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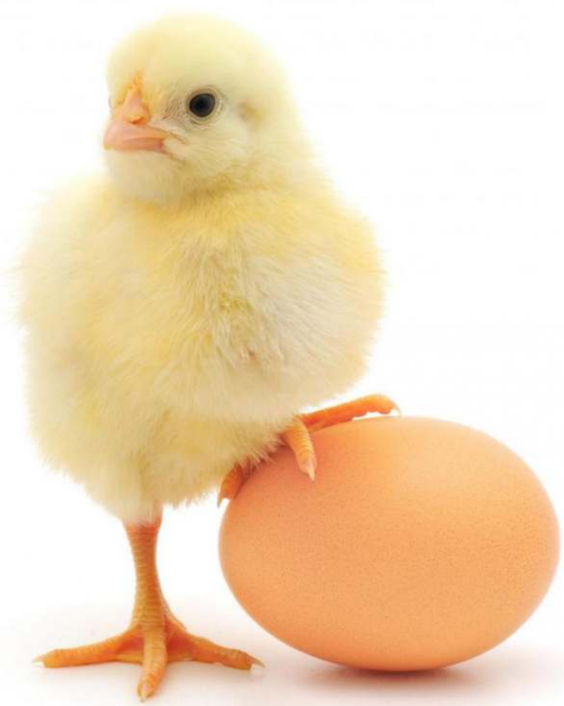

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Research Article
Effects of Transportation Duration on Broiler Chicken Physiology and Performance Factors

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Abstract
Background and Objective: In Indonesia, broiler chicken transportation traditionally occurs post-harvest and entails travel from farm to chicken slaughter house. Transportation usually takes place during the day without shade or isolation, which causes stress in the chickens, leading to body weight reduction and increased mortality. Therefore, the purpose of this study was to evaluate the effects of transportation duration on broiler chicken physiology. **Methodology:** A total of 45 broiler chicken (35-40 days old) were used in this study (average body weight: 1.40-1.90 kg/32). Chickens were transported from farms to a slaughter house in Pondok Rumput, Bogor across either in 1, 2 or 3 h. The variables measured include red blood and white blood profiles, stress index (descriptively analyzed) and chicken performance (statistically analyzed). **Results:** High temperatures during transport and density of transported chicken affected lymphocytes (H/L: 1.25) and blood glucose concentration (lowest: 135.47 mg dL^{-1}). However, there were no significant effects on body weight (3.32-3.93%) across time points and no deaths occurred. **Conclusion:** Three hours of travel time resulted in the highest levels of stress but did not affect body weight or mortality.

Key words: Travel time, broiler chicken, blood profile, stress index, chicken performance

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

INTRODUCTION

The poultry industry produces the animal protein needs of Indonesia. Badan Pusat Statistik[1] has reported that the broiler population has increased sharply to more than 1.4 billion. This large population requires an integrated management chain from upstream to downstream to ensure meat quality. The management chains of broiler chickens include production process in the farm, distribution from farm to slaughter house or from slaughter house to marketing place and marketing process of broiler chickens from distributor to consumer. This distribution process is closely related to transportation.

According to SNI[2], broiler chickens are transported in a basket made of strong, durable construction materials equipped with insulators. TAS[3] reported that transport basket size should correspond with the size of a comfortable chamber with a protective roof and should be equipped with a ventilator (inhaust and exhaust). However, in daily practice, 15-16 broiler chickens are often transported without food, water or shade, in baskets measuring $0.94\times0.58\times0.27$ m^3. These conditions cause stress and greatly impact the broilers. Moberg[4] reported that stress can disrupt homeostasis, negatively affecting the welfare of livestock. Stressful transportation conditions can cause harm to farmers and consumers as well as chicken health. The health status of chickens can be evaluated measuring physiological parameters, such as blood profile and stress index.

Blood profile can be an indicator of the effect of transportation on chicken and other livestock and can be measured by evaluating nutrients, oxygen, carbon dioxide, metabolites, hormones and heat as immune mediators[5]. Blood contains erythrocytes, leukocytes and thrombocytes and erythrocytes play a role in the exchange and distribution of nutrients and gases in the cells, which are necessary for metabolism, while leukocytes play a role in body immunity[6,7]. Tian *et al*.[8] found that decreased erythrocyte levels can decrease hemoglobin, which causes anemia.

