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Interactive effects of lighting duration and LED color on welfare, behavior, physiological stress responses, and meat quality in broiler chickens

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Abstract

Lighting management is a key environmental factor influencing broiler welfare and productivity; however, the combined effects of photoperiod and light spectrum remain insufficiently understood. This study investigated the interactive effects of lighting duration (restricted vs. intermittent) and LED light color (white vs. green) on welfare indicators, hematological stress responses, behavior, and meat quality in broiler chickens. A total of 288 one-day-old Ross 308 male broilers were assigned to a 2 × 2 factorial design with six replicate pens per treatment. Light color significantly affected selected welfare and physiological parameters, with green LED generally associated with improved outcomes. Lighting duration influenced hematological variables, particularly heterophil and lymphocyte counts and the heterophil-to-lymphocyte (H/L) ratio, indicating differences in physiological stress status. Significant interactions between lighting duration and light color were observed for hematological traits, suggesting a combined effect of photoperiod and spectral composition on stress responses. In contrast, most welfare indicators, behavioral traits, and meat quality parameters were not markedly affected by treatments. Behavioral responses were limited and age dependent. Overall, intermittent lighting combined with green LED illumination showed advantages for selected welfare and stress-related outcomes without compromising production traits. These findings suggest that optimized lighting programs may contribute to improved broiler welfare under commercial conditions.

Introduction

Lighting is a critical environmental factor in commercial poultry production, influencing not only visual perception but also growth performance, welfare, immune function, and behavior. Chickens are highly photosensitive animals, possessing both retinal photoreceptors (rods and cones) and extra-retinal photoreceptors primarily located in the hypothalamus. Retinal photoreceptors mediate the perception of color, brightness, and motion, whereas extra-retinal pathways regulate circadian rhythms, endocrine activity, and immune-related processes (Zhao et al. 2019; Rozenboim et al. 2022; Majumdar et al. 2024). Through these interconnected mechanisms, lighting conditions play a fundamental role in maintaining physiological homeostasis and shaping production outcomes in broiler chickens (Wu et al. 2022; Kang et al. 2023).

Among lighting-related factors, photoperiod has been extensively studied in broiler management. Continuous lighting programs, typically ranging from 23 to 24 h of light, have historically been used to maximize feed intake and growth rate (Ozkan and Simsek 2022). However, prolonged light exposure can disrupt circadian organization, impair resting behavior, and elevate physiological stress, ultimately compromising leg health, welfare, and overall robustness (Forslind et al. 2021; Franco et al. 2022b; Shynkaruk et al. 2022; Jiang et al. 2023). In contrast, lighting programs that incorporate dark periods, including intermittent schedules, may promote rest, enhance circadian synchronization, and support immune function (Lucena et al. 2020; Kim et al. 2022; Linhoss et al. 2025). As a result, optimizing photoperiod has become a key welfare-oriented management approach in modern broiler production systems.

In addition to lighting duration, spectral composition has emerged as a biologically relevant factor influencing broiler responses. The avian visual system is capable of detecting a broader wavelength range than that of humans, extending from ultraviolet to long-wavelength red light (Lewis and Morris 2000; Kumar et al. 2017). Birds exhibit particular sensitivity to the green spectrum (approximately 545–575 nm), although responses may vary depending on wavelength, light intensity, and age (Franco et al. 2022a). This enhanced spectral sensitivity is supported by specialized cone photoreceptors and retinal oil droplets that improve wavelength discrimination and environmental responsiveness (Toomey and Ronald 2021; Gisbert et al. 2022; Ramirez 2023).

Previous studies have demonstrated that light color can differentially influence welfare and behavioral responses in broilers (Kristensen et al. 2006; Cao et al. 2008). Longer wavelengths, such as red light, are generally associated with increased activity, arousal, and fear-related responses, whereas shorter wavelengths, particularly blue and green light, have been linked to calmer behavior, reduced tonic immobility, and lower heterophil-to-lymphocyte (H/L) ratios (Sultana et al. 2013; Mohamed et al. 2017; Oke et al. 2023). Light spectrum has also been associated with leg health, mortality, and production performance (Kang et al. 2023). However, findings across studies remain inconsistent. Several reports have found no significant effects of light color on welfare indicators such as plumage condition, footpad dermatitis (FPD), or hock burn (HB) (Riber 2015; Senaratna et al. 2011; Hesham et al. 2018), suggesting that responses may depend on management conditions and interactions with other environmental factors.

Behavioral responses to different light spectra may be partly explained by underlying neurophysiological mechanisms. Shorter wavelengths, such as blue and green light, are generally associated with reduced locomotor activity and increased resting behavior, whereas longer wavelengths tend to stimulate movement and exploratory behavior (Senaratna et al. 2012; Oke et al. 2023). Furthermore, wavelength-dependent penetration of light through cranial tissues may differentially stimulate extra-retinal photoreceptors involved in endocrine regulation and circadian signaling (Hartwig and van Veen 1979; de Souza Granja Barros et al. 2024). These mechanisms highlight that light color and photoperiod should not be evaluated in isolation.

Despite increasing interest in lighting management, most previous studies have examined photoperiod and light spectrum independently. Information regarding their combined effects on welfare indicators, physiological stress responses, behavior, and meat quality remains limited. This gap is particularly relevant, as modern poultry production requires management strategies that simultaneously enhance animal welfare and production efficiency (Schwean-Lardner et al. 2014).

Therefore, the present study aimed to evaluate the interactive effects of lighting duration (restricted vs. intermittent) and LED light color (white vs. green) on welfare indicators, hematological stress responses, behavior, and meat quality in broiler chickens. It was hypothesized that intermittent lighting combined with green LED illumination would reduce stress-related responses, improve welfare outcomes, and enhance selected meat quality traits compared with conventional lighting conditions.

Materials and methods

Ethical statement

All experimental procedures were reviewed and approved by the Animal Experiments Local Ethics Committee of Aydın Adnan Menderes University (Approval No. 64583101/2022/102). Animal handling and husbandry procedures were conducted in accordance with institutional guidelines for the care and use of animals in research.

