.05 | 0.05* | 0.01 | 0.02* | 0.00 | 104.0A | 12.4 | 96.8A | 17.5 | 98.18A | 9.8 |
| 29 - 35 | A | 0.5 | 0.14 | 0.15b | 0.01 | NA | NA | 113.9c | 32.7 | 155.6a | 22.5 | NA | NA |
| | B | 0.7 | 0.14 | 0.21a | 0.02 | 0.20a | 0.01 | 136.6b | 19.2 | 139.8b | 19.2 | NA | NA |
| 36 - 42 | A | 1.1 | 0.14 | 0.05* | 0.01 | NA | NA | 112.0b | 40.1 | 110.1b | 39.9 | NA | NA |
| | B | 1.1 | 0.12 | 0.08* | 0.01 | NA | NA | 156.8a | 45.3 | 149.3a | 35.4 | 143.40a | 16.6 |
| 43 - 49 | A | 1.0 | 0.18 | 0.07b | 0.01 | NA | NA | 135.9B | 40.1 | 134.5B | 27.1 | NA | NA |
| | B | 1.2 | 0.22 | 0.21a | 0.01 | NA | NA | 146.4A | 26.0 | 136.6B | 28.3 | NA | NA |

R=right foot; L=left foot; SE=standard error; Gait score varied from 0 – 2;

* = not significant; Capital letter = significant at 90%; Small letter = significant at 95%; NA = not available.

Capital letter and small letter within columns at a period of growth means difference in results. BW=Body weight



Table 4 – Summary of leg abnormalities and lesions (tibial dyschondroplasia (TD), femoral head necrosis (FHN), spondylolisthesis (S) and total valgus – varus deformities (VVDT).

| Rearing period (d) | Treatment | TD (%) | FHN (%) | S (%) | VVDT (%) | Mean number of leg lesions | | Mean number of cervical lesions | |
|--------------------|-----------|--------|---------|-------|----------|----------------------------|-----|---------------------------------|-----|
| | | | | | | Value | SE | Value | SE |
| 21 - 28 | A | 40.0 | 30.0 | 0.0 | 17.6 | 1.43 | 4.6 | 0.2 | 0.1 |
| | B | 80.0 | 20.0 | 0.0 | 0.0 | 0.50 | 0.2 | 0.3 | 0.1 |
| 29 - 35 | A | 42.9 | 71.4 | 0.0 | 35.0 | 1.50 | 0.3 | 0.3 | 0.2 |
| | B | 30.8 | 46.2 | 0.0 | 15.0 | 1.30 | 0.4 | 0.0 | 0.0 |
| 36 - 42 | A | 51.9 | 58.3 | 20.0 | 10.4 | 1.80 | 0.4 | 0.5 | 0.1 |
| | B | 16.7 | 37.0 | 0.0 | 10.3 | 0.80 | 0.2 | 0.3 | 0.1 |
| 43 - 49 | A | 20.0 | 100.0 | 23.5 | 20.0 | 2.67 | 0.4 | 1.3 | 0.1 |
| | B | 0.0 | 65.0 | 30.8 | 0.0 | 1.47 | 0.2 | 0.9 | 0.1 |

The effect of mean ambient temperature on the total lesions was not significant ($p = 0.06$). However, when testing TLs at maximum ambient temperature, it was found that TLs in the control group increased proportionally to the increase in maximum house temperature ($p = 0.01$). An increase of 1 °C in maximum ambient temperature led to an estimated increase of one lesion in the total number of lesions per bird (Table 4).

The findings of the present study indicate that the use of dietary vitamin D₃ reduces lameness in fast-growing broiler chickens exposed to harsh environments. Lameness is reported to be associated with age and body weight (BW) (Kestin *et al.*, 2001; Sorensen *et al.*, 2000; Bessei, 2006), and the birds that ingested vitamin D presented fewer lesions than those in the control group, independently of age and BW. Mean walking speed was higher in the group fed vitamin D, and at different ages, the broilers from the control group presented unbalanced walking due to distinct vertical pressures on the right and left feet. According to Camacho *et al.* (2004), a group of broilers fed 5% of a supplement containing amino acids, vitamins, and minerals presented a significant decrease in leg problems. Other authors (Whitehead *et al.*, 2004; Atencio *et al.*, 2005, 2006) have shown improvements in tibial ash content and skeletal integrity in broilers when the breeders are fed high values of vitamin D₃, thereby minimizing the incidence of TD.

