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Research Article
Interactive Effects of Ambient Temperature and Light Sources at High Relative Humidity on Growth Performance and Blood Physiological Variables in Broilers Grown to 42 Day of Age

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Abstract

Objective: The objective of the study is to evaluate the interactive effects of ambient temperature and light sources at high relative humidity on growth performance and blood physiological reactions in broilers grown to 42 days of age. **Methodology:** The experiment consisted of two levels (Moderate = 21.1°C and high = 26.7°C) of temperatures and two light sources (ICD and PSF-LED) at high relative humidity (80%). A total of 532 1 day-old Ross 708 chicks were randomly distributed into 8 environmentally-controlled rooms (22 males and 22 females chicks per room). Feed and water were provided *ad libitum*. Light treatment started from 1 day, while temperature and RH were applied continuously from 22 days through 42 days. Both feed intake and bird's weight were recorded on 1 and 21day (before initiation of the treatments), 28 and 42 day for the growth performance. Also, wing brachial vein blood (3 mL) samples were collected on weigh days from six (3 males/3 females) randomly selected chicks from each room. **Results:** Treatments with only high ambient temperature significantly ($p<0.05$) reduced Body Weight (BW), Body Weight Gain (BWG) and Feed Intake (FI), but no treatments effect were observed for Feed Conversion Ratio (FCR) and mortality. Also, there was no effect of light sources and high humidity on all examined production variables. In addition, only treatments with high ambient temperature significantly ($p<0.05$) increased Hb, Hct, McHc and Osmo along with significantly ($p<0.05$) reduced Na^+. However, light sources and high humidity had no effect on all examined blood physiological variables. All acid-base changes during these combined treatments were still within the normal acid-base homeostasis and physiological ranges. Plasma corticosterone concentrations were not affected by treatments. **Conclusion:** It was concluded that the LED sources evaluated in this study may be suitable for replacement of ICD light source in commercial poultry facilities along with moderate temperature with or without high relative humidity to reduce energy cost and optimize production efficiency without compromising the welfare of broilers.

Key words: Light sources, temperature, humidity, acid-base balance, broilers

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Study in poultry production has been displayed through the genetic selection of breeds for high productivity. However, this genetic potential will not be fully realized until microenvironmental constraints (temperature, humidity, light intensity and air velocity etc.) have been fully researched and optimized. Exposure of poultry to inadequate environmental conditions during the course of poultry production has an adverse impact on production efficiency (BW, BWG and FCR), meat yield, immune response, mortality and welfare[1,2]. The body temperature of an adult chicken is 40.6-41.7°C and the thermoneutral zone which allows chickens to maintain their body temperature is 18-24°C. It has been reported that the currently recommended thermoneutral zone (18-24°C) that allows chickens to maintain their body temperature to avoid heat stress will not be able to dissipate enough heat to maximize performance in fast-growing broilers and will therefore result in chickens being subjected to poor performance and physiological heat stress[3-5]. For instance, when ambient temperature is high, chickens have higher energy (feed) needs than when in thermoneutral environments. Major losses result from a less efficient conversion of feed to meat which can detrimentally impact poultry health and productivity. It is estimated that 1% improvement in feed conversion would save U.S. poultry producers more than $50 million per year.

Light is one of the major microclimate factors that influences bird activity, behavior, physiology, immune response and growth rate and has been used to alleviate mortality issues related to metabolic disease. Based on the Energy Independence and Security Act[6], 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 light bulbs are high efficiency, long operating life, moisture resistance and availability in differing peak wavelengths[7]. However, choosing the correct one can be difficult since some do not dim very well.

Blood analyses along with other biochemical evaluations are routinely used to determine various influences of environmental, nutritional and pathological factors[2,8]. Changes in acid-base balance may signal early symptoms of disease and influence the early manifestation of clinical signs and therapeutic effectiveness in both domestic animals and human beings[9,10]. 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[11].