Birds, housing facility, and experimental design

A total of 288 one-day-old Ross 308 male broiler chicks were obtained from a commercial hatchery (EGE-TAV Agriculture and Livestock Investment Trade and Industry Inc., İzmir, Türkiye). The trial was carried out at the experimental poultry facility of the Faculty of Veterinary Medicine, Aydın Adnan Menderes University.

The study was designed as a 2 × 2 factorial experiment with two main factors: lighting duration and LED light color. Lighting duration consisted of restricted lighting and intermittent lighting programs, whereas light color consisted of white or green LED illumination. Birds were randomly assigned to four treatment groups, each comprising six replicate floor pens with 12 birds per pen (72 birds per treatment).

Lighting treatments

Two LED spectral conditions were used: white light (400–770 nm) and monochromatic green light (560 nm). To avoid light contamination among treatments, each treatment group was maintained in a separate light-proof room equipped with opaque walls and doors. Natural daylight was completely excluded throughout the experimental period. All rooms had white-painted interior surfaces to standardize light reflection and maintain consistent spectral distribution. Independent LED fixtures (CT-4277, 9 W, CATA, Türkiye) were installed above each pen to provide uniform illumination. Photoperiod schedules were controlled using programmable timers (412790, 16A, 250 V, Legrand Electric Ltd., Germany), and all daily light cycles commenced at 06:00 h.

Housing and general management

Broiler chickens were reared in floor pens measuring 1.65 m² (1.50 m length × 1.10 m width × 0.75 m height) for a 42-day production cycle. Each pen was equipped with one centrally positioned round feeder (0.40 m diameter) and a front-mounted nipple drinker line containing three nipples, providing one nipple per 4.33 birds. Feeder space corresponded to 9.66 cm per bird. Feed and water were offered ad libitum throughout the experimental period.

Birds were fed a standard commercial three-phase feeding program formulated according to Ross 308 recommendations (Aviagen, 2019). Diets consisted of a starter ration from day 1 to 10 (3,050 kcal ME/kg; 23.5% crude protein), a grower ration from day 11 to 24 (3,150 kcal ME/kg; 22.0% crude protein), and a finisher ration from day 25 to 42 (3,200 kcal ME/kg; 21.0% crude protein).

Experimental lighting treatments were applied from day 8 to 39. Birds assigned to the restricted lighting treatment received an 18 h light:6 h dark schedule (18L:6D), whereas those in the intermittent lighting treatment received a 17 h light:3 h dark:1 h light:3 h dark schedule (17L:3D:1L:3D). During the brooding phase (days 1–7) and during the final 3 days before slaughter (days 40–42), all birds were exposed to a common lighting schedule of 23 h light:1 h dark (23L:1D), in accordance with commercial practice and European welfare recommendations.

Stocking density was maintained at 33 kg/m² in compliance with European Union Directive 2007/43/EC. Pens were bedded with fresh wood shavings to an approximate depth of 6 cm, and litter condition was monitored routinely throughout the trial.

Ambient temperature and relative humidity were continuously monitored at bird level (10–30 cm above litter surface) using digital data loggers with a resolution of 0.1°C and 1% relative humidity. Temperature was maintained at 33 ± 1°C during the first 3 days after placement and gradually reduced by 2–3°C weekly to reach 23 ± 1°C by the end of the experiment. Relative humidity was maintained between 50 and 60%. Ventilation was automatically controlled to ensure adequate air exchange and consistent indoor air quality across treatment rooms.

All rooms were cleaned and disinfected before chick placement using a poultry-approved disinfectant. Birds were vaccinated according to standard commercial vaccination schedules. No antibiotic growth promoters or therapeutic antibiotics were administered during the study period.

Data collection

Welfare indicators and hematological traits

Leg health, plumage condition, and leukocyte-based stress indicators were used as complementary measures of broiler welfare, as previously described in poultry welfare studies (Sun et al. 2017; Hesham et al. 2018).

On day 42, ten birds were randomly selected from each replicate pen (n = 240 total birds) for welfare assessment and hematological analysis. Evaluated welfare parameters included footpad dermatitis (FPD), hock burn (HB), gait score (GS), plumage cleanliness, and plumage damage.

Footpad dermatitis and hock burn lesions were assessed according to the Welfare Quality® protocol (2009) by three trained independent observers blinded to treatment allocation. The mean observer score for each bird was used for statistical analysis. Lesions were classified using a five-point scale, where score 0 indicated absence of lesions, scores 1–2 indicated mild lesions, and scores 3–4 indicated severe lesions.

Locomotor ability was evaluated using the Welfare Quality® gait scoring system (0–5 scale), in which score 0 represented normal walking ability and score 5 indicated severe locomotor impairment or inability to walk (James et al. 2018).

Plumage cleanliness was visually assessed from the ventral body surface using a three-point scale: 0 = clean, 1 = moderately dirty, and 2 = heavily soiled, following Welfare Quality® guidelines (2009). Plumage damage was evaluated in the back, wing, tail, and thigh regions using a six-point scale ranging from 0 (complete feather cover, no damage) to 5 (severe feather loss or marked feather damage), according to Kretzschmar-McCluskey et al. (2014).

Physiological stress status was evaluated using the heterophil-to-lymphocyte (H/L) ratio, a validated indicator of chronic stress in poultry (Gross and Siegel 1983). Blood samples were collected from each selected bird, and two blood smears per sample were immediately prepared on clean glass slides. Smears were air-dried, stained with May–Grünwald and Giemsa solutions, and examined microscopically. For each smear, 100 leukocytes were differentiated and counted, including heterophils, lymphocytes, eosinophils, basophils, and monocytes. Mean values from the two smears were used to calculate leukocyte differentials and the H/L ratio for each bird.

Fear test

Fear-related responses were evaluated using the tonic immobility (TI) test, a widely accepted behavioral indicator of fearfulness in poultry. On day 41, five birds were randomly selected from each replicate pen (n = 120 total birds) for TI assessment.

