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Research Article

# Effects of lighting schedule and ascorbic acid on performance and tibiotarsus bone characteristics in broilers

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**Abstract:** This study was conducted to determine the effects of 2 lighting programs (continuous: 24 h light:0 h dark; intermittent: 12 h daylight followed by 3 cycles of 1 h light, and then 3 h dark during the night) and 3 ascorbic acid supplementations (0, 200, and 400 mg/L added to water) on broiler performance, bone characteristics, and the incidence of tibial dyschondroplasia. One-day-old male commercial (Ross $PM_3$) broiler chicks (600) were divided into 6 treatment groups (2 × 3) with random replicates (4 replicates per treatment). At the end of 6 weeks data revealed that the lighting program, which was one of the main factors tested, affected the feed conversion ratio and live weight gain ($P < 0.05$), while ascorbic acid (the other main factor) and the interactions of both factors did not significantly influence broiler performance characteristics. Length and weight of the tibiotarsus were positively affected by the use of intermittent lighting, whereas ascorbic acid had a positive effect only on the weight of the bone. Continuous lighting improved cortical thickness of the tibiotarsus ($P < 0.01$) and decreased the incidence of tibial dyschondroplasia insignificantly. There was not a lighting × supplemental ascorbic acid interaction for tibial dyschondroplasia.

The results of the present study suggest that use of intermittent lighting and supplementation of ascorbic acid (200 mg/L) can efficiently prevent leg problems, and increase bone strength and broiler performance.

**Key words:** Broiler, lighting, ascorbic acid, performance, tibiotarsus

## Aydınlatma programı ve askorbik asitin broilerlerde performans ve tibiotarsus özellikleri üzerine etkileri

**Özet:** Çalışmada sürekli ve kesintili (sürekli; 24 saat aydınlık:0 saat karanlık, kesintili; 12 saat gün ışığına ilave olarak 1 saat aydınlık:3 saat karanlık toplam 4 saat ve 3 siklus ile 12 saat tamamlanarak gece ışıklandırması) olmak üzere 2 farklı aydınlatma programı ve içme sularına farklı düzeylerde (0, 200, 400 mg/L) ilave edilen askorbik asidin broyler performansı, kemik özellikleri ve tibial dyschondroplasia üzerine olan etkileri incelendi. Bu amaçla 600 adet günlük yaşta broyler civciv (Ross $PM_3$) kullanılarak, 2 × 3 faktöriyel tertipte 4'er tekrarlı toplam 6 grup oluşturuldu. Çalışmada

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6. hafta sonunda, ana faktörlerden aydınlatmanın canlı ağırlık ve yemden yararlanma oranı üzerine olumlu etkisi saptanırken (P < 0,05), diğer temel etken olan askorbik asit ile bunların kombinasyonlarının performans özellikleri üzerine etkisinin olmadığı görüldü. Tibiotarsus'un ağırlık ve uzunluğu üzerine kesintili aydınlatmanın pozitif etkisi saptanırken, diğer temel etken olan askorbik asidin kemiğin sadece ağırlığını olumlu yönde etkilediği görüldü. Sürekli aydınlatmanın tibiotarsus kortikal kalınlığını arttırırken tibial dyschondroplasia'yi istatistiksel olarak önemsiz düzeyde (P < 0,01) azalttığı saptandı. Aydınlatma ve askorbik asit kombinasyonlarının tibial dyschondroplasia üzerine etkisinin olmadığı görüldü.

Sonuç olarak, kesintili aydınlatma ve içme suyuna ilave edilen 200 mg/L askorbik asidin bacak problemlerini azalttığı, kemik direnci ve broyler performansını arttırdığı saptandı.

