

## Effect of Perch Cooling and Litter Thickness on Leg Health and Bone Characteristics in Broilers

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### ABSTRACT

The study was aimed at assessing the leg health variables (footpad dermatitis, hock burn, tibial dyschondroplasia, gait score), tarsometatarsus asymmetry, and physical properties of tibiatarsus of broilers reared with perch application and different litter thicknesses. A total of 459 one-day old Ross-308 broiler chicks were allocated into 27 pens, each containing 17 chicks, in a 3 (perch treatments) x 3 (litter treatments) experimental design with three replications. The position of both cooled and non-cooled perches was set to make animals pass over the perch for feeding and drinking. The perch temperature was set to 10°C for cooled perches while litter thicknesses were 1, 7, and 14 cm. Results showed that cooled perches decreased the severity of footpad and gait score, and tibial dyschondroplasia in broilers. Footpad dermatitis and hock burn score decreased in broilers reared at 14 cm litter. It was determined that the length and width of tarsometatarsus at 42 day of age was increased in cooled perch and 1 cm litter group. Tibia length and robustness index in cooled perch group was found higher than those in non-cooled and no-perch groups. The perch application has no effect on the weight-length and tibio-tarsal indexes. Tibia lengths in 1 cm (103.81 mm) litter thickness group were higher than in both 7 and 14 cm groups. The bones from 1 cm litter thickness group were found heavier (21.55 g) than in 7 cm litter group. These results indicated that cooled perch and 14 cm litter thickness has a beneficial effect on broilers' well-being in hot weathers.

**Keywords:** Footpad dermatitis, Hot weather, Ross-308 broiler chicks, Tibio-tarsal indexes.

### INTRODUCTION

Intense genetic selection and strict management practices have led to a fast gain of muscle weight and this excessive load on tibial bones makes them prone to various disorders (Charuta *et al.*, 2011; Azizian *et al.*, 2013). The strength of leg bones is not only genetically determined but it also depends on the sex, age, health, nutrition, and environment (Rath *et al.*, 1999; Cook, 2000; Mabelebele *et al.*, 2017; Karaarslan and Nazligul, 2018; Kwiatkowska *et al.*, 2018; Süzer *et al.*, 2019). Intensive genetic selection may have increased the broilers

sensitivity to heat stress. In a previous study, it was demonstrated that broilers exposed to acute heat stress and decreased feed intake (FI) had a physiological response in order to decrease metabolic heat production and to maintain body homeostasis, which resulted in a significant decrease in Body Weight (BW) (Mello *et al.*, 2015). In this context, cooled perch usage is attracting attention as a new alternative system in hot weathers. Hu *et al.* (2016) determined that cooled perches reduced thermoregulatory behaviors during heat stress, but did not affect BW, FI, and physiological traits in laying hens reared at ambient temperature imposed during the study.

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Broiler chickens are generally reared on floors covered with litter, and birds spend most of their lifetime in contact with litter. Therefore, litter thickness on floors is another important factor that affects broilers' welfare and performance (Nagaraj *et al.*, 2007). Shao *et al.* (2015) found that broilers reared on thicker litter scored better on hock swelling and Footpad Dermatitis (FPD). Broiler chicken welfare audits generally include leg health problems such as FPD and Hock Burn (HB), Tibial Dyschondroplasia (TD), and lameness [as defined by Gait Score (GS)], and asymmetry [Fluctuating Asymmetry (FA)] and relative Fluctuating Asymmetry (rFA) (Cook, 2000). The rFA provides information on the level of responses of birds to non-optimal environmental conditions. The genetic and environmental stress factors may also increase the asymmetry of bilateral traits in poultry species (Yalcin *et al.*, 2003). Kaukonen *et al.* (2017) recently assessed leg health of broilers to gait scoring, as the presence and severity of TD, and as bone ash and mineral contents.

This study was designed to determine the effects of litter thickness and the provision of a perch cooling on leg health parameters, asymmetry of tarsometatarsus (FA and rFA of tarsometatarsus), and physical properties of tibiotarsus (the weight, length, diameter, Robustness Index (RI), and weight-length index, tibio-tarsal index, total, medullar and cortical areas, moment of inertia, Mediolateral (ML) and Craniocaudal (CrCau) cortical thicknesses) within the scope of welfare in broilers. Besides, the correlations among live BW and bone properties in broilers were also investigated.