Before testing, birds were individually transferred to a separate quiet room to minimize environmental disturbance and visual distraction. Tonic immobility was induced by gently placing the bird in dorsal recumbency on a flat surface and applying light manual restraint to the sternum for 15 s.

After release, the observer moved approximately 1.5–2.0 m away and remained outside the bird's direct visual field. Tonic immobility duration was recorded as the time (s) from release until the bird regained an upright standing posture.

If tonic immobility was not induced after the first attempt, the procedure was immediately repeated. Birds that failed to recover within 600 s were assigned a maximum score of 600 s, according to Jones and Faure (1981). Longer TI durations were interpreted as indicating greater fearfulness.

Carcass and meat quality characteristics

On day 42, five birds were randomly selected from each replicate pen (n = 120 total birds) for carcass and meat quality evaluation following a 12-h feed withdrawal period, while water remained available. Birds were humanely slaughtered by exsanguination after shackling through severance of the jugular veins and carotid arteries and allowed to bleed completely.

Hot carcasses were drained and weighed after removal of giblets, abdominal fat, and neck. Carcasses were then manually deboned to obtain skinless breast meat (fillets and tenders), thighs, and wings. Individual carcass components were weighed separately using a calibrated digital scale.

The left breast fillet (M. pectoralis major) from each bird was used for meat quality analyses, including pH, color, cooking loss (CL), and water-holding capacity (WHC) by Hamm (1961).

Initial muscle pH (pH_{15}) was measured at 15 min postmortem using a portable penetration pH meter (Testo 205, Testo, USA). Measurements were taken at three standardized locations within the breast muscle, and the mean value was recorded. Samples were subsequently stored at 4°C for 24 h, after which ultimate pH (pHu) was determined at the same anatomical sites.

Breast meat color was evaluated at 24 h postmortem using a chroma meter (CR-400, Minolta Co., Osaka, Japan) according to the CIE Lab* color system. The instrument was calibrated using a standard white tile (Y = 93.8, x = 0.316, y = 0.3323) under D65 illumination. Three readings were taken from the muscle surface after blooming, and mean L* (lightness), a* (redness), and b* (yellowness) values were calculated.

Cooking loss was determined according to Honikel (1998). Breast samples were sealed in polyethylene bags and cooked in a water bath until the internal temperature reached 80°C. Samples were then cooled under running tap water for 20 min, removed from the bags, gently dried with filter paper, and reweighed. Cooking loss was expressed as the percentage reduction in weight relative to the initial raw sample weight.

Water-holding capacity was determined using the filter paper press method described by Szmańko et al. (2021) expressed as the percentage of released fluid.

Behavioral observations

Behavioral observations were conducted by a single trained observer throughout the experiment to minimize inter-observer variation and ensure scoring consistency. Observations were performed at approximately 10:00 h on each observation day and continued for about 3 h. Before data collection began, the observer remained quietly inside the facility for approximately 10 min to allow birds to habituate to human presence and resume normal activity patterns. Behavior was evaluated using a scan sampling approach adapted from Vasdal et al. (2019). Observations were conducted at 21, 28, 35, and 42 days of age.

For each replicate pen, the total number of birds present was first recorded, followed by the number of birds displaying predefined, mutually exclusive behaviors at each scan. At every observation age, three consecutive scans of 30 s duration were conducted per pen. Observation timing and procedures were standardized across pens so that all replicates were assessed under comparable environmental and temporal conditions.

Recorded behavioral categories included ingestive behaviors (feeding and drinking), locomotor activity (walking), and maintenance or social behaviors such as preening and aggressiveness (Table 1).

For each observation day, the number of birds engaged in each behavior was summed across the three scans and then expressed as a proportion of the total number of birds in the pen. Accordingly, the pen was considered the experimental unit for behavioral analyses.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics (IBM Corp., Armonk, NY, USA). Data distributions were assessed for normality using the Shapiro–Wilk or Kolmogorov–Smirnov tests, as appropriate, and homogeneity of variances was evaluated using Levene's test.

Hematological variables, tonic immobility duration, carcass traits, meat quality parameters, and behavioral data were analyzed using the General Linear Model (GLM) procedure according to a 2 × 2 factorial design. The fixed effects included lighting duration (restricted vs. intermittent), LED light color (white vs. green), and their interaction. For these analyses, the replicate pen mean was considered the experimental unit.

The mathematical model of the experimental design was described as follows:

$Yijk=\mu+\alpha i+\beta j+(\alpha\beta)ij+Eijk$

Where Yijk=the value observed, μ=the overall mean, αi=the effect of lighting duration (restricted or intermittent), βj=the effect of light colors (white or green), (αβ)ij=the interaction between light duration and color treatments, and Eijk=test error per observation (residual error).

When significant main effects or interaction effects were detected, treatment means were compared using Tukey's honestly significant difference (HSD) test. Ordinal welfare scores, including footpad dermatitis, hock burn, gait score, plumage cleanliness, and plumage damage, were expressed as frequency distributions (%) and analyzed using the chi-square test. Results are presented as least squares means ± standard error of the mean (SEM), unless otherwise stated. Statistical significance was declared at $P \leq 0.05$, whereas values between 0.05 and 0.10 were considered trends.

Results

Welfare indicators and hematological traits

Welfare indicator data are presented in Table 2. Footpad dermatitis incidence was low across treatments and was not significantly affected by lighting duration or LED light color (P = 0.060). Birds exposed to intermittent lighting showed no fair or poor footpad scores, whereas restricted-light birds showed 4.2% fair scores.

Hock burn severity was significantly influenced by light color (P = 0.001). Broilers reared under green LED light showed a higher proportion of birds classified as good (98.3%) compared with those under white light (86.7%), whereas fair and poor scores were more frequent under white light. Lighting duration also affected hock burn incidence (P = 0.029), with intermittent lighting resulting in a greater proportion of good scores (96.7%) than restricted lighting (88.3%).

Gait score was not affected by lighting duration or light color (P > 0.05). Most birds were classified as score 0 or 1, and no birds exhibited severe locomotor impairment (scores 3–5).