**Anahtar sözcükler:** Broyler, aydınlatma, askorbik asit, performans, tibiotarsus

## Introduction

It is generally accepted that the principal factor negatively affecting broiler health and welfare is leg problems. Today, broilers reach slaughter weight in around 41 days, but the supporting structure of their legs fails to keep pace with the rapid body growth and can buckle under the strain of supporting an overgrown body. As a result, broilers suffer from painful, sometimes crippling leg disorders (1,2). The incidence of leg weakness in commercial flocks can be very high. A national survey conducted in Bursa, Turkey, indicated that broiler flocks experience 15% additional mortality due to leg abnormalities (3). Because no pathogens are directly involved, good management and husbandry practices play a key role in preventing leg problems. In recent years there has been increasing interest in the relationship between lighting and feeding, and skeletal disorders in broilers (4,5). Field data and test results show that supplementation with ascorbic acid (AA) is necessary for decreasing the incidence of leg weakness (6). AA is an activator of the enzyme 25-hydroxyl vitamin $D_3$-1 hydroxylase, which is required for the regulation of calcium absorption and excretion. Thus, AA nutrition influences calcium and phosphorus metabolism in young chicks. Birds are normally able to synthesize adequate amounts of AA; however, there is much evidence showing that they cannot produce enough AA for their metabolic needs (7). Kutlu and Forbes (8) reported that the amount of AA to be added to feed for improving broiler health was not clear. Broiler performance, and skeletal and metabolic disorders are also affected by lighting programs (9,10). Intermittent lighting is better than continuous lighting for broiler growth performance (5,11).

In order to reduce the incidence of leg weakness in broilers, special attention should be given to the interactive effects of the factors affecting broiler growth. The present study aimed to investigate the interactive effect of lighting and supplemental AA on broiler performance, tibiotarsus bone characteristics, and the incidence of tibial dyschondroplasia (TD), which accounts for a large proportion of leg weakness seen in commercial broilers.

## Materials and methods

The experimental procedures employed in this study complied with the principles and guidelines set out by the Faculty of Veterinary Medicine, Committee of Animal Care. This experiment was conducted at the Livestock Research Center at the Faculty of Veterinary Medicine in Bursa, Turkey, in April. Daylight was 12 h during this period in the region. One-day-old male chicks (600, Ross $PM_3$) obtained from a commercial hatchery were reared in 2 windowed houses with usual brooding techniques until they were 6 weeks old. The chicks were randomly divided into 6 treatment groups (2 levels of lighting × 3 levels of supplemental AA), consisting of 100 chicks per treatment based on the lighting (continuous: 24 h light (L):0 h dark (D), or intermittent: 12 h daylight followed by 3 cycles of 1 h L:3 h D during the night period) and supplemental AA (0, 200, and 400 mg/L added to water) conditions. Each treatment group consisted of 4 replicates of 25 birds each, housed in 1 × 3-m pens.

Newly hatched chicks in all treatments were reared under the same environmental conditions (floor space, bird density, and feeder and drinker space) in deep litter pens. Birds in all trials consumed a commercial maize-based broiler starter ration from 1 to 21 days of age (ME = 12.50 MJ/kg, 220 g/kg total protein), a broiler grower ration from 22 to 35 days of age (ME = 12.70 MJ/kg, 200 g/kg total protein), and a broiler finisher ration (ME = 12.92 MJ/kg, 180 g/kg total protein) from 36 to 42 days. Within each pen, water was provided via a hanging automatic bell drinker, and feed was provided ad libitum via a hanging tube feeder. Broilers received natural day light during daytime and artificial light (continuous or intermittent) during the night. For intermittent lighting an automatic timer was used. AA was supplemented by adding AA in powder form to water (200 or 400 mg/L of water).

