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# Research Article
# Effects of Light Sources and Intensity on Broilers Grown to Heavy Weights: Hematophysiological and Biochemical Assessment

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## Abstract

**Background:** Most governments around the world including the USA have passed measures to phase out incandescent light bulbs in favor of more energy-efficient lighting alternatives. Research is limited on blood physiological variables of broilers grown to heavy weights (>3 kg) under these new light sources to ensure health and welfare of broilers. **Objective:** We investigated the effects of light sources and intensity on blood physiological and biochemical variables of broilers grown to heavy weights. **Methodology:** In each of 4 trials having 2 replicates per trial, 960 1 day old ross×ross 708 chicks were randomly distributed into 16 rooms (30 male and 30 female per room). A 4×2 factorial treatment structure evaluated 4 light sources [incandescent (ICD, standard), compact fluorescent (CFL), Light Emitting Diode (LED) and poultry specific filtered LED (PSF-LED)] from day 1-56 and 2 levels of light intensities (5 and 20 lx) from day 22-56 of age at 50% RH. Feed and water were provided *ad libitum*. Blood samples were collected from wing brachial vein on day 14, 21, 28, 42 and day 56 of age and analyzed immediately. **Results:** Light sources had effect ($p<0.05$) on BW, pH, $pCO_2$, $pO_2$, $SaO_2$, electrolytes, MCHC and Osmo. However, all these changes were still within the acid-base homeostasis and physiological ranges. There was no effect of light intensity and no difference between 5 and 20 lx on almost all examined variables. Plasma corticosterone and blood glucose concentrations were not affected by treatment. **Conclusion:** It was concluded that the light sources evaluated in this study might be suitable for replacement of ICD light source in commercial poultry facilities at the light intensities used in this study to reduce energy cost and optimize production efficiency without compromising welfare of broilers grown to heavy weights.

Key words: Light sources, light intensity, acid-base balance, broilers, welfare

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Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Light is one of the most important microclimate factors for poultry production that influences growth development and physiological functioning. Artificial lighting is extensively used in raising commercial poultry. Lighting programs have a central purpose of slowing the early growth rate of broilers, which allows birds to achieve physiological maturity prior to maximal rate of muscle mass accretion. It is a powerful exogenous factor that influences bird activity, behavior, physiology, immune response, growth rate and has been used to alleviate mortality issues related to metabolic disease. Based on the Energy Independence and Security Act[1], incandescent (ICD) bulbs within the marketplace are being phased out in favor of more energy-efficient lighting alternatives in poultry houses. Many new lighting technologies that exceed energy efficiency requirements are currently being developed by different companies as potential replacements for ICD light sources, including Cold Cathode Fluorescent Lamps (CCFL), Compact Fluorescent Lamps (CFL) and Light Emitting Diodes (LED) among others. The major benefits of these bulbs are high efficiency, long operating life, moisture resistance and availability in differing peak wavelengths[2]. However, choosing the correct one can be difficult since some do not dim very well.

The chicken is typical of avian species that possess 7 photoreceptor cell types consisting of 1 rod and 6 cones[3]. Tetrachromatic color vision is mediated by 4 types of single cones, which are maximally responsive to violet, blue, green and red light[4,5]. However, double cones consist of pairs of closely apposed principal and accessory members that act as a single functional unit and are thought to mediate luminance detection, which is used for motion perception[6,7]. Light signals are perceived by the avian brain through both the eyes (retinal) and direct penetration to the skull tissue (extra-retinal)[8,9]. Chickens showed peak sensitivity at blue to green light range (455-571 nm)[5,10]. Moreover, the avian brain also has 3 advanced light receptors within the brain that play a major role in biological and physiological functions[11,12]. Exposure to different light sources could alter the physiological state by altering the rhythmicity of several hormones including glucocorticoids[13], adrenocorticotropic hormone, corticotrophin releasing factor[14] and melatonin[15].

Blood analyses along with other biochemical evaluations have been used to assess the health status of animals including the chicken[16,17]. The changes in selected major blood variables are routinely used to determine various influences of environmental, nutritional and pathological factors[17]. Changes in acid-base balance may signal early symptoms of diseases and influence the early manifestation of clinical signs and therapeutic effectiveness in both domestic animals and human beings[18,19]. The basal corticosterone levels that increase in response to stress have been found to be consistently and significantly higher in birds housed under UV deficient lighting[20]. Stress responses are also integrally involved with acid-base balance in several species[17,21].

There are a number of studies focusing on the effect of differing light sources and schedules on broiler growth performance, welfare, meat quality and muscle tissue accretion with conflicting reports. Evaluation of CFL, CCL and LED among others is needed based on energy use, duration and cost on broiler growth performance and physiological responses. It is well known that lighting programs can affect many aspects of avian physiology, welfare, behavior and other factors, including blood chemistry, ocular development and behavioral rhythms[17,22,23]. Most of the studies on broiler acid-base balance especially $pO_2$ and $pCO_2$ are on broilers under 3 kg b.wt.[24,25]. The principal organ systems (lung, kidneys, gastrointestinal, cardiovascular) used in acid-base balance also influences respiratory and metabolic activities of these modern heavy weight broilers. Hence, more studies are needed on how these differing light sources in combination with light intensity impact blood physiological and biochemical variables welfare indices of broilers grown to heavy weights (>3.0 kg) to further ensure the health and welfare of broilers. The results from a previous study indicated that LED light bulbs might be a better potential replacement light source for ICD on broiler growth performance and yields[26,27]. The objective of the present study was to evaluate the effects of light sources (CFL, LED, PSF-LED bulbs) and light intensity (5 and 20 lx) in the presence of ICD bulbs on blood physiological and biochemical variables that will not compromise the welfare of broilers grown to heavy weights (>3.0 kg). It is hypothesized that the use of differing LED light sources and varying light intensities will not adversely affect blood physiological and biochemical variables of modern heavy broilers grown to heavy weight.