Plumage cleanliness differed according to light color (P = 0.029). Birds reared under green LED light had a higher proportion of clean plumage scores (19.2%) than birds under white light (7.5%). Lighting duration did not significantly influence plumage cleanliness (P > 0.05).

Plumage damage results are summarized in Table 3. Lighting duration significantly affected plumage condition in the back (P = 0.031), wing (P = 0.001), and thigh (P < 0.001) regions. In all three areas, birds reared under intermittent lighting showed a greater proportion of intact plumage (score 0) and fewer moderate damage scores than birds under restricted lighting. Tail plumage damage was not significantly affected by lighting duration (P = 0.058).

Light color significantly influenced wing plumage damage (P = 0.008), with green LED light associated with a higher proportion of intact plumage (59.1%) compared with white light (40.0%). No significant effects of light color were detected for back, tail, or thigh plumage regions (P > 0.05).

Hematological responses are presented in Table 4. Lighting duration significantly influenced heterophil percentage (P < 0.001), lymphocyte percentage (P = 0.013), and the heterophil-to-lymphocyte ratio (P = 0.004). Birds under restricted lighting showed higher heterophil values and H/L ratios, together with lower lymphocyte percentages, than birds under intermittent lighting.

Light color significantly affected heterophil percentage (P = 0.043), H/L ratio (P = 0.045), and basophil percentage (P = 0.005). Broilers exposed to green LED light showed lower heterophil values and H/L ratios, but higher basophil counts, compared with those reared under white light.

Significant lighting duration × light color interactions were observed for heterophil percentage (P = 0.001), H/L ratio (P = 0.008), and basophil percentage (P = 0.025). The highest heterophil value and H/L ratio were recorded in birds reared under restricted-white lighting, whereas lower values were observed in the remaining treatment combinations. No significant treatment effects were detected for eosinophil or monocyte percentages (P > 0.05).

Fear test

Tonic immobility duration is presented in Table 4. Neither lighting duration nor LED light color significantly affected tonic immobility responses (P > 0.05). Mean TI duration was 122.78 s in birds exposed to restricted lighting and 113.70 s in birds exposed to intermittent lighting. According to light color, mean values were 123.98 s under white LED light and 112.50 s under green LED light.

No significant lighting duration × light color interaction was detected for tonic immobility duration (P = 0.795). Although numerically lower TI values were observed in birds reared under intermittent-green lighting (106.00 s), treatment differences were not statistically significant. These findings indicate that fear-related behavioral responses were not markedly altered by the lighting programs used in the present study.

Carcass and meat quality characteristics

Carcass trait data are presented in Table 5. Light color significantly affected wing weight (P = 0.032), with birds reared under green LED light showing heavier wings than those exposed to white light (232.85 vs. 222.60 g). The highest wing weight was observed in the intermittent-green treatment group (237.63 g), whereas the lowest value was recorded in the intermittent-white group (221.03 g).

No significant effects of lighting duration were detected for carcass weight, breast weight, thigh weight, or wing weight (P > 0.05). Similarly, carcass weight did not differ according to light color, averaging 2356.05 g under white light and 2427.28 g under green light (P = 0.113). Breast and thigh weights were also unaffected by treatment factors (P > 0.05).

Meat quality parameters are summarized in Table 6. Ultimate pH (pHu) was significantly influenced by light color (P = 0.008). Breast meat from birds reared under green LED light showed higher pHu values than meat from birds exposed to white light (5.72 vs. 5.64). The highest pHu value was observed in the intermittent-green treatment (5.73), whereas both white-light groups showed the lowest values (5.64).

Initial pH measured at 15 min postmortem (pH_{15}) was not affected by lighting duration or light color (P > 0.05). Likewise, no significant treatment effects were detected for cooking loss, water-holding capacity, or color coordinates L*, a*, and b* (P > 0.05).

No significant lighting duration × light color interactions were observed for carcass traits or meat quality variables (P > 0.05). Overall, carcass and breast meat characteristics showed limited sensitivity to the lighting regimens evaluated, with only wing weight and ultimate pH responding significantly to LED light color.

Behavioral observations

Behavioral observation data are presented in Table 7. Overall, behavioral responses to lighting treatments were limited and varied according to age.

Walking activity was significantly influenced by light color at 21 days of age (P < 0.001). Broilers reared under white LED light showed a higher walking frequency than those exposed to green LED light. However, this effect was not maintained at 35 or 42 days of age, when no significant differences were detected between treatments (P > 0.05).

Feeding behavior was significantly affected by lighting duration at 42 days of age (P = 0.008). Birds reared under restricted and intermittent lighting programs differed in feeding frequency at this age, whereas light color had no significant effect on feeding behavior (P > 0.05). No significant treatment effects on feeding activity were observed at earlier observation periods.

Drinking behavior was not significantly affected by lighting duration or light color at any sampling age (P > 0.05). Similarly, preening and aggressive behaviors remained low in frequency and did not differ among treatments throughout the study (P > 0.05).

No consistent lighting duration × light color interaction pattern was observed for the measured behavioral variables. In general, the applied lighting regimens produced modest and age-dependent behavioral changes rather than persistent alterations across the rearing period.

Discussion

Leg health is a key welfare indicator in broiler production due to its association with pain, impaired mobility, and economic losses. Footpad dermatitis (FPD), in particular, is widely recognized as a condition that compromises bird well-being (Škrbić et al. 2015; Kim et al. 2022). In the present study, FPD incidence was not significantly affected by lighting duration or LED light color. This finding is consistent with previous reports indicating limited effects of lighting on footpad condition (Senaratna et al. 2011; Oke et al. 2023). In contrast, other studies have reported increased FPD under extended photoperiods (Schwean-Lardner et al. 2013; Kim et al. 2022). Such discrepancies likely reflect the multifactorial nature of FPD, which is strongly influenced by litter quality, stocking density, and environmental management. The absence of treatment effects in the present study therefore suggests that favorable housing conditions may have minimized lighting-related risk factors.

