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Research Article

Water Spraying Prior to Transportation Reduces Transportation Stress and Maintain the Meat Quality of Broiler Chickens

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Abstract

Background and Objective: Transportation of broiler chickens triggers the stress responses and reduces the quality of meat. The aim of this study was to evaluate the effects of water spraying of broiler chickens prior to transportation on the hematological status and the quality of broiler meat. **Methodology:** The study was designed with a one-way completely randomized design using 45 female broilers aged 30 days. The broilers were divided into 3 treatment groups and each group consisted of 15 chickens. Treatment I (control) consisted of broilers chickens without water spraying and transportation. Treatment II consisted of broilers chickens without water spraying prior to transportation. Treatment III consisted of broilers chickens that were water sprayed prior to transportation. Experimental broilers chickens in Treatments II and III were transported using an open vehicle from 09.00 am-12.00 pm. **Results:** Transportation of experimental broilers chickens increased rectal temperature, erythrocyte level, leukocyte level, heterophile percentage and H/L ratio and decreased lymphocyte percentage, decreased meat pH, water holding capacity, cooking loss and meat tenderness ($p<0.01$). The water spraying of the broilers chickens shortly prior to transportation resulted in the same hematological status and meat quality as control broilers without water spraying and transportation ($p>0.05$). **Conclusion:** Transportation causes the experimental broilers suffer stress and water spraying of the broilers shortly prior to transportation minimizes the stress effect.

Key words: Broiler, transportation, water spraying, hematology, meat quality

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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

Broiler chicken is the main meat supplier in Indonesia. In 2017, 1848.1 t of broiler meat was produced, far higher compared to the other types of meat, like beef meat (531.8 t), buffalo meat (32.3 t), goat meat (70 t), lamb (54.9 t), pork (344.2 t) and non-broiler poultry meat (459.2 t)[1]. The contribution of broiler chicken has increased (1497.9 t) significantly in 2014. However, regardless of the high production level, the quality of broiler meats in Indonesia is substantially low.

Transportation of broiler chickens from farm to slaughtering locations is one of the aspects in broiler production that highly affects the quality of meat produced. During the production process, transportation is actually a short duration process. However, this short duration process often causes a big loss due to the stress triggered by the transportation process[2-4]. The losses come from the high mortality rates during transportation and the reduction of meat quality due to the stress[2,3,5,6].

There are several factors triggering the emergence of stress during transportation, including feed withdrawal, capturing process, loading and unloading process, handling process when moving from cage to boxes, poultry density in boxes, social disturbances, movement restrictions, heat radiation, wind, noise and vibration[7,8]. The effect of transportation is worsen by the fact that chickens do not have sweat glands and all parts of their body are covered with the feathers,[7,9-14] causing chickens suffering from acute heat stress during the transportation. As a result, glycogen is then broken down excessively and lactic acid is piled up, making the meat produced to have low pH, pale color, soft texture and watery[2,5,15,16]. Therefore, to maintain the normal quality of broiler meat during the transportation process, all factors causing the chicken to suffer from heat stress should be minimized.

Resting the chickens for 12-24 h after being transported has become a common strategy to help them recover from the heat stress[17]. However, this strategy is not practical because 12-24 h of resting after transportation is a long waiting time. Therefore, it is necessary to find the other strategies to reduce the danger of stress, so the chickens are ready to be slaughtered shortly after reaching the chicken slaughterhouse. One practice that has been widely used by farmers is water spraying of the chickens shortly prior to transportation to minimize the negative effects of solar radiation and heat load from the body metabolism of transported chickens. Heat loads in the bodies of transported chickens can be dissipated through the conduction process, i.e., the body heat is conducted to water on the surface of skin and feathers that further evaporated the water through evaporative process. However, scientifically, the practice effects on hematological status and meat quality of the chickens have not been identified. Therefore, this study was designed to evaluate the effects of water spraying prior to transportation on hematological parameters and meat qualities of transported chickens.