Feed consumed by each pen was recorded and birds in each pen were weighed individually at 42 days of age to calculate the feed conversion ratio (kg of feed intake per kg of body weight gain) and average body weight. Survival was recorded on a per-pen basis as it occurred, and the survival rate was calculated in each group by dividing the number of live animals by the total number of birds at the beginning of experiment. Gait analysis was used to characterize the walking ability of the birds on day 42, as described by Kestin et al. (12), and then the craniocaudal (CCD) and latero-lateral (LLD) diameters of the knee joint were measured with a digital caliper (Mitutoyo Corporation, Kawasaki, Japan). The joints were placed in a normal posture position for anatomical investigation. The legs were manually palpated and scored as normal or abnormal (varus and valgus).

Bone properties (weight, length, volume, and cortical thickness of the tibiotarsus) and TD occurrence were measured on day 42. For this purpose birds were humanely killed by decapitation (13). Each tibiotarsus was immediately dissected from the surrounding tissues and kept frozen in a plastic bag at −20 °C until measured. Each frozen tibiotarsus was later thawed at room temperature for 1 h (14), and bone weight (Precisa XB 4200C, Zurich, Switzerland), length, and cortical thickness were measured with a digital caliper; volume was measured by immersing into a liquid-filled graded cylinder. There was a positive correlation between bone strength and cortical thickness; therefore, cortical thickness was measured from the front border after cutting the tibiotarsus along its central length with a fret saw (15,16).

The incidence of TD was evaluated based on the occurrence of a cartilage plug in the proximal head of the tibiotarsus, as illustrated by Kestin et al. (15) and Edwards and Veltman (17). The scoring system is from 0 to 3: 0) normal cartilage, 1) cartilage is thickened or shows considerably irregularities, 2) cartilage is thickened with evidence of persisting prehypertrophic cartilage, and 3) a large mass of cartilage is present in the proximal end of the tibiotarsus. For histological examination sections from the proximal head of the tibiotarsus, which were visually scored, were fixed in buffered neutral formalin before decalcification in formic acid/formalin. Paraffin-embedded sections (6 mm thick) were then prepared, stained with hematoxylin and eosin, and examined with light microscopy. Assessment of dyschondroplasia by histological examination was based on microscopic changes in the cells and matrix that are considered characteristic of TD (18).

Body weight, feed conversion ratio, and bone properties of the broilers were analyzed using ANOVA, using the general linear model procedure in SPSS® v.11.5 (SPSS Inc., Chicago, IL, USA). When differences between the groups were significant, means were separated using Tukey's test. Differences in the survival rate and incidence of TD between groups were evaluated using the chi-square test (19). Lighting and supplemental AA were the main effects. Results presented in the tables for body weight and bone properties are expressed as mean values ± SEM.

## Results

The main and interactive effects of lighting and supplemental AA on broiler performance are presented in Table 1. The body weights and feed conversion ratios in broilers receiving intermittent lighting were significantly better ($P < 0.05$) than in those receiving continuous lighting. No significant effects due to AA supplementation were observed on body weight or feed conversion ratio. The main effects, lighting and supplemental AA treatment, did not have any significant influence on the survival rate. There was no lighting × supplemental AA effect on body weight, feed conversion ratio, or survival rate.

Table 1. The main and interactive effects of lighting and supplemental AA on broiler performance (mean ± SEM).

