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# Effect of Varying Light Intensity on Welfare Indices of Broiler Chickens Grown to Heavy Weights

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**Abstract:** The effects of varying light-intensity on ocular, immune, fear and leg health of broiler chickens grown to heavy weights under environmentally controlled conditions were evaluated. Four identical trials were conducted with two replications per trial. In each trial, 600 Ross 308 chicks were randomly distributed into 10 environmentally controlled chambers (30 males and 30 females chicks/chamber) at 1 d of age. Each chamber was randomly assigned one of five light intensities (25, 10, 5, 2.5 and 0.2 lx) from d 22 to 56 d of age. Feed and water were provided *ad libitum*. Humoral immune response was determined on d 28, while ocular health and general well-being assessments were performed on d 42 and 49, respectively. Results indicated that total Anti-Sheep Red Blood Cells (SRBC) antibody was not significantly ($p>0.05$) affected by the treatments, but there was significant ($p\leq0.05$) sex effects under 25 and 2.5 lx treatments. There were no differences among treatments for either ocular weight relative to BW, ocular assessments, gait scoring test or tonic immobility responses, suggesting that these levels of light intensities did not compromise welfare of the birds. This study shows the positive impact on profits to commercial poultry facilities that are using low lighting environment to reduce hyperactivity, pecking damage and energy costs without compromising the welfare of the broilers.

**Key words:** Light-intensity, broiler, eye, welfare

## INTRODUCTION

Animal welfare has generated concerns from the domestic and global market sectors. International animal welfare concerns have included the effects of temperature and lighting programs on broilers (Food Marketing Institute and National Council of Chain Restaurants, 2003; National Chicken Council, 2005). Welfare of birds is to a large extent regulated by various intrinsic and extrinsic factors, among which lighting programs play a crucial role. Research development in poultry production has been displayed through the genetic selection of high productivity breeds. However, this genetic potential will not be fully realized until microenvironmental constraints (temperature, humidity, light intensity, air velocity, etc.) have been fully addressed. Low light intensities have shown benefits in broiler growth (Charles *et al*., 1992). However, welfare consultants have expressed concerns that low light intensity may cause damage to the eye lens or lead to blindness (Cummings *et al*., 1986; Chiu *et al*., 1975). Manipulation of normal light perception in birds has been shown to be associated with several eye conditions including avian glaucoma, which is induced by prolonged exposure to continuous bright light (Jensen and Matson, 1957) and avian macrophthalmos from prolonged exposure to darkness or dim light (Berkovitz *et al*., 1972; Lauber and Kinnear, 1979). Modern commercial poultry facilities are dimly lit to optimize feed conversion and minimize the incidence of skin scratches associated with higher illuminance and activity. Although the effects of lighting, particularly photoperiod, on poultry production are well understood, knowledge of the light intensity on broiler visual abilities and its involvement in the welfare of the bird itself is shallow by comparison. Cummings *et al*. (1986) reported blindness in pullets exposed to low light intensity (3 lx). The researchers did not measure ammonia concentration, which may have confounded the results. A previous study (Olanrewaju *et al*., 2007) indicated that light intensities lone yielded no significant eye lesions, but ammonia treatments induced eye lesions and the interaction of ammonia concentration on eye lesions. Most of this research has indicated that short photoperiod enhances both cellular and humoral immune responses (Moore and Siopes, 2000; Raghavendra *et al*., 2000).

The objective of the present study was to evaluate the effects of light intensity over time on ocular, immune response, Tonic Immobility (TI) and Gait Score (GS) in modern heavy (>2.5 kg) broiler chickens. It was

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hypothesized that exposure to varying light intensities would adversely affect eye health and the general welfare of modern heavy broiler chickens.