Poultry production requires a sustainable housing environment condition that provides adequate lighting, temperature, ventilation and relative humidity among others. Several researchers have studied the effects of microenvironment factors including temperature[4,5], temperature-humidity-index[12], lighting program[13] and air velocity[14] on broiler growth performances and physiological responses. Evaluation of the interactive effects of temperature, light source and relative humidity are critical in determining the bird's ability to dissipate heat and avoid losses caused by heat stress under light source. Therefore, the objective of the present study was to evaluate the interactive effects of ambient temperature and light sources in the presence of high relative humidity on growth performance and blood physiological variables in broilers grown to 42 days of age while not compromising their welfare.

MATERIALS AND METHODS

Bird husbandry: All procedures relating to the use of live birds in this study were approved by the USDA-ARS Institutional Animal Care and Use Committee at the Mississippi State location. A total of 352 (176 males and 176 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 8 environmentally-controlled rooms (22 male and 22 female chicks per room). Each environmentally-controlled room was 1.5×3.0 m and a floor area of 4.5 m^2 with a room volume of 11.3 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 (1-42 days of age). All birds were fed the same diet throughout the study. Birds were provided a three phases feeding program (starter: 1-14 days, grower: 15-28 days and finisher: 29-42 days of age). Diets were formulated to meet or exceed[15] 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 RH on 1 day were maintained at

Table 1: Treatment allocation

	Treatment combination		
Treatments	Temperature (°C)	Light source	RH (%)
A	21.1	ICD	80
B	21.1	PSF-LED	80
C	26.7	ICD	80
D	26.7	PSF-LED	80

ICD: Incandescent bulb (Standard, 2,010 k), PSF-LED: Cool poultry specific filtered LED bulb, A: 21.1°C, ICD, 80% RH, B: 21.1°C, PSF-LED, 80% RH, C: 26.7°C, ICD, 80% RH and D: 26.7°C, PSF-LED, 80% RH

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.

Experimental treatments: A 2×2 factorial treatments structure was used to evaluate two light sources [incandescent (ICD, 2100k; Standard) and cool poultry specific filtered LED (Cool-PSF-LED, 5000k)] from 1-42 days of age and two levels (Moderate = 21.1°C and high = 26.7°C) of ambient temperatures at high relative humidity (80%) were commenced from 22-42 days of age (Table 1). Each of the two light source treatments was paired with one of the two temperature treatments so that each room represented a particular light source: Temperature level combination for a total of 8 rooms of two replications. Photoperiod consisted of continuous (24 L:0 D) lighting at 20 lx from placement to 7 day with 20 L:4 D at 10 lx from 8-21 days and temperatures and high relative humidity treatments from 22 through 42 days. The 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[16]. The light spectra of the light sources and ICD bulbs utilized in this study have been shown in a previous report[13]. Light intensity settings were verified from the center and 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

Growth performance: Birds and feed were weighed on 1 and 21 day (before initiation of treatments), 28 and 42 day of age for the computation of Body Weight (BW) and Feed Intake (FI). Cumulative Feed Intake (FI) was calculated by subtracting the remaining feed weights in the feeders from the initial feed-added weights in the feeders. Also, cumulative Body Weight (BW) was recorded from each room at biweekly intervals. The incidence of mortality was recorded daily. Necropsies and cause of death (when determined) were performed on all birds that died during the trials. Cumulative Body Weight Gain (BWG) was calculated by subtracting initial (1 day) Body Weight (BW) from the current BW of the birds. Feed Conversion Ratio (FCR) was calculated by dividing FI with BWG and it was corrected for mortality.

Blood collections and chemical analyses: On 21 day (before initiation of treatments), 28 and 42 day blood samples were collected between 800 and 900 h on sampling day from wing brachial vein of 6 (3 male and 3 female chicks per 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[17]. 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-80Flex-CO-OX, 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]. Also, arterial oxygen saturation (SaO_2) which is the amount of oxyhemoglobin (O_2 Hb) in blood expressed as a percent of the total amount of hemoglobin able to bind oxygen (O_2 Hb)+deoxyhemoglobin was calculated using the standard formula [$SaO_2 = 100 \times O_2$ Hb/(O_2 Hb+ deoxyhemoglobin)]. 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-Tek Instruments Inc., Winooski, VT) with ELISA reagent assay test kits (EIA-CS Kit, Enzo Life Sciences, Farmingdale, NY), according to the manufacturer's instructions.

