ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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OPEN ACCESS International Journal of Poultry Science

ISSN: 1682-8356
DOI: 10.3923/ijps.2017.288.295

Research Article
Effects of Light Ingress through Ventilation Fan Apertures on Selected Blood Variables of Male Broilers

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Abstract

Background and Objective: Increasing broiler house size and ventilation capacity have resulted in increased light ingress through ventilation system component apertures. The effective photoperiod for broilers may create local increases in light intensity, which may also impact broiler' body homeostasis. This study was conducted to investigate the influence of aperture light ingress in grow-out houses on selected blood variables of male broilers reared to 63 days of age. **Materials and Methods:** Sixty male broiler chicks were randomly distributed to each of 16 environmentally-controlled rooms (960 total birds). Birds were provided a diet formulated to meet or exceed NRC recommendations with feed and water provided *ad libitum*. The two treatments consisted of a constant (C) light intensity at 2.5 lx at 16L:8D based on prescriptive intensities in typical heavy broiler lighting programs and a variable (V) light intensity and photoperiod program that was based on field measurements near tunnel fans in a solid walled broiler house. The lighting program to 35 days was identical for both treatments and the treatments were initiated at 36 days. Blood samples were collected from the wing brachial vein of 6 birds per room on day 35 (before treatments) and 63, which were then analyzed immediately for whole blood physiological variables. Selected blood plasma biochemistry, enzyme activities and electrolyte levels were evaluated. **Results:** In comparison to broilers exposed to a 'C' light intensity of 2.5 lx, broilers exposed to 'V' lighting that mimicked areas near tunnel fans, had significantly lower levels of pH, Ca^{2+}, K^{+}, CK and higher levels of angap, which were within physiological acid-base ranges. Also, age have significant contributor effects on most selected variables. In addition, blood glucose and plasma corticosterone concentrations were not affected by treatment, suggesting an absence of physiological stress and an uncompromising welfare of the birds. **Conclusion:** It is concluded that there may be a need to mitigate light ingress through ventilation system components to improve live performance of broilers.

Key words: Lighting, biochemistry, enzyme, blood gases, broilers

Received: May 19, 2017 Accepted: June 22, 2017 Published: July 15, 2017

Citation: H.A. Olanrewaju, J.L. Purswell, S.D. Collier and S.L. Branton, 2017. Effects of light ingress through ventilation fan apertures on selected blood variables of male broilers. Int. J. Poult. Sci., 16: 288-295.

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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

Lighting programs for broiler production are formulated partially to provide some control over growth trajectory and to minimize adverse behaviors[1]. However, the lighting environment within a modern solid-walled broiler house can vary with construction practices (fan placement, evaporative pad length, interior reflectivity, etc.) as well as environmental control methods (tunnel vs. perimeter ventilation) and may alter the effectiveness of the prescribed lighting program. Fairchild *et al.*[2] found that light leakage through tunnel fans resulted in delayed onset of drinking as broilers responded primarily to sunlight, rather than the photoperiod of artificial lighting. Increasing broiler house size and ventilation capacity have resulted in increased light ingress through ventilation system components. Light ingress may alter the effective photoperiod for broiler chickens and create local increases in light intensity. This shift in photoperiod may act to result in increased bird activity, restrict feed consumption and thus negatively affect live performance and house uniformity. Conversely, the change in the length of the photoperiod can be an alternative way to improve the welfare, immune response and consequently, the performance of birds. However, it is important to recognize that many environmental variables including photoperiod can potentially influence blood reference ranges from which birds are sampled. It has been reported that physical stress induced by photoperiod, prolonged handling, or restraint can result in acidosis, increased creatine kinase (CK), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) activities, elevated potassium (K^+) levels and decreased glucose (GLU) concentrations in the blood[3,4]. Exposure of poultry to inadequate micro-environmental factors during the course of poultry production has an adverse impact on physiological variables such as blood acid-base balance, electrolytes and metabolites, which can lead to production inefficiencies, compromised animal well-being and increased animal morbidity and mortality[3,5].