## MATERIALS AND METHODS

**Bird husbandry:** A randomized complete block experimental design was utilized in the 4 trials conducted for this study. In each of 4 trials with 2 replicates per trial, 600 1-d-old Ross 308 chicks were purchased from a commercial hatchery (Aviagen, Inc., Huntsville, AL) and upon arrival, the chicks were sexed and then group-weighed. All procedures relating to the use of live birds were approved by a USDA-Agricultural Research Service Animal Care and Use Committee at the Mississippi State location. Chicks were vaccinated for Marek's, Newcastle and infectious bronchitis diseases at the hatchery. At 12 d of age, birds received a Gumboro vaccination via water administration. Chicks were randomly distributed into 10 environmentally controlled chambers (30 males and 30 females chicks/chamber). Each chamber was randomly assigned one of five light intensities (25, 10, 5, 2.5 and 0.2 lx). Each environmentally controlled chamber had a floor area of 6 $m^2$ (2.3 m width x 2.6 m depth) with a chamber volume of 15.3 $m^3$ (2.5 m height). Each chamber contained 7.62 cm depth of new pine shavings, tube feeders and a 7-nipple watering system. The chicks remained in their respective chambers from 1-d-old throughout the experimental period (56 d of age). Birds were provided a 4-phase feeding program (starter: 1 to 14 d; grower: 15 to 28 d; finisher: 29 to 42 d; withdrawal: 43 to 56 d). Diets were formulated to meet or exceed NRC (1994) nutrient recommendations. Starter feed was provided as crumbles and subsequent feeds were provided as whole pellets. Feed and water were offered *ad libitum*. Temperature and relative humidity were the same across all treatments. Chamber temperature was 32°C at the initiation of experimentation and reduced by 2°C at weekly intervals until 15.6°C on d 49 of age with 50% RH.

**Experimental treatments:** Light intensity treatments commenced on d 22. Lighting in each chamber was set to a light intensity typical of those found in commercial production (25, 10, 5, 2.5 and 0.2 lx). Each chamber was equipped with incandescent bulbs, which peaked in the red portion of the visible spectrum (750 nm) and were controlled by a dimmer and digital timer, typical of commercial housing. Light intensity settings were verified at bird level using a photometric sensor with NIST-traceable calibration (403125, Extech Instruments, Waltham, MA) for each intensity and adjusted weekly when necessary. The light fittings and tubes were cleaned weekly in order to minimize dust build-up, which would otherwise reduce the intensity. Photoperiod in each chamber consisted of continuous lighting (24L:0D) at 20 lx from placement to 7 d, 20L:4D at 10 lx from 8 to 21 d, 20L:4D from 23 through 53 d and 24L:0D from 54 to 56 d of age.

### Experimental measurements

**Humoral immune response:** On day 28, 6 (3 males and 3 females) birds per chamber were randomly selected and intravenously injected via the wing vein with a 3 % solution of Sheep Red Blood Cells (SRBC). Birds were bled 7 days later via the wing vein to collect serum that was used for evaluating primary antibody response. Antibody titers were determined as described by Thornton *et al.* (2006) with little modification. Briefly, serum complement was inactivated via incubation in a water bath for 30 min at 56°C. Twenty-five microliters of a 0.85% saline solution was added to the wells of a 96-well microtitre plate. Serum samples (25 µl) were then added to the first well and serially diluted from 2- to 1,024 fold. A 2.5% sheep red blood cell suspension at 25 µl was added to each well. The plate was sealed and incubated for 2 hrs at 37°C. The results of the microhemagglutination assay were determined by recording the last dilution at which complete agglutination was apparent in the well. Antibody titers were reported as a $\log_2$ of the recorded dilutions.

### Ocular assessments

**Eye examination:** On day 42, eye scoring and intraocular pressure were evaluated on 10 (5 males and 5 females) birds from each chamber by veterinary ophthalmologist. The ophthalmologist did not know the treatment origin of any bird examined. Biomicroscopy was performed using a Kowa SL-14 portable slit-lamp (KOWA Company Ltd., Tokyo, Japan). During weekly exams, signs of clinical keratoconjunctivitis and anterior uveitis were recorded. Corneal lesions assessed by biomicroscopy were assigned injury scores similar to Thoft's classification (Thoft, 1979). The numerical scale for grading corneal lesions was 0 = normal cornea; 0.5 = not normal but less than 1; 1 = diffuse corneal edema generally over greater than three quarters of the corneal surface; 2 = 1 + a focal superficial corneal ulcer measuring less than one quarter of the corneal surface; 3 = 1 + a corneal ulcer of half or more of the corneal surface and extending into the anterior chamber; 4 = 3 + deeper extension into the stromal layers and 5 = corneal perforation. This scale is also dependent on the definition of a flare, which is the breakdown of the blood-eye barrier or protein leakage across this barrier into the anterior chamber thereby creating cloudiness. Therefore, the anterior chamber was further assessed as either 0 = normal anterior chamber; 0.5 = not normal but less than 1; 1 = flare is visible; 2 = flare is easily visible; 3 = flare is easily visible with neovascularization on the iris surface; or 4 = flare is easily visible with hyphema clearly evident and diffuse iris neovascularization.

