

## The Effect of Stocking Density on Stress Reaction in Broiler Chickens during Summer

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**Abstract:** The aim of this study was to investigate the effect of stocking density on the heterophil to lymphocyte ratio (H:L), blood corticosterone concentration, immune response, and some performance parameters in broilers during summer. In all, 300 1-day-old commercial broiler chicks (Ross 308) were housed at densities of 15, 20, and 25 birds/m<sup>2</sup>, with 2 replicates of each. H:L ratio and blood corticosterone concentration were used to measure the level of stress in the birds. Newcastle disease (ND) antibody titers were also analyzed to determine the level of immune response with a commercial ELISA kit. Mean H:L ratio, corticosterone concentration, and Newcastle disease antibody titer (log<sub>10</sub>) on day 42 were 0.41, 0.43, and 0.45, 3.81, 4.13, and 4.39 ng/ml, 3.99, 4.10, and 3.88, respectively, for birds housed at 15, 20, and 25 birds/m<sup>2</sup>. Stocking density had no significant effect on H:L ratio, blood corticosterone concentration, immune response, and some performance parameters in broiler chickens. Consequently, high yield per unit area could be achieved with different stocking densities (15, 20, and 25 birds/m<sup>2</sup>) in broiler production during summer.

**Key Words:** Broiler chicken, corticosterone, heterophil to lymphocyte ratio (H:L), immune response, stress

### Yaz Mevsiminde Yerleşim Sıklığının Broilerlerde Stres Oluşumu Üzerine Etkileri

**Özet:** Bu çalışmanın amacı yaz mevsiminde broilerlerde yerleşim sıklığının heterofil/lenfosit oranı (H:L), kan kortikosteron düzeyi, bağışık yanıt oluşumu ve bazı performans parametreleri üzerine etkilerinin incelenmesidir. Bir günlük yaşta toplam 300 ticari broiler civivi (Ross-308) hayvan yoğunluğu 15, 20 ve 25 broiler/m<sup>2</sup> olacak şekilde üç ayrı gruba ayrılmış ve çalışma iki tekrar şeklinde düzenlenmiştir. H:L oranı ve kan kortikosteron yoğunluğu hayvanlardaki stresin düzeyini ölçmede kullanılmıştır. Hayvanlarda oluşan bağışık yanıtın düzeyi ise Newcastle antikor düzeyinin ELISA kiti ile ölçülmesiyle saptanmıştır. Kırkikinci gün ortalama H:L oranları, kortikosteron yoğunlukları ve antikor titreleri (log<sub>10</sub>) 15, 20 ve 25 broiler/m<sup>2</sup> için sırasıyla 0.41, 0.43 ve 0.45; 3.81, 4.13 ve 4.39 ng/ml; 3.99, 4.10 ve 3.88 olmuştur. Bu çalışmanın bulguları yaz mevsiminde yerleşim sıklığının H:L, kan kortikosteron, bağışık yanıt oluşumu ile bazı performans parametreleri üzerine istatistiksel olarak önemli etkileri olmadığını göstermiştir. Sonuç olarak, broiler yetiştiriciliğinde değişik yerleşim sıklıkları (15, 20 ve 25 broiler/m<sup>2</sup>) ile yaz mevsiminde birim alandan yüksek miktarda ürün alınabilir.

**Anahtar Sözcükler:** Broiler, kortikosteron, heterofil lenfosit oranı (H:L), bağışık yanıt, stres

### Introduction

Stocking density plays an important role, especially during summer, in broiler production. Higher mortality, lower meat production, greater incidence of leg disorders, and cannibalism occur at higher stocking densities in broilers.

Zulkifli et al. (1) reported that the heterophil to lymphocyte ratio (H:L) is a reliable indicator of avian stress. Broilers exposed to heat stress in summer show an

increase in heterophils and a decrease in lymphocytes, which leads to an increase in the H:L ratio. McFarlane and Curtis (2) reported that the H:L ratio increased with heat stress in broiler chicks. Elevation of the H:L ratio with increasing stocking density was reported to indicate that high stocking density in broiler production is stressful (3,4). Spinu et al. (5) reported that there was no difference in the H:L ratio between different stocking densities in broiler breeders in summer.