## MATERIALS AND METHODS

**Birds husbandry:** All procedures relating to the use of live birds in this study were approved by the USDA-ARS Animal Care and Use Committee at Mississippi State location. In each of 4 trials with 2 replicates per trial, 960 (480 males and 480 females) 1 day old ross×ross 708 chicks were purchased from a commercial hatchery. Upon arrival, the chicks were sexed and group-weighed. Chicks were randomly distributed into 16 environmentally controlled rooms (30 male and

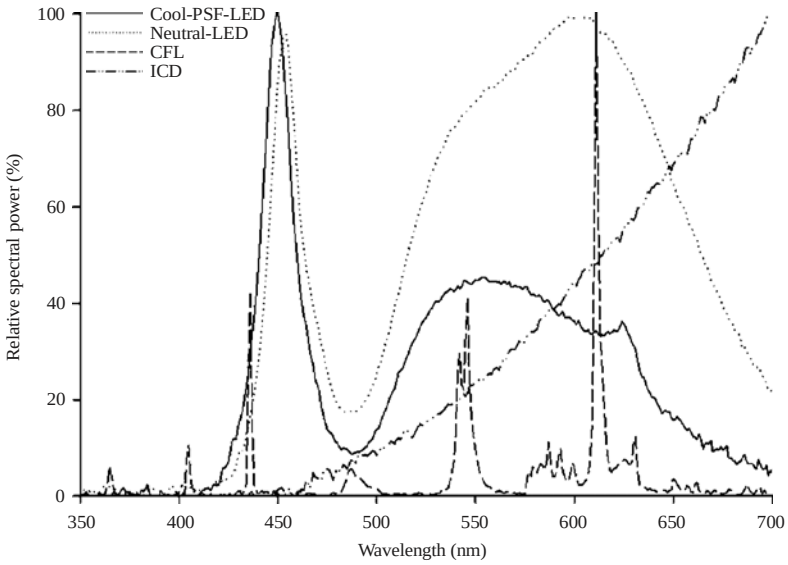


Fig. 1: Spectral content of the bulbs used in this study: COOL-PSF-LED: Cool poultry specific filtered LED (5000 k) purchased from once-innovation agrishif, Neutral-LED: Neutral-LED (3500 k) were purchased from green watt, CFL: Compact fluorescent (2700 k) and ICD: Incandescent bulb (2010 k)

30 female chicks/room). Each environmentally controlled room had a floor area of 6 $m^2$ (42 kg $m^{-2}$) with a room volume of 15.3 $m^3$ (2.5 m height). Each room contained approximately 7.62 cm depth of fresh pine shavings, tube feeders and a 7-nipple watering system. Chicks were vaccinated for Marek's, Newcastle and infectious bronchitis diseases at the hatchery. At 12 day of age, birds received a Gumboro vaccination via water administration. The chicks remained in their respective rooms from 1 day old throughout the experimental period (1-56 days of age). All birds were fed the same diet throughout the study. Birds were provided a 4-phase feeding program (starter: 1-14 days, grower: 15-28 days, finisher: 29-42 days and withdrawal: 43-56 days of age). Diets were formulated to meet or exceed NRC[28] nutrient recommendations for each feeding phase. 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 (RH) on day 1 were maintained at 32±1.1°C and 50±5%, respectively and RH was held constant across all treatments. Temperature was decreased as the birds progressed in age until 15.6°C was reached at 49 days of age where it remained through day 56.

**Experimental treatments:** A 4×2 factorial treatments structure was used to evaluate 4 light sources [incandescent (ICD, 2100 k; standard), compact fluorescent (CFL, 2700 k), neutral light emitting diode (Neutral-LED, 3500 k), cool poultry specific filtered LED (Cool-PSF-LED, 5000 k)] from day 1-56 of age and 2 levels (5 and 20 lx) of light intensities commenced from day 22-56 of age at 50% RH. Each of the 4 light source treatments was paired with one of the 2 light intensities treatments so that each room represented a particular light source: Light-intensity level combination for a total of 16 rooms. Photoperiod consisted of continuous (24L:0D) lighting at 20 lx from placement to 7 days with 20L:4D at 10 lx from 8-21 days and light intensity treatments from 22 through day 56. Neutral-LED were purchased from NexGen Illumination Inc. (Fayetteville, AR), CFL light bulbs were purchased from Osram Sylvania (Danvers, MA) and cool poultry specific filtered LED (Cool-PSF-LED) light bulbs, made specifically for poultry were purchased from once-innovation agrishift (Plymouth, MN). The light sources were adjusted to equal intensity according to the spectral sensitivity of broilers[10]. The light spectra of the light sources and ICD bulbs utilized in this study are presented in Fig. 1, which have been shown in our previous report under the same experimental condition[27]. We selected 5 and 20 lx in this study since American poultry industries use 5 lx while European poultry industries use 20 lx. Light intensity settings were verified from the center and from the four corners of each room at bird level (30 cm) to maintain a uniform intensity using a photometric sensor from National Institute of Standards and Technology-Traceable calibration (403125, Extech Instruments, Waltham, MA) for each intensity adjustment. The light bulbs were cleaned weekly in order to minimize dust build-up, which would otherwise reduce the intensity.