Blood analyses along with other biochemical evaluations have been used to assess the health status of animals[6,7]. Changes in major selected blood variables are routinely used to determine various influences of environmental, nutritional and pathological factors[5,8]. 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[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]. Stress responses are also integrally involved with acid-base balance in several species[1,12]. Furthermore, changes in the levels of various blood chemistry parameters as a function of age represent another set of physiological alterations which can be observed for variables such as alkaline phosphatase (ALP) and total cholesterol[13]. It has been documented that green muscle disease in broilers is associated with large increase in plasma CK and AST activities[14], which can have either nutritional or genetic causes[15]. In this study, the influence of light-ingress through fan apertures on selected blood physiological variables, plasma biochemistry and enzyme activities of broilers were examined. The same individual birds, treatments and conditions were used as part of an investigation of male broilers reared to 63 days of age and that were exposed to either a constant light intensity of 2.5 lx for 18L:6D or a variable light intensity to mimic light ingress through tunnel fans reported in a previous manuscript[16].

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, Mississippi location. A total of 960 male 1-day-old Ross × Ross 708 chicks were purchased from a commercial hatchery. Upon arrival, chicks were weighed and allocated to 16 groups with equated BW; initial BW was 44 g and was not different between treatments ($p = 0.33$). Chicks were randomly distributed into 16 environmentally-controlled rooms (60 chicks/room). Each environmental room had a floor area of 2.3 × 2.6 m (5.98 m^2) with a room volume of 14.95 m^3 (ceiling height = 2.5 m). Each room contained 3 cm deep 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 days of age, birds received a Gumboro vaccination via water administration. The chicks remained in their respective rooms from 1-63 days of age (experimental period). Birds were provided a 4-phase feeding program (starter: 1-14 days, grower: 15-28 days finisher: 29-42 days and withdrawal: 43-63 days). Diets were formulated to meet NRC[17] 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 day 1 were maintained at 32 ± 1.1°C and 50 ± 5%, respectively and were held constant across all treatments. Temperature was decreased as the birds progressed in age until it reached 15.6°C at 49 days of age.

Experimental treatments: A completely randomized experimental design with room serving as the experimental unit was used and included 2 treatments with 8 replicates treatment. Lighting was provided with a broad spectrum dimmable 6W LED bulb in a typical A19 shape. Light intensity was controlled with a phase-control electronic dimmer and light intensity switching was controlled with a programmable logic controller. The lighting program employed to days 35 was identical for both treatments. The treatment period was initiated at day 36 to mimic onset of tunnel ventilation use and consisted of two treatments: constant (C) light intensity at 2.5 lx at 16L: 8D based on prescriptive intensities in typical heavy broiler lighting programs and a variable (V) light intensity and photoperiod program based on field measurements near tunnel fans in a solid walled broiler house at 15 min intervals over a 7 days period. The resulting photoperiod was 18L:6D with a peak intensity of 35 lx. Lighting was changed to 5 lx at 23L: 1D 48 h prior to processing for the C lighting program and the minimum light intensity for the V lighting program was also increased to 5 lx. Light intensity settings were verified from the center and four corners of each room at bird level (30 cm) using a photometric sensor with 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 their intensity.

Measurements

Blood collections and chemical analyses: On days 35 (day before initiation of the treatments) and 63, blood samples were collected from a brachial vein of 6 randomly selected chicks from each room. The birds were then returned to the appropriate rooms without unnecessary discomfort using proper housing and handling techniques, as described by the NRC[18]. All bleedings were completed within 45 sec after birds were caught. Blood samples (3 mL) were collected directly into heparinized (50 IU mL^{-1}) monovette syringes and 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, glucose (Gluc), anion gap (angap), osmolality (Osmo) 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 g dL^{-1} was calculated using the standard formula:

$$\frac{Hb \times 100}{Hct}$$

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 following standard formula:

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

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 rpm 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. In addition, analyses of the plasma concentrations and activities of albumin (ALB), total bilirubin (TBILI), blood urea nitrogen (BUN), creatinine (CREAT), total protein (TP), uric acid (UA), cholesterol (CHOL), low density lipoprotein (LDL-C), high density lipoprotein (HDL), triglycerides (TRIG), alanine aminotransferase (ALT), alkaline phosphatase (ALP), amylase (AMYL), aspartate aminotransferase (AST), creatine kinase (CK), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH) and lipase (LIP) were measured using ACE-AXCEL (Alfa Wassermann DiagnosticTech, West Caldwell, NJ) through biochemistry and enzymatic rate reactions. Some of these blood parameters are indicators of internal organ health and systemic homeostasis.