## MATERIALS AND METHODS

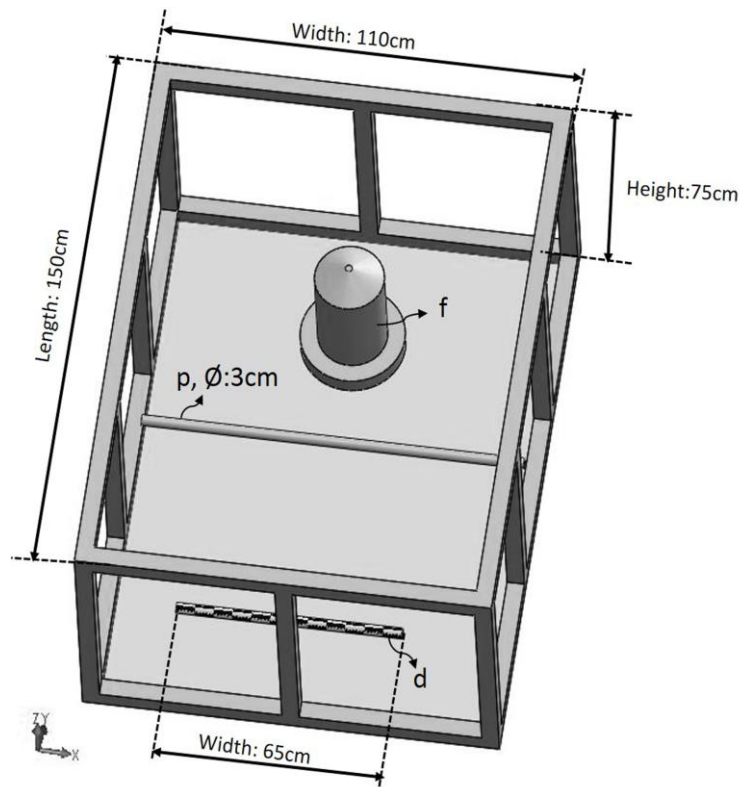
### Birds and Husbandry

This study was conducted at the Poultry Breeding Unit of the Department of Animal Science, University of Adnan Menderes, Aydin. The climate during the period of

study was characterized by dry to hot season. Aydin district is situated at 37° 51' 27" N latitude and 27° 51' 14" E longitudes with an altitude range between 50-200 m above sea level. Aydin has the Mediterranean climate (Kirmaci and Agcagil, 2009). A total of 459 one-day-old Ross 308 male broiler chickens were purchased from a local commercial hatchery. Broiler chicks were randomly assigned to perch and litter thickness treatments with three replications with 3×3 factorial arrangement of 3 perch treatments (no perch, non-cooled perch i.e. the same temperature with the ambient temperature, and cooled perch) and 3 litter thickness treatments (1, 7, and 14 cm litter thicknesses of pine wood shavings). Chicks of each treatment were placed in separate floor pens (in a total of 27 pens (3×3= 9 main groups, 9×3 Replicates= 27 pens)) measuring 110×150 cm, width×length, respectively. A total of 27 pens were settled in the same room. All chicks were fed with complete diets (1-14 days; 3,050 kcal ME kg<sup>-1</sup>, 23.5% crude protein, 15-28 day; 3,150 kcal ME kg<sup>-1</sup>, 22% crude protein, and 29-42 days; 3,200 kcal ME kg<sup>-1</sup>, 21% crude protein). Feed and water were ensured *ad libitum* throughout the study. According to the recent EU guidelines (EU Council Directive, 2007), photoperiod lengths were applied 24:0 (L:D) from 1 to 7 days, 18:6 (L:D) from 8 to 39 days, followed by 24:0 (L:D) from 40 to 42 days. The brooding temperature was 34°C for the first 3 d and was reduced gradually to around 30°C at 1 week of age. Then, all the birds were subjected to ambient temperatures, which were recorded daily. The humidity varied from 50 to 60%. The experiment lasted for 42 days.

### Experimental Design

Before conducting the experiment, ethical clearance was obtained from the Institutional Animal Ethics Committee of Adnan Menderes University (File No:



**Figure 1.** Schematics of pen with perch layouts (non-cooled and cooled perch): d= Drinker, f= Feeder, p= Perch.

64583101/2017/049). In terms of perch application, 18 galvanized steel perches (110 mm length and 30 mm in diameter) were horizontally attached 10 cm above the pen litter as experimental design. For the motivation of birds, the position of both cooled and non-cooled perches was set to make chicks to pass over the perch for feeding and drinking (Figure 1). However, in two weeks of their life, because of their small bodies, all chicks had a chance to reach drinkers and feeders without passing over the perches. For cooling treatment, 9 out of the 18 perches were connected to the water cooler (Cihso 2000, Cihan Cooling Ltd. Co., Turkey), which cooled the water to 10°C and circulate 5 L min<sup>-1</sup> through the pipes. Water temperature in cooled perches was kept between 9.8-10.2°C by thermosensors and controlled twice daily with gauges installed in the system. Because of the height of the pen ceiling (4.0 m), there was a little possibility that cooling perches

cooled the pen at the same time. Chickens were exposed to perches from 1<sup>st</sup> day, and the cooling process was initiated on the 14<sup>th</sup> day and lasted until end of the experiment.