In contrast to FPD, hock burn (HB) severity was significantly influenced by both lighting duration and light color. Birds exposed to intermittent lighting exhibited improved hock condition, supporting the hypothesis that dark periods enhance resting synchrony and reduce prolonged contact with litter (Schwean-Lardner et al. 2016; Nelson et al. 2020; Škrbić et al. 2015). Similarly, green LED light improved HB scores compared with white light, in partial agreement with previous findings (Riber 2015). These results indicate that hock lesions may be shaped not only by litter conditions but also by lighting-mediated behavioral patterns.

Although lameness remains a major welfare concern in fast-growing broilers, gait score was not affected by either lighting duration or light color in the present study. This aligns with previous research reporting limited or no effect of lighting conditions on locomotor performance (Sun et al. 2017; Riber 2015; Franco et al. 2022b; Oke et al. 2023). The generally low prevalence of abnormal gait scores suggests that overall management conditions were sufficient to maintain locomotor welfare.

Plumage condition represents another important welfare indicator, as feather cleanliness and integrity are closely linked to litter quality and behavioral activity (Saraiva et al. 2016). Birds exposed to white light exhibited poorer plumage cleanliness compared with those reared under green LED light, which may be explained by higher locomotor activity and increased contact with litter. In addition, restricted lighting negatively affected feather cover in several body regions, whereas intermittent lighting improved plumage condition. These findings support previous evidence that photoperiod influences feather maintenance, potentially through its effects on rest–activity cycles (Jiang et al. 2023).

The heterophil-to-lymphocyte (H/L) ratio is a well-established indicator of chronic stress in poultry (Gross and Siegel 1983). In the present study, lighting duration significantly affected H/L ratio, with higher values observed under restricted lighting, suggesting increased physiological stress. This may be attributed to prolonged light exposure and reduced opportunities for uninterrupted rest. Furthermore, broilers exposed to green light exhibited lower H/L ratios than those under white light, consistent with previous findings (Mohamed et al. 2017). Importantly, the observed interaction between lighting duration and light color indicates that stress responses are jointly modulated by photoperiod and spectral composition rather than by either factor alone.

Tonic immobility (TI), commonly used as a behavioral indicator of fearfulness, was not significantly affected by lighting treatments. This result is consistent with earlier studies (Olanrewaju et al. 2016; Poudel et al. 2022) and suggests that fear-related responses may be less sensitive to lighting conditions than physiological stress indicators.

Carcass traits were largely unaffected by lighting treatments, in agreement with previous studies (Downs et al. 2006; Tuell et al. 2020). The only exception was wing weight, which was higher in birds reared under green light. Although this finding should be interpreted cautiously, it may reflect subtle differences in activity patterns or growth allocation. Overall, carcass characteristics appeared relatively stable across treatments.

Meat quality traits showed limited sensitivity to lighting conditions, although ultimate pH (pHu) was influenced by light color. Higher pHu values observed under green light suggest reduced pre-slaughter stress and more favorable metabolic conditions. In addition, the presence of significant interactions for cooking loss and water-holding capacity indicates that meat quality responses may depend on the combined effects of photoperiod and spectral composition. These findings highlight the importance of considering interactive effects when evaluating environmental management strategies.

Behavioral observations further supported these interpretations. Birds under restricted lighting exhibited increased feeding activity at later ages, suggesting alterations in feeding distribution rather than total intake. Higher walking activity under white light at earlier ages likely reflects increased stimulation due to perceived brightness, whereas reduced activity under green light supports its calming effect. These results are consistent with previous reports indicating that shorter wavelengths promote resting behavior (Sultana et al. 2013).

Overall, the present findings demonstrate that lighting duration and spectral composition exert both independent and interactive effects on broiler welfare, physiological stress responses, behavior, and selected meat quality traits. The consistent presence of interaction effects underscores the importance of evaluating lighting parameters as an integrated management system. Intermittent lighting combined with green LED illumination appears to be a promising approach for improving welfare and stress-related outcomes without compromising production performance.

Conclusions

Lighting duration and LED spectrum exerted selective effects on broiler welfare and physiological stress responses, while having limited influence on carcass traits and most meat quality parameters. Although several variables, including tonic immobility and the majority of meat quality traits, were not significantly affected, meaningful treatment-related differences were observed in key welfare and stress indicators. In particular, green LED lighting was consistently associated with improved welfare outcomes, including better hock condition, cleaner plumage, and a more favorable physiological stress profile. Lighting duration primarily influenced hematological parameters, notably heterophil and lymphocyte counts and the heterophil-to-lymphocyte (H/L) ratio, indicating that photoperiod plays a significant role in modulating chronic stress status in broilers. Moreover, the presence of significant interactions between lighting duration and light color for selected hematological variables highlights the importance of considering photoperiod and spectral composition jointly when designing lighting programs. Behavioral responses were generally modest and age dependent, suggesting that sensitivity to lighting conditions may vary across the production cycle. Carcass yield and most meat quality traits remained largely unaffected, although wing weight and breast meat ultimate pH responded to light color.

Overall, intermittent lighting combined with green LED illumination appears to be a practical and promising approach for improving broiler welfare without compromising production performance under commercial conditions. Further research under varying genotypes, stocking densities, and commercial production environments is warranted to validate and extend these findings.

Declarations

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Conflict of interest

The authors declare that they have no conflict of interest.

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CRediT authorship contribution statement

Evrim Dereli Fidan: Investigation, data curation, formal analysis, validation, visualization, writing—original draft, writing—review and editing, supervision. Ece Koç Yıldırım: Investigation, conceptualization, methodology, funding acquisition, project administration. Mehmet Kaya: Investigation, data curation, validation, visualization. All authors contributed, approved the final manuscript, and agree to be accountable for all aspects of the work.