**Ocular development and histopathologic examination:** On d 42, 6 birds (3 males and 3 females) from each chamber were weighed individually. Subsequently, birds were euthanatized by cervical dislocation according to the USDA Animal Care and Ethics Committee for blood sampling and organ collection procedures. The right eyeball was dissected out, trimmed of extraneous tissue and weighed to the nearest 0.01 g. Assuming bilateral symmetry, only the right eye was excised and its weight doubled to give an estimate of total eye weight and calculation of the total eyes weight to BW ratio was determined. The dissected right eyeball was placed inside 10% buffered formalin for gross anatomical anomalies and histopathological evaluation by a veterinary pathologist using Kristensen (1948) method. Briefly, after fixing for at least 72 hr in formalin, the eyes were placed in Kristensen's decalcifying solution (1:1 mixture of 8 N formic acid and 1 N sodium formate) for 3 days. Two sections were prepared from each eye as follows. The eye was held in a normal postural position and cut vertically approximately 4 mm lateral to the center of the cornea. A second cut was made through the center of the cornea and then a third cut was made approximately 4 mm medial to the center of the cornea. All cuts were made completely through the eye. The two trimmed sections were placed in a single cassette such that the center of the cornea was face-down for each section. Following this, the cassettes were washed in gently running water for 24 hr to remove residual acid and then placed in 10% buffered neutral formalin until processed. All tissues were processed routinely, embedded in paraffin, sectioned at 6 μm and stained with hematoxylin and eosin. We have used this procedure previously (Olanrewaju *et al*., 2007). The examining pathologist was unaware of bird treatment origin. The iris and ciliary body were scored for the presence (+) or absence (-) of heterophils, diffuse lymphocytic infiltrates and nodular lymphocytic infiltrates. In addition, the presence (+) or absence (-) of increased cellularity along the rostral surface of the iris was also noted and the corneal epithelium was scored for the presence (+) or absence (-) of ulceration.

**General well-being:** On day 49, 10 (5 males and 5 females) birds from each chamber were randomly selected for assessment of their general welfare using three different protocols as described previously (Olanrewaju *et al*., 2007). Welfare locomotive ability was assessed using a modification of the Kestin Gait Scoring System (Kristin *et al*., 1994) as described in the American Humane Welfare Standard. Fear and frustration was assessed by determining tonic immobility index time (American Humane Welfare Standard). In addition, unnecessary discomfort to the birds was also avoided by using proper housing and handling techniques (National Research Council, 1996).

**Gait Scoring (GS) test:** From 10 (5 males and 5 females) randomly selected birds from each chamber, 2 (1 male and 1 female) chicks at a time were allowed to walk freely (1.52 m) within an interior enclosed floor area of 1.83 m x 3.66 m that contained new pine shavings. Gait score performance was evaluated according to the Kestin Gait Scoring System (Kristin *et al*., 1994) and modified by Dawkins *et al*. (2004) on a scale ranging from 0 to 2. Score 0 represented no detectable impairment of walking, score 1 indicated birds with no detectable walking impairment and able to walk at least 5 feet without sitting down, while score 2 indicated severe impairment of walking ability with birds being unable to walk 5 feet without sitting down again. Chicks assigned a score 2 were unable to walk. Each chick was observed for 2 to 3 min. If the chick hesitated or remained immobile, it was touched with a long stick to encourage it to walk.

**Tonic Immobility (TI):** Ten (5 males and 5 females) birds from each chamber were also randomly selected for TI assessment. Tonic immobility was induced by inverting the bird on its back and restraining it for 10 s in a U-shaped wooden cradle covered with a layer of cloth. One hand was used to cover the birds head and the other hand was placed on the sternum, as described by Jones and Waddington (1992). Eye contact was completely avoided between the bird and the experimenter after the experimenter removed his hands from the cradle. A stopwatch was used to record latencies until the bird righted itself (getting to its feet again). The time was measured from withdrawal of the hand until the bird straightened up. If the bird righted itself in less than 10 s, then TI was not considered to have been induced. If TI was not induced after 3 attempts, the duration of TI was considered to be 0 s and the restraining procedure had to be repeated. If the bird did not show a righting response over the 10 s test period, then a maximum score of 600 was given for righting time. The number of inductions required to attain TI was also recorded for each bird.