| Lighting | AA (mg/L) | Body Weight (g) | Feed Conversion Ratio | Survival Rate (%) |
|---|---|---|---|---|
| **Main Effects** | | | | |
| Continuous | | 2272.48 ± 13.30[b] | 1.791 ± 0.026[a] | 96.292 ± 1.082 |
| Intermittent | | 2317.83 ± 13.38[a] | 1.697 ± 0.026[b] | 96.566 ± 1.085 |
| | 0 | 2295.50 ± 16.39 | 1.774 ± 0.032 | 95.775 ± 1.325 |
| | 200 | 2307.63 ± 16.28 | 1.731 ± 0.032 | 96.755 ± 1.320 |
| | 400 | 2282.34 ± 16.35 | 1.727 ± 0.032 | 96.756 ± 1.324 |
| **Interactive Effects** | | | | |
| Continuous | 0 | 2278.83 ± 23.18 | 1.824 ± 0.046 | 95.365 ± 1.874 |
| Continuous | 200 | 2267.86 ± 22.85 | 1.782 ± 0.046 | 97.218 ± 1.872 |
| Continuous | 400 | 2270.69 ± 23.07 | 1.767 ± 0.046 | 96.293 ± 1.873 |
| Intermittent | 0 | 2312.10 ± 23.18 | 1.723 ± 0.046 | 96.185 ± 1.874 |
| Intermittent | 200 | 2347.40 ± 23.18 | 1.681 ± 0.046 | 96.293 ± 1.874 |
| Intermittent | 400 | 2293.98 ± 23.18 | 1.686 ± 0.046 | 97.220 ± 1.870 |
| **ANOVA** | | | | |
| Lighting | | 0.017 | 0.021 | 0.860 |
| AA (mg/L) | | 0.548 | 0.537 | 0.835 |
| Lighting × AA (mg/L) | | 0.429 | 0.969 | 0.858 |

[a,b]Means with different superscripts differ in columns.

The main and interactive effects of lighting and supplemental AA on bone properties and diameters of the knee joint are presented in Tables 2 and 3, respectively. The weight and length of the tibiotarsus in broilers that received intermittent lighting were significantly better ($P < 0.05$) than in those that received continuous lighting. Cortical thickness was significantly affected by continuous lighting ($P < 0.05$). Although supplemental AA treatment did not significantly affect the length, volume, or cortical thickness, tibiotarsus weight increased significantly in the 200 and 400 mg/L AA groups ($P < 0.001$). There were lighting × AA interactions on the length of the tibiotarsus ($P < 0.05$). Based on gait analysis, genu varum was observed in 3 chicks. The main effect (lighting) affected LLD of the knee joint in broilers. The other main effect (AA supplementation) significantly affected both CCD and LLD of the knee joint ($P < 0.05$ and $P < 0.001$, respectively). There were lighting × AA interactions on CCD of the knee joint ($P < 0.05$).

Birds with abnormal legs in each treatment group varied from 1 to 2. There were no significant differences in the incidence of TD and lesion score between the light and AA supplementation treatments. Total mean percentage of TD in response to intermittent light was 6.66%, as compared to 3.33% in response to continuous light treatment. The interaction between lighting × AA supplementation had no significant effect on the incidence of TD.

The main and interactive effects of lighting and supplemental AA on the incidence of TD and TD score are presented in Table 4. Neither AA supplementation nor lighting had any significant effect on TD. Histopathological examination revealed that the irregular cartilage mass, which was situated below the epiphyseal growth plate and extended into the metaphysis, was composed of transitional (prehypertrophic) chondrocytes, which is typical of TD. The number of transitional chondrocytes increased markedly in the proximal head of the tibiotarsi with TD. The cartilage contained smaller lacunae and more extracellular matrix than normal hypertrophic cartilage. The lacunae were ovoid and slightly flattened. The chondrocytes had pyknotic nuclei with scant cytoplasm.

Table 2. The main and interactive effects of lighting and supplemental AA on tibiotarsus bone properties (mean ± SEM).