### Traits Measured

At 42 day, for each pen, seven randomly selected broilers (a total of 189 broilers) were assessed for FPD, HB and GS (Welfare Quality®, 2009). The FPD was assigned to 0 of 4 scores: 0= Footpads with no visible lesions, 1 and 2= Footpads with mild superficial lesions, 3 and 4= Footpads with severe ulcerative lesions. HB mild superficial lesions (score 1 and 2) were judged having no trouble or disorder and were combined with category 0 (not affected). The ulcerative lesions (score 3 and 4) were assigned as a painful condition. Gait score was determined by using the 0-to-5 scale (0: Excellent gait and 5: Deficiency



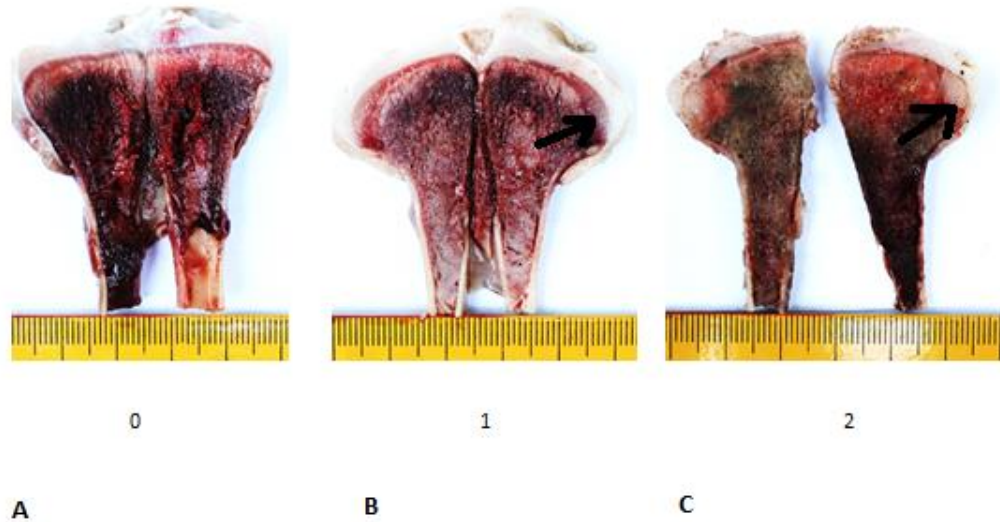
stand). Also, same birds were used for the measurement of claw length, FA and rFA of tarsometatarsus. Additionally, selected birds were weighted individually for live BW. Length and width of the left and right tarsometatarsus were measured with a digital caliper for calculation of FA and rFA of tarsometatarsus length and width (Moller *et al.*, 1999). FA was defined as the absolute difference between the Left (L) and Right (R) tarsometatarsus. The rFA was obtained by dividing FA with the mean of length and width of L and R tarsometatarsus. Mean rFA was defined as the mean rFA of the tarsometatarsus different traits (Moller *et al.*, 1999). Claw length was measured with a digital caliper. Chickens randomly selected for leg health variables, GS, and symmetry measurements were checked for TD and physical properties of tibiotarsus when slaughtered by cutting the jugular veins at 42 days of age. Left tibias were collected, surrounding tissues were dissected, tibia bones were weighed, and stored at  $-20^{\circ}\text{C}$  until analysis. The head of the tibiotarsal bone was cut with a sharp knife horizontally from the midpoint on proximal tibiotarsus, and scoring the amount of cartilage tissue on the cut (0= No cartilage, 1= Some to one third of the cut with cartilage, 2= From a third to half of the cut with cartilage, 3= More than a half of the cut consisting of cartilage) (Moller *et al.*, 1999). Tibia bone weight was measured by using 0.01 g sensitive digital scale (Scaltec, SBP 52, Germany). Other physical properties of tibia bones i.e. tibia weight, tibia length, the medullar canal (intracortical) and diaphysis (extracortical) diameters were measured using a digital caliper (Mitutoyo, Model: CD-15CP, Code: 500-181 U, Japan). Robustness index, weight-length index, and tibio-tarsal index were calculated as described by Kara (2002). The RI (Robustness Index): Bone length/cubic root of bone weight, Weight-length index: Bone weight/bone length ( $\text{mg mm}^{-1}$ ), and Tibio-tarsal index (Cortical index) (%): [(Diaphysis diameter-Medullar canal diameter)/Diaphysis diameter] $\times 100$  were

calculated. In this study, the area values were calculated according to Vitorović *et al.* (2009). Also, the cross section of the bone was accepted as a hollow ellipse and the moment values of inertia were calculated according to Lopez and Markel (2000). TA-Total area (cross sectional diaphyseal area)=  $\pi/4 (DD_{\text{CrCau}} \times DD_{\text{ML}})$ , MA-Medullar area (cross sectional medullar canal area)=  $\pi/4 (MD_{\text{CrCau}} \times MD_{\text{ML}})$ , CA-Cortical area (cross sectional medullar canal area)= TA-MA, and I-Moment of inertia=  $\pi/64 [(DD_{\text{ML}} \times DD_{\text{CrCau}}^3) - (MD_{\text{ML}} MD_{\text{CrCau}}^3)]$ .

Broiler chicken behaviors were evaluated by using a video recording program. Daily perching behaviors of birds were monitored and recorded with this program from the day of 22 to 42 with cameras that were placed on the top of each pen. In this assessment, if the bird was standing, sitting, or walking with both feet on the perch and staying at least 2 s, it was defined as perching behavior (Zhao *et al.*, 2013). However, if the chicken was not perching, but was in physical contact with the perch (perch-contacting), these animals were evaluated as animals being in contact with the perch. No perching and perch-contacting behaviors were accepted as other behaviors (e.g. eating, drinking, walking, and resting on the floor).