Stress index is a parameter that can determine the level of animal welfare; among others are the ratio of heterophil-lymphocytes (H/L) and blood glucose levels. Blood profile and stress index may change according to temperature and other physiological conditions. Hematological values can be used to estimate chicken performance, including body weight loss and death. Therefore, this study aimed to evaluate the hematological values and performance of broiler chickens transported traditionally across different time points.

MATERIALS AND METHODS

Locations: This research was performed at broiler farms in Kemang, Leuwiliang in Sukabumi and at chicken slaughter houses in Pondok Rumput, Bogor. Blood analysis was performed at the Physiology Laboratory, Faculty of Veterinary Medicine at Bogor Agricultural University.

Materials: This study used 45 commercial broiler chickens (35-40 days old) with average body weight ranging from 1.4-1.9 kg/bird. Materials included 70% alcohol, cotton, EDTA (ethylene diamine tetra acetic acid) anticoagulants, Rees and Ecker modified solutions, potassium hexacyanoferrate (III) reagents and aquades.

Procedures

Transportation: Broiler chickens were transported from Kemang, Leuwiliang and Sukabumi farms to a slaughter house in Pondok Rumput, Bogor using truck (constant speed of 25 km h^{-1}) at 1, 2 or 3 h of duration. Transportation was carried out at the beginning of the first, second and third week. Transportation from each region included 5 broiler chickens with numbered legs. They were placed in one basket, where they traveled with chickens that were not part of the study. The size of basket was $0.94\times0.58\times0.27$ m^3. Each basket was filled 15 chickens (body weight: approximately 1.4-1.9 kg bird^{-1}). Thus, the density of chicken in the basket was approximately 41.27-52.27 kg m^{-2} (Fig. 1).

Transportation began at 14.00 Western Indonesian Time. Prior to transportation, chickens were weighed and blood samples were taken in the brachial vein (1 mL). After arrival at the slaughter house in Pondok Rumput, chickens were weighed and blood samples were taken again. The temperature and humidity were measured during each trip, as well as weather and road conditions.

Performance observation: Performance parameters included body weight and mortality during transportation. Data were calculated based on the percentage of broiler chicken body weight differences after transportation. The number of broiler chickens that died was recorded and mortality rate was calculated based on the number of chickens that died.

Hematological analysis: Blood profile parameters (red blood cells/erythrocytes and white blood cells/leukocytes) and stress index (H/L and glucose levels) were measured. This analysis was conducted at the Animal Physiology Laboratory, Faculty of Veterinary Medicine at Bogor Agricultural University.

Fig. 1(a-b): Chickens transport through (a) Truck and (b) Chicken basket

Table 1: Average temperature and humidity during transportation

Travel times (h)	Departure		Arrival	
	Temperature (°C)	Humidity (%)	Temperature (°C)	Humidity (%)
1	33.5	67	32.1	70
2	33.0	70	30.7	73
3	32.8	73	29.4	80

Analysis of the red blood profile included concentrations of erythrocytes, hematocrit and hemoglobin levels. Furthermore, from the red blood profile data, the erythrocyte indices were calculated. The erythrocytes index consists of the following: mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH). Analysis of white blood profile included the number of leukocytes and their differentiation (heterophils, monocytes, lymphocytes, eosinophils and basophils), then H/L was calculated. These values were analyzed according to the method described by Giemsa[9]. Analysis of blood glucose levels was performed after transportation using a glucose kit (GlucoDr).

Statistical analysis: This experiment used a randomized block design. The “treatment” was the travel time of the broiler chickens to the slaughter house. This treatment consists of 3 levels (1, 2 or 3 h) and, as a group, the transport periods (in the first, second and third week). The mathematical models used included:

$$Y_{ij} = \mu + Ki + Pj + \varepsilon_{ij}$$

Where:

Y_{ij} = Value of observation
μ = Average middle value of observations
Ki = Effect of grouping transportation
Pj = Effect of travel times
ε_{ij} = Effect of experimental error

Data on hematological values were analyzed descriptively, while data on broiler performance were analyzed statistically using analysis of variance[10].

RESULTS AND DISCUSSION

General condition: Broiler chickens were transported at a constant speed of 25 km h^{-1}; thus it can be estimated that the distance between broiler farms in Kemang, Leuwiliang and Sukabumi were 25, 50 and 75 km, respectively to slaughter houses in Pondok Rumput, Bogor. Transportation occurred during the day which at approximately 14.00. Temperature and humidity during transportation are presented in Table 1.