**Statistical analysis:** A randomized complete block design was used in this study with two replications per trial. Analyses were conducted using ANOVA followed by the least significant difference test comparing treatment means using the MIXED procedure of SAS software (SAS Institute, 2008). Chambers used were switched within trials to remove chamber effects so that treatments are not confounded. All mortality data were subjected to arc sine transformation before analysis. Chamber was considered as the experimental unit and treatments were replicated in time. Four trials were repeated over time where trial serves as the blocking factor. In addition to the treatment effect, the statistical model also incorporated the sex and day effects. Log transformation

Table 1: Clinical Corneal Lesion Score (CLS) and Anterior Chamber Score (ACS) of broilers exposed to varying light intensity from d 22 to d 49 of age[1]

| Treatments | CLS[2] | ACS[3] |
|---|---|---|
| 25 lx | 0.02 | 0.02 |
| 10 lx | 0.01 | 0.00 |
| 5 lx | 0.00 | 0.02 |
| 2.5 lx | 0.00 | 0.00 |
| 0.2 lx | 0.00 | 0.00 |
| SEM[4] | 0.013 | 0.012 |
| p-value | 0.758 | 0.897 |

[1]Pooled SEM for each of the treatments (n = 8).
[2]Numerical scale for grading corneal lesions: 0 = normal cornea, ½ = not normal but <1, 1 = diffuse corneal edema generally over >3/4 of the corneal surface, 2 = 1 + a focal superficial corneal ulcer measuring <¼ of the corneal surface, 3 = 1 + a corneal ulcer of ½ or more of the corneal surface and extending into the anterior stroma and 4 = 3 + deeper extension into the stromal layers, 5 = corneal perforation.
[3]Numerical scale for grading anterior chamber: 0 = normal anterior chamber, ½ = not normal but <1, 1< flare is visible, 2 = flare is easily visible, 3 = flare is easily visible with neovascularization on the iris surface and 4 = flare is easily visible with hyphema clearly evident and diffuse iris neovascularization

of the raw scores was used because of the large range among the data. Geometric means are presented (Table 1) for the corneal and anterior chamber scores. The histopathologic eye tissue evaluations (presented as percent of occurrence in Table 3) required arcsine transformation before analysis. For each of the eye tissue, the presence or absence of lymphocytic or heterophilic infiltrates in iris and ciliary body was given as a positive or negative score. If the number of samples with a positive score was 3 out of 4 for a particular treatment, the percentage of occurrence was 75%. Means comparisons were assessed by least significant differences and the level of significance was fixed at $p \leq 0.05$ unless otherwise stated.

## RESULTS

**Eye examination:** Effects of the exposure to varying light-intensity from d 22 to d 42 on corneal lesion scores and anterior chamber scores of broiler chickens are summarized in Table 1. There was no significant ($p \geq 0.05$) difference among the treatments for corneal lesions scores and anterior chamber scores. There was a significant ($p \leq 0.040$) effect of varying light intensity on eye weight (Table 2). Broilers reared under 2.5 and 10 lx had the lowest eye weights as compared with birds reared under 0.2, 5 and 25 lx. In addition, male broilers had significant higher live BW ($p \leq 0.000$) and eye ($p \leq 0.000$) weights in comparison to female broilers. However, there was no effect of treatments on the ratio of eye weight to live BW indicating that eye weight was directly proportional to the live BW. Table 3 presents histopathologic examination due to the varying light intensity on rostral surface, lymphocytes and heterophils in the iris stroma and ciliary body of broiler chickens. There were no statistically significant treatment differences due to light-intensity among iris and ciliary body lymphocytic and heterophilic infiltrates. Table 4 represents results of varying light intensity on Tonic Immobility (TI) and Gait Scores (GS). Tonic immobility and gait scores were not significantly affected by light intensity. The overall GS values were less than 1 and no

Table 2: Influence of light-intensity on live body weights, eye weights and relative eye weight at 42 d of age[1]

| Item | Live BW (kg) | Eye WT (g) | Eye WT:BW (g/kg) |
|---|---|---|---|
| **Light-intensity treatment (lx)** | | | |
| 25 | 3.011 | 6.117[a] | 2.032 |
| 10 | 3.053 | 5.676[b] | 1.859 |
| 5 | 3.028 | 6.317[a] | 2.086 |
| 2.5 | 3.028 | 5.776[b] | 1.907 |
| 0.2 | 2.985 | 6.326[a] | 2.119 |
| Pooled SEM[2] | 0.060 | 0.105 | 0.087 |
| **Sex** | | | |
| Male | 3.45[a] | 6.93[a] | 2.008 |
| Female | 3.19[b] | 5.96[b] | 1.981 |
| Pooled SEM[2] | 0.039 | 0.080 | 0.042 |
| Source of variation | ---------- | p-value | ---------- |
| Light intensity | 0.129 | 0.040 | 0.062 |
| Sex | 0.000 | 0.000 | 0.624 |