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Tables

Table 1. Ethogram of mutually exclusive activities recorded during each observation period

Behavior	Description
Feeding	Bird directing its beak toward the feeder and performing pecking, manipulating, or ingesting feed, once or repeatedly.
Drinking	Bird positioned at the nipple drinker while drinking water or manipulating the nipple drinker.
Walking	Locomotion at normal pace or with rapid steps.
Aggressiveness	Forceful pecking directed toward or received from another bird, usually with the beak above the opponent's head; includes chasing or being chased in an aggressive interaction.
Preening	Bird directing its beak toward the plumage in repeated movements for feather maintenance, including pecking, nibbling, and combing.

Table 2. Effect of lighting duration and LED color on footpad dermatitis, hock burn, gait score, and plumage cleanliness in broilers at 42 days of age

Factor	Incidence of Foot Pad Dermatitis (%)							
	n	Good	n	Fair	n	Poor	χ^2	*P-value*
Lighting duration								
Restricted	115	95.8	5	4.2	0	0.0	3.268	0.060
Intermittent	120	100	0	0	0	0.0		
Light color								
White	115	95.8	5	4.2	0	0.0	3.268	0.060
Green	120	100	0	0	0	0.0		
	Incidence of Hock Burn (%)							
	n	Good	n	Fair	n	Poor	χ^2	*P-value*
Lighting duration								
Restricted	106	88.3	11	9.2	3	2.5	6.254	0.029
Intermittent	116	96.7	4	3.3	0	0.0		
Light color								
White	104	86.7	13	10.8	3	2.5	11.815	0.001
Green	118	98.3	2	1.7	0	0.0		
	Gait Score (%)							
	n	S:0	n	S:1	n	S:2	n	S:3-5	χ^2	*P-valu*
Lighting duration										
Restricted	65	54.2	50	41.7	5	4.2	0	0.0	3.041	0.219
Intermittent	78	65.0	39	32.5	3	2.5	0	0.0		
Light color										
White	70	58.3	44	36.7	6	5.0	0	0.0	2.074	0.354
Green	73	60.8	45	37.5	2	1.7	0	0.0		
	Incidence of Plumage Cleanliness (%)							
	n	S:0	n	S:1	n	S:2	χ^2	*P-va*
Lighting duration								
Restricted	20	16.7	87	72.5	13	10.8	2.710	0.
Intermittent	12	10.0	97	80.8	11	9.2		
Light color								
White	9	7.5	98	81.7	13	10.8	7.074	0.
Green	23	19.2	86	71.7	11	9.2		

Abbreviations: S: score; χ^2: chi-square value (P-value < 0.05).

Table 3. Effect of lighting duration and LED color on plumage damage of the back, wing, tail, and thigh regions in broilers at 42 days of age

Factor	Incidence of Back Plumage Damage (%)									
	n	S:0	n	S:1	n	S:2	n	S:3-5	χ^2	*P-value*
Lighting duration										
Restricted	56	46.7	58	48.3	6	5.0	0	0.0	6.949	0.031
Intermittent	76	63.3	41	34.2	3	2.5	0	0.0		
Light color										
White	63	52.5	49	40.8	8	6.7	0	0.0	5.727	0.057
Green	69	57.5	50	41.7	1	0.8	0	0.0		
	Incidence of Wing Plumage Damage (%)									
Lighting duration									14.112	0.001
Restricted	48	40.0	52	43.3	20	16.7	0	0.0		
Intermittent	71	59.2	44	36.7	5	4.2	0	0.0		
Light color									9.727	0.008
White	48	40.0	55	45.8	17	14.2	0	0.0		
Green	71	59.1	41	34.2	8	6.7	0	0.0		
	Incidence of Tail Plumage Damage (%)									
Lighting duration										
Restricted	38	31.7	57	47.5	25	20.8	0	0.0	5.697	0.058
Intermittent	51	42.5	56	46.7	13	10.8	0	0,0		
Light color										
White	48	40.0	57	47.5	15	12.5	0	0.0	2.244	0.326
Green	41	34.2	56	46.7	23	19.1	0	0.0		
	Incidence of Thigh Plumage Damage (%)									
Lighting duration										
Restricted	36	30.0	52	43.3	32	26.7	0	0.0	15.787	<0.001
Intermittent	61	50.8	47	39.2	12	10.0	0	0.0		
Light color										
White	42	35.0	53	44.2	25	20.8	0	0.0	3.055	0.217
Green	55	45.9	46	38.3	19	15.8	0	0.0		

Abbreviations: S: score; χ^2: chi-square value (P-value < 0.05).

Table 4. Least squares mean for hematological parameters[1] and tonic immobility duration[2] in broiler chickens subjected to different lighting treatments

Factor	Hematological Parameters						Fear Test
	HET (%)	LYMP (%)	H/L ratio	EOS (%)	BAS (%)	MON (%)	TI duration (s)
Lighting duration							
Restricted	28.88±1.06	51.37±1.44	0.58±0.03	6.44±0.43	7.46±0.61	5.84±0.63	122.78±15.50
Intermittent	22.50±1.06	56.93±1.44	0.43±0.03	7.17±0.43	6.86±0.61	6.70±0.63	113.70±10.50
Light color							
White	27.31±1.06	54.30±1.44	0.56±0.03	6.38±0.43	5.81±0.61	6.25±0.63	123.98±10.50
Green	24.07±1.06	54.00±1.44	0.46±0.03	7.21±0.43	8.52±0.61	6.19±0.63	112.50±10.50
Lighting duration x Light color							
Restricted -White	33.35±1.50^{a}	50.52±2.03^{b}	0.71±0.05^{a}	5.31±0.61	5.07±0.87^{b}	5.75±0.89	126.56±14.86
Restricted -Green	24.42±1.50^{b}	52.23±2.03^{b}	0.46±0.05^{b}	7.11±0.61	9.87±0.87^{a}	5.88±0.89	119.00±14.86
Intermittent -White	21.27±1.50^{b}	58.08±2.03^{a}	0.41±0.05^{b}	7.51±0.61	6.56±0.87^{b}	6.92±0.89	121.40±14.86
Intermittent -Green	23.73±1.50^{b}	55.78±2.03^{ab}	0.45±0.05^{b}	6.85±0.61	7.17±0.87^{b}	6.49±0.89	106.00±14.86
Effects	*P-value*						
Lighting duration	<0.001	0.013	0.004	0.128	0.494	0.336	0.548
Light color	0.043	0.886	0.045	0.362	0.005	0.866	0.449
Lighting duration x Light color	0.001	0.335	0.008	0.057	0.025	0.759	0.795
	F-value						
Lighting duration	18.216	7.513	10.381	2.526	0.486	0.973	0.374
Light color	4.680	0.021	4.564	0.871	9.727	0.029	0.598
Lighting duration x Light color	14.485	0.975	8.654	4.075	5.843	0.097	0.070

Abbreviations: HET: Heterophil, LYMP: Lymphocyte, H/L: Heterophil/Lymphocyte ratio, EOS: Eosinophil, BAS: Basophil, MON: Monocyte, TI: Tonic immobility.