Among the studied leg abnormalities related to lameness, TD and VVD were positively correlated with the absence of vitamin D, which is consistent with the findings of Whitehead *et al.* (2004) and Angel (2007), who stated that the addition of vitamin D₃ alleviates TD clinical signs primarily by inducing chondrocyte maturation. Whitehead *et al.* (2004) and Mitchell *et al.* (1997) showed that high dietary concentrations of vitamin D₃ can prevent TD in chickens presenting

a high genetic tendency for this condition. The authors concluded that the vitamin D₃ requirements of broilers up to 14 d of age are much higher than earlier estimates and may be related to the higher calcium requirements of the modern broiler genotypes. The results of the present study are also in agreement with the conclusions obtained by other investigators who, using diets with unbalanced Ca: P levels to induce TD, observed a reduction in the leg disorders when using, in particular, the active metabolite 1,25-dihydroxyvitamin D₃ (Rennie *et al.*, 1993; Edwards Jr. *et al.*, 1994, 2000; Fritts & Waldroup, 2003). It is likely that, when TD is induced by hypocalcaemia or vitamin-deficient diets, this condition may be secondary to rickets, which might be mitigated by correcting calcium homeostasis. However, the findings of the present study were different from the results obtained by Rath *et al.* (2007), where the use of dietary vitamin D did not prevent the incidence of induced TD.

Older birds (> 21 d) generally presented unbalanced mean vertical pressures. The weight load is usually evenly distributed between each limb in normal bipeds functioning under biomechanical principles. According to Corr *et al.* (2007), when the limb is painful to use or it is biomechanically unsound, the bird is less willing to bear weight on it, resulting in unbalanced and/or unaligned walking.

All birds were exposed to heat stress in the present study, especially at market weight (42-49 d). This may have influenced the response to the vitamin, especially as it was administered diluted in drinking water. However, no difference in water consumption was found ($p > 0.05$) between the tested groups. Complex metabolic, dietary and management issues are often involved in leg disorders. When exposed to heat stress, broilers are likely to reduce their feed intake, aggravating their leg problems (Bessei, 2006). According to Rennie *et al.* (1993), heat stress is known to interfere with



the conversion of vitamin D₃ to its metabolically active form, 1,25(OH)₂D₃; as a result, higher dietary levels may be required during hot periods. The active form of vitamin D₃ is involved in the synthesis of calcium-binding protein, which is essential for calcium and phosphorus homeostasis (Teeter & Belay, 1995; Borges *et al.*, 2003). It was demonstrated that flocks with a high incidence of endochondrial ossification defects have significantly lower bone ash and 1,25 (OH)₂D₃ levels compared with mildly-affected flocks, and it seems probable that higher systemic concentrations of 1,25(OH)₂D₃ will enhance the ability of broiler chickens to effectively mineralize the cartilaginous growth plates in the appendicle skeleton during early bone maturation (Vaiano *et al.*, 1994). Dietary calcium or phosphorus contents below the suboptimal level in modern broiler genotypes directly influence vitamin D₃ requirements (Kling, 1985; Baker *et al.*, 1998; Waldensted, 2006). On the other hand, due to the importance of preventing excessive phosphorus supply to minimize pollution, dietary contents sometimes fail to meet the specifications (Whitehead *et al.*, 2004).

Heat stress reduces calcium intake and the conversion of vitamin D₃ to its metabolically active form (1,25(OH)₂D₃). In effect, calcium requirements of birds increase when environmental temperatures are high. It is recommended to supply extra calcium to broilers at the rate of 1 g per bird during hot months to overcome this effect. Livestock diets that are deficient in vitamin D have been implicated in a number of growth abnormalities, including seizures, bone fracturing, thin or soft eggshells, decreased egg production and hatching, and liver and kidney diseases. Other stressful conditions, such as high bird density, mycotoxicosis, enteritis, malabsorption syndromes, and specific immune disorders may impair cholecalciferol absorption or liver hydroxylation, which is one of the reasons for the use of Vitamin D metabolites in broiler feed (Whitehead *et al.*, 2004; Waldensted, 2006; Leeson, 2007).

Qualitative feed restriction under heat stress may affect weight gain as well as bone formation (Bruno, 2002; Moraes *et al.* 2002; Pelicano *et al.*, 2005). However, research carried out by Yalçin *et al.* (1996) did not identify any effect of rearing temperature on femur or tibia width. Moraes *et al.* (2002) reported differences in bones length when broilers were reared in temperatures above the thermoneutral zone, which may lead to lameness.

At 4 weeks of age, leg weakness was a relatively minor problem in the present study; only a few severely lame birds presented gait score (GS) 4 or 5. However, two weeks later, their walking ability was significantly poorer, and it worsened when birds were seven weeks

old. At all ages, males exhibited greater leg weakness than females, and the percentage of severely lame birds increased with age, in agreement with the findings of Sorensen *et al.* (2000).

Light intensity remained relatively constant during the experiment, as the birds were reared in open-sided houses. Broilers had access to natural lighting and indirect UV light, as it is the case of most broiler houses in Brazil, which may have enhanced the vitamin D status. Several authors (Edwards Jr. *et al.*, 1994; Edwards Jr., 2003) established the optimal doses and methods of secure use of UV light to chicks to increase their vitamin D status under laboratory conditions. This may provide an alternative to supplying high levels of vitamin D in the starter diets. In addition, dietary imbalances that may occur under commercial setting, leading to leg weakness, should be prevented.

CONCLUSIONS

The use of dietary vitamin D is expected to reduce the leg abnormalities in broilers reared in harsh environments. Broilers that are not fed vitamin D present higher chances of suffering from lameness, which is caused mainly by skeletal lesions.

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