The environmental temperature increased above a comfortable temperature for chickens (20-24°C)[11]. Moreover, the broiler chickens placed in baskets at a very high density (41.27-52.27 kg m^{-2}), so the chickens may have experienced very high stress that interferes with physiological processes.

Red blood profile: Red blood profiles of the broiler chickens before and after transportation are presented in Table 2 and the erythrocytes index is presented in Table 3.

Table 2: Concentrations of erythrocytes, hematocrit and hemoglobin of broiler chickens across different time points

Travel times (h)	Variable	Before transportation	After transportation	Normal range*	Delta**
1	Erythrocytes (10^6 cells mm^{-3})	2.68±0.31	2.87±0.40	2.5-3.50	0.19
	Hematocrit (%)	23.44±3.29	24.61±2.27	22.0-35.0	1.17
	Hemoglobin (g%)	7.82±1.91	8.17±1.23	7.0-13.0	0.35
2	Erythrocytes (10^6 cells mm^{-3})	3.16±0.79	3.24±0.74	2.5-3.50	0.08
	Hematocrit (%)	27.48±7.85	27.38±7.94	22.0-35.0	-0.10
	Hemoglobin (g %)	9.18±2.57	9.24±2.62	7.0-13.0	0.06
3	Erythrocytes (10^6 cells mm^{-3})	2.92±0.36	2.93±0.36	2.5-3.50	0.01
	Hematocrit (%)	23.43±2.60	23.79±1.64	22.0-35.0	0.36
	Hemoglobin (g %)	11.50±0.89	11.66±0.69	7.0-13.0	0.16

*25 (1993). **Difference between variables before and after transportation

Table 3: Index of erythrocytes of broiler chickens at different travel times

Travel times (h)	Variable	Before transportation	After transportation	Normal range*	Delta**
1	MCV (fL)	88.07±14.49	86.97±11.61	90-140	-2.90
	MCHC (g mL^{-1})	34.21±10.39	33.55±6.62	26-35	-0.66
	MCH (fg)	29.47±8.29	28.90±5.58	33-47	-0.57
2	MCV (fL)	86.94±10.90	83.86±10.49	90-140	-3.08
	MCHC (g mL^{-1})	33.55±1.44	33.85±2.65	26-35	0.30
	MCH (fg)	29.09±3.60	28.33±3.69	33-47	-0.76
3	MCV (fL)	82.82±10.29	80.25±11.25	90-140	-2.57
	MCHC (g mL^{-1})	49.36±3.77	49.11±3.01	26-35	-0.25
	MCH (fg)	39.81±4.93	40.36±5.80	33-47	0.55

*25 (1993). **Difference between variables before and after transportation

Erythrocytes: Erythrocytes are one of the most abundant cell components in the blood. The main function of erythrocytes is to transport hemoglobin and carry oxygen from lungs to cells and carry carbon dioxide from cells to lungs[7]. Bijanti *et al*.[12] reported that another function of erythrocytes is to maintain the body's osmotic balance and to form ATP. Erythrocytes in poultry have an oval form and have cell nuclei and are size in larger than in mammals.

The results showed that the erythrocytes at all travel times were in the normal range. Across all time points, the number of erythrocytes before and after transportation increased because transportation occurred during the day with temperatures of approximately 33°C. Schalm[13] reported that increased erythrocytes are caused by a lack of oxygen (hypoxia state) during transportation. The state of hypoxia stimulates the formation of erythrocytes (erythropoiesis) characterized by the release of the hormone erythropoietin[14]. The erythropoietin hormone serves to stimulate erythropoiesis by triggering the production of proerythroblasts from hemopoietic cells in bone marrow[15]. Hoffband and Moss[16] found that hypoxia induces hypoxia-induced factors (HIF-2α and β) and stimulates erythropoietin production, resulting in the formation of new erythrocytes.

Hematocrit: The hematocrit value is the percentage of red blood cells in 100 mL of blood and the higher the hematocrit value, the higher the blood viscosity, so that friction between cells is also higher[7]. All hematocrit values in this study were in the normal range. Higher hematocrit values occurred in broiler chickens transported for 1 h. Aengwanich[17] stated that broiler chickens and other livestock transported at high temperatures will have higher blood viscosity, which slows blood flow in the capillaries and improves the heart. This deviation in hematocrit values has an important effect on the blood's ability to transport oxygen[18].