MATERIALS AND METHODS

Research location

Chicken rearing: The rearing process of experimental broiler chickens was conducted in Applied Laboratory of Faculty of Animal Science, University of Mataram in Lingsar Village, West Lombok Regency, using an open cage with litter. The chickens were kept in the cage from day old chick (DOC) age to 1 month old.

Treatment of transportation stress: The transportation treatment was started from teaching farm of Faculty of Animal Science. The experimental chickens were transported around West Lombok Regency and Central Lombok Regency for 3 h from 09.00 am until 12.00 pm.

Measurement of hematology variables: Measurements of hematology variables were performed at the Immunobiology Laboratory of the University of Mataram.

Measurement of meat quality: Meat quality measurement was carried out at the Laboratory of Animal Product Processing Technology, Faculty of Animal Science of the University of Mataram.

Experimental design: This study was designed using a one-way complete randomized design using 45 female broilers strain MB 202 aged 4 weeks. The experimental chickens were divided into 3 groups of treatment and each treatment group consisted of 15 chickens (each chicken acted as a repetition). Treatment I (control) consisted of chickens that were not water sprayed and transported. This group of experimental chickens was maintained in the cage without water spraying. Treatment II consisted of experimental chickens that were not water sprayed prior to transportation for 3 h. Treatment III, consisted of experimental chickens that were water sprayed prior to transportation for 3 h. Water spraying was conducted by spraying water until all the feathers were wet. Treatment groups II and III were transported using an open vehicle for 3 h.

Variables observed: The variables observed in this study were rectal temperatures, hematological parameters and meat quality.

Rectal temperature: Rectal temperatures of experimental chickens were conducted shortly after the completion of the transportation treatment. Measurements of rectal temperatures were conducted by inserting the digital thermometer through the anus and then read.

Shortly after finishing the measurement of rectal temperature, blood samples were taken through the wing veins using a 1 cc insulin syringe. The samples were then transferred into 5 mL EDTA tube. The hematological variables observed were (1) total erythrocytes, (2) hemoglobin concentrations, (3) hematocrit, (4) total leucocytes, (5) leukocyte differentiation (percentage of heterophile, eosinophil, basophils, monocytes and lymphocytes) and H/L ratio. Measurement of the hematological variables referred to the method summarized by Kolmer *et al.*[18] as used by Tamzil *et al.*[19].

Erythrocyte concentrations: Measurements of erythrocyte concentrations were performed using the Count Rooms method[18] as was used by Tamzil *et al.*[19]. Twenty microliters of blood mixed with EDTA was put into 4000 µL Hayem solution using a micropipette. This blood mixture was then rinsed, mixed thoroughly and incubated for two minutes. The mixture was then placed in the Improved Neubauer count chamber. The number of erythrocytes was calculated from five erythrocyte squares with 40x enlargement of objective lens. The number of erythrocytes was calculated by multiplying the number of counted erythrocytes by 10,000 mm^3.

Hemoglobin concentrations: The hemoglobin concentrations were determined using spectrophotometer method[18] as used by Tamzil *et al.*[19]. Twenty microliters of blood sample mixed with EDTA was added to Drabkin solution using a micropipette. The mixture was rinsed and mixed thoroughly and was incubated for 3 min. Absorbance was then read at 540 nm wavelengths using a spectrophotometer (UV Visible). Hemoglobin concentration was calculated by multiplying the absorbance by a factor of g dL^{-1}.

Hematocrit: Measurements of hematocrit values were performed using the Microhematocrit method[18] as used by Tamzil *et al.*[19]. Blood sample was inserted into a microhematocrit tube and the bottom of the tube was closed using wax. The tube was placed in a hematocrit centrifuge (Hettick) and centrifuged at 15000 rpm for 5 min. The percentage of blood could be read using a hematocrit measuring instrument.

Leukocytes: The total number of leukocytes was calculated using the Counting chamber method[18], as used by Tamzil *et al.*[19]. A total of 380 µL of Turk solution was put into a glass tube using a micropipette. Twenty microliters of blood sample mixed with EDTA was transferred into a Turk solution using a micropipette, then rinsed and mixed thoroughly and further incubated for 2 min. The mixture was placed in the Improved Neubauer count chamber and leucocytes were calculated from four planes using 10x enlargement of objective lens. The number of leukocytes was determined by multiplying the number of leukocytes by 50 mm^3.