## Measurements

**Blood collections and chemical analyses:** On day 14, 21 (day before initiation of light intensity treatments), 28, 42 and 56, blood samples were collected between 0800 and 0900 h on sampling day from wing brachial vein of 6 (3 male and 3 female chicks/room) randomly selected birds from each room. The birds were then returned to the appropriate rooms without unnecessary discomfort to the birds using proper housing and handling techniques, as described by the NRC[29]. Blood samples (3 mL) were collected directly into heparinized (50 IU $mL^{-1}$) monovette syringes. All bleedings were completed within 45 sec after birds were caught. Blood samples were drawn directly from the syringes into a blood gas electrolyte analyzer (ABL-80 CO-OX Flex, Radiometer America, Westlake, OH) for immediate analysis of $pCO_2$, $pO_2$, $HCO_3^-$, pH, Hct, Hb, $SO_2$ and electrolytes ($Na^+$, $K^+$, $Ca^{2+}$ and $Cl^-$). This ABL-80 CO-OX Flex blood gas electrolyte analyzer was set to reflect a broiler body temperature of 41.5°C as per the manufacturer's instructions. The Mean Corpuscular Hemoglobin Concentration (MCHC) in grams per deciliter was calculated using the standard formula [(Hb×100)/Hct]. In addition, arterial oxygen saturation ($SaO_2$), which is the amount of oxyhemoglobin ($O_2Hb$) in blood expressed as a percent of the total amount of hemoglobin able to bind oxygen ($O_2Hb$)+deoxyhemoglobin was calculated using the standard equation:

$$SaO_2 = \frac{O_2Hb}{O_2Hb+\text{deoxyhemoglobin}} \times 100$$

The needle mounted on each monovette syringe was then removed, a cap was placed over the needle port and the syringes containing the blood samples were plunged into ice. After all birds were bled, the iced samples were transferred to the laboratory and centrifuged at 4,000×g for 20 min at 4°C. Two milliliters of each of the plasma samples from the syringes were stored in 2.5 mL graduated tubes at -20°C for later chemical analyses. Plasma samples were removed from the freezer, thawed and analyzed for corticosterone using a universal microplate spectrophotometer (Bio-Tec Instruments Inc., Winooski, VT) with ELISA reagent assay test kits (EIA-CS Kit, Enzo Life Sciences, Farmingdale, NY) according to the manufacturer's instructions. Plasma triiodothyronine ($T_3$) and thyroxine ($T_4$) concentrations were measured according to the manufacturer's instructions using a universal microplate spectrophotometer (Bio-Tec Instruments Inc.) with ELISA reagent assay test kits from ALPCO Diagnostics (Salem, NH).

**Statistical analysis:** The experimental design was a randomized complete block design. Treatment structure was a 4×2 factorial arrangement with the main factors being 4 light sources (ICD, CFL, neutral-LED, cool-PSF-LED) and 2 levels (5 and 20 lx) of light intensities with 2 replicates per trial. Individual sample data within each of the replicate units were averaged before analysis and data from the 4 trials were pooled and analyzed together. Analyses were conducted using the PROC MIXED procedure of SAS software[30]. Trial was a random effect, whereas, the light sources and light intensity were the fixed effect. Room was considered the experimental unit and treatments were replicated over time. Rooms used were switched randomly between trials to remove room effects so that treatments were not confounded. Main effects of light sources, light intensity and the interaction of the 2 factors were tested. Means comparisons on day 14, 21, 28, 42 and 56 were assessed by least significant differences and statements of significance were based on $p<0.05$ unless otherwise stated. Analyses of variance combined across days were performed to obtain treatment comparisons averaged across days and to test for treatment interactions with equal variances between days.

## RESULTS

Table 1 shows the combined main effects of light sources and light intensity on major selected blood physiological variables. In comparison with ICD light, birds in the CFL group had higher $Na^+$ ($p<0.001$) and Osmo ($p<0.001$), birds reared under neutral-LED light sources had lower pH ($p<0.023$), $pO_2$ ($p<0.035$), $SaO_2$ ($p<0.041$) and higher $pCO_2$ ($p<0.026$), $K^+$ ($p<0.035$), while birds reared under cool-PSF-LED light sources had higher MCHC ($p<0.036$) and BW ($p<0.011$). There was no effect of light intensity and no difference between 5 and 20 lx on all examined variables. In addition, no main effects of light sources, light intensity, or their interaction on $HCO_3^-$, Hct, Hb, $Ca^{2+}$, angap, GLU, CORT, $T_3$ and $T_4$ were observed.