Statistical analysis: Data were analyzed using PROC MIXED in SAS[19] in a completely randomized design with room serving as the experimental unit and with 8 replicates treatment. Treatment and age served as fixed effects and room served as a random effect in analysis of the main and interactive effects of treatment and age. Means comparisons on days 35 and 63

were separated using Fisher's least significant differences, with significance considered at $p \leq 0.05$ unless otherwise stated.

RESULTS

Table 1 shows the main effects of treatment and age on major selected blood physiological variables. In comparison with broilers reared under the 'C' treatment, broilers reared under the 'V' treatment had significantly lower levels of pH ($p<0.015$), Ca^{2+} ($p<0.041$), K^+ ($p<0.001$) and higher levels of angap ($p<0.026$). There was a major influence of age on most examined blood physiological variables. Broilers reared up to 63 days of age in comparison to those at 35 days of age, apart from BW, had higher levels of pCO_2 ($p<0.043$), Ca^{2+} ($p<0.041$), K^+ ($p<0.001$), angap ($p<0.026$) and Osmo ($p<0.044$), along with reduced levels of pH ($p<0.015$), pO_2 ($p<0.032$), sO_2 ($p<0.014$), SaO_2 ($p<0.027$), Na^+ ($p<0.036$), Cl^- ($p<0.032$) and GLU ($p<0.042$) with a small increase in HCO_3^-. However, no effect of treatment or age was noted on Hb, Hct, McHc and CORT on any of the sampling days. The influence of treatment and age on selected blood plasma biochemistry variables are presented in Table 2. As shown in Table 2, there was no effect of treatments noted on selected blood plasma biochemistry variables. However, broilers reared up to 63 days of age in

Table 1: Combined main effects of fan-induced photoperiod on selected blood physiological variables of male broilers reared to 63 days of age[1]

	Days				
	35	63			
Variables	Baseline	Constant[2]	Variables[3]	SEM	p-value
Body Weight (Kg)	2.165[b]	4.927[a]	4.921[a]	0.103	0.028
pH	7.40[a]	7.32[b]	7.29[c]	0.008	0.015
pCO_2 (mmHg)	57.02[b]	60.34[a]	63.52[a]	0.130	0.043
pO_2 (mmHg)	69.56[a]	51.39[b]	47.61[b]	1.052	0.032
HCO_3^- (mmHg)	27.57	28.55	27.88	0.327	0.058
sO_2 %	52.23[a]	47.80[b]	44.13[b]	1.726	0.014
SaO_2 %	76.38[a]	73.36[b]	70.75[b]	1.595	0.027
Hb (g dL^{-1})	8.87	8.79	8.63	0.103	0.275
Hct (%)	27.87	27.28	26.81	0.309	0.290
McHc (g dL^{-1})	32.22	32.23	32.21	0.013	0.116
Ca^{2+} (mEq L^{-1})	2.976[c]	3.102[a]	3.008[b]	0.011	0.041
Na^+ (mEq L^{-1})	153.15[a]	151.60[b]	152.31[b]	0.914	0.036
K^+ (mEq L^{-1})	4.131[c]	4.548[a]	4.231[b]	0.063	0.001
Cl^- (mEq L^{-1})	111.01[a]	109.46[b]	109.36[b]	0.271	0.032
Anion Gap (mEq L^{-1})	18.02[c]	18.20[b]	19.28[a]	0.324	0.026
GLU (mg dL^{-1})	273.57[a]	262.69[b]	254.56[b]	2.97	0.042
Osmo (mOs kg^{-1})	316.23[b]	333.62[a]	319.97[a]	4.90	0.044
CORT (pg mL^{-1})	1994.36	2014.25	1987.46	156.4	0.158

[a-b]Means within a row not sharing a common superscript are significantly different at $p \leq 0.05$, [1]Table values are least square means of eight replicate pens for each treatment, [2]Constant = Broilers subjected to a constant light intensity of 2.5 lx, [3]Variable = Broiler subjected to a variable light intensity to mimic light leakage through tunnel fans. Effective photoperiod was 18L: 6D with a peak intensity of 35 lx

Table 2: Influence of fan induced photoperiod on selected blood plasma biochemistry of male broilers reared to 63 days of age[1]

