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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Effect of Varying Light Intensity on Blood Physiological Reactions of Broiler Chickens Grown to Heavy Weights

H.A. Olanrewaju, J.L. Purswell, S.D. Collier and S.L. Branton
*USDA, Agricultural Research Service, Poultry Research Unit,
P.O. Box 5367, Mississippi State, MS 39762-5367, USA

Abstract: This study investigated effects of varying levels of light intensities (25, 10, 5, 2.5 and 0.2 lx) from 22 to 56 d of age at 50% RH on blood acid-base balance, metabolites and electrolytes of heavy broilers reared under environmentally controlled conditions. Four identical trials were conducted with two replications per trial. In each trial, 600 1-d-old Ross 308 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 from d 22 to 56 d of age. Birds were provided a four phase-feeding program (starter: 1 to 14 d, grower: 15 to 28 d, finisher: 29 to 42 d and withdrawal: 43 to 56 d). Feed and water were provided *ad libitum*. Venous blood samples were collected on d 21 (base line), 28, 42 and 56. The lowest light intensity of 0.2 lx significantly ($p \leq 0.05$) increased pH, Na^+, K^+, Cl^- and reduced pCO_2, Hb and Hct. However, all these acid-base changes are still within the normal acid-base homeostasis physiological ranges. In addition, exposure of modern heavy broilers to varying light intensity produced no significant effect on pO_2, sO_2, Ca^{2+}, mOsm, McHc, Angap, T_3, T_4 and CS. Acid-base regulation during light intensity exposure did not deteriorate despite a lower pCO_2 which consequently increased blood pH that resulted in a compensatory mechanism for mild alkalosis. 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 the induction of physiological stress effects on broiler welfare.

Key words: Acid-base balance, blood gases, broiler, light-intensity, well-being

INTRODUCTION

In order to develop a profitability program useful to most broiler growers, microenvironmental factors (air, temperature, humidity, light) recommendations are important to optimize profitability and welfare of broiler production. However, information is sparse on these factors and establishing proper welfare practices are central to international trade negotiations of meat products. High light intensity will increase activity, while low light intensity reduces hyperactivity and minimizes skin scratches (Classen and Riddell, 1989; Buyse *et al*., 1996; Manser, 1996). Our previous review article reported that light intensity influences bird activity, behavior, physiology, immune response, growth rate and has been used to alleviate mortality issues related to metabolic disease (Olanrewaju *et al*., 2006b).

Changes in acid-base balance may occur during the early phase of many diseases and they have an influence on the early manifestation of clinical signs and the effectiveness of therapeutics in both domestic animals and human being (Brobst, 1983; Gunnerson, 2005). The limited research on acid-base balance, specifically as it relates to animal welfare, made it necessary to conduct research of this kind. An evaluation of blood pH, electrolytes, blood gases and metabolites could elucidate acid-base disturbances and differentiate between metabolic and respiratory disorders in broilers exposed to various microenvironmental factors. In addition, circulating concentration of triiodothyronine (T_3) has been found to be linearly related to weight gain and feed intake in chickens (Yahav *et al*., 1995; 1998). However, the association between light intensity and plasma triiodothyronine (T_3) and thyroxine (T_4) are not well defined physiologically in broiler chickens grown to heavy weights (>2.5 kg). Determination of these factors are essential so that therapeutic or nutritional strategies can be applied to reduce negative effects, if any and thereby optimize the environment in broiler houses to maximize the genetic potential of birds while reducing production costs. Most studies have not evaluated gradient levels of light intensity at ranges typically used in commercial practice on blood physiology with modern early- and late-developing broiler production systems. Previous studies demonstrated partial effects of light intensity on blood physiological parameters in the presence of ammonia (Olanrewaju *et al*., 2008) and temperature (Olanrewaju *et al*., 2010). We do not know if these two microenvironmental constraints masked the effect of light intensity or whether the range of light intensity utilized was too narrow. To address this

Corresponding Author: H.A. Olanrewaju, USDA, Agricultural Research Service, Poultry Research Unit, P.O. Box 5367, Mississippi State, MS 39762-5367, USA

knowledge gap, the objective of this current study was to evaluate the specific effects of varying light intensity on various key physiological parameters in broilers grown to heavy weights under environmentally controlled conditions.

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. 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 7-8 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 constant across all treatments. Chamber temperature was 32°C at the initiation of experimentation and reduced by 2°C per week until it reached 15.6°C on d 49 d of age with 50% RH.