Hemoglobin: Hemoglobin (Hb) is an erythrocyte pigment that consists of 2 pairs of polypeptide chains and 4 heme groups containing an iron atom[12]. Heme is a protein derivative that contains Fe and binds O_2. Reece[5] found that hemoglobin fills one-third of the erythrocyte components after water and stroma. Hemoglobin carries oxygen to the tissues and secretes CO_2 from the tissues, producing the red color of blood cells[18].

The Hb levels of each group were in the normal range but increased slightly after transportation; this increase is in line with an increase in the amount of erythrocytes stimulated by hypoxia due to heat stress. An increase in hemoglobin levels leads to better oxygen-carrying capacity in cells and more efficient CO_2 excretion, resulting in better cellular function. Tian *et al*.[8] reported that age, species, environment, feed, presence or absence of erythrocyte damage and handling at the time of examination affect hemoglobin levels[8].

Table 4: Concentration of leukocytes before and after transport

Travel times (h)	Before transportation (10^3 cells mm^{-1})	After transportation (10^3 cells mm^{-1})	Normal range*	Delta**
1	9.59±3.49	9.69±5.89	12-30	0.10
2	14.40±11.60	16.18±5.75	12-30	1.78
3	11.95±6.83	14.89±4.18	12-30	2.94

*25 (1993). **Delta was the difference value between the variables before and after the transportation

Table 5: Percentages of heterophils, monocytes, lymphocytes, eosinophils and basophils before and after transport

Travel times (h)	Differentiation leukocytes (%)	Before transportation	After transportation	Normal range*	Delta**
1	Heterophils	30.78±19.34	28.64±15.53	9-56	-2.14
	Monocytes	1.86±1.96	1.71±1.07	0-30	-0.15
	Eosinophil	0.64±1.01	0.50±1.87	0-7	-0.14
	Basophil	-	-	0.4	-
	Lymphocytes	66.71±18.79	69.21±15.12	24-84	2.50
2	Heterophils	42.53±20.47	43.93±19.81	9-56	1.40
	Monocytes	3.40±2.35	3.60±2.47	0-30	0.20
	Eosinophil	0.80±0.86	0.87±1.13	0-7	0.07
	Basophil	-	-	0.4	-
	Lymphocytes	52.67±22.92	50.87±22.59	24-84	-1.80
3	Heterophils	30.60±11.80	48.40±15.64	9-56	17.80
	Monocytes	2.00±0.93	2.40±1.18	0-30	0.40
	Eosinophil	0.80±1.01	0.13±0.35	0-7	-0.67
	Basophil	-	-	0.4	-
	Lymphocytes	66.60±9.83	49.00±15.79	24-84	-17.60

*25 (1993). **Difference between variables before and after transportation

Erythrocyte index: Mean corpuscular volume (MCV) was used to measure the average volume of the red blood cells. Low MCV values indicate microcytic cells were small. Normal MCV values indicate normocytic cells were large[12]. The size of red blood cells is affected by age, ambient temperature and nutrients[15]. MCV values of each treatment were below the normal range. The red blood cells of broiler chickens were small and categorized as microcytic anemia.

Mean corpuscular hemoglobin concentration (MCHC) is the average concentration of hemoglobin in 1 erythrocyte cell or the mean ratio of Hb with erythrocyte volume. Red blood cells with normal hemoglobin concentrations are called normochromic and red blood cells with low hemoglobin concentrations are called hypochromic[13]. Broiler chickens transported across 3 h had high MCHC values greater than normal that decreased after transportation. During transport, especially under high temperatures in a hypoxic state, broilers produce red blood cells in the form of reticulocytes with a smaller volume in large quantities[7]. Thus, hemoglobin continues to be synthesized to form mature erythrocytes, resulting in an increase in MCHC values. After transportation, the chicken slowly returns to its former state, the production of hemoglobin decreases and MCHC values decrease.