	Days				
	35	63			
Chemistry assays	baseline	Contant[2]	Variables[3]	SEM	p-value
Albumin (ALB), g dL^{-1}	0.906[b]	1.049[a]	1.057[a]	0.058	0.018
Bilirubin, or total (TBILI), mg dL^{-1}	0.546	0.557	0.420	0.083	0.118
Blood urea nitrogen (BUN), mg dL^{-1}	0.987[a]	0.733[b]	0.700[b]	0.282	0.017
Creatinine (CREAT), mg dL^{-1}	0.148[b]	0.179[a]	0.185[a]	0.097	0.038
Total protein (TP), g dL^{-1}	2.210[b]	2.870[a]	2.993[a]	0.195	0.032
Uric acid (UA), mg dL^{-1}	6.733[a]	5.874[b]	5.773[b]	0.275	0.003
Cholesterol (CHOL), mg dL^{-1}	85.28[b]	99.23[a]	104.20[a]	7.906	0.028
Low density lipoprotein (LDL-C), mg dL^{-1}	16.21[b]	26.20[a]	25.47[a]	2.818	0.004
High density lipoprotein (HDL), mg dL^{-1}	71.67[a]	60.67[b]	63.63[b]	4.887	0.016
Triglycerides (TRIG), mg dL^{-1}	33.13[b]	46.63[a]	43.37[a]	4.483	0.002

[a-b]Means within a row not sharing a common superscript are significantly different at $p \leq 0.05$, [1]Table values are least square means of eight replicate pens for each treatment, [2]Constant = Broilers subjected to a constant light intensity of 2.5 lx, [3]Variable = Broiler subjected to a variable light intensity to mimic light leakage through tunnel fans Effective photoperiod was 18L: 6D with a peak intensity of 35 lx

Table 3: Influence of fan induced photoperiod on selected blood plasma enzymes activities of male broilers reared to 63 day of age[1]

	Days				
	35	63			
Enzyme assays	baseline	Contant[2]	Variables[3]	SEM	p-value
Alanine aminotransferase (ALT), U L^{-1}	3.45[a]	2.15[b]	2.19[b]	1.310	0.025
Alkaline phosphatase (ALP), U L^{-1}	13417[a]	954.93[b]	1063[b]	139.230	0.044
Amylase (AMYL), U L^{-1}	495.65[a]	438.07[b]	434.57[b]	12.850	0.042
Aspartate aminotransferase (AST), U L^{-1}	256.23[b]	329.33[a]	408.43[a]	56.060	0.015
Creatine kinase (CK), U L^{-1}	21,362.0[c]	25,593.0[a]	21,578.0[b]	379.580	0.031
Gamma-glutamyl transferase (GGT), U L^{-1}	10.256[b]	12.567[a]	13.700[a]	0.743	0.014
Lactate dehydrogenase (LDH), U L^{-1}	984.73[b]	1,221[a]	1,325[a]	20.010	0.035
Lipase (LIP), U L^{-1}	19.56[a]	13.35[b]	11.733[b]	1.938	0.024

[a-b]Means within a row not sharing a common superscript are significantly different at $p \leq 0.05$, [1]Table values are least square means of eight replicate pens for each treatment, [2]Constant = Broilers subjected to a constant light intensity of 2.5 lx, [3]Variable = Broiler subjected to a variable light intensity to mimic light leakage through tunnel fans, Effective photoperiod was 18L: 6D with a peak intensity of 35 lx

comparison to those at 35 days of age had higher levels of ALB ($p<0.018$), CREAT ($p<0.038$), TP ($p<0.032$), CHOL ($p<0.028$) and LDL-C ($p<0.004$), TRIG, ($p<0.002$) along with reduced levels of plasma UA ($p<0.003$) and HDL ($p<0.016$). There were no effects of treatment or age noted on TBILI. The influence of treatment and age on blood plasma enzymes activities are presented in Table 3. In comparison with broilers reared under C treatment, broilers reared under the V treatment only had lower activity levels of CK. Broilers reared up to 63 days of age in comparison to those at 35 days of age had higher enzyme activity levels of AST ($p<0.015$), CK ($p<0.031$), GGT ($p<0.014$), land LDH ($p<0.035$) along with reduced activity levels of ALT ($p<0.025$), AMYL ($p<0.042$) and LIP ($p<0.024$).