Unlike Table 1, which was ANOVA combined effects of treatments over day, Table 2-4 represented separate ANOVAs for each sampling day. The influence of light sources, light intensity and their interaction on whole blood $pO_2$ and $SaO_2$ is presented in Table 2. As shown in Table 2, light sources had an effect on $pO_2$ on day 56 ($p<0.010$), where birds reared under CFL had higher $pO_2$ in comparison with other light sources. There was no main effect of light intensity found on any of the other sampling days.

Table 1: Combined main effects of light sources and light-intensity on blood physiological selected variables of broilers grown to heavy weights*

| | Light sources | | | | | Light intensity (lx) | | | |
|---|---|---|---|---|---|---|---|---|---|
| Variables | ICD | CFL | Neutral-LED | PSF-LED | p-value | 5 | 20 | p-value | Pooled SEM |
| BW (kg) | 4.080$^{b}$ | 4.119$^{ab}$ | 4.114$^{ab}$ | 4.226$^{a}$ | 0.011 | 4.162 | 4.123 | 0.213 | 0.014 |
| pH | 7.39$^{a}$ | 7.38$^{ab}$ | 7.37$^{b}$ | 7.39$^{a}$ | 0.023 | 7.38 | 7.37 | 0.065 | 0.003 |
| $pCO_2$ (mmHg) | 49.37$^{b}$ | 49.51$^{b}$ | 50.79$^{a}$ | 50.24$^{b}$ | 0.026 | 49.42 | 50.01 | 0.494 | 0.698 |
| $pO_2$ (mmHg) | 41.85$^{a}$ | 42.03$^{a}$ | 40.10$^{b}$ | 40.38$^{ab}$ | 0.035 | 41.19 | 41.19 | 0.792 | 0.520 |
| $HCO_3^-$ (mmHg) | 28.10 | 27.96 | 27.53 | 27.83 | 0.059 | 27.88 | 27.83 | 0.722 | 0.194 |
| $SaO_2$ (%) | 73.15$^{a}$ | 72.98$^{ab}$ | 70.24$^{b}$ | 70.94$^{ab}$ | 0.041 | 72.25 | 71.9 | 0.601 | 0.473 |
| Hct (%) | 24.70 | 24.67 | 24.92 | 24.72 | 0.728 | 24.89 | 24.62 | 0.113 | 0.170 |
| Hb (g $dL^{-1}$) | 7.93 | 7.92 | 8.00 | 7.94 | 0.738 | 7.99 | 7.91 | 0.120 | 0.120 |
| MCHC (g $dL^{-1}$) | 32.06$^{b}$ | 32.07$^{b}$ | 32.11$^{ab}$ | 32.13$^{a}$ | 0.036 | 32.11 | 32.10 | 0.361 | 0.010 |
| $Ca^{2+}$ (meq $L^{-1}$) | 3.46 | 3.48 | 3.45 | 3.45 | 0.896 | 3.46 | 3.46 | 0.986 | 0.094 |
| $Na^{+}$ (meq $L^{-1}$) | 148.36$^{b}$ | 149.15$^{a}$ | 148.49$^{b}$ | 148.28$^{b}$ | 0.001 | 148.65 | 148.49 | 0.274 | 0.144 |
| $K^{+}$ (meq $L^{-1}$) | 4.66$^{b}$ | 4.67$^{b}$ | 4.91$^{a}$ | 4.82$^{ab}$ | 0.035 | 4.89 | 4.84 | 0.386 | 0.054 |
| $Cl^{-}$ (meq $L^{-1}$) | 104.92$^{ab}$ | 105.51$^{a}$ | 105.13$^{ab}$ | 104.81$^{b}$ | 0.024 | 105.2 | 105.0 | 0.444 | 0.172 |
| Angap (mmol $L^{-1}$) | 20.81 | 20.68 | 20.82 | 20.47 | 0.529 | 20.56 | 20.57 | 0.877 | 0.275 |
| GLU (mg $dL^{-1}$) | 233.99 | 233.97 | 231.49 | 230.30 | 0.064 | 232.93 | 232.0 | 0.384 | 0.796 |
| Osmo (mmol $kg^{-1}$) | 309.71$^{b}$ | 311.29$^{a}$ | 309.84$^{b}$ | 309.37$^{b}$ | 0.001 | 310.23 | 309.88 | 0.204 | 0.222 |
| CORT (pg $mL^{-1}$) | 1,719.25 | 1,849.18 | 2,026.93 | 1,938.4 | 0.182 | 1,926.0 | 1,839.9 | 0.403 | 72.650 |
| $T_3$ (ng $mL^{-1}$) | 3.04 | 3.08 | 3.00 | 3.00 | 0.633 | 3.01 | 3.06 | 0.338 | 0.053 |
| $T_4$ (µg $dL^{-1}$) | 2.07 | 2.08 | 2.02 | 2.07 | 0.811 | 2.08 | 2.04 | 0.369 | 0.048 |

