Rest Before Slaughtering Alleviates Transportation Stress and Improves Meat Quality in Broiler Chickens

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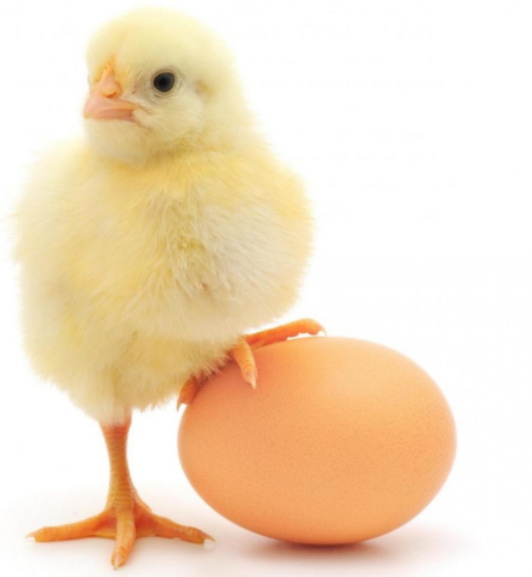

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Research Article
Rest Before Slaughtering Alleviates Transportation Stress and Improves Meat Quality in Broiler Chickens

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Abstract

Background and Objectives: Transportation of broilers generally induces stress to the birds and may lead to decrease meat quality. The study aimed to determine the effects of resting time given to the broilers after being transported prior to slaughtering on meat quality. **Materials and Methods:** A Completely Randomized Design was laid out using 60 female broiler chickens, aged 33 days. The broilers were divided into three groups, in which every group was given different treatments. Each group consisted of 20 female chickens as replicates. Group I (control) was not transported. Group II was transported for 3 h and after that immediately slaughtered. Group III was transported for 3 h and then slaughtered after being rested for 12 h. Shortly before and after the broilers were transported, their rectal temperature was measured and blood was taken for hematology test. Variables observed were body temperature, erythrocyte total number, hemoglobin levels, hematocrit values, total number of leukocytes, leukocyte differentiation, H/L ratio, mortality rate and meat quality. Data obtained were analyzed using Variant Analysis and LSMEAN. **Results:** Three hour-long transportation increased the rectal temperature, erythrocyte level, leukocyte level, heterophile percentage, H/L ratio, mortality rate and meat pH and decreased lymphocyte percentage, water holding capacity and cooking loss ($p<0.01$). A 12 h resting time after transportation, however, reduced the adverse effect of transportation stress on meat quality. **Conclusion:** The lower quality of meat owing to transportation stress can be alleviated by giving the birds rest for 12 h after transportation and prior to slaughtering.

Key words: Hematology, meat pH, meat quality, resting time, transportation stress

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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

According to Kementan[1], poultry meat production in Indonesia during 2018 reached 2,302,580 t. Broiler meat accounted for 80%, of this and the remaining came from domestic chicken meat (12.86%), laying chicken meat (4.95%), duck meat (1.87%), quail meat (0.04%) and pigeon meat (0.01%)[1]. What drives this high proportion of broiler meat production is its high demand. Broiler meat production, however, faces various challenges. One of them is associated with the transport of the birds to the slaughter houses. The window available for transporting is usually very short, especially in comparison to other processes such as the preparation of housing facilities and the process of chicken production. The transport process often causes considerable bird mortality, besides a decrease in the quality of meat.

In Indonesia, especially in Lombok Island, broilers are transported conventionally using trucks and even using motor cycles. These conventional modes of transportation causes great discomfort to the chickens and can act as a potential stressor that harms the broiler industry. Some studies[2-5] have stated that the trigger factors for the emergence of stress during transportation are the methods employed for catching the birds, loading and unloading into the trucks, handling when transporting from cages to boxes, density of broilers in boxes, social disturbances, motion restrictions, heat radiation, wind-blows, noise and vibration. All these stressors affect the physiology of the broilers simultaneously, causing them to suffer acute stress during the transportat.

Broilers are homeothermic animal meaning their body temperatures range from 40.5-41.5 °C that do not have sweat glands and almost all parts of their body are covered with fur[6]. In a high-temperature environment, broilers find it difficult to release their body heat, triggering heat stress[7-10]. In addition, broilers are classified as poultry which are nervous and requires special treatment during the production and post-production period; otherwise it will cause broilers to feel unsafe and disturb the homeostasis process in the body[11]. Therefore, broilers must be maintained in a thermo-neutral zone with careful handling that does not disturb the physiological processes of the birds. When the broilers are maintained under above thermo-neutral conditions and handled roughly, they will suffer from stress, which will cause glycogen breakdown in the muscles and lactic acid buildup, producing meat that is high in pH, pale, soft and exudative (PSE) after slaughtering[12].

However, poultries suffering from stress will try to restore their homeostasis states to the condition before the stress by activating the neurogenic and nerves systems[13]. This will occur automatically when the poultries are rested after experiencing acute stress[14]. This study was conducted to determine the effect of giving rest to the animals after transportation on several hematological variables and their effect on meat quality of broiler chickens.

MATERIALS AND METHODS

Location: Rearing of the chickens and acute transportation stress induction were conducted at Teaching Farm. Hematological analysis was done at the Laboratory of Immunology whereas meat quality analysis was conducted at the Laboratory of Technology of Animal Husbandry Processing the farm and laboratories; all facilities belonged to the University of Mataram.

Experimental design: The study was conducted using a Completely Randomized Design using 60 female broiler chickens, aged 33 days. The chickens were categorized into three different treatment groups in which every group was given different treatments. Each group consisted of 20 female chickens as replicates. Group I was used as a control group, which was not transported. Group II was transported for 3 h and then immediately slaughtered. Group III was transported for 3 h like group II but was given a resting time for 12 h before they were slaughtered.

Treatment: Chickens of group I were put in a cage. Chickens of group II and III were transported for 3 h starting from 9 to 12 am. After being transported, rectal temperatures of chickens from group II were measured directly by inserting a digital thermometer into the rectum. Blood sample for hematological variables was collected from the wing vein using a 1 cc insulin syringe and then slaughtered. The chickens from group III were given rest for 12 h and slaughtered. Shortly before slaughtering, rectal temperature was measured and blood sample was sampled as before.

Sampling for meat quality test: All broilers from treatment groups were slaughtered by cutting the respiratory tract and digestive tracts in the neck area. After that, the experimental broilers were put into hot water so that their feathers could be pulled out easily. Further, the legs and heads of the broilers were cut and the offal of the broilers were removed as well. For the quality of meat test, the left side of breast meat was used.