### Statistical Analyses

Data were analyzed using the Statistical Package for the Social Sciences for Windows (SPSS) 22.0 (SPSS Inc, Chicago, IL, USA). Analysis of variance was performed with GLM procedure to reveal the effects of cooled perch and litter thickness on footpad and hock health, GS, TD, FA and rFA of tarsometatarsus, claw length, physical properties of tibiotarsus, and tibia robustness, weight-length, and tibio-tarsal indexes. The interaction among perch and litter thickness was not significant for the investigated traits and, therefore, it was excluded from the analysis. Comparisons among subclass means were carried out by Duncan's multiple range test. FPD, HB, GS,



**Figure 2.** Tibia bones without TD lesion (A; Score 0), with mild TD lesion (B; Score 1), and severe TD lesion (C; Score 2).

and TD scores were square root transformed, and significant values were calculated using square root transformed data. Chi-square test was performed for perching behaviors. The correlations between live BW and tibia bone properties were calculated using the Person's correlation coefficients.

## RESULTS

In the study, maximum average diurnal temperatures in the house was 33.4°C from 3 to 6 weeks of age, daily mean ambient temperature ranged from 30.4 to 31.9°C, and maximum diurnal ambient temperatures were in the range of 31.4 to 34.2°C for dry bulb in the house from 3 to 6 weeks of age, which are characteristic for Aydin region in Turkey. The effects of perch and litter thickness in broilers on FPD, HB, GS, and TD scores and claw length, measurements of tarsometatarsus are represented in Table 1. Perch had a significantly positive effect on

FPD, GS, and TD (Table 2). The provision of a perch cooling reduced the score and severity of TD in broiler chickens ( $P < 0.01$ ) compared to those without perch and non-cooled perch (Figure 2). Tarsometatarsus length and width was greater ( $P < 0.05$ ) in broiler chickens subjected to the cooled perch compared to no perch. However, there was no effect of perch on the FA and rFA of the tarsometatarsus. Tibia length and RI were measured higher in the cooled perch group than no perch and normal perch groups ( $P < 0.001$ ) (Table 2). The weight and length of tibia and RI were found the highest in 1 cm litter thickness group. The litter thickness had no statistically significant effect on the medullar canal and diaphysis diameters of tibia, weight-length index, and tibio-tarsal index. The effects of perch and litter thickness in broilers on total area, medullar area, cortical area, the moment of inertia, ML cortical thickness, and CrCau cortical thickness of tibiotarsus are represented in Table 3. The increase in total

**Table 1.** FPD, HB, GS, and TD scores, claw length, length, width, FA, and rFA of tarsometatarsus in relation to perch and litter thickness based on random effect ANOVA GLM models.<sup>a</sup>

| Treatment main effects  | GS                |                   |                    |                   | TD               |                                |       |       | Length of tarsometatarsus (mm) |       |       |                               | Width of tarsometatarsus (mm) |       |          |  |
|-------------------------|-------------------|-------------------|--------------------|-------------------|------------------|--------------------------------|-------|-------|--------------------------------|-------|-------|-------------------------------|-------------------------------|-------|----------|--|
|                         | FPD               | HB                | GS                 | TD                | Claw length (mm) | Length of tarsometatarsus (mm) | FA    | rFA   | Length of tarsometatarsus (mm) | FA    | rFA   | Width of tarsometatarsus (mm) | FA                            | rFA   | Mean rFA |  |
| Expected mean ( $\mu$ ) | 0.20              | 0.76              | 0.29               | 0.48              | 13.36            | 86.28                          | 2.39  | 0.03  | 86.28                          | 2.39  | 0.03  | 13.36                         | 0.57                          | 0.04  | 0.04     |  |
| Perch treatment         |                   |                   |                    |                   |                  |                                |       |       |                                |       |       |                               |                               |       |          |  |
| No perch                | 0.44 <sup>a</sup> | 0.90              | 0.43 <sup>a</sup>  | 0.60 <sup>a</sup> | 13.31            | 85.53 <sup>b</sup>             | 2.90  | 0.04  | 85.53 <sup>b</sup>             | 2.90  | 0.04  | 13.22 <sup>b</sup>            | 0.64                          | 0.05  | 0.04     |  |
| Non-cooled perch        | 0.12 <sup>b</sup> | 0.68              | 0.32 <sup>ab</sup> | 0.57 <sup>a</sup> | 13.34            | 86.07 <sup>ab</sup>            | 2.01  | 0.02  | 86.07 <sup>ab</sup>            | 2.01  | 0.02  | 13.29 <sup>ab</sup>           | 0.51                          | 0.04  | 0.03     |  |
| Cooled Perch            | 0.05 <sup>b</sup> | 0.68              | 0.13 <sup>b</sup>  | 0.27 <sup>b</sup> | 13.43            | 87.24 <sup>a</sup>             | 2.26  | 0.03  | 87.24 <sup>a</sup>             | 2.26  | 0.03  | 13.57 <sup>a</sup>            | 0.55                          | 0.04  | 0.03     |  |
| Litter thickness        |                   |                   |                    |                   |                  |                                |       |       |                                |       |       |                               |                               |       |          |  |
| 1 cm                    | 0.52 <sup>a</sup> | 1.05 <sup>a</sup> | 0.27               | 0.52              | 13.26            | 87.536 <sup>a</sup>            | 2.16  | 0.02  | 87.536 <sup>a</sup>            | 2.16  | 0.02  | 13.620 <sup>a</sup>           | 0.45                          | 0.03  | 0.03     |  |
| 7 cm                    | 0.07 <sup>b</sup> | 0.73 <sup>b</sup> | 0.24               | 0.48              | 13.26            | 85.292 <sup>b</sup>            | 2.92  | 0.04  | 85.292 <sup>b</sup>            | 2.92  | 0.04  | 13.335 <sup>b</sup>           | 0.68                          | 0.05  | 0.04     |  |
| 14 cm                   | 0.02 <sup>b</sup> | 0.49 <sup>c</sup> | 0.37               | 0.44              | 13.57            | 86.011 <sup>b</sup>            | 2.09  | 0.02  | 86.011 <sup>b</sup>            | 2.09  | 0.02  | 13.123 <sup>b</sup>           | 0.57                          | 0.04  | 0.03     |  |
| SEM                     | 0.03              | 0.04              | 0.04               | 0.04              | 0.06             | 0.27                           | 0.30  | 0.01  | 0.27                           | 0.30  | 0.01  | 0.06                          | 0.04                          | 0.00  | 0.00     |  |
| Treatment effects       |                   |                   |                    |                   |                  |                                |       |       |                                |       |       |                               |                               |       |          |  |
| Perch                   | 0.000             | 0.055             | 0.004              | 0.003             | 0.699            | 0.033                          | 0.471 | 0.458 | 0.033                          | 0.471 | 0.458 | 0.037                         | 0.376                         | 0.309 | 0.213    |  |
| Litter thickness        | 0.000             | 0.000             | 0.365              | 0.829             | 0.067            | 0.003                          | 0.467 | 0.415 | 0.003                          | 0.467 | 0.415 | 0.003                         | 0.071                         | 0.053 | 0.071    |  |