$^{ab}$Means within a row and treatment that lack common superscripts differ significantly ($p \leq 0.05$), *Values are least squares of 8 replicate rooms with 60 birds per room, BW: Body weight, $pCO_2$: Partial pressure of $CO_2$, $pO_2$: Partial pressure of $O_2$, $HCO_3^-$: Bicarbonate, $SaO_2$: Saturated $O_2$, Hct: Haematocrit, Hb: Hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, GLU: Glucose, Osmo: Osmolality, Angap: Anion gap, CORT: Corticosterone, $T_3$: Triiodothyronine, $T_4$: Thyroxine, ICD: Incandescent light (Standard), CFL: Compact fluorescent light, Neutral-LED: Light emitting diode, Cool-PSF-LED: Poultry specific filtered LED, Light intensity: 1 = 5 lx, 2 = 20 lx

Table 2: Influence of light sources and light-intensity on blood $pO_2$ (mmHg) and $SaO_2$ (%) of broilers grown to heavy weights*

| | $pO_2$ (mmHg) | | | | $SaO_2$ (%) | | | |
|---|---|---|---|---|---|---|---|---|
| Items | 21 days | 28 days | 42 days | 56 days | 21 days | 28 days | 42 days | 56 days |
| **Light sources** | | | | | | | | |
| ICD | 41.48 | 42.20 | 40.80 | 42.93$^{ab}$ | 73.26 | 73.26 | 71.57 | 74.52 |
| CFL | 39.09 | 42.86 | 42.20 | 43.96$^{a}$ | 69.43 | 73.99 | 72.63 | 75.86 |
| Neutral-LED | 39.68 | 41.66 | 39.85 | 40.79$^{b}$ | 69.76 | 72.73 | 70.03 | 72.43 |
| Cool-PSF-LED | 38.94 | 41.47 | 40.44 | 40.69$^{b}$ | 69.34 | 72.55 | 69.69 | 72.20 |
| **Light intensity (lx)** | | | | | | | | |
| 5.0 lx | 40.11 | 42.07 | 40.75 | 41.84 | 70.68 | 73.16 | 71.01 | 74.17 |
| 20.0 lx | 39.49 | 42.02 | 40.90 | 42.34 | 70.22 | 73.11 | 70.95 | 73.34 |
| Pooled SEM | 1.120 | 1.115 | 1.083 | 0.812 | 1.250 | 1.411 | 1.552 | 0.773 |
| **Light sources-light intensity** | | | | | | | | |
| ICD+5.0 lx | 42.48 | 43.41 | 42.00 | 43.85$^{ab}$ | 74.73$^{a}$ | 74.18 | 73.64 | 76.55$^{ab}$ |
| ICD+20.0 lx | 40.48 | 41.35 | 39.60 | 42.00$^{abc}$ | 71.79$^{ab}$ | 72.35 | 69.50 | 72.49$^{bc}$ |
| CFL+5.0 lx | 37.83 | 42.27 | 42.56 | 44.77$^{a}$ | 66.35$^{c}$ | 72.58 | 73.12 | 77.50$^{a}$ |
| CFL+20.0 lx | 40.35 | 43.44 | 41.83 | 43.14$^{abc}$ | 72.50$^{ab}$ | 75.40 | 72.13 | 74.23$^{abc}$ |
| Neutral-LED+5.0 lx | 42.00 | 42.48 | 39.94 | 40.33$^{cd}$ | 73.02$^{ab}$ | 74.33 | 70.40 | 72.37$^{bc}$ |
| Neutral-LED+20 lx | 37.36 | 40.83 | 39.77 | 41.25$^{bcd}$ | 66.50$^{c}$ | 71.14 | 69.67 | 72.25$^{bc}$ |
| Cool-PSF-LED+5.0 lx | 38.12 | 40.48 | 38.50 | 38.40$^{d}$ | 68.62$^{bc}$ | 71.53 | 66.89 | 70.25$^{c}$ |
| Cool-PSF-LED+20.0 lx | 39.75 | 42.46 | 42.38 | 42.98$^{abc}$ | 70.02$^{abc}$ | 73.57 | 72.49 | 74.15$^{abc}$ |
| Pooled SEM | 0.661 | 0.606 | 1.122 | 0.601 | 1.767 | 1.996 | 2.195 | 2.234 |
| Source of variation | p-value | | | | | | | |
| Light sources | 0.359 | 0.817 | 0.471 | 0.010 | 0.084 | 0.89 | 0.508 | 0.058 |
| Light intensity | 0.579 | 0.866 | 0.893 | 0.536 | 0.711 | 0.925 | 0.866 | 0.451 |
| Light sources×light intensity | 0.093 | 0.539 | 0.216 | 0.020 | 0.003 | 0.363 | 0.165 | 0.045 |

$^{a-c}$Means within a column and effect that lack common superscripts differ significantly ($p \leq 0.05$), *Values are least squares of 8 replicate rooms with 60 birds per room, ICD: Incandescent light (standard), CFL: Compact fluorescent light, Neutral-LED: Light emitting diode, Cool-PSF-LED: Poultry specific filtered LED

In addition, light sources×light intensity interaction was noted on $pO_2$ only on day 56. Moreover, no main effect of light sources or light intensity was observed on $SaO_2$ on any of the sampling days. However, light sources×light intensity interaction was noted on $SaO_2$ only on day 21 and 56. Only birds reared under 20 lx light intensity had reduced