Experimental treatments: Light intensity treatments commenced on d 22. Lighting in each chamber was set to a light intensity in the range that is typically of those used in commercial production (25, 10, 5, 2.5 and 0.2 lx). Each chamber was equipped with incandescent bulbs, which peak 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 when necessary weekly. 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.

Blood collections and chemical analyses: On d 21 (d before initiation of the treatments), 28, 42 and 56, blood samples were collected anaerobically between 0800 and 0900 h on sampling d from a brachial vein of 6 (3 male and 3 female chicks/chamber) randomly selected birds from each chamber and the birds were then returned to the appropriate chambers by using our standard handling procedure (Olanrewaju *et al*., 2008). These sampled birds were identified so as not to be sampled again. In addition, unnecessary discomfort to the birds was avoided by using proper housing and handling techniques, as described by the NRC (1996). Blood samples were collected directly into heparinized (50 IU/mL) monovette syringes within 45 s after birds were caught. The blood samples were immediately drawn directly from the syringes into a blood gas electrolyte analyzer (ABL-80 Flex, Radiometer America, Westlake, OH) for immediate analysis of partial pressure of CO_2 (pCO_2), partial pressure of O_2 (pO_2), oxygen saturation (sO_2), pH, hematocrit (Hct), hemoglobin (Hb) and electrolytes (Na^+, K^+, Ca^{2+}, HCO_3^- and Cl^-). In addition, glucose (Glu), osmolality (mOsm) and anion gap (Angap) were analyzed simultaneously. The pH, pCO_2, pO_2 and HCO_3^- values were corrected to reflect a body temperature of 41.5°C (Burnett and Noonan, 1974). The mean corpuscular hemoglobin concentration (McHc) was calculated using the standard formula [(Hb*100)/Hct]. 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, centrifuged at 4,000 x g for 20 min at 4°C. Two mL of each of the plasma samples from the syringes were stored in 2.0 mL graduated tubes at -20°C for later chemical analyses.

Plasma samples were removed from the freezer, thawed and analyzed for Corticosterone (CS) using a universal microplate spectrophotometer (Bio-Tek Instruments Inc., Winooski, VT) with ELISA reagent assay test kits from Assay Designs (EIA-CS Kit, Assay Designs Inc., Ann Arbor, MI), according to the manufacturer's instructions. We have previously used this kit methodology per manufacturer's instructions in broilers (Olanrewaju *et al*., 2008; 2010).

Levels of plasma T_3 and T_4 concentrations were measured using a universal microplate spectro-photometer (Bio-Tek Instruments Inc., Winooski, VT) with ELISA reagent assay test kits from Assay Designs (EIA-CS Kit, Assay Designs Inc., Ann Arbor, MI) according to

the manufacturer's instructions. Standards, samples and controls were added to the appropriate wells of a microtiter plate precoated with anti-T_3 or anti-T_4 antibodies. The microtiter plate was then incubated for 60 min with T_3 conjugated with horseradish peroxidase or T_4 conjugated with horseradish peroxidase. Following incubation, the plate was washed 5 times and then incubated with the horseradish peroxidase substrate 3,3',5,5'-tetramethyl benzidine for 20 min. Absorbance was measured at 450 nm following the addition of stop solution to each well. The concentrations of T_3 and T_4 were calculated using standard curves. This methodology of the kit as it relates to the manufacturer's instructions has been used previously (Dozier *et al*., 2011).

Statistical analysis: A randomized complete block design was used in this study with two replications per trial. Analyses were conducted using ANOVA followed by least significant difference test comparing treatment means using the MIXED procedure of SAS software (SAS Institute, 2008). 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. Chambers used were switched within trials to remove chamber effects so that treatments are not confounded. Means comparisons were assessed by least significant differences and the level of significance was fixed at $p \leq 0.05$ unless otherwise stated.