<sup>a</sup> SEM: Standard Error Mean; FPD: Footpad Dermatitis; HB: Hock Burns; GS: Gait Score, TD: Tibial Dyschondroplasia; FA: Fluctuating Asymmetry, rFA: Relative Fluctuating Asymmetry. (a-b) Means with different superscript letters in the same column differ (P<0.05).

**Table 2.** Effects of perch and litter thickness on some physical properties of tibiotarsus and RI, weight-length index, tibio-tarsal index in broiler chickens.<sup>a</sup>

| Treatment main effects  | Tibia weight (g)    | Tibia length (mm)   | Medullar canal diameter (mm) | Diaphysis diameter (mm) | RI                 | Weight-length index (mg mm <sup>-1</sup> ) | Tibio-tarsal index |
|-------------------------|---------------------|---------------------|------------------------------|-------------------------|--------------------|--|--------------------|
| Expected mean ( $\mu$ ) | 21.01               | 102.18              | 4.98                         | 8.21                    | 3.71               | 205.38                                     | 39.19              |
| Perch treatment         |                     |                     |                              |                         |                    |  |                    |
| No perch                | 20.94               | 100.92 <sup>b</sup> | 4.90                         | 8.15                    | 3.67 <sup>b</sup>  | 207.23                                     | 39.77              |
| Non-cooled perch        | 20.78               | 101.24 <sup>b</sup> | 4.94                         | 8.11                    | 3.69 <sup>b</sup>  | 205.21                                     | 38.90              |
| Cooled Perch            | 21.29               | 104.37 <sup>a</sup> | 5.11                         | 8.36                    | 3.77 <sup>a</sup>  | 203.71                                     | 38.91              |
| Litter thickness        |                     |                     |                              |                         |                    |  |                    |
| 1 cm                    | 21.55 <sup>a</sup>  | 103.81 <sup>a</sup> | 5.07                         | 8.22                    | 3.74 <sup>a</sup>  | 207.32                                     | 38.12              |
| 7 cm                    | 20.57 <sup>b</sup>  | 100.77 <sup>b</sup> | 4.89                         | 8.21                    | 3.68 <sup>b</sup>  | 203.97                                     | 40.44              |
| 14 cm                   | 20.90 <sup>ab</sup> | 101.96 <sup>b</sup> | 4.99                         | 8.19                    | 3.70 <sup>ab</sup> | 204.86                                     | 39.02              |
|                         | 0.15                | 0.30                | 0.04                         | 0.05                    | 0.01               | 1.11                                       | 0.44               |
| Treatment effects       |                     |                     |                              |                         |                    |  |                    |
| Perch                   | 0.343               | 0.000               | 0.079                        | 0.059                   | 0.000              | 0.433                                      | 0.652              |
| Litter thickness        | 0.024               | 0.000               | 0.225                        | 0.974                   | 0.027              | 0.445                                      | 0.099              |

<sup>a</sup> Data presented as the least square means. SEM: Standard Error Mean, RI: Robustness Index. <sup>ab</sup> Values with different superscripts in a column differ significantly (P<0.05).