Table 3: Influence of light sources and light-intensity on blood $Na^+$ and $Cl^-$ of broilers grown to weights*

| | $Na^+$ (meq $L^{-1}$) | | | | $Cl^-$ (meq $L^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| Items | 21 days | 28 days | 42 days | 56 days | 21 days | 28 days | 42 days | 56 days |
| **Light sources** | | | | | | | | |
| ICD | 146.50 | 147.63$^{ab}$ | 148.29 | 151.01$^{b}$ | 101.95 | 103.76 | 105.92 | 108.06$^{b}$ |
| CFL | 147.13 | 148.09$^{a}$ | 149.27 | 152.13$^{a}$ | 102.31 | 104.15 | 106.22 | 109.75$^{a}$ |
| Neutral-LED | 146.71 | 147.57$^{b}$ | 148.42 | 151.28$^{b}$ | 102.44 | 104.10 | 106.08 | 107.90$^{b}$ |
| Cool-PSF-LED | 146.62 | 147.26$^{b}$ | 148.41 | 150.82$^{b}$ | 102.07 | 103.61 | 105.8 | 107.76$^{b}$ |
| **Light intensity (lx)** | | | | | | | | |
| 5.0 lx | 146.85 | 147..64 | 148.66 | 151.45 | 102.33 | 103.81 | 105.95 | 108.55 |
| 20.0 lx | 146.62 | 147.65 | 148.54 | 151.17 | 102.06 | 104.01 | 106.06 | 107.99 |
| Pooled SEM | 0.183 | 0.169 | 0.434 | 0.283 | 0.281 | 0.295 | 0.440 | 0.336 |
| **Light sources-light intensity** | | | | | | | | |
| ICD+5.0 lx | 146.69 | 147.81$^{abc}$ | 148.15$^{bcd}$ | 150.69 | 102.25 | 103.83 | 105.88 | 108.34 |
| ICD+20.0 lx | 146.31 | 147.46$^{bcd}$ | 148.44$^{bcd}$ | 151.33 | 101.65 | 103.69 | 105.96 | 107.70 |
| CFL+5.0 lx | 147.27 | 147.94$^{ab}$ | 147.98$^{bcd}$ | 152.56 | 102.23 | 103.77 | 106.08 | 109.85 |
| CFL+20.0 lx | 146.61 | 148.25$^{a}$ | 150.65$^{a}$ | 151.69 | 102.40 | 104.53 | 106.35 | 108.86 |
| Neutral-LED+5.0 lx | 146.83 | 147.25$^{cd}$ | 149.50$^{ab}$ | 151.67 | 102.73 | 104.08 | 105.38 | 107.96 |
| Neutral-LED+20 lx | 146.58 | 147.90$^{abc}$ | 147.33$^{d}$ | 150.89 | 102.14 | 104.13 | 105.79 | 107.83 |
| Cool-PSF-LED+5.0 lx | 146.63 | 147.54$^{bcd}$ | 149.08$^{abc}$ | 150.90 | 102.10 | 103.54 | 105.48 | 108.04 |
| Cool-PSF-LED+20.0 lx | 146.61 | 146.98$^{d}$ | 147.73$^{cd}$ | 150.75 | 102.04 | 103.69 | 106.12 | 107.48 |
| Pooled SEM | 0.258 | 0.239 | 0.614 | 0.401 | 0.400 | 0.417 | 0.622 | 0.475 |
| **Source of variation (p-value)** | | | | | | | | |
| Light sources | 0.089 | 0.008 | 0.358 | 0.008 | 0.604 | 0.508 | 0.913 | 0.004 |
| Light intensity | 0.202 | 0.851 | 0.785 | 0.313 | 0.339 | 0.503 | 0.814 | 0.101 |
| Light sources-light intensity | 0.915 | 0.043 | 0.001 | 0.212 | 0.708 | 0.730 | 0.795 | 0.838 |

$^{a-d}$Means within a column and effect that lack common superscripts differ significantly ($p \leq 0.05$), *Values are least squares of 8 replicate rooms with 60 birds per room, ICD: Incandescent light (Standard), CFL: Compact fluorescent light, LED: Light emitting diode, PSF-LED: Poultry specific filtered LED

Table 4: Influence of light sources and light-intensity on blood $Ca^{2+}$ and $Cl^-$ of broilers grown to heavy weights*