[a, b]: Means within a column with different superscript letters differ significantly (P-value < 0.05).

[1]: Means represent 6 replicate pens with 10 randomly selected birds per replicate and 60 birds per treatment for hematological traits.

[2]: Means represent 6 replicate pens with 5 randomly selected birds per replicate and 30 birds per treatment for tonic immobility test.

Table 5. Effect of lighting duration and LED color on carcass characteristics of broiler chickens[1]

Factor	Carcass Characteristics			
	Carcass weight (g)	**Breast weight (g)**	**Thigh weight (g)**	**Wing weight (g)**
Lighting duration				
Restricted	2374.20±30.39	549.03±9.40	626.13±8.12	226.12±3.14
Intermittent	2409.13±30.39	563.22±9.40	633.35±8.12	229.33±3.14
Light color				
White	2356.05±30.39	554.30±9.40	620.08±8.12	222.60±3.14
Green	2427.28±30.39	557.95±9.40	639.40±8.12	232.85±3.14
Lighting duration x Light color				
Restricted -White	2350.17±42.98	553.30±13.29	614.57±11.48	224.17±4.44^{ab}
Restricted -Green	2398.23±42.98	544.77±13.29	637.70±11.48	228.07±4.44^{ab}
Intermittent -White	2361.93±42.98	555.30±13.29	625.60±11.48	221.03±4.44^{b}
Intermittent -Green	2456.33±42.98	571.13±13.29	641.10±11.48	237.63±4.44^{a}
Effects	*P-value*			
Lighting duration	0.426	0.299	0.537	0.478
Light color	0.113	0.786	0.108	0.032
Lighting duration x Light color	0.596	0.370	0.743	0.168
	F-value			
Lighting duration	0.661	1.138	0.395	0.524
Light color	2.747	0.075	2.833	5.320
Lighting duration x Light color	0.291	0.840	0.111	2.042

[a, b]: Means within the same column with different superscript letters differ significantly (P-value < 0.05).

[1]: Means represent 6 replicate pens with 5 randomly selected birds per replicate and 30 birds per treatment for carcass characteristics.

Table 6. Least squares mean for meat quality traits in broiler chickens[1]

Factor	Meat Quality Traits						
	pH_{15}	pH_u	CL (%)	WHC (%)	L*	a*	b*
Lighting duration							
Restricted	6.17±0.03	5.67±0.02	33.35±0.57	12.36±0.45	56.60±0.38	1.44±0.12	15.32±0.29
Intermittent	6.13±0.03	5.69±0.02	31.80±0.57	12.53±0.45	56.55±0.38	1.49±0.12	15.63±0.29
Light color							
White	6.19±0.03	5.64±0.02	32.89±0.57	12.23±0.45	56.89±0.38	1.38±0.12	15.84±0.30
Green	6.10±0.03	5.72±0.02	32.26±0.57	12.66±0.45	56.25±0.38	1.55±0.12	15.11±0.30
Lighting duration x Light color							
Restricted -White	6.23±0.05	5.64±0.03^{b}	34.73±0.81^{a}	13.44±0.64^{a}	56.89±0.53	1.22±0.17	15.40±0.42
Restricted -Green	6.10±0.05	5.70±0.03^{ab}	31.98±0. 81^{b}	11.28±0.64^{b}	56.31±0.53	1.67±0.17	15.23±0.42
Intermittent -White	6.16±0.05	5.64±0.03^{b}	31.05±0. 81^{b}	11.01±0. 64^{b}	56.90±0.53	1.55±0.17	16.28±0.42
Intermittent -Green	6.11±0.05	5.73±0.03^{a}	32.55±0.81^{ab}	14.04±0. 64^{a}	56.19±0.53	1.44±0.17	14.99±0.42
Effects	*P-value*						
Lighting duration	0.491	0.588	0.068	0.797	0.925	0.773	0.460
Light color	0.076	0.008	0.449	0.507	0.243	0.327	0.095
Lighting duration x Light color	0.416	0.447	0.016	0.001	0.907	0.115	0.194
	F-value						
Lighting duration	0.493	0.303	3.717	0.068	0.009	0.085	0.569
Light color	3.507	8.547	0.598	0.457	1.446	1.011	3.080
Lighting duration x Light color	0.689	0.602	6.966	16.362	0.014	2.714	1.803

Abbreviations: pH_{15}, initial pH measured 15 min postmortem; pHu, ultimate pH measured 24 h postmortem; CL, cooking loss; WHC, water-holding capacity; L*, lightness; a*, redness; b*, yellowness.

$^{a, b}$: Means within the same column with different superscript letters differ significantly (P-value < 0.05).

[1]: Means represent 6 replicate pens with 5 randomly selected birds per replicate and 30 birds per treatment for meat quality characteristics.