**Table 3.** Effects of perch and litter thickness on total area, medullar area, cortical area, the moment of inertia, ML cortical thickness, CrCau cortical thickness of tibiotarsus in broilers.<sup>a</sup>

| Treatment main effects  | Total area          | Medullar area       | Cortical area | Moment of inertia   | ML cortical thickness | CrCau cortical thickness |
|-------------------------|---------------------|---------------------|---------------|---------------------|-----------------------|--------------------------|
| Expected mean ( $\mu$ ) | 52.52               | 19.62               | 32.90         | 153.17              | 1.92                  | 1.31                     |
| Perch treatment         |                     |                     |               |                     |                       |                          |
| No perch                | 51.27 <sup>a</sup>  | 18.96 <sup>a</sup>  | 32.04         | 144.81 <sup>a</sup> | 1.88                  | 1.29                     |
| Non-cooled perch        | 51.77 <sup>ab</sup> | 19.23 <sup>ab</sup> | 32.80         | 148.20 <sup>a</sup> | 1.92                  | 1.30                     |
| Cooled Perch            | 54.52 <sup>b</sup>  | 20.66 <sup>b</sup>  | 33.86         | 166.49 <sup>b</sup> | 1.95                  | 1.33                     |
| Litter thickness        |                     |                     |               |                     |                       |                          |
| 1 cm                    | 52.881              | 20.312              | 32.569        | 155.818             | 1.879                 | 1.275                    |
| 7 cm                    | 52.381              | 18.950              | 33.432        | 150.401             | 1.984                 | 1.330                    |
| 14 cm                   | 52.307              | 19.600              | 32.708        | 153.286             | 1.890                 | 1.318                    |
| SEM                     | 0.58                | 0.32                | 0.74          | 3.71                | 0.03                  | 0.02                     |
| Treatment effects       |                     |                     |               |                     |                       |                          |
| Perch                   | 0.042               | 0.048               | 0.290         | 0.039               | 0.524                 | 0.773                    |
| Litter thickness        | 0.909               | 0.228               | 0.728         | 0.837               | 0.187                 | 0.618                    |

<sup>a</sup> Data presented as the least square means. SEM: Standard Error Mean; ML: Mediolateral, CrCau: Craniocaudal. (a-b) Values with different superscripts in a column differ significantly ( $P < 0.05$ ).

area and the cortical area was 6%, the medullary area was increased by 9%, and the cortex thickness increased by only 3% at both axes. In addition, the lengths, areas and diameters of the bones showed alterations as no perch < non-cooled perch < cooled perch. The largest values were observed in the cooled perch group while the smallest values were seen in the no perch group. Although there was no statistical difference, cross-sectional parameters increased with the increase in litter thickness. It is noteworthy that the length of the tibia and the medullar diameter were found largest at the 1 cm height of litter and the smallest on the 7 cm thick litter. The RI, which is an index based on diameter and length, showed similar results.

In this study, perching frequency in non-cooled and cooled perch treatments were 7.5 and 5.3%, respectively. However, the perch-contact frequency was greater in the cooled perch group (39.2%) than non-cooled perch (13.7%) at 6 weeks of age ( $\chi^2 = 882.980$ ,  $df = 2$ ), ( $P < 0.001$ ). The frequency of perching increased in broiler chickens subjected to 1 cm litter thickness in comparison with those subjected to 7 and 14 cm litter thickness (7.9 vs. 5.4% and 1.5%) at 6 wk. The perch-contacting frequency was higher ( $P < 0.001$ )

in broiler chickens subjected to the 14 cm litter thickness (59.1%) compared to 1 and 7 cm litter thicknesses (25.2 and 35.4%, respectively) ( $\chi^2 = 294.764$ ,  $df = 2$ ), ( $P < 0.001$ ). In this study, live BW had generally highly significant ( $P < 0.01$ ) positive correlations with length and thickness of tarsometatarsus, weight, length, medullar and diaphysis diameters of the tibia, and weight-length index (0.388, 0.434, 0.721, 0.577, 0.194, 0.387, and 0.594, respectively). However, low, positive and no significant correlations were shown between live BW and RI (0.074) and tibio-tarsal index (0.089).

## DISCUSSION

In this study, results revealed that perch cooling resulted in decreased FPD and GS and TD conditions. In a study about the effect of perches and platform on leg health, Kaukonen *et al.* (2017) reported better GS for birds with access to platforms. Zhao *et al.* (2012; 2013) reported that cooled perch decreased the incidence of FPD and HB and improved welfare. It was determined that non-cooled perches increased these lesions and affected welfare, therefore, perch type is



quite important. These results were similar to other studies performed with a different type of perches by Pettit-Riley and Estevez (2001), Ventura *et al.* (2010), and Bailie and O'Connell (2015). This can be explained by the fact that the use of cooled perch decreased foot and leg disorders, indicating improved bird's welfare. It can be also said that perch cooling would increase the perch-contact frequency, and bird's welfare. Accordingly, our findings suggest that poor leg health could be increased with decreasing litter thickness. Another study has reported similar results that the best scores occur in pens with 12 cm of litter (Shao *et al.*, 2015). Haslam *et al.* (2007) and Shao *et al.* (2015) reported that broilers reared on thicker litter had lower FPD, HB, hock swelling and breast blister scores. Previous research has shown that high litter moisture increases the score of footpad lesions (Dozier *et al.*, 2006). It was thought that there was a developing trend in foot and leg lesions related to decreasing litter thickness. When broilers had prolonged contact with thinnest litters, their feet health was affected and increased sensitivity to litter thickness, which could probably increase footpad and hock scores.