Leukocyte differentiation: The value of leukocyte differentiation was calculated using the Rapid method[18] as used by Tamzil *et al.*[19]. Five microliters of blood sample was transferred using a micropipette on the tip of the glass object and the blood was led to stick and spread on the edge of the sliding glass. The blood was spread with a slope of 35°. The preparation was then dried and fixed with methanol. After that, the preparation was stained using eosin for 20-30 sec. Then, the preparation was stained again with eosin for 15-30 sec. Then, the stained preparation was rinsed using flowing water until clean and then it was dried. The preparations can be read under a microscope using emersion oil. The percentage of each leukocyte cell was calculated using hand counter.

Meat pH: Meat pH in this study was measured by following the procedure used by Tamzil *et al.*[4]. Ten grams of breast meat of experimental broiler chicken was put into a 100 mL cup glass. Ten milliliters of distilled water was added into the glass. The mixture was stirred and then stored for 2 h at 5°C. After that, the mixture was equilibrated at 23°C. The pH of the mixture was measured by using digital pH meter.

Water holding capacity: Water holding capacity of broiler meat was measured using a method summarized by Soeparno[20] and used by Tamzil *et al.*[4] Three hundred milligrams (0.3 g) of the meat sample was placed under a load of 35 kg on a filter paper placed between two glass plates for 5 min. The pressing result was drawn on a transparent plastic. The wet area outside the pressed meat was measured with the assistance of millimeter block paper (expressed in cm^2). The water load of the meat (mg) is expressed by the following equation:

$$\text{mg } H_2O = \frac{\text{wet area } (cm^2)}{0.0948} - 8.0$$

$$\text{Water holding capacity} = \text{Total water percentage} (\%) - \frac{\text{mg } H_2O \times 100\%}{300 \text{ mg}}$$

Cooking loss: The cooking loss data were determined using a method summarized by Soeparno[20] used by Tamzil *et al*.[4]. Ten grams of sample (X) was put inside a plastic bag. The sample was boiled for 1 h at 80°C. After that, the sample was removed from the plastic bag and separated from the broth. The sample was then wiped with tissue papers without being pressed. Lastly, it was weighed (Y). Cooking loss was determined using the following equation:

$$\frac{X-Y}{x}100\%$$

Meat tenderness: Meat tenderness in this study was measured by using a method used by Tamzil *et al*.[4]. Meat samples of broiler chicken with the sizes of 1 cm^3 were placed at the bottom of a penetrometer. The pointer was set so that the surface of the meat met the pointer tip and the pointer was at zero position. A load weighing 50 g (a) were released at the same time with the timer was pressed for 10 sec. The depth of the pointer was seen on the penetrometer scale (b). The tenderness of the meat was calculated by:

$$\frac{b}{a}/t\,(mm/dt)$$

Statistical analysis: The data obtained were tabulated and analyzed using the Analysis of Variance, LSMEAN Advanced Test and GLM procedures using SAS[21] software.

RESULTS AND DISCUSSION

The effects of water spraying of the broiler chickens prior to transportation on rectal temperature and hematological status are presented in Table 1. It can be seen that the body temperatures of all the treatment groups before transportation did not show any difference ($p>0.05$). The body temperatures of the experimental broiler chickens prior to transportation ranged from 40.5-41.5°C[22]. However, after transportation, the body temperature increased significantly. The increased body temperatures during transportation were caused by the decreased heat dissipation from the broilers' body to the environment. Accumulation of body heat loads from body metabolic process and the solar radiation eventually increases body temperature. The ambient temperature during the 3 h transportation ranged from 29.8-36.7°C. This range is in the range of ambient temperature when Tamzil *et al*.[4] conducted a study on broiler transportation (29.9-36.4°C). Both of the ambient temperatures could be categorized as a critical temperature for poultries, as the comfortable temperature for poultries ranges from 26-27°C[23]. The increase in body temperatures of broiler chickens during transportation was reported by Tamzil *et al*.[4], who found that body temperature of stressed broiler increased from 41.29-43.17°C. During the transportation process several factors are considered as stressors like capturing process, moving chickens into the box, intense crowd in the box, wind and heat stress[3,8].