| Lighting | AA (mg/L) | Weight (g) | Length (mm) | Volume ($cm^3$) | Cortical Thickness (mm) |
|---|---|---|---|---|---|
| **Main Effects** | | | | | |
| Continuous | | 12.56 ± 0.22[b] | 102.04 ± 0.25[b] | 14.30 ± 0.58[a] | 25.64 ± 1.43[a] |
| Intermittent | | 13.25 ± 0.36[a] | 103.33 ± 0.66[a] | 13.61 ± 0.21[b] | 20.40 ± 1.04[b] |
| | 0 | 11.99 ± 0.27[b] | 102.21 ± 0.48 | 13.75 ± 0.26 | 24.40 ± 1.76 |
| | 200 | 13.09 ± 0.31[a] | 103.07 ± 0.62 | 14.17 ± 0.65 | 21.96 ± 1.54 |
| | 400 | 13.64 ± 0.46[a] | 102.78 ± 0.27 | 13.95 ± 0.14 | 22.70 ± 1.93 |
| **Interactive Effects** | | | | | |
| Continuous | 0 | 12.02 ± 0.39 | 102.46 ± 0.69 | 13.80 ± 0.37 | 29.63 ± 2.49 |
| Continuous | 200 | 12.34 ± 0.56 | 101.67 ± 0.66 | 13.70 ± 0.11 | 23.45 ± 2.65 |
| Continuous | 400 | 13.32 ± 0.84 | 101.98 ± 0.95 | 13.35 ± 0.73 | 23.85 ± 2.36 |
| Intermittent | 0 | 11.96 ± 0.67 | 101.96 ± 0.84 | 13.70 ± 0.76 | 19.18 ± 2.15 |
| Intermittent | 200 | 13.84 ± 0.44 | 104.47 ± 0.37 | 14.65 ± 0.99 | 20.48 ± 2.19 |
| Intermittent | 400 | 13.95 ± 0.31 | 103.58 ± 0.26 | 14.55 ± 0.15 | 21.55 ± 2.76 |
| **ANOVA** | | | | | |
| Lighting | | 0.033 | 0.023 | 0.026 | 0.011 |
| AA (mg/L) | | 0.001 | 0.450 | 0.520 | 0.606 |
| Lighting × AA (mg/L) | | 0.144 | 0.058 | 0.182 | 0.198 |

[a,b]Means with different superscripts differ in columns.

Table 3. The main and interactive effects of lighting and supplemental AA on knee joint diameter (mean ± SEM).

| Lighting | AA (mg/L) | Knee Joint CCD (mm) | Knee Joint LLD (mm) |
|---|---|---|---|
| **Main Effects** | | | |
| Continuous | | 54.62 ± 0.47 | 37.92 ± 0.33[b] |
| Intermittent | | 54.15 ± 0.65 | 38.88 ± 0.67[a] |
| | 0 | 53.67 ± 0.57[b] | 36.86 ± 0.41[b] |
| | 200 | 55.64 ± 0.42[a] | 39.05 ± 0.92[a] |
| | 400 | 53.84 ± 0.87[b] | 39.29 ± 0.66[a] |
| **Interactive Effects** | | | |
| Continuous | 0 | 54.23 ± 0.81 | 36.33 ± 0.58 |
| Continuous | 200 | 54.80 ± 0.21 | 38.01 ± 0.88 |
| Continuous | 400 | 54.84 ± 0.46 | 39.40 ± 0.17 |
| Intermittent | 0 | 53.46 ± 0.34 | 37.39 ± 0.63 |
| Intermittent | 200 | 56.48 ± 0.96 | 40.08 ± 0.49 |
| Intermittent | 400 | 52.51 ± 0.08 | 39.17 ± 0.35 |
| **ANOVA** | | | |
| Lighting | | 0.479 | 0.045 |
| AA (mg/L) | | 0.031 | 0.000 |
| Lighting × AA (mg/L) | | 0.049 | 0.146 |

[a,b]Means with different superscripts differ in columns.

Table 4. The main and interactive effects of lighting and supplemental AA on the incidence of TD (%) and TD score (%).