In this study, average tarsometatarsus length and width was affected by the perch and litter thickness. Likewise, Bizeray *et al.* (2002) reported birds maintained in pens provided with barriers had wider tibias compared with the controls. Length and width of tarsometatarsus of birds reared under the cooled perch were significantly longer and wider than the no perch birds. Cooled perches had a beneficial effect on FA and rFA of tarsometatarsus, suggesting that greater FA and rFA of tarsometatarsus may serve birds to maintain developmental stability under the environmental stress factors. Perch and litter thickness did not have statistically significant influence on the FA, rFA and mean rFA of tarsometatarsus length and width and claw length. Similarly, Ventura *et al.* (2010) found that the difference in the level of FA and rFA between barrier perch groups (simple,

complex, and no barriers (control)) were not statistically significant. In contrast, Moller *et al.* (1999) reported that environmental factors (e.g. inadequate food intake, poorer health and diseases, stocking density, and photoperiod) increased levels of rFA in the chickens. Moller *et al.* (1999) also indicated that rFA was positively related to the increasing score of TD and GS and thus may ensure lameness sign. In the present study, the TD score of broilers reared under the cooled perch was significantly lower than the non-cooled and absence of perches birds. Similarly, Kaukonen *et al.* (2017) reported a significant decrease in the incidence and severity of TD in broiler chickens subjected to platform-equipped houses. On the other hand, some studies reported that different perch treatments did not significantly affect the TD score in broilers (Bizeray *et al.*, 2002; Tablante *et al.*, 2003). It is suggested that perch cooling can help to improve leg health as an indicator of welfare in broiler chickens.

The physical inspection of tibia revealed that broilers in cooled perch groups had heavier, longer, and wider in diameter tibia bones than non-used and non-cooled perch counterparts, while perch cooling has a significant effect on tibia length but not significant effect on tibia weight, medullar canal and diaphysis diameters. Similarly, Tolon and Yalcin (1997) reported that caged systems did not affect tibia weight. Gonzalez-Ceron *et al.* (2015) reported the weight, length and diameter of the tibia as 13.14 g, 100.26 mm and 7.49 mm for Arkansas random-bred chicken population, respectively. In another study, Harvey *et al.* (2015) reported that average tibia length at the 42<sup>nd</sup> day was 120.0 mm for the control group in broilers. Kwiatkowska *et al.* (2018) also reported the range of tibia length and weight as 110.5 mm to 112.1 mm and 21.91 g to 23.45 g, respectively, in a study on the effect of Fe-Glycinate chelate in the diet for broiler chickens. However, the same results were not obtained for tibia weight (18.0 g) and diameter (10.9 mm). Rath *et al.* (1999) reported that diaphyseal diameter was 8.50



and 7.49 mm for male broilers at the 7<sup>th</sup> week of age. In the present study, tibia length was affected by the perch treatment. Our results suggested that bone development was significantly affected by the housing system because the chickens were kept in cooled perch, so, they had better growing condition. In terms of the physical properties of the tibia, it can be said that if the bone length continues to grow without the increase in bone width, this could make bones prone to breakage. In the present study, RI was greater in broilers subjected to cooled perch compared to non-perch and non-cooled perches. There were no effects of perch treatment on weight-length and tibio-tarsal indexes. Mabelebele *et al.* (2017) reported the RI and weight-length index as 4.35-254.84 for male, respectively. The difference between the two studies could arise from the age of animals. Similar to the results of this study, Karaarslan and Nazligul (2018) reported that perch usage had no significant effects on the weight-length index (189.0 for perch, 186.89 for non-perch). In addition, they reported that perch usage had no significant effects on RI (4.10 for perch, 4.11 for non-perch). Yildiz *et al.* (2003) suggested that significant differences were observed in cortical areas of the humerus between caged, aviary, perchery and litter housing systems. That study stated the larger cortical area in aviary, perchery and litter was caused by the greater activity of birds in these housing systems. Reiter and Bessei (1995) also reported that active birds had a thicker and denser cortical bone in the tibiotarsus than less active controls. Süzer *et al.* (2019) reported that there was no significant difference in terms of the cortical area among housing systems, whereas fast-growing broilers had significantly larger cortical area than slow-growing broilers. However, similar movement possibilities of the chickens in experimental housing systems did not significantly affect the tibio-tarsal index (bone cortical development). In Church and Johnson (1964) studies, the BW in chickens showed a significant increase from 4-5 to 15-16 weeks of age. They also stated that the development of tibia was quite fast in the first three weeks. The growth plate of the

tibia in the proximal region closed at the age of 18 weeks, and the growth plate in the distal region closed at the 14<sup>th</sup> week of age, while the diameters of the bone in the mid-shaft increased at similar periods with the elongation of the bone. Thus, it is seen that the tibia grows both in length and diameter in a similar time span. In this study, animals were weighed because they were euthanized on day 42 and, according to the Church and Johnson (1964) study, bone growth continues as the growth plate is not yet closed in the bones on the 42<sup>nd</sup> day. Besides, there is a positive correlation between the BW, the bone weight and the morphometric characteristics. The weights of the bones differ in both groups of the perch and the litter thickness groups. In parallel to this, the length and diameter of the bones also showed similar differences.