Table 1: Effect of water spraying prior to transportation on hematological status and meat quality of broiler chickens

	Treatment			
Variables	I	II	III	p-value
Rectal temperature (°C)				
Before transportation	40.970	40.460	40.570	<0.0954
After transportation	-	41.060^a	40.940^b	<0.0001
Hematological status				
Erythrocyte ($\times 10^6$ mm^{-3})	5.572^a	6.607^b	5.809^a	<0.0001
Hemoglobin (%)	8.854	9.108	9.042	<0.071
Hematocrit value (%)	29.067	29.667	29.061	<0.08
Leukocyte ($\times 10^3$ mm^{-3})	34.060^a	31.758^b	33.980^a	<0.0002
Leukocyte differentiation				
Heterophile (%)	31.170^a	33.010^b	32.100^a	<0.0001
Eosinophil (%)	0.300	0.280	0.300	<0.08
Basophil (%)	-	-	-	-
Lymphocyte (%)	68.030^a	65.670^b	66.400^a	<0.0001
Monocyte (%)	0.500	1.040	1.200	<0.06
Ratio H/L	0.460^a	0.500^b	0.480^a	<0.0001
Meat quality				
pH	6.527^a	6.240^b	6.340^b	<0.0001
Water holding capacity (%)	14.430^a	12.629^b	13.059^a	<0.0001
Cooking loss (%)	0.450^a	0.413^b	0.425^a	<0.0001
Tenderness	2.631^a	2.420^b	2.533^a	<0.0401

Different subscripts in the same row indicate a significant difference ($p<0.01$). I: Control treatment (no water spraying and transportation), II: No water spraying prior to transportation, III: Water sprayed prior to transportation

Thus, it can be concluded that the transportation process causes stress in broiler chickens. It was also observed that the water spraying of broiler chickens shortly prior to transportation could lower the rectal temperature ($p<0.01$). The decreased rectal temperature in water sprayed broilers was caused by the increased heat dissipation from the body. This result indicates that water spraying of experimental broiler chickens shortly prior to transportation decreases the effects of transportation stress.

The data in Table 1 also display that the transportation process increased erythrocyte and decreased leukocyte concentrations ($p<0.01$). However, the treatment did not affect hemoglobin and hematocrit parameters ($p>0.05$). The increase in erythrocyte concentrations was caused by the stress during transportation. The stress was triggered by the deprivation of oxygen (hypoxic state) during transportation due to heat stress and the crowded condition in the broiler boxes during transportation. Hypoxic state stimulates erythropoiesis process and the release of erythropoietin hormone (a hormone that serves to stimulate erythropoiesis) by stimulating the pro-erythroblast production from hematopoietic cells in the bone marrow[24]. Hypoxia induces hypoxia-induced factors (HIF-2α and β) and stimulates the production of erythropoietin to produce new erythrocytes[25].

However, Table 1 also shows that the watering treatment can actually maintain blood erythrocyte levels. Hypoxic condition is eliminated by the presence of water covering the body of broiler chicken. Water could act as a conductor of heat release to the air. This means that during the transportation, the broilers do not experience hypoxic because of solar radiation and heat produced from metabolic process. Thus, the water spraying treatment can maintain erythrocyte level in the blood of the broiler during the 3 h of transportation in a hot temperature.

The data in Table 1 show that the 3 h transportation decreases the blood leukocyte concentrations ($p<0.01$). However, when the broiler chickens were water sprayed shortly prior to transportation the decrease in leukocyte concentration could be eliminated ($p>0.05$). The decrease in leukocyte concentrations in broiler chickens without water spraying prior to transportation is caused by the increases in glucocorticoids hormones in plasma[3,14,19] that decreases leukocyte concentrations[26]. When the experimental broiler chickens were water sprayed prior to transportation, leukocyte concentrations could be brought back to normal level due to the decreased heat stress condition. The decreased heat stress condition in water-sprayed broiler chickens prior to transportation is caused by the increased body heat dissipation by the water covering the body and feathers. The body heat load is released through conduction process using water sprayed on the surface of the broilers' skin and feathers.