[1]Means within a column and effect that lack common superscripts differ significantly ($p \leq 0.05$).
[2]Pooled SEM (n = 8)

Table 3: Histological changes noted in the iris and ciliary of broilers exposed to varying light intensity from d 22 to d 49 of age[1]

| | Iris | | | Ciliary body | |
|---|---|---|---|---|---|
| Treatments | Rostral surface[2] | Diffuse lymphocytic infiltrates[3] | Heterophilic infiltrates[4] | Diffuse lymphocytic infiltrates[3] | Heterophilic infiltrates[4] |
| 25 lx | 72.20 | 50.50 | 30.30 | 25.60 | 20.60 |
| 10 lx | 61.60 | 49.70 | 30.10 | 25.70 | 20.40 |
| 5 lx | 50.90 | 48.30 | 20.10 | 20.30 | 20.00 |
| 2.5 lx | 46.80 | 48.50 | 20.10 | 20.30 | 20.10 |
| 0.2 lx | 40.50 | 42.10 | 22.30 | 20.30 | 20.00 |
| SEM[5] | 7.453 | 7.618 | 6.251 | 2.652 | 2.131 |
| p-value | 0.458 | 0.524 | 0.364 | 0.856 | 0.749 |

[1]Pooled SEM for each of the treatments (n = 8).
[2]Observed increased cells along the roatral surface of the iris, which may have been the result of epithelial/endothelial hyperplasia, lymphocytic infiltrates, or both.
[3]Indicates the presence of lymphocytes in the iris stroma or ciliary body but does not include lymphocytes that may be present in a nodular aggregate. There were no observations of nodular aggregates of lymphocytes in the iris or ciliary body.
[4]Indicates the presence of heterophils in the iris stroma or ciliary body.

Table 4: Tonic immobility (TI) and gait-score (GS) of broilers exposed to varying light intensity from d 22 to d 49 of age[1]

| Treatments[1] | TI (s) | GS (%) |
|---|---|---|
| 25 lx | 195.400 | 15.400 |
| 10 lx | 181.400 | 15.710 |
| 5 lx | 177.400 | 15.400 |
| 2.5 lx | 182.700 | 10.800 |
| 0.2 lx | 178.400 | 10.420 |
| SEM[1] | 5.436 | 1.862 |
| p-value | 0.759 | 0.864 |

[1]Pooled SEM for each of the treatments (n = 8)

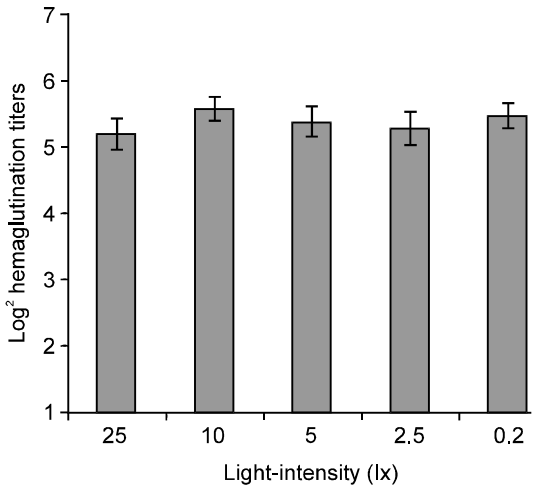


Fig. 1A: Effects of varying light intensity on total anti-SRBC of 28- to 35-d-old broilers

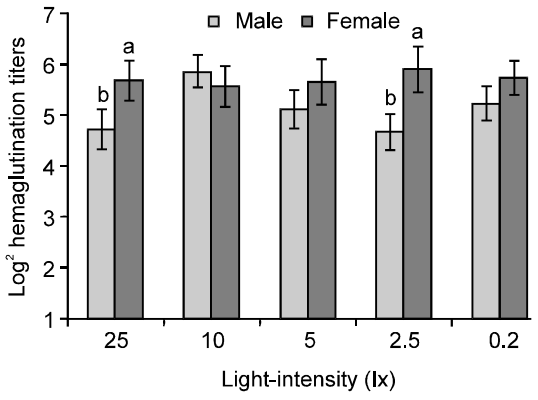


Fig. 1B: Sex effect of varying light intensity on total anti-SRBC of 28- to 35-d-old broilers

birds were found to have GS ≥2. There was no significant effect of the light intensity treatment on antibody production against SRBC (Fig. 1A). However, the highest value of antibody titer was obtained in the female group that received 10 lx and 0.2 lx compared to male broilers under the same light intensity treatments (Fig. 1B).