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Maxwell et al. (6) reported that food restriction is associated with a significant increase in the H:L ratio on day 42; however, Hocking et al. (7) claimed that some stressors, such as food restriction and high air temperature, have a small effect on the H:L ratio in broiler breeders. Maxwell (8) suggested that both genotype and environmental factors affect hematological traits in broilers.

Blood corticosterone concentration has been widely used as a measure of environmental stress in broilers (1,2). Lin et al. (9) reported that corticosterone concentration in broilers that had been exposed to acute heat stress (32 °C and 40% relative humidity) for 6 h varied from 3.17 to 4.28 ng/ml on day 42. Sandilands et al. (10) indicated that changes in the nutrient status of food increased corticosterone concentration from 1.73 to 4.63 ng/ml in broiler breeders aged 7 to 42 days. It was also reported that heat and cold stress cause the blood corticosterone level to increase in cockerels (11).

Immune response to a given antigen is another parameter that has been widely used in assessing the stress level of broiler chickens. In this context, Erişir and Erişir (12) reported that there was a significant decrease in immune response with an increase in stocking density in Japanese quails.

Altan et al. (13) reported that increasing cage density to 5 birds/cage in white layers decreased egg production and the Haugh unit, whereas egg shell quality and egg weight were not affected. It is well established that stressed chickens change their behavior patterns. New behaviors generally result in an increase in energy expenditure (14). In broilers that are usually reared at a high stocking density social factors may be more important than environmental factors in causing stress and affecting behavior patterns (15,16). Keeling and Duncan (17) reported that aggressiveness is relatively higher in small flocks than in large flocks, as birds adopt strategies to avoid negative social interactions.

The goal of this study was to investigate the effects of stocking density on the H:L ratio, blood corticosterone concentration, immune response, and some performance parameters (body weight, total feed consumption, feed conversion ratio, and mortality) in broilers during summer.

## Materials and Methods

This study was carried out at the Poultry Unit of the Faculty of Veterinary Medicine, Adnan Menderes University, Aydın, Turkey. Aydın is a city that has a very hot and humid climate in summer. In all, 300 1-day-old commercial broiler chicks (Ross 308) were obtained from a local hatchery. On day 1 the chicks were individually weighed, wing-tagged, and allocated to 1 of 3 groups with stocking densities of 15 (group 1, n = 50), 20 (group 2, n = 50), and 25 (group 3, n = 50) birds/m<sup>2</sup> in cages with deep litter of wood shavings; each density was replicated twice. These stocking densities were chosen to simulate the usual rearing conditions in the region.

Minimum and maximum air temperature was recorded daily and relative humidity was measured at 0900 and 1500 hours. A constant photo-period of 24 h was provided. The feed supply was changed from starter (3060 kcal ME/kg, 23% crude protein) to finisher pellet (3200 kcal ME/kg, 22% crude protein) at 14 days of age. Chicks were administered live and inactive Newcastle disease vaccine (Hipraviar ND Clone 30) via drinking water on day 7 and 21 to determine their immune response against stress caused by the stocking density.

Blood samples were collected at the 4<sup>th</sup>, 5<sup>th</sup>, and 6<sup>th</sup> weeks of the study. A total of 25 randomly selected chickens from each group were gently removed from their rooms and blood samples (0.5 ml) were taken into EDTA tubes for heterophil and lymphocyte counts. Blood smears were prepared using May-Grünwald-Giemsa stain, and heterophil and lymphocytes were counted to a total of 60 cells (18). For corticosterone analysis, blood samples were kept at room temperature until the serum separated. Then, the serum samples were immediately assayed for corticosterone concentration with a commercial ELISA kit (Assay Designs, catalogue no: 900-097).

Newcastle disease (ND) antibody titers were analyzed with a commercial ELISA kit (BioCheck) and blood samples taken for the corticosterone analysis.