The data in Table 1 show that the transportation affects the percentage of heterophile and lymphocytes as well as the H/L ratio ($p<0.01$). However, water spraying of the experimental broiler chickens shortly prior to transportation restored the percentage of heterophile and lymphocytes and the H/L ratio to normal levels similar to control broiler chickens without transportation ($p>0.05$). The increase in heterophile percentage and the decrease in lymphocytes percentage cause the increase in H/L ratio. The effects of transportation on these parameters are caused by the increase in heat stress that eventually triggers the synthesis and secretion of glucocorticoid hormone[3,4,14,27]. Zhang *et al.*[2] reported that transportation stress can stimulate the secretion of glucocorticoid in the plasma. A study using three different types of chickens (kampong chicken, Arabic chicken and laying chicken) by Tamzil *et al.*[14] and Kolmer *et al.*[18] found that there was a positive correlation between corticosteroid hormones and heterophile percentage and H/L ratio both with r-value of 0.9, where as the correlation between corticosteroid and lymphocyte percentage was negative (f = -0.9). A similar study in goats also identify that goats exposed to transportation stress have higher blood cortisol concentrations compared to control goats without transportation stress[17]. The differences in leukocyte, lymphocyte and H/L ratio are caused by the decrease in body temperature to a more comfort zone in broiler chickens water sprayed prior to transportation. The decrease in body temperature to a more comfort range is caused by the increase in body-heat dissipation through the conduction and evaporation of water sprayed prior to transportation.

Table 1 shows the effects of the transportation on the meat quality of broiler chicken. Transportation decreased pH, water holding capacity and cooking loss ($p<0.01$) as well as meat tenderness ($p<0.05$). This observation confirms that transportation triggers stress in broiler chickens. Zulkifli *et al.*[7] and Tamzil[8] identified that the source of stress during transportation was the process of catching, loading and unloading into the truck, handling in boxes, social disturbances, restrictions on movement, heat radiation, wind, noise and vibrations. Poultries suffering from stress in the body will experience break down of muscle glycogen and buildup of lactic acid and will subsequently produce meat with a low pH, pale color, soft and watery[2,5].

The water-spraying treatment given in this study could minimize the effects of heat stress on meat quality, as was shown by pH, water holding capacity, cooking loss and tenderness of the meats of water-sprayed broiler chickens prior to transportation similar to those of control broiler chickens. Water spraying prior to transportation minimizes the influence of stressor, especially the influence of solar radiation and heat produced from chicken metabolic process. The sprayed water acts as a conductor for body-heat dissipation to the environment. The result is the maintenance of body temperature in a comfortable condition that is similar to the condition of control broiler chickens without transportation. As a result, meat quality of the water-sprayed broiler chickens prior to transportation was similar with those of control broiler chickens without transportation. The results of this study confirm that transportation reduces the quality of meat and the negative effects of transportation stress can be minimized by water spraying of broilers chickens shortly prior to transportation.

CONCLUSION

It can be concluded that transportation process causes the broiler to suffer from stress. Transportation increased body temperature, erythrocyte content, leukocyte, heterophile percentage and H/L ratio as well as decreased percentage of lymphocyte, meat pH, water holding capacity, cooking loss and meat tenderness ($p<0.01$). Water spraying of broiler chickens shortly prior to transportation can minimize the effect of transportation stress. The water spraying treatment gives similar hematological and meat quality conditions to the control meat ($p>0.05$). Therefore, to minimize the effect of transportation stress on meat quality, it is advisable to give water spraying prior to transportation.

SIGNIFICANCE STATEMENT

The study found that water spraying treatment shortly prior to transportation can reduce the negative effects of transportation stress. The hematological status and the meat quality of water-sprayed broiler chickens shortly prior to transportation were relatively similar to those of control broiler chicken without transportation. Therefore, the results of this study can be used as a practical guide to improve the decreased quality of meat due to transportation stress.

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