Statistical analysis: The experimental design was a randomized complete block design. Treatment structure was a 2×2 factorial arrangement with the main factors being two light sources (ICD and Cool-PSF-LED) and two levels (Moderate = 21.1°C and high = 26.7°C) of ambient temperatures at high relative humidity (80%) with two replicates. Individual sample data within each of the replicate units were averaged before analysis. Analysis were conducted using the PROC MIXED procedure of SAS software[18]. Means comparisons on 21, 28 and 42 days were assessed by least significant differences and statements of significance were based on $p<0.05$ unless otherwise stated. Analysis 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 2 shows that the interactive effect of ambient temperature exposure and light sources in the presence of high Relative Humidity (RH) on live production performance at 42 days of age. Birds reared under treatments A and B with moderate temperature had a significantly ($p<0.05$) higher, BW, BWG and FI when compared with those birds reared under treatments C and D with high ambient temperature. Furthermore, birds reared under treatments A and B with moderate temperature had a significantly ($p<0.05$) lower FCR when compared with those birds reared under treatments C and D with high ambient temperature. Mortality was not significantly different between treatments but rather variable and did not appear to be either temperature, light sources, RH or their interaction dependent.

Table 3 shows that the birds reared under treatments A and B with moderate temperature had a significantly ($p<0.05$) higher pCO_2, HCO_3^-, Hb, Hct, McHc, Na^+ and K^+ along with significantly ($p<0.05$) lower Glu and Osmo in comparison with birds reared under treatments C and D with high ambient temperature. There were no effects of treatments on pH, pO_2, SO_2, SaO_2, Ca^{2+}, Cl^-, Angap and CORT but these values were within normal ranges.

Table 2: Interactive effects of treatments on growth performance at 42 day of age

Treatments	BW	BWG	FI	FCR	Mortality
	----------(kg)----------				------(%)-----
A	2.71[a]	2.67[a]	4.33[a]	1.62[b]	1.14
B	2.71[a]	2.67[a]	4.31[a]	1.61[b]	1.14
C	2.55[b]	2.51[b]	4.24[b]	1.69[a]	0.00
D	2.58[b]	2.53[b]	4.22[b]	1.67[a]	2.41
SEM	0.027	0.026	0.038	0.011	1.841
p-value	0.002	0.002	0.001	0.418	0.963

Means within a column and treatment that lack common superscripts differ significantly $p<0.05$, A: 21.1°C, ICD, 80% RH, B: 21.1°C, PSF-LED, 80% RH, C: 26.7°C, ICD, 80% RH, D: 26.7°C, PSF-LED, 80% RH and pooled SEM for treatments effects (n = 2)

Table 3: Interactive effect of treatments on selected blood physiological variables of broilers grown at 42 day of age

Variables	Treatments				SEM	p-value
	A	B	C	D		
pH	7.33	7.33	7.33	7.32	0.007	0.806
pCO_2 (mmHg)	58.53[a]	58.56[a]	57.06[b]	57.25[b]	0.930	0.613
pO_2 (mmHg)	54.58	51.81	51.86	49.67	1.269	0.070
HCO_3^- (mmHg)	27.43[a]	28.11[a]	27.46[b]	27.23[b]	0.310	0.223
SO_2 (%)	34.25	31.92	31.72	29.16	1.554	0.163
SaO_2 (%)	79.44	77.20	78.33	76.77	0.836	0.119
Hb (g dL^{-1})	8.51[a]	8.58[a]	8.07[b]	8.07[b]	0.121	0.033
Hct (%)	26.43[a]	25.44[a]	25.13[b]	25.11[b]	0.364	0.034
McHc (g dL^{-1})	32.19[a]	32.44[a]	32.13[b]	32.14[b]	0.017	0.045
Ca^{2+} (meq L^{-1})	2.98	2.98	2.95	2.94	0.017	0.216
Na^+ (meq L^{-1})	148.72[a]	148.75[a]	147.36[b]	147.55[b]	0.396	0.023
K^+ (meq L^{-1})	5.14[a]	5.10[a]	4.88[b]	4.82[b]	0.112	0.210
Cl^- (meq L^{-1})	108.08	108.06	107.31	106.92	0.477	0.239
Angap (mmol L^{-1})	18.35	17.59	17.48	18.23	0.337	0.180
GLU (mg dL^{-1})	255.14[b]	244.64[b]	247.44[a]	250.75[a]	4.870	0.468
OSmo (mmol kg^{-1})	308.46[b]	308.50[b]	311.61[a]	311.08[a]	0.909	0.034
CORT (pg mL^{-1})	2170.60	2745.70	2235.00	1923.20	661.300	0.656