| | $Ca^{2+}$ (meq $L^{-1}$) | | | | Osmo (mmol $kg^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| Items | 21 days | 28 days | 42 days | 56 days | 21 days | 28 days | 42 days | 56 days |
| **Light sources** | | | | | | | | |
| ICD | 3.84 | 3.86 | 3.06 | 3.08$^{a}$ | 306.49 | 308.23$^{ab}$ | 309.42 | 314.69$^{b}$ |
| CFL | 3.85 | 3.91 | 3.07 | 3.07$^{a}$ | 307.50 | 309.27$^{a}$ | 311.50 | 316.91$^{a}$ |
| Neutral-LED | 3.85 | 3.85 | 3.07 | 3.05$^{ab}$ | 306.72 | 307.90$^{b}$ | 309.67 | 315.08$^{b}$ |
| Cool-PSF-LED | 3.85 | 3.84 | 3.06 | 3.04$^{b}$ | 306.50 | 307.22$^{b}$ | 309.64 | 314.10$^{b}$ |
| **Light intensity (lx)** | | | | | | | | |
| 5.0 lx | 3.85 | 3.87 | 3.07 | 3.05 | 307.09 | 308.24 | 310.21 | 315.38 |
| 20.0 lx | 3.84 | 3.87 | 3.07 | 3.07 | 306.51 | 308.07 | 309.91 | 315.02 |
| Pooled SEM | 0.185 | 0.269 | 0.020 | 0.012 | 0.350 | 0.376 | 0.873 | 0.553 |
| **Light sources-light intensity** | | | | | | | | |
| ICD+5.0 lx | 3.85 | 3.87 | 3.05 | 3.07$^{ab}$ | 306.93 | 308.70 | 309.15$^{bcd}$ | 314.07 |
| ICD+20.0 lx | 3.84 | 3.85 | 3.08 | 3.09$^{a}$ | 306.04 | 307.76 | 309.70$^{bcd}$ | 315.31 |
| CFL+5.0 lx | 3.85 | 3.97 | 3.45 | 3.05$^{ab}$ | 307.76 | 309.06 | 308.62$^{bcd}$ | 317.81 |
| CFL+20.0 lx | 3.86 | 3.92 | 3.10 | 3.10$^{a}$ | 307.24 | 309.48 | 314.37$^{a}$ | 316.02 |
| Neutral-LED+5.0 lx | 3.86 | 3.83 | 3.10 | 3.08$^{a}$ | 307.14 | 307.31 | 312.06$^{ab}$ | 315.62 |
| Neutral-LED+20 lx | 3.83 | 3.88 | 3.04 | 3.02$^{b}$ | 306.29 | 308.48 | 307.28$^{d}$ | 314.55 |
| Cool-PSF-LED+5.0 lx | 3.84 | 3.86 | 3.07 | 3.02$^{b}$ | 306.54 | 307.88 | 311.02$^{abc}$ | 314.00 |
| Cool-PSF-LED+20.0 lx | 3.85 | 3.83 | 3.05 | 3.05$^{ab}$ | 306.46 | 306.55 | 308.27$^{cd}$ | 314.21 |
| Pooled SEM | 0.370 | 0.380 | 0.029 | 0.018 | 0.495 | 0.532 | 1.234 | 0.782 |
| **Source of variation (p-value)** | | | | | | | | |
| Light sources | 0.782 | 0.787 | 0.874 | 0.035 | 0.143 | 0.002 | 0.304 | 0.003 |
| Light intensity | 0.724 | 0.876 | 0.733 | 0.320 | 0.100 | 0.656 | 0.727 | 0.521 |
| Light sources-light intensity | 0.789 | 0.778 | 0.249 | 0.024 | 0.833 | 0.072 | 0.001 | 0.224 |

$^{a-d}$Means within a column and effect that lack common superscripts differ significantly ($p \leq 0.05$), *Values are least squares of 8 replicate rooms with 60 birds per room, ICD: Incandescent light (Standard), CFL: Compact fluorescent light, LED: Light emitting diode, PSF-LED: Poultry specific filtered LED

blood pH on day 56 ($p<0.008$) in comparison with those reared under 5 lx light intensity and no main effect of light sources or light sources×light intensity interaction was observed on pH on any of the sampling days.

Table 3 shows the effects of light sources and light intensity on blood concentrations of $Na^+$ and $Cl^-$. Birds reared under CFL had increased blood concentration of $Na^+$ on day 28 ($p<0.008$) and 56 ($p<0.008$). Furthermore, birds in the CFL group had increased blood concentration of $Cl^-$ on day 56 ($p<0.004$). There was no main effect of light intensity on any of the other sampling days. There was only light sources×light intensity interaction on blood concentration of $Na^+$ on day 28 ($p<0.043$) and 42 ($p<0.001$).

Table 4 shows the influence of light sources and light intensity on blood concentrations of $Ca^{2+}$ and Osmo. In comparison with ICD, birds reared under Cool-PSF-LED had reduced blood concentrations of $Ca^{2+}$ on day 56 ($p<0.035$). Furthermore, there was an effect of light sources on blood concentration of Osmo on day 28 ($p<0.002$) and 56 ($p<0.003$). There was no main effect of light intensity on any of the other sampling days. There was only light sources×light intensity interaction on blood of $Ca^{2+}$ on day 56 ($p<0.024$) and Osmo on day 42 ($p<0.001$).

In addition, no effects of light sources, light intensity or strain×light intensity were observed on $pCO_2$, Hct, Hb, $K^+$, anion gap and glucose concentrations on any of the sampling days. Furthermore, no effect of treatments on plasma concentrations of $T_3$, $T_4$ and corticosterone were observed on any of the sampling days.

## DISCUSSION

Most of the previous studies on the effects of lighting program on blood physiological variables of broilers grown to heavy weights (>3 kg) used an ICD light source[31-33]. These previous studies demonstrated partial effects of light intensity on blood physiological parameters[34] and in combination with ammonia[35], temperature[31], photoperiod[32] and genetics[33]. In addition, we reported the effects of color temperature (Kelvin) of LED light bulbs on blood physiological variables of broilers grown to heavy weights[26]. We recently reported the effects of light sources and intensity on growth performance, carcass characteristics and welfare indices of broilers grown to heavy weights[27]. The present study investigated the effects of light sources and intensity on blood physiological and biochemical variables of broilers grown to heavy weights from the same individual birds included in our previous manuscript[27]. The results indicated that in comparison with ICD light, CFL had higher $Na^+$ and Osmo, neutral-LED sources had lower pH, $pO_2$, $SaO_2$ and higher $pCO_2$ and $K^+$ while cool-PSF-LED light sources had higher MCHC and BW, but these values were within normal ranges.