The birds were weighed on day 42 for final body weight. Mortality, total feed intake, and feed conversion ratio (FCR) were also determined.

Data for corticosterone concentrations and Newcastle disease antibody titers were normalized using logarithmic transformation prior to analysis. Data on the mortality rate were analyzed by chi-square test. One-way analysis

of variance was used to test the effect of density on the H:L ratio, antibody titers, corticosterone concentration, and body weight. Comparisons among weeks were analyzed with the repeated measures module of the general linear model (19).

## Results

Mean minimum and maximum air temperatures were 24.7 °C and 29.7 °C, respectively. Mean relative humidity was 64.3% at 0900 and 54.3% at 1500 (Table 1).

Mean H:L ratio, corticosterone concentration, and immune response values are presented in Table 2.

No difference was found in the H:L ratio between the 3 density groups. Mean H:L ratio on day 42 was 0.41, 0.43, and 0.45 for 15, 20, and 25 birds/m<sup>2</sup>, respectively. There was no difference between the weeks.

Blood corticosterone concentration was 3.78, 3.76, and 3.65, 4.00, 4.05, and 4.15, and 3.81, 4.13, and 4.39 for 15, 20, and 25 birds/m<sup>2</sup> at the 4<sup>th</sup>, 5<sup>th</sup>, and 6<sup>th</sup> weeks, respectively (Table 2). As in the case of the H:L ratio, blood corticosterone concentration was not affected by stocking density or production period (4<sup>th</sup>, 5<sup>th</sup>, and 6<sup>th</sup> weeks).

Newcastle disease antibody titers (log<sub>10</sub>) were 3.99, 4.10, and 3.88 for 15, 20, and 25 birds/m<sup>2</sup>, respectively, on day 42. These results indicated that stocking density had no significant effect on immune response in broilers in summer.

Body weight on day 42 was 2157.10, 2012.32, and 1895.78 g for 15, 20, and 25 birds/m<sup>2</sup>, respectively (Table 3). Total feed intake was 3485.22, 3410.10 and 3062.17 g, and FCR was 1.62, 1.69, and 1.62 for 15, 20, and 25 birds/m<sup>2</sup>, respectively (Table 3). Stocking density had no effect on any of the performance parameters or the mortality rate.

## Discussion

This study showed that the H:L ratio was not stocking density dependent. The H:L ratio of 0.41, 0.43, and 0.45 for 15, 20, and 25 birds/m<sup>2</sup>, respectively, in the present study were comparable to the H:L ratio of 0.39, 0.49, 0.55, and 0.58 for 5, 7, 9, and 11 birds/m<sup>2</sup>, respectively, for broiler breeders at the 6<sup>th</sup> week of age (5), and 0.25 and 0.43 for control and heat-stressed broilers (20). In contrast to these observations, according to Cravener et al. (21) there is a negative correlation between the H:L ratio and stocking density ( $P < 0.05$ ). Accordingly, the H:L ratio was 0.45, 0.28, and 0.30 for 11.1, 14.3, and 20.0 birds/m<sup>2</sup>, respectively, for broiler breeder hens (21).

The discrepancy observed between the findings of the present study and those of Cravener et al. (21) might have originated from the relatively small population used for the present study, genotype, and the methodology used. In this context, Keeling and Duncan (17) stated that aggressiveness is higher in small flocks than in large flocks. On the other hand, environmental factors, such as temperature, humidity, and gas emissions (CO<sub>2</sub>, CO, NH<sub>3</sub>, and H<sub>2</sub>S), which cannot be controlled, may cause different

Table 1. Minimum, maximum, and mean temperature and relative humidity according to week.