Means within a row and treatment that lack common superscripts differ significantly $p<0.05$, A: 21.1°C, ICD, 80% RH, B: 21.1°C, PSF-LED, 80% RH, C: 26.7°C, ICD, 80% RH, D: 26.7°C, PSF-LED, 80% RH and pooled SEM for treatments effects (n = 2)

Table 4 shows that the interactive effect of treatments by sex on selected blood physiological variables of broilers grown to 42 days of age. Male broiler chickens had significantly ($p<0.05$) higher pCO_2, HCO_3^- and GLU in comparison with female birds but these values were within normal ranges. There were no effects of treatments on the rest examined variables.

DISCUSSION

When considering bird's microenvironments, temperature, relative humidity and light are among the major factors. These factors along with others (air velocity and stocking density) affect the bird's metabolism which in turn is

Table 4: Interactive effects of treatments by sex on selected blood physiological variables of broilers grown to 42 day of age

	Sex			
Variables	Male	Female	SEM	p-value
pH	7.32	7.33	0.005	0.203
pCO_2 (mmHg)	59.11[a]	56.11[b]	0.779	0.012
HCO_3^- (mmHg)	27.95[a]	27.16[b]	0.183	0.006
pO_2 (mmHg)	51.53	25.43	0.982	0.523
SO_2 (%)	30.84	32.69	1.101	0.246
SaO_2 (%)	77.40	78.47	0.613	0.233
Hct (%)	25.38	25.53	0.195	0.581
Hb (g dL^{-1})	8.16	8.21	0.065	0.577
Na^+ (meq L^{-1})	148.06	148.14	0.266	0.828
K^+ (meq L^{-1})	5.03	4.89	0.590	0.111
Ca^{2+} (meq L^{-1})	2.98	2.95	0.010	0.066
Cl^- (meq L^{-1})	107.35	107.83	0.218	0.128
McHc (g dL^{-1})	32.14	32.16	0.009	0.424
GLU (mg dL^{-1})	255.14[a]	243.85[b]	2.386	0.003
OSmo (mmol kg^{-1})	310.27	309.65	0.559	0.437
Angap (mmol L^{-1})	17.79	18.04	0.230	0.453
CORT (pg L^{-1})	1980.50	2015.40	72.635	0.357

Means within a row and treatment that lack common superscripts differ significantly $p<0.05$, A: 21.1°C, ICD, 80% RH, B: 21.1°C, PSF-LED, 80% RH, C: 26.7°C, ICD, 65% RH, D: 26.7°C, PSF-LED, 65% RH and pooled SEM for treatments effects (n = 2)

responsible for maximizing growth performance and body heat to maintain normal physiological processes and functions. The current study clearly demonstrates that among the three microenvironment factors examined, high ambient temperatures markedly affect the production performance of the bird as shown by reduced BW, BWG, FI and increased FCR of chickens during the growth period that had a significant negative impact on the efficiency of production. It has been shown that the chicken is most comfortable, more productive and stress is minimized when the ambient temperature is in the thermoneutral zone[19]. Further, the present study supports the general concept that over the growing period, the ambient temperature range of 15.6 and 21.1°C is more suitable for modern heavy broilers as indicted in previous studies[5,20]. The results of the current study indicated that with or without differing light sources, high RH and high ambient temperature had a significant impact on the metabolism of modern heavy broiler chickens. The intake and metabolism of feed have a thermogenic effect. In the current study, the depression in the growth rate and body weight gain at high ambient temperatures (26.7°C) might be due to many factors which include decreasing feed consumption[21], inefficient digestion[22], impaired metabolism[23] and temperature *per se*[24]. This is supported by the results of Leeson *et al.*[25] wherein the optimum environmental temperature range in which broilers are able to perform to their maximum genetic potential is between 12.7 and 26.7°C from 4-9 weeks of age.