RESULTS

The influence of varying light intensity on plasma pH and HCO_3^- is presented in Table 1. In comparison with other treatments, only the lowest light intensity (0.2 lx) significantly ($p \leq 0.005$) increased pH at 56 d of age. Furthermore, females had significantly ($p \leq 0.001$) higher pH in comparison with males on 56 d of age. There was no effect of light intensity on HCO_3^- on any of the sampling days. As shown in Table 2, there was only an effect of varying light intensity on pCO_2 on d 56. Lowest light intensity significantly ($p \leq 0.001$) decreased pCO_2 on d 56 and females concurrently had significantly ($p \leq 0.053$) lower pCO_2. No effect of light intensity on pO_2 was found on any of the sampling days, but the highest value was under the lowest light intensity of 0.2 lx, which has the lowest value of pCO_2. Table 3 shows the effect of varying light intensity on blood Hb and Hct. Light intensity of 0.2 lx significantly decreased Hb ($p \leq 0.000$) and Hct ($p \leq 0.000$) on d 28 in comparison with 2.5 and 25 lx, respectively. In addition, males had significantly higher Hb ($p \leq 0.048$) and Hct ($p \leq 0.044$) concentrations in comparison with females on d 42. Table 4 shows the effects of light intensity on plasma Na^+ and Cl^-. Lowest level of light intensity significantly increased blood Na^+ ($p \leq 0.003$) and Cl^- ($p \leq 0.003$) levels on d 42, respectively. No sex effect of light intensity on blood Na^+ and Cl^- was found on any of the sampling days. Table 5 shows the influence of varying light intensity on blood K^+ and Ca^{2+}. Light intensities of 0.2 and 2.5 lx significantly ($p \leq 0.054$) increased blood K^+ concentration on d 42 in comparison with 5 lx, but there was no effect of varying light intensity on blood Ca^{2+} concentration on any of the sampling days. There were no effects of varying light intensity on mOsm, sO_2, Angap, McHc, T_3 and T_4 on any of the sampling days (data not shown). Furthermore, blood glucose levels and plasma CS concentrations were not significantly affected by treatments on any of the sampling days in the present study (data not shown).

DISCUSSION

Previous studies demonstrated partial effects of light intensity on blood physiological parameters in the presence of ammonia (Olanrewaju *et al*., 2008) and temperature (Olanrewaju *et al*., 2010). We do not know if these two microenvironmental constraints masked the effect of light intensity or the range of light intensity utilized was too narrow. Therefore, the present study examined varying levels of light intensity on blood physiological parameters of broilers grown to heavy weights. The results indicated that exposure of broilers to the lowest light intensity of 0.2 lx significantly affected the acid-base balance especially starting from 28 d of age, which was in agreement with our previous report (Olanrewaju *et al*., 2008). For instance, 0.2 lx significantly increased pH, Na^+, K^+, Cl^- and significantly reduced pCO_2, Hb and Hct. However, all these acid-base changes were still between the normal acid-base homeostasis and physiological ranges for broiler chickens. In addition, exposure of modern heavy broilers to varying light intensity produced no significant effect on pO_2, sO_2, Ca^{2+}, mOsm, McHc, Angap, T_3, T_4 and CS.

The finding that pH was elevated and blood pCO_2 was decreased along with slightly increased pO_2 indicates minimal respiratory alkalosis. Results suggest an increased ventilation rate in broilers exposed to the lowest light intensity, which might be related to their BW and less physical activity. As was observed in this study, the decreased pCO_2 may account for respiratory hypopnea, which prevents pO_2 from decreasing. It is generally agreed that a decrease in blood pH results in increased stimulation ventilation capacity (Davenport, 1950). 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 (Gesell, 1925). Changes in the acid-base balance are a source of information about the early phase of diseases and they influence the clinical signs, disease development and the effectiveness of therapeutics (Brobst, 1983). The systems controlling acid base

Table 1: Influence of light-intensity on plasma pH and HCO_3^- of broilers grown to heavy weights

	pH				HCO_3^- (mmHg)			
Item	21 d	28 d	42 d	56 d	21 d	28 d	42 d	56 d
Light-intensity treatment (lx)								
25	7.35	7.32	7.35	7.33[b]	26.24	26.00	25.53	26.38
10	7.36	7.34	7.37	7.32[b]	26.53	26.34	26.45	26.86
5	7.35	7.33	7.37	7.33[b]	25.94	25.55	26.56	26.19
2.5	7.35	7.32	7.37	7.32[b]	26.26	26.13	26.39	26.91
0.2	7.39	7.34	7.36	7.37[a]	27.14	26.26	25.39	26.08
SEM[1]	0.017	0.018	0.010	0.010	0.485	0.457	0.493	0.326
p-value	0.073	0.863	0.705	0.005	0.074	0.763	0.287	0.257
Sex								
Male	7.37	7.34	7.36	7.32[b]	26.58	26.86	26.57	27.08
Female	7.35	7.32	7.37	7.35[a]	26.27	25.25	25.56	25.89
SEM[1]	0.007	0.005	0.006	0.006	0.180	0.589	0.382	0.398
p-value	0.064	0.221	0.462	0.001	0.223	0.060	0.066	0.102

[ab]Means within a column and effect that lack common superscripts differ significantly ($p \leq 0.05$).
[1]SEM = Standard error of the mean (n = 8)