The acid-base status of poultry is challenged daily by environmental factors such as light, temperature, humidity and air quality, as well as by other factors including nutrition that influence respiratory and metabolic activities. The principal organ systems used in acid-base homeostasis in birds are the lungs and kidneys, supported by the gastrointestinal tract[36]. The cardiovascular system also participates in thermoregulatory processes through modulation of heat dissipation on the one hand and by oxygen transport on the other. The pH of the blood is maintained within a very narrow range because sudden changes can result in cellular damage via protein ionization[37]. However, any speculation regarding the role of blood pH in the regulation of respiration must be tempered by a consideration of other factors that are also influential in the chemical control of respiration[38]. Moreover, the carbonic acid-bicarbonate system is the most important buffer for maintaining blood acid-base balance.

Higher blood Osmo observed in birds reared under CFL in comparison with ICD light may be associated with the higher blood $Na^+$ concentration observed. This high osmolality that is associated with high $Na^+$ is characterized by a water shift from Intra Cellular Fluid (ICF) to Extra Cellular Fluid (ECF) as reported by Freda *et al.*[39]. High blood Osmo can also be caused by several other conditions, including dehydration and high sodium (hypernatremia). Blood sodium concentration is maintained by a homeostatic mechanism that involves thirst, vasopressin secretion, the renin-angiotensin-aldosterone feedback system and the renal handling of sodium. Sodium is the main determinant of plasma osmolality and water moves toward body compartments with higher osmolality[39]. The pH of blood affects the distribution of ions throughout the body and changes in pH may be associated with changes in ion concentration, which may have effects on body systems. The presence of ions affects the amount of $H^+$ and $HCO_3^-$ produced, as electrochemical neutrality must be maintained in the body. Short-term control of the blood pH is exerted via the respiratory system. Increased ventilation will drop the $pCO_2$ and thus increase the pH within minutes, while decreased ventilation will have the opposite effect. The respiratory center is driven by the pH of the cerebrospinal fluid (CSF). Any drop in CSF pH will result in an increase in respiratory drive with increased ventilation and loss of $CO_2$. Conversely, an increase in CSF pH will tend to decrease the respiratory drive.

Birds reared under neutral-LED in comparison with those reared under ICD light had higher $pCO_2$, $K^+$ and lower pH, $pO_2$ and $SaO_2$. Respiratory acidosis is characterized by a low pH with a high $pCO_2$, due to retention of $CO_2$ as observed in the present study. These changes may be due to acute respiratory acidosis. In acute respiratory acidosis, the $HCO_3^-$ will be normal, but in prolonged respiratory acidosis, there is a tendency for the $HCO_3^-$ to gradually increase from renal compensation. It is important to note that the body will never overcompensate, so if the pH is normal or elevated in the face of an elevated $pCO_2$, it means that there is concomitant metabolic alkalosis. However, all observed acid-base changes in this present study are still within the normal acid-base homeostasis and physiological ranges for this species.

The reduced $pO_2$ and $SaO_2$ observed in broilers reared under neutral-LED in comparison those reared under ICD may be due to inadequate blood oxygenation and hypoxemia, which may increase the risk of hypoxia[40]. These changes include reductions in the systemic venous $pO_2$, $SaO_2$ and increased[24,41] $pCO_2$ which may lead to acute respiratory acidosis due to hydrogen ion ($H^+$, acid) accumulation. Heavy weight modern broilers are able to consume large quantities of feed and grow rapidly due to genetic selection, resulting in high oxygen demand. When oxygen intake is low (low $pO_2$, $SaO_2$) relative to BW, the heart essentially pushes the blood through the lungs with more pressure to increase the amount of oxygen available for the bird's metabolism. However, because the lung volume and cardiovascular volume within the lung tissue are fixed in birds, unlike in mammals, eventually a point is reached whereby the lungs may no longer accommodate more blood being supplied by the heart and this may result in negative effects on the body (poor oxygenation).

The monovalent ions ($Na^+$, $K^+$ and $Cl^-$) are the key minerals involved in the acid-base balance of the body fluids[42] because they have a higher permeability and greater absorption than divalent ions[43] such as $Ca^{2+}$. Body fluid electrolyte concentrations, such as $Na^+$, $K^+$ and $Cl^-$ and acid–base balance are interconnected and are associated with the condition producing acidosis or alkalosis in mammals, which may also be true in birds[44]. Concentrations of certain plasma hormones, enzymes and metabolites such as glucose and corticosterone among others have been suggested to be sensitive indicators of stress levels in broiler chickens[45,46]. No significant effect of treatments on blood glucose, thyroid hormones, along with that of plasma corticosterone concentrations in the present study, suggesting that these examined light sources did not present stressors to broilers grown to heavy weights.

## CONCLUSION

It was concluded that the light sources evaluated in this study might be suitable for replacement of ICD light source in commercial poultry facilities at the light intensities used in this study to reduce energy cost and optimize production efficiency without compromising welfare of broilers grown to heavy weights.

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