| Weeks   | Temperatures (°C) |      |      | Relative humidity (%) |      |      |
|---------|-------------------|------|------|-----------------------|------|------|
|         | Min               | Max  | Mean | Min                   | Max  | Mean |
| 1       | 24.5              | 29.7 | 27.8 | 50.7                  | 61.3 | 55.2 |
| 2       | 23.7              | 28.3 | 25.3 | 51.4                  | 62.8 | 58.0 |
| 3       | 25.1              | 30.4 | 26.4 | 53.3                  | 63.4 | 58.8 |
| 4       | 24.9              | 29.1 | 27.9 | 56.2                  | 65.5 | 59.7 |
| 5       | 25.4              | 31.0 | 28.5 | 55.4                  | 63.6 | 60.3 |
| 6       | 24.3              | 29.5 | 27.3 | 58.5                  | 69.3 | 62.9 |
| General | 24.7              | 29.7 |      | 54.3                  | 64.3 |      |

Table 2. The effect of stocking density on the H:L ratio, corticosterone concentration, and immune response at the 4<sup>th</sup>, 5<sup>th</sup>, and 6<sup>th</sup> weeks.<sup>1</sup>

|  | Stocking density<br>(birds/m <sup>2</sup> ) | 4 <sup>th</sup> week<br>$\bar{X} \pm S\bar{x}$ | 5 <sup>th</sup> week<br>$\bar{X} \pm S\bar{x}$ | 6 <sup>th</sup> week<br>$\bar{X} \pm S\bar{x}$ | P |
|--|---|--|--|--|---|
| H:L ratio                                  | 15 (group 1, n = 25)                        | 0.40 ± 0.02                                    | 0.42 ± 0.01                                    | 0.41 ± 0.02                                    | - |
|  | 20 (group 2, n = 25)                        | 0.37 ± 0.01                                    | 0.40 ± 0.02                                    | 0.43 ± 0.02                                    | - |
|  | 25 (group 3, n = 25)                        | 0.40 ± 0.02                                    | 0.43 ± 0.03                                    | 0.45 ± 0.03                                    | - |
| P  | -   | -  | -  |  |   |
| Corticosterone<br>concentration<br>(ng/ml) | 15 (group 1, n = 25)                        | 3.78 ± 0.20                                    | 4.00 ± 0.21                                    | 3.81 ± 0.23                                    | - |
|  | 20 (group 2, n = 25)                        | 3.76 ± 0.21                                    | 4.05 ± 0.21                                    | 4.13 ± 0.22                                    | - |
|  | 25 (group 3, n = 25)                        | 3.65 ± 0.21                                    | 4.15 ± 0.23                                    | 4.39 ± 0.35                                    | - |
| P  | -   | -  | -  |  |   |
| Immune<br>response <sup>2</sup>            | 15 (group 1, n = 25)                        | 3.90 ± 0.01                                    | 3.82 ± 0.04                                    | 3.99 ± 0.05                                    | - |
|  | 20 (group 2, n = 25)                        | 4.01 ± 0.02                                    | 4.06 ± 0.04                                    | 4.10 ± 0.09                                    | - |
|  | 25 (group 3, n = 25)                        | 3.97 ± 0.02                                    | 4.00 ± 0.03                                    | 3.88 ± 0.04                                    | - |
| P  | -   | -  | -  |  |   |

<sup>1</sup>Values are means of 2 replicates of 25 chickens each.

<sup>2</sup>Newcastle disease antibody titres (log<sub>10</sub>) were measured with ELISA.

-: not significant.

Table 3. Some performance parameters according to group.<sup>1</sup>

| Parameters                           |        | Group 1<br>(15 birds/m <sup>2</sup> )<br>$\bar{X} \pm S\bar{x}$ | Group 2<br>(20 birds/m <sup>2</sup> )<br>$\bar{X} \pm S\bar{x}$ | Group 3<br>(25 birds/m <sup>2</sup> )<br>$\bar{X} \pm S\bar{x}$ | P |
|--------------------------------------|--------|---|---|---|---|
| Body weight (g)                      | Day 1  | 44.39 ± 0.38  | 44.57 ± 0.40  | 45.07 ± 0.33  | - |
|                                      | Day 42 | 2157.10 ± 19.20   | 2012.32 ± 18.13   | 1895.78 ± 32.21   | - |
| Total feed intake (g)<br>(1-42 days) |        | 3485.22   | 3410.10   | 3062.17   |   |
| FCR                                  |        | 1.62  | 1.69  | 1.62  |   |
| Mortality (%) <sup>2</sup>           |        | 6.00  | 8.00  | 12.00   | - |

<sup>1</sup>Values are means of 2 replicates of 50 chickens each.