The Mean corpuscular hemoglobin (MCH) value is the amount of hemoglobin in total erythrocytes[19]. The MCH value is an indicator of the color quantity of cells in the blood. Broiler chickens transported across 3 h had the highest MCH levels but they were in the normal range (39.81-40.36fg). This condition due to the MCHC value of broiler on 3 h travel times were also highest than the other treatments. Bashar *et al.*[20] suggested that small erythrocytes have low MCH values. Lower MCH values are due to the high transport temperatures (32.1-33.5°C). Heat stress results in the reduction of micro nutrient intake in livestock needed in the synthesis of hemoglobin and erythropoiesis[17]. Heat stress also increases the secretion of urine and feces, resulting in the breakdown of hemoglobin, especially the porphyrin portion into bilirubin (bile pigment).

White blood profile: The white blood profile observed in this study consisted of leukocytes concentration (Table 4). Leukocyte differentiation (heterophils, lymphocytes, monocytes, eosinophils and basophils) is presented in Table 5.

Leukocytes: The concentration of broiler leukocytes was below the normal range after 1 h of transportation; however, there was an increase in the number of leukocytes after transportation. The highest increase was found in chickens transported across 3 h (2.94×10^3 cells mm^{-1}) due to the high temperature. The high of chicken density in the baskets, as well as low air circulation during transport and transported across 3 h caused broiler chickens to experience the highest levels of heat stress and hypoxic state. This situation causes chickens to increase leukocytes production[7].

Table 6: H/L values of broiler chickens across different time points

Travel times (h)	Before transportation	After transportation	Normal*	Delta**
1	0.69±0.98	0.48±0.34	0.45-0.5	-0.21
2	1.29±1.24	1.24±0.99	0.45-0.5	-0.05
3	0.49±0.24	1.25±0.91	0.45-0.5	0.76

*25 (1993). **Difference between variables before and after transportation

Table 7: Blood glucose levels of broiler chickens across different time points

Travel times (h)	Blood glucose levels (mg dL^{-1})	Normal*
1	181.00±30.63	230-370
2	162.79±14.49	230-370
3	135.47±10.20	230-370

*Sulistyoningsih[27]

Leukocyte differentiation: Heterophils are mature leukocytes in blood circulation and play a role in responding to cell infection by phagocytosis[14]. The heterophil percentage was highest (17.80%) after transportation across 3 h because these broiler chickens experienced the highest levels of stress; this stress can stimulate the adrenal glands to release glucocorticoid hormones which induce the formation and release of young heterophils in the bone marrow, resulting in increased heterophils[21]. An increase in the number of heterophils occurs due to exposure from epinephrine and corticosteroids, as well as trauma and infection.

Monocytes are macrophage precursors, which play a role in forming non-specific immune responses through phagocytosis[22]. In this study, the percentage of monocytes in broiler chickens transported across time points was in the normal range[23].

Eosinophils are leukocytes cells that can prevent parasitic phagocytosis. The eosinophil percentage in this study was low (0.06-0.87%) but values were in the normal range, meaning that parasite infection rates were low. Sanitation of the maintenance environment was well preserved[16].

Basophils are a type of leukocyte that can change into mast cells. Basophils have many dark cytoplasmic granules covering the nucleus and contain heparin and histamine[16]. Heparin plays a very important role in preventing freezing in blood circulation. Histamine serves to overcome allergies that play a role in inflammation[24]. In this study, no basophils in the blood were observed; however, basophils may have been present in the blood at levels less than 1%.

Lymphocytes are a type of leukocytes that mediate the formation of a specific immune response through the formation of antibodies and cellular immunity[22]. The lymphocyte percentage of broiler chickens transported for 1, 2 or 3 h, both before and after transportation was within the normal range[23]. However, at 2 and 3 h there was a decrease in lymphocytes. The highest reduction occurred at the travel times of 3 h (17%). This reduction was likely due to nutrient deficiency (especially in Zn minerals) and stress conditions[25]. Under a state of stress, the cortisol hormone suppresses the growth of lymphoid organs[26].

Stress index: Table 6 shows the observations of stress index parameters included H/L values and Table 7 shows the blood glucose levels.

H/L value: H/L is an indication of stress, especially heat stress. Transportation across 3 h in this study caused the broiler chickens to experience stress (H/L = 0.49), with an H/L value of approximately 1.25 (Table 6). Stress can stimulate the adrenal glands to release glucocorticoid hormones and induce the formation and release of young heterophils in the bone marrow[21]. In addition, under chronic stress, the glucocorticoid hormone also inhibits heterophil to the tissues; otherwise, it encourages lymphocytes to the tissues, so that it decreases lymphocytes[17].