Table 2: Influence of light-intensity on plasma pCO_2 and pO_2 of broilers grown to heavy weights

	pCO_2 (mmHg)				pO_2 (mmHg)			
Item	21 d	28 d	42 d	56 d	21 d	28 d	42 d	56 d
Light-intensity treatment (lx)								
25	48.67	51.08	48.00	51.75[a]	35.64	37.14	34.92	28.42
10	49.00	50.53	47.22	52.78[a]	38.28	39.56	35.58	30.59
5	48.19	50.00	47.50	51.33[a]	30.19	39.59	37.00	29.50
2.5	49.11	52.28	47.64	52.83[a]	38.86	40.44	36.17	30.39
0.2	46.03	50.42	47.78	46.89[b]	37.31	42.64	36.78	33.42
SEM[1]	1.032	1.499	1.315	1.049	2.962	1.398	1.198	2.058
p-value	0.127	0.846	0.995	0.001	0.081	0.109	0.727	0.519
Sex								
Male	49.39	51.11	49.36	53.44[a]	37.79	40.23	36.69	30.82
Female	47.01	50.62	45.90	48.79[b]	34.32	39.51	35.49	30.10
SEM[1]	0.863	0.948	1.162	0.663	1.198	0.884	0.758	1.301
p-value	0.071	0.711	0.065	0.053	0.065	0.566	0.268	0.697

[ab]Means within a column and effect that lack common superscripts differ significantly ($p \leq 0.05$).
[1]SEM = Standard error of the mean (n = 8)

balance are interlinked. The principal organ systems used in acid-base homeostasis in birds are the lungs and kidneys and these are supported by the gastrointestinal tract (Long, 1982). Therefore, relatively small changes in ventilation can have a profound effect on hydrogen ion concentration and pH. Thus, a change in plasma pH or pCO_2 results in a change in ventilation within minutes. Disturbances in venous blood acid-base status (pCO_2 and pH) observed in this study may be attributed to relative differences in the body sizes and ages of birds. The older broilers exhibited greater resting venous blood pCO_2 tensions and were relatively more acidotic (hypercapnic acidosis). This may be due to age-dependent differences in ventilation rate or may reflect the consequences of an increased metabolic demand in the larger birds (Korte *et al*., 1999). Birds exposed to the lowest light intensity exhibited higher respiratory rates, which may be due to their BW or lack of activities. The increased respiratory rate disrupted their acid-base balance because of excessive CO_2 losses (Toyomizu *et al*., 2005).

The essential electrolytes for the maintenance of the acid-base balance are sodium (Na^+), potassium (K^+) and chlorine (Cl^-). However, K^+ is more involved in many metabolic processes, including the acid-base balance (Borges *et al*., 2007). The number of positive and negative ions in the plasma must balance at all times. Aside from the plasma proteins, HCO_3^- and Cl^- are the two most abundant negative ions (anions) in plasma. The plasma K^+, Na^+ and Cl^- concentration results in the current study are consistent with our earlier reports (Olanrewaju *et al*., 2008). This may be attributed to mild

Table 3: Influence of light-intensity on plasma hemoglobin (Hb) and hematocrit (Hct) of broilers grown to heavy weights

	Hb (g/dL)				Hct (%)			
Item	21 d	28 d	42 d	56 d	21 d	28 d	42 d	56 d
Light-intensity treatment (lx)								
25	7.54	8.08[a]	8.33	8.93	23.53	25.17[a]	25.92	27.67
10	7.30	7.73[ab]	8.04	8.90	23.06	24.08[ab]	25.03	27.58
5	7.30	7.69[ab]	8.16	8.73	22.81	23.95[ab]	25.36	27.08
2.5	7.56	7.79[a]	7.85	9.04	23.61	24.28[a]	24.42	28.06
0.2	7.16	7.32[b]	8.27	8.75	22.39	22.89[b]	25.69	27.17
SEM[1]	0.141	0.110	0.132	0.197	0.482	0.326	0.392	0.588
p-value	0.069	0.000	0.098	0.791	0.069	0.000	0.075	0.770
Sex								
Male	7.36	7.73	8.25[a]	9.21	23.00	24.08	25.64[a]	28.53
Female	7.38	7.72	8.01[b]	8.53	23.16	24.07	24.92[b]	26.49
SEM[1]	0.064	0.069	0.083	0.276	0.179	0.206	0.248	0.682
p-value	0.838	0.916	0.048	0.083	0.540	0.968	0.044	0.078