<sup>2</sup>Chi-Square test was used for mortality analysis.

-: not significant.

levels of stress in broilers. El-Lethey et al. (22) also reported that the H:L ratio was influenced by housing condition and food form.

Although there was a trend toward increasing corticosterone concentration with higher stocking density, no statistical significance was found in blood corticosterone concentration between the stocking density groups. However, mean corticosterone concentration on day 42 indicated an increase from 3.81 to 4.39 ng/ml with increased stocking density. Lin et al. (9) and Sandilands et al. (10) reported that exposing broilers to high temperature and food restriction is associated with higher corticosterone concentration than in control counterparts. Furthermore, there was no interaction among blood corticosterone concentration at 4, 5, and 6 weeks of age. Hocking et al. (23) reported that mean corticosterone concentration in broiler breeders at 6 weeks of age was 0.5 ng/ml under usual stocking density (9 birds/m<sup>2</sup>). Hocking et al. (24) also reported that plasma corticosterone concentration was similar in both control and food-restricted broiler breeder groups at 36, 48, and 60 weeks of age. Management practices, genotypes, sex, and age of birds may be some of the major reasons for the differences between studies.

Newcastle disease antibody production (ND titers (log<sub>10</sub>) of 3.99, 4.10, and 3.88 for 15, 20, and 25 birds/m<sup>2</sup>, respectively) determined in the present study demonstrated that stocking density had no effect on immune response in broilers, which is in agreement with other studies (1,18). Hocking et al. (24) reported that immune function was not affected by food restriction in broiler breeders; however, Erişir and Erişir (12) observed a significant decrease in immune response with an increase in stocking density in Japanese quails. Tufft and Nockels (25) also reported that a decrease in space allowance made broilers more susceptible to infections.

Stocking density had no significant effect on body weight, total feed intake, or FCR; however, the average body weight (2157.10 g) in group 1 was higher than that

reported by Feddes et al. (3) (1985 g), and Pettit-Riley and Esteves (26) (1917 g) for broilers at the same stocking density on day 42. This result suggests that the growth of breast muscle was more susceptible to corticosterone concentration. This may be related to the lower use of nutrient factors and/or a higher priority of protein catabolism. FCR of 1.62-1.69 in the present study was lower than the previously reported 1.71-1.83 for broilers on day 42 (26). Shanawany (27) reported that high stocking density (above 20 birds/m<sup>2</sup>) has significant adverse effects on total feed intake in broiler chickens; however, Thomas et al. (28) claim that mean body weight and total feed intake in broilers on day 35 were not affected by stocking density ( $P < 0.05$ ). These results would indicate that high corticosterone concentration in blood resulted in high feed consumption and low FCR in broilers. The difference among the present study and the others may be due to the level of corticosterone in blood, genotype, age of birds, and the methodology.

Stocking density had no significant effect on mortality in broilers in summer ( $X^2 = 2.36$ ). İşcan et al. (29) reported that mortality for 15 and 20 birds/m<sup>2</sup> was 7.1 and 6.4 %, respectively. As in our study, stocking density in broilers had no significant effect on mortality (21,30). Increased mortality can be explained by decreased animal welfare, such as bad air and litter quality, poor immune response, and poor feed intake.

In conclusion, stocking density had no significant effect on the H:L ratio, blood corticosterone concentration, and antibody production in broiler chickens. In addition, some important performance parameters, such as final body weight, total feed intake, FCR, and mortality did not appear to be affected by stocking density in the summer season. Finally, high yield per unit area could be achieved with different stocking densities (15, 20, and 25 birds/m<sup>2</sup>) in broiler production in the summer season.

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