There are some limitations in this study. The most important one is that only the robusticity index was used to calculate the bone strength. Therefore, it could not be discussed in detail. It needs application of mechanical testing techniques for the exact measurement of bone strength. In this study, the bone weight was highest in the groups of cold perch and the group having 1 cm litter thickness. Furthermore, the bone length and diameter values were the largest in the same groups. The primary role of the long bones is to bear skeleton loads (Van Der Meulen *et al.*, 2001). In order to compensate for the bone load, it increases in diameters (Masse *et al.*, 2003). Thus, structural integrity is ensured by increasing the dimensions of the bone (Van Der Meulen *et al.*, 2001). Mabelebele *et al.* (2017) stated that if bone length grows and continues to grow without increasing bone width, susceptibility to increased leg health problems in animals would increase.

## CONCLUSIONS

In conclusion, perch cooling was very beneficial for FPD, GS, and TD in broilers. The thicker litter reduced the development of FPD and HB. The results indicate that 14 cm litter thickness has a favorable effect on

leg health of broilers reared at high temperatures. It can be said that if litter thickness was set properly, perch application and perch cooling have great advantages for the motivation of fast growing broilers for activity, which are prone to bone disorders. It can be also said that the morphometric properties of the bone were much more affected by the litter than the perch characteristics. Potential beneficial effects on birds' welfare status and bone morphometric properties of cooled perch in broilers raised in thicker litter need to be further investigated.

### ACKNOWLEDGEMENTS

The authors would like to thank the Adnan Menderes University Research Foundation (ADU-BAP) for financial support of this work (grant number VTF 17-053).

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### تأثیر خنک کردن نشیمن گاه طیور و ضخامت لاشبرگ بستر روی سلامت پا و ویژگی های استخوان در جوجه های گوشتی

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#### چکیده

هدف این پژوهش ارزیابی متغیرهای سلامت پا (درماتیت پا، سوختگی مفصل=hock burn، دیسکندروپلازی تیبیا، امتیاز مربوط به طرز راه رفتن)، نامتقارنی کف پا، و خواص فیزیکی Tibiatarus جوجه های گوشتی پرورش یافته در شرایط وجود نشیمن گاه(perch) همراه با ضخامت های مختلف لاشبرگ بستر (litter) بود. در کل، ۴۵۹ جوجه یکروزه Ross-308 در ۲۷ جایگاه جدا سازی (pen) که هر کدام ۱۷ جوجه داشت قرار داده شد و آزمایش با ۳ تیمار نشیمن گاه ۳ X تیمار ضخامت لاشبرگ بستر، در سه تکرار طراحی و اجرا شد. موقعیت هر دو نشیمن گاه خنک شده و خنک نشده طوری بود که جوجه ها برای غذا خوری و نوشیدن آب ناچار از عبور از روی آن بودند. درجه گرما برای نشیمن گاه خنک ۱۰ درجه سانتی گراد بود و ضخامت لاشبرگ بستر برابر ۱، ۷، و ۱۴ سانتی متر. نتایج نشان داد که نشیمن گاه خنک شدت صدمات پا (footpad) و امتیاز مربوط به طرز راه رفتن (gait score) و دیسکندروپلازی تیبیا را در جوجه های گوشتی کاهش داد. درماتیت پا و سوختگی مفصل در جوجه هایی که در تیمار ضخامت ۱۴ سانتی متر لاشبرگ بستر قرار داشتند کاهش یافت. نیز مشخص شد که طول و عرض استخوان مچ و کف پا (tarsometatarsus) در سن ۴۲ روزه در تیمار با نشیمن گاه خنک و ضخامت ۱ سانتی متر لاشبرگ بستر افزایش داشت. طول تیبیا و شاخص استواری (robustness index) در گروه دارای نشیمن گاه خنک بیشتر از تیمارهای دیگر بود. استفاده از نشیمن گاه تأثیری روی شاخص های وزن-طول و tibio-tarsal نداشت. طول تیبیا در تیمار با ضخامت لاشبرگ ۱ سانتی متری (برابر ۱۰۳/۸۱ میلی متر) بیشتر از ضخامت های ۷ و ۱۴ سانتی متری بود. استخوانها در تیمار دارای ضخامت بستر ۱ سانتی متری سنگینتر (۲۱/۵۵ گرم) از تیمار ۷ سانتی



متری بود. این نتایج حاکی از آن است که در شرایط هوای گرم، نشیمن گاه خنک همراه با ضخامت لاشبرگ ۱۴ سانتی متری تاثیر مفیدی روی سلامت جوجه های گوشتی دارد.