DISCUSSION

The live performance and processing yields results of male broilers reared to 63 days of age that were exposed to either 'C' or 'V' treatments indicated that feed consumption and feed conversion were significantly increased for the 'V' light treatment birds but, BW gain and mortality were not different between treatments, indicating that mitigating light ingress may reduce feed requirements[16]. The present study in which the same individual birds, treatments and conditions were used indicated that baseline acid-base status of broilers reared under the V treatment had only significantly lower levels of pH, Ca^{2+}, K^+ and higher levels of angap in comparison with broilers reared under the C treatment. However, these blood variables values were within normal physiological ranges. There was a major influence of broiler age on the variables examined. The levels of most examined blood physiological variables at 63 days of age differed significantly from those at 35 days. There was a significant increase in venous pCO_2 with concomitant decrease in pO_2 in birds at 63 days of age. This may have been due to tissue metabolism requirement of the growing and mature birds, while the small increase in HCO_3^- may have contributed to a shift toward metabolic alkalosis. They would subsequently trigger respiratory renal compensation via an increase in pCO^2 [20]. The reduced pO_2 and sO_2 observed in broilers reared up to 63 days of age, may be due to inadequate blood oxygenation and hypoxemia, which may increase the risk of hypoxia[21]. These changes include reductions in systemic venous pO_2, sO_2 and increased pCO_2 associated with a small increase in HCO_3^- [22]. The combination of these changes may lead to acute respiratory acidosis due to hydrogen ion (H^+, acid) accumulation. Modern broiler chickens 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, sO_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 both lung and cardiovascular volume within lung tissue is fixed in birds, unlike in mammals, a point is eventually reached whereby the lungs may no longer accommodate more blood being supplied by the heart and this may have a negative effect on the body (poor oxygenation). However, observed acid-base changes in the present study remained within normal acid-base homeostasis and physiological ranges for this species.

Although respiratory acidosis does not always have an appreciable effect on plasma or serum electrolyte levels, it may sometimes cause some small changes in Ca^{2+} and K^+ levels. As shown in Table 1 in this study, birds reared up to 63 days of age in comparison to those at 35 days of age had higher K^+, which may be associated with a higher BW, since K^+ ion concentration have been shown to be involved in many metabolic processes, including amino acid absorption and transport, protein synthesis and acid-base balance[23]. It has

been documented that K^+ is the monovalent cation which regulates protein synthesis in the cell and that relatively small decreases in intracellular K^+ result in a reduction in protein synthesis[24]. It has also been reported that older birds have higher blood Ca^{2+} concentrations than younger ones[25]. A significant decrease in plasma Cl^- concentration may be related to an increase in HCO_3^- as part of an attempt to assure electro-neutrality according to Stewart's theory of strong Ion difference (SID). In this case, after a pulmonary response to increasing pCO_2, renal response starts in an effort to regulate Na^+ and Cl^- ion concentrations which are major SID determinants. The subsequent Na^+, Cl^- and K^+ levels were significant difference in the two age groups where Na^+ and Cl^- concentrations were higher in 35 days old broilers, whereas K^+ concentrations were higher in 63 days old broilers.