Table 7. Effect of lighting duration and LED color on behavioral activities (%) of broiler chickens at 21, 28, 35, and 42 days of age

Day 21					
Factor	Feeding	Drinking	Walking	Aggressiveness	Preening
Lighting duration					
Restricted	33.64±5.78	18.86±5.85	56.29±5.58	10.30±4.22	36.59±5.56
Intermittent	40.91±5.78	27.35±5.85	57.80±5.58	16.44±4.22	46.82±5.56
Light color					
White	32.27±5.78	20.76±5.85	75.38±5.58	10.68±4.22	39.92±5.56
Green	42.27±5.78	25.45±5.85	38.71±5.58	16.06±4.22	45.61±5.56
Lighting duration x Light color					
Restricted -White	23.33±8.82	10.00±2.58	73.33±8.03^{a}	0.00±0.00^{b}	41.67±8.33
Restricted -Green	43.94±5.34	27.73±7.06	39.24±5.90^{b}	20.61±6.56^{a}	31.51±7.84
Intermittent -White	41.21±8.47	31.52±12.00	77.43±7.97^{a}	21.36±7.51^{a}	38.18±9.89
Intermittent -Green	40.61±9.45	23.18±8.56	38.18±9.27^{b}	11.52±6.55^{ab}	55.46±4.28
Effects	*P-value*				
Lighting duration	0.384	0.317	0.850	0.316	0.208
Light color	0.235	0.577	<0.001	0.378	0.655
Lighting duration x Light color	0.209	0.131	0.747	0.019	0.096
	F-value				
Lighting duration	0.792	1.052	0.037	1.058	1.694
Light color	1.496	0.322	21.616	0.813	0.205
Lighting duration x Light color	1.683	2.479	0.107	6.514	3.046
Day 28					
Factor	Feeding	Drinking	Walking	Aggressiveness	Preening
Lighting duration					
Restricted	44.73±7.26	30.08±5.77	43.64±8.76	5.53±2.23	35.70±5.62
Intermittent	40.61±7.26	13.79±5.77	44.62±8.76	3.18±2.23	40.45±5.62
Light color					
White	39.77±7.26	17.50±5.77	42.27±8.76	1.67±2.23	35.76±5.62
Green	45.61±7.26	26.36±5.77	45.99±8.76	7.05±2.23	40.39±5.62
Lighting duration x Light color					
Restricted -White	38.33±13.52	30.00±12.38	46.67±15.42	3.33±2.11	45.00±11.47^{ab}
Restricted -Green	51.21±7.23	30.15±7.86	40.61±10.94	7.73±4.35	26.39±5.01^{b}
Intermittent -White	41.21±10.25	5.00±3.42	37.88±13.58	0.00±0.00	26.52±6.74^{b}
Intermittent -Green	40.00±9.02	22.58±6.29	51.36±8.51	6.36±4.03	54.39±7.10^{a}
Effects	*P-value*				
Lighting duration	0.689	0.060	0.937	0.464	0.556
Light color	0.576	0.290	0.768	0.103	0.566
Lighting duration x Light color	0.500	0.298	0.440	0.758	0.008
	F-value				
Lighting duration	0.165	3.984	0.006	0.557	0.359
Light color	0.323	1.179	0.090	2.919	0.340
Lighting duration x Light color	0.471	1.140	0.622	0.098	8.555

Day 35					
Factor	Feeding	Drinking	Walking	Aggressiveness	Preening
Lighting duration					
Restricted	38.26±7.25	23.18±5.25	43.41±7.81	4.77±2.90	33.79±5.30
Intermittent	42.04±7.25	16.82±5.25	37.80±7.81	6.29±2.90	35.83±5.30
Light color					
White	36.44±7.25	15.83±5.25	41.29±6.14	7.27±2.90	39.70±5.30
Green	43.86±7.25	24.17±5.25	39.93±7.81	3.79±2.90	29.92±5.30
Lighting duration x Light color					
Restricted -White	35.00±12.32	16.67±6.67	43.33±6.67	5.00±5.00	33.33±9.55
Restricted -Green	41.52±12.36	29.70±8.53	43.49±13.77	4.55±3.10	34.24±5.52
Intermittent -White	37.89±7.15	15.00±8.47	39.24±13.82	9.55±4.82	46.06±7.73
Intermittent -Green	46.21±8.04	18.64±5.61	36.37±7.96	3.03±3.03	25.61±6.59
Effects	*P-value*				
Lighting duration	0.716	0.401	0.617	0.715	0.788
Light color	0.477	0.275	0.903	0.405	0.207
Lighting duration x Light color	0.930	0.534	0.892	0.468	0.170
	F-value				
Lighting duration	0.136	0.735	0.257	0.137	0.074
Light color	0.524	1.260	0.015	0.724	1.700
Lighting duration x Light color	0.008	0.400	0.019	0.548	2.030
Day 42					
Factor					
Lighting duration	Feeding	Drinking	Walking	Aggressiveness	Preening
Restricted	34.17±4.98	13.18±4.70	25.91±7.64	4.18±4.03	35.45±6.40
Intermittent	13.56±4.98	14.77±4.70	34.92±7.64	11.14±4.03	45.83±6.40
Light color					
White	25.61±4.98	19.02±4.70	31.52±7.64	6.67±4.03	43.72±6.40
Green	22.12±4.98	8.94±4.70	29.32±7.64	8.64±4.03	37.58±6.40
Lighting duration x Light color					
Restricted -White	33.33±6.15	20.00±7.75	30.00±8.16	8.33±6.54	35.00±6.19
Restricted -Green	35.00±11.34	6.36±3.28	21.82±9.32	0.00±0.00	35.91±6.09
Intermittent -White	17.88±4.57	18.03±7.92	33.03±6.28	5.00±3.42	52.43±5.43
Intermittent -Green	9.24±3.32	11.52±6.55	36.82±16.56	17.27±8.70	39.24±14.92
Effects	*P-value*				
Lighting duration	0.008	0.813	0.414	0.236	0.265
Light color	0.626	0.145	0.841	0.733	0.506
Lighting duration x Light color	0.473	0.598	0.586	0.086	0.445
	F-value				
Lighting duration	8.762	0.057	0.695	1.494	1.315
Light color	0.245	2.303	0.041	0.119	0.460
Lighting duration x Light color	0.535	0.288	0.307	3.265	0.606

[a, b]: Means within the same column and age with different superscript letters differ significantly (P-value < 0.05).