Blood glucose levels: According to Sulistyoningsih[27], broiler glucose level is 230-270 mg dL^{-1}. Table 7 shows that blood glucose levels at different travel times were below the normal range. The lowest blood glucose levels were found in chickens that traveled for 3 h. During transportation, broiler chickens were stressed and did not receive food. Without food, blood glucose levels decrease, since the chicken performs metabolic processes that require glucose as a source of energy.

If the state of stress continues or under chronic stress conditions, blood glucose levels may also increase. Virden and Kidd[28] suggested that stress stimulates the neurogenic system so the hypothalamus produces corticotrophin releasing factor (CRF), which in turn stimulates the anterior pituitary to release adrenocorticotropic hormone (ACTH)[28]. ACTH secretion causes adrenal cortex tissue cells to proliferate and secrete glucocorticoid hormones that play an important role in accelerating gluconeogenesis, the process of forming new sugars from non-glycogen compounds. Glucocorticoids hydrolyze proteins and convert them into amino acids or fats into fatty acids that are transported to the liver. In the liver, amino acids or fatty acids are converted into glucose, which increases blood glucose levels.

Table 8: Body weight reduction and mortality of broiler chickens across different time points

	Body weight (g/32)		Shrinkage		
Travel times (h)	Start	Final	(g/32)	(%)	Mortality (%)
1	1 819.33±158.72	1 748.67±119.57	70.67±54.70	3.93±2.96[a]	0
2	1 906.67±172.03	1 848.00±191.51	58.67±39.07	3.32±2.33[a]	0
3	1 539.33±105.79	1 487.33±98.16	52.00±31.21	3.50±2.15[a]	0

[a]$p>0.05$

Broiler chicken performance: Table 8 shows the chicken performance included body weight reduction and mortality.

Body weight: There was no significant decrease in body weight, although broiler chickens transported for 3 h experienced the greatest stress. Chickens do not have sweat glands. Sensible and insensible heat loss occurred during transport[29]. Sensible heat loss is the loss of body heat through radiation, conduction and convection, while insensible heat loss is the loss of body heat through panting, broiler chicken behavior that occurs under heat stress. Chickens try to reduce body temperature by evaporating water from the throat by panting (gular fluttering), a rapid oscillation movement of the mouth and throat[30]. This mechanism causes chicken body weight to decrease during transport.

Mortality: Death commonly occurs during transport due to temperature, wind and high basket density. Nijdam *et al.*[31] reported that basket density is one of the biggest factors that cause deaths during transport. Traditionally, transport is performed by placing the broiler chickens in a basket with size of $0.94\times0.58\times0.27$ m^3. Each basket is filled with 15-16 broiler chickens with an average weight of 1.4-1.9 kg. Estevez[32] stated that densities above 34-38 kg m^{-2} cause disturbances in the health and welfare of broiler chickens. The density of baskets in this study was quite high (41.27-52.27 kg m^{-2}) but there were no deaths observed during transport, indicating that transportation times up to 3 h are within the limit of broiler chicken tolerance. Factors affecting chickens mortality include high environmental temperature, high density and low air circulation as well as hunger and exhaustion.

Although there were no deaths or reductions in body weight found across transportation times, broiler chickens transported across 1 h exhibited physiological stress based on laboratory analysis. Thus, this traditional transportation method does not meet animal welfare standards. To resolve this problem, it is recommend 3 methods of transportation after harvest: 1) transportation should occur at night, 2) the chicken truck should be covered by shade during the day to avoid direct sunlight and 3) the density of chicken in the box should be reduced.

CONCLUSION

It is concluded that broiler chickens suffered hypoxia and microcytic anemia across all transportation time points evaluated. At a travel time of 3 h, broiler chickens at the age of harvest (35-40 days) experienced the highest stress level. Nevertheless, transportation up to 3 h did not significantly affect body weight or mortality.

SIGNIFICANCE STATEMENT

This study discovers the effects of transportation duration on broiler chicken physiology and performance factors. This study will help the researchers to uncover the critical area of animal handling and transportation that many researchers were not able to explore. Thus, a new theory on a good animal handling during transportation may be arrived at.

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