There was no effect of treatment noted on any of the selected blood plasma biochemistry variables. However, in comparison to those boilers at 35 days of age, boilers at 63 days of age had higher levels of ALB, CREAT, TP, CHOL, LDL, TRIG and lower levels of BUN, UA and HDL. It has been documented that certain plasma and serum biochemical variables including ALB, TP and CHOL levels were lower in younger broiler chickens in comparison to adult birds[8]. The levels of TP have been reported to be influenced by age, environment and physiological state, among other factors[26]. In addition, the age-related increases in TP concentrations may be the reflection of a more prolific protein synthesis process as the birds grow older. Albumin constitutes a large part of the protein fraction of plasma and the age-related changes in ALB values in the present study were consistent with those of TP, since ALB will increase when protein intake increases. This specifically occurs after the protein requirement for maintenance and growth is met. In the present study, the concentration of CREAT increased with age, which is similar to other reports[27]. It has been reported that the CREAT concentration is directly proportional to muscle mass, which is further related to age and physical activity[28]. In addition, Bell[26] indicated that 50% of the calcium present in blood is bound to ALB, which may explain the higher concurrent TP, ALB and Ca^{2+} levels in broilers at 63 days of age. The higher levels of CHOL, LDL and TRIG observed in 63 days old broilers have been reported in other studies[29]. These higher values correspond to their low mobilization by tissues and to their intense synthesis by the liver[28]. On the other hand, the lower TRIG and CHOL levels at 35 days of age may be due to the bird's high energy requirement at that age in association with an increase in the rate of body development[30]. In the present study, levels of UA decreased with age, which agrees with other studies[8,28]. Any changes in protein catabolism are mainly reflected in serum UA concentrations. Earlier studies on poultry revealed age-dependent changes in blood UA concentrations and a direct relationship between the amount of ingested protein and serum UA levels has been reported[28]. The decrease in the levels of UA observed in the broilers at 63 days of age in the present study is supported by the finding of Szabo *et al.*[28]. In the present study, the change in diet from finisher (29-42 days) to withdrawal (43-63 days) for older birds, results in a significant decrease in plasma UA, which may be due to reduced protein percentage values in broiler of 63 days of age diet? Szabo *et al.*[28] and Bowes *et al.*[31] showed a direct relationship between the amount of ingested protein and blood UA levels. Bowes *et al.*[31] reported concurrent concentrations of CREAT and BW weight in chickens and suggested that CPK activity declines as metabolic rate decreased. Similar to our results, Peebles *et al.*[32] also detected the tendency of HDL levels to decrease with age in meat-type chickens. A tendency of serum TRIG to increase with age, were also observed in chickens by Peebles *et al.*[33]. The significant increase in both TRIG and GLUC concentrations with age observed in broilers at 63 days of age may be due to an increase in BW in birds, as reported by Alonso *et al.* [34]. The relative decrease in metabolic rate at older age may result in a reduced intake of GLUC and TRIG from the blood.

In the present study, the plasma activity of all (ALT, ALP, AMYL, AST, CK, GGT, LDH and LIP) enzymes changed as a function of broiler age. The plasma activities of AST, CK, GGT and LDH progressively increased with age, which is consistent with the observations of other studies[35]. These changes may be due to an increase in liver metabolism and to significant changes in muscle development, as observed by Szabo *et al.*[28]. On the other hand, the plasma activities of ALT, ALP, AMYL and LIP decreased at an older age, which may due to increased bone development. The significant age-dependent activity of the plasma enzymes in the present study may be due to the biochemical changes of different organ systems, whose enzyme activities increase with age[36]. Tissue development and modification during the growing period could induce high plasma enzyme activity. For instance, increased activity of ALP in 35 days old broilers may be due to rapid bone development associated with osteoblastic activity rather than liver injury. Alkaline phosphatase activity also displayed high activity values in 35 days old broilers compared with 63 days broilers, which is in agreement with Costa *et al.*[25]. This enzyme is normally higher in young animals when bone metabolism is intense relative to older animals. The higher levels of LDH and K^+ in 63 days broilers may be a reflection of their larger muscle mass. An age-related decrease in LDH activity has been reported in chickens[37]. The more intense

basal metabolism of 35 days old broilers could explain the higher plasma activity of AMYL and LIP than 63 days old broilers. Lower serum Ca^{2+} and K^{+} concentrations confirm this hypothesis since it has been reported that when these enzyme activities increase, Ca^{2+} and K^{+} concentrations decrease[38]. Finally, the values found for each selected variables (blood physiological variables, plasma biochemistry and enzyme activities) are in broad agreement with those reported in the literature would be very useful in detecting not only environmental welfare conditions of broilers but also metabolic-nutritional disorders of broilers.

CONCLUSION

From a physiological perspective, the significant age-related (days 35 and 63) variations of some selected blood physiological variables, plasma biochemistry and enzyme activities observed are in broad agreement with those reported in the literature and contribute by enhancing our knowledge of homeostasis variation and the range of various blood metabolites in developing male broilers.

SIGNIFICANCE STATEMENTS

It was observed significant increases in FCR and feed consumption for the 'V' lighting treatment under growth performance as reported in the previous manuscript[16] and an uncompromising welfare of these birds in the present study, the authors suggest there may be a need to mitigate light ingress through ventilation system components to improve live performance of broiler chickens.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to the animal care staff and engineering technicians at USDA-ARS Poultry Research Unit for their efforts during the conduct of this experiment.

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