| Lighting | AA (mg/L) | Number of birds with TD | Distribution of birds according to TD score | TD incidence (%) |
|---|---|---|---|---|
| **Main Effects** | | | | |
| Continuous | | 10 | 1 | 3.33 |
| Intermittent | | 20 | 1.2 | 6.66 |
| | 0 | 10 | 1 | 5.00 |
| | 200 | 5 | 1 | 2.50 |
| | 400 | 15 | 1.2 | 7.50 |
| **Interactive Effects** | | | | |
| Continuous | 0 | 5 | 1 | 5.00 |
| Continuous | 200 | 0 | 0 | 0.00 |
| Continuous | 400 | 5 | 1 | 5.00 |
| Intermittent | 0 | 5 | 1 | 5.00 |
| Intermittent | 200 | 5 | 1 | 5.00 |
| Intermittent | 400 | 10 | 1.2 | 10.00 |
| **ANOVA** | | | | |
| Lighting | | 0.156 | - | - |
| AA (mg/L) | | 0.874 | - | - |
| Lighting × AA | | 0.712 | - | - |

## Discussion

The growth performance in broilers reared under intermittent lighting was better than in those reared under continuous lighting (5,11,20). Intermittent lighting significantly increased both body weight and the feed conversion ratio in broilers ($P < 0.05$). The efficiency of feed utilization in broilers that received intermittent lighting improved because of the short feeding period, followed by a longer period of time for digestion. It has been reported that birds eating under continuous lighting excreted more protein than did birds given a meal at regular intervals under intermittent lighting (21). Contrary to the findings obtained by Doan and Giang (6), McKee and Harrison (22), and Whitehead and Keller (23), Petek et al. (20) reported that supplemental AA had no effect on any of the growth parameters measured in broilers.

While the continuous lighting program affected volume and cortical thickness, intermittent lighting had a significant effect on tibiotarsal weight and length. Previous researchers failed to demonstrate the effect of intermittent lighting on tibiotarsal length and weight (5,20,24). The increase in bone length and weight observed in our study could be due to the increase in the activity of the birds during the intermittent period. Contrary to the findings obtained by Ingram et al. (5) and Petek et al. (20), continuous lighting had an effect on cortical thickness in broilers. This could be due to the stimulating effect of light on bone development. Although bone characteristics have been reported to improve with supplemental AA (1,22,25,26), in this study supplemental AA had no effect on length or volume, but did have an effect on tibiotarsal weight. The effect of supplemental AA on cortical thickness of the tibiotarsus, one of the main indicators of leg weakness, was not significant. The combined application of lighting and supplemental AA positively influenced tibiotarsal length, indicating that birds receiving intermittent light exhibited greater resistance to leg weakness in the presence of 200 mg/L of supplemental AA. Bone weight of the tibiotarsi in this group was also higher than in the other groups.

AA positively affects the CCD of the knee joint (an indicator of leg weakness) (15).

Although the continuous lighting program had numerically lower TD, light treatment had no significant effect on the incidence of TD and TD scores were 0, 1, and 2. This may suggest that the increase in activity of the birds during extended periods of light may have improved skeletal bone development. While these results are supported by the findings of Elliot and Edwards (27), they are not in concordance with the findings of Ingram et al. (5) or Renden et al. (28). In general, the percentage of TD in broilers subjected to different light treatments was lower than reported by Renden et al. (28). In our study the lowest incidence of TD was seen in broilers treated with 200 mg/L of supplemental AA ($P > 0.05$); TD scores were 1 and 1.2. These data are similar to those obtained by Doan and Giang (6), McKee and Harrison (22), and Bains et al. (29), who reported that AA supplementation decreased mortality and the percentage of chickens with TD. A higher proportion of birds that scored 1 and 2 were in the group treated with intermittent light and 400 mg/L of AA supplementation. The higher incidence of TD in this group might be explained by the fact that supplemental AA is more effective under continuous light. All of the birds in the continuous light × 200 mg/L of supplemental AA had a score of 0.

Nonetheless, due to better broiler performance and tibiotarsus characteristics, intermittent lighting was determined to be more suitable. Addition of 200 mg/L of AA in drinking water may have beneficial effects on tibiotarsus characteristics and prevent diseases. In conclusion, it can be suggested that intermittent lighting + 200 mg/L of AA supplementation results in better performance in Ross PM3 broilers.

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