The principal organ systems used in acid-base homeostasis in birds are the lungs and kidneys and these are supported by the gastrointestinal tract[26]. In addition, the cardiovascular system also participates in thermoregulatory processes through modulation of heat dissipation on the one hand and by oxygen transport on the other. In the present study, exposure of broilers to treatments C and D that contained high ambient temperature significantly affected acid-base balance with and without RH and light source. These two treatments C and D in comparison with treatments A and B that contained moderate temperature, significantly decreased pCO_2, HCO_3^-, Hb, Hct, McHc, Na^+ and K^+ along with significantly ($p<0.05$) higher Glu and Osmo.

The results indicated an increased respiratory rate in broilers exposed to the treatments C and D with higher ambient temperature with or without differing light sources and high RH in an effort to dissipate heat by evaporation. This would suggest that thermoregulatory efforts (panting and CO_2 elimination) were much greater in the birds reared under treatments C and D. Evaporative heat loss through panting is the most important mechanism used to control body temperature under heat stress. The increased respiratory rate disrupted their acid-base balance due to excessive carbon dioxide (CO_2) losses[27]. Decreases in circulating partial CO_2 pressures (pCO_2) cause a decrease in the concentration of carbonic acid (H_2CO_3) and hydrogen (H^+). In response to this, the kidneys increase HCO_3^- excretion and reduce H^+ excretion in an attempt to homeostatically control the bird's acid-base balance. This change in acid-base balance is known as respiratory alkalosis[28].

Increased electrolyte excretions through urine and faces along with continuous loss of water through panting have been shown to further disrupt acid-base balance[29]. The degree of water loss in intracellular fluids has been associated with losses of intracellular K^+. This loss of extracellular fluid has been linked to a loss in plasma Na^+. Plasma Na^+, K^+ and Cl^- levels were affected by heat stress in this study. It has been shown that plasma K^+ and Na^+ concentrations decrease as temperature rises[30,31]. This might contribute to blood acidification which in turn, seems to be an appropriate response to alkalosis. The plasma K^+ and Na^+ concentration results in the current study are consistent with earlier reports[4].

It has been reported that haematocrit values can decrease with increasing rearing temperature[32,33]. Hematological examinations in the present study have also shown that total amount of hemoglobin in blood can decrease with increased rearing temperature, which can result in lower metabolic rate as shown by others[5,34]. Concentrations of certain plasma hormones, enzymes and metabolites such as corticosterone

(CS) have been suggested to be sensitive indicators of stress levels in broiler chickens[35,36]. Non-significant increases in plasma CS as observed in this present study indicate that birds were not stressed. Another response from this study was the increase in whole blood glucose concentration which was suggest of a stimulation of gluconeogenetic processes in direct response to increased epinephrine, norepinephrine and glucocorticoid secretion[31,37]. Hyperthermia has been reported to induce hyperglycemia, whereas hypothermia can cause hypoglycemia in domestic fowls[38].

Studies have been conducted to examine the effect of temperature-humidity index for modern broilers on the homeostasis and thermal levels within birds[12,39]. However, the interactive effects of temperature, light source and relative humidity are critical in determining the modern broiler's ability to dissipate heat and avoid losses caused by heat stress.

CONCLUSION

The results of this study supplement current knowledge of the hematology and biochemistry of plasma in modern heavy weight broiler chickens during the growth period and provide warning that even relatively small changes in the ambient temperature from the thermoneural zone 18-24°C can have a negative impact on metabolism and performance. In addition, the light source and moderate ambient temperatures at high relative humidity as used in this study apparently did not act together or separately to affect plasma corticosterone concentrations, suggesting that these factors may not pose as stressors to modern heavy broiler chickens.

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