[ab]Means within a column and effect that lack common superscripts differ significantly ($p \leq 0.05$).
[1]SEM = Standard error of the mean (n = 8)

Table 4: Influence of light-intensity on plasma sodium (Na^+) and Chloride (Cl^-) of broilers grown to heavy weights

	Na^+ (mEq/L)				Cl^- (mEq/L)			
Item	21 d	28 d	42 d	56 d	21 d	28 d	42 d	56 d
Light-intensity treatment (lx)								
25	147.3	147.8	149.9[b]	152.9	105.0	105.6	108.7[b]	110.1
10	147.1	147.8	149.4[b]	152.4	104.4	105.9	108.1[b]	109.6
5	147.3	148.5	150.1[b]	153.1	104.8	106.7	108.7[b]	110.0
2.5	146.8	147.6	150.0[b]	154.5	104.5	105.8	109.3[b]	111.4
0.2	147.1	148.2	151.7[a]	152.8	105.0	106.7	110.5[a]	110.8
SEM[1]	0.218	0.365	0.533	0.518	0.251	0.354	0.365	0.519
p-value	0.448	0.417	0.003	0.068	0.266	0.133	0.003	0.148
Sex								
Male	146.8	147.8	150.4	154.5	104.4	105.6	108.8	111.1
Female	147.4	148.1	150.0	151.8	105.1	106.7	109.3	109.6
SEM[1]	0.238	0.231	0.236	0.928	0.959	0.424	0.271	0.528
p-value	0.070	0.326	0.179	0.080	0.085	0.071	0.198	0.061

[ab]Means within a column and effect that lack common superscripts differ significantly ($p \leq 0.05$).
[1]SEM = Standard error of the mean (n = 8)

Table 5: Influence of light-intensity on plasma potassium (K^+) and Calcium (Ca^{2+}) of broilers grown to heavy weights

	K^+ (mEq/L)				Ca^{2+} (mEq/L)			
Item	21 d	28 d	42 d	56 d	21 d	28 d	42 d	56 d
Light-intensity treatment (lx)								
25	4.57	4.74	4.50[ab]	5.11	3.18	3.18	3.17	3.16
10	4.32	4.75	4.51[ab]	4.9	3.15	3.18	3.16	3.15
5	4.10	4.65	4.25[b]	4.68	3.13	3.18	3.17	3.17
2.5	4.75	5.12	4.78[a]	5.39	3.17	3.21	3.20	3.21
0.2	4.44	5.02	4.79[a]	4.84	3.15	3.22	3.21	3.15
SEM[1]	0.231	0.183	0.134	0.275	0.014	0.015	0.016	0.018
p-value	0.073	0.308	0.054	0.084	0.129	0.192	0.057	0.114
Sex								
Male	4.51	4.85	4.60	5.02	3.17	3.20	3.20	3.20
Female	4.37	4.86	4.50	4.96	3.15	3.19	3.17	3.14
SEM[1]	0.083	0.116	0.085	0.111	0.009	0.010	0.012	0.021
p-value	0.228	0.935	0.713	0.691	0.187	0.511	0.068	0.069

[ab]Means within a column and effect that lack common superscripts differ significantly ($p \leq 0.05$).
[1]SEM = Standard error of the mean (n = 8)

metabolic alkalosis, which has been associated with a minor increase of pH and reduction of pCO_2 concentration, along with sustained plasma Ca^{2+}.

Hematological examinations in these studies have also proved that the total amount of Hb and Hct in blood can decrease with decreased rearing light intensity, which can result in lower metabolic rate. Decreases in Hb and Hct observed in this study, may be related to the decreased metabolic activity, leading to a decrease in erythropoiesis and subsequent oxygen-carrying capacity in the blood. The lack of effect of light intensity on sO_2 in the present study still supports this finding, since it measures the percent of hemoglobin that is fully combined with oxygen. Concentrations of certain plasma hormones, enzymes and metabolites such as CS have been suggested to be sensitive indicators of stress levels in broiler chickens (Puvadolpirod and Thaxton, 2000; Olanrewaju *et al*., 2006a). The nonsignificant effect on plasma CS observed in the present study indicates that birds were not stressed.

The results of this study supplement current knowledge of the hematology and biochemistry of plasma in modern chickens with heavy BW during the growth period using varying light intensities of 25, 10, 5, 2.5 and 0.2 lx. In addition, treatments did not affect plasma corticosterone. Also, results imply that sex and day represent significant contributors of variation in levels of several blood physiological parameters that should be considered in the interpretation of the laboratory test results in broiler chickens. 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 physiological stress effects on broiler welfare.

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