## DISCUSSION

It is known that light intensity can affect many aspects of avian physiology, welfare and behavior that include skeletal, blood chemistry, blood gases, ocular development and behavioral rhythms (Nelson and Demas, 1997; Reiter, 2003; Olanrewaju *et al*., 2006). There are conflicting reports on the effects of lighting programs on the welfare, performance and immune status of birds. The present results that indicate significant effects of low light intensity on eye weights are similar to the studies reported by others (Harrison *et al*., 1968; Blatchford *et al*., 2009; Deep *et al*., 2010), except that increased eye weight was not evaluated in proportion to the bird BW in those prior studies. The increased eye weights observed in this study due to varying light-intensity are proportional to their BW. Broilers reared in dim lighting (0.2 lx) had heavier eye weight than broilers reared under higher illumination of 25 lx, however, these differences in eyes weights were neutralized when reported on a BW basis.

Results on both GS and TI in the present study were generally normal with no significant differences occurring among treatments. This indicates that intensive selection against skeletal abnormalities in modern broiler chickens has improved the skeletal condition and in agreement with industry awareness and recent reports by Classen *et al*. (2004). Prayitno *et al*. (1997) speculated that this improvement in leg health may have been due to the increased activity of the broilers reared with high intensity red light, but in other studies no differences in leg health were observed even when greater light intensities were associated with greater activity levels (Kristensen *et al*., 2006). The duration of TI was similar for all the treatments. Duration of TI has been described as a good predictor of the level of fearfulness in domestic chickens (Jones, 1986). Unlike our present results, the incidence of leg problems has been shown to be influenced by light intensity (Newberry *et al*., 1988), photoperiod (Wilson *et al*., 1984) and light color (Prayitno *et al*., 1997). Lighting treatments had no effects on gait scores, which signify that the levels of light intensity in the present study had no impact on their overall leg health. Leg abnormalities and fear in broilers are both economic and welfare concerns in poultry production. The economic costs associated with leg weakness include culling and condemnations or downgrading at processing plant. However, recent reports indicated that Gait Scores (GS) and the incidence of leg weakness may have improved over time (Classen *et al*., 2004).

The differences in the antibody titer in all light intensity treatments were not significant. However, the value of antibody titer obtained in the female group that received the 10 lx and 0.2 lx light program were significantly higher compared to the male group under the same light intensity treatments. This could be due to increased

estradiol levels in the females or to levels of testosterone in the males because it has been reported that humoral immunity is enhanced following estradiol treatment and suppressed following testosterone treatment (Chao, 1996; Savita and Rai, 1998). It has been suggested that the reproductive system plays an important role in the regulation of the immune system in females compared with males independent of hormonal condition (Bilbo and Nelson, 2001). Kirby and Froman (1991) compared the immune responses of White Leghorn cockerels reared with constant light or with a 12L:12D regimen (both at a photophase intensity of 40 to 45 lx). Chickens in the constant light group had significantly less anti-SRBC antibody titers and a less delayed hypersensitivity response in the wattle than the 12L:12D group, which indicates a potential impairment of immune system response. Similar results have been observed in Japanese quail (Moore and Siopes, 2000) reared with constant light or on short (8L:16D) or long (16L:8D) days.

The findings in this investigation suggest that exposure to varying levels of light intensities ranging from 0.2 to 25 lx had no significant effect on most welfare indices evaluated in broilers grown to heavy weights, suggesting that these levels of light intensities did not pose as anti-welfare to these birds. Also, results imply that sex represents a significant contributor of variation in levels of humoral immune response in broiler chickens. We conclude that there are potential benefits associated with providing lower light intensity during rearing. This study shows the positive impact on profits to commercial poultry facilities that are using low lighting environment to reduce hyperactivity, pecking damage and energy costs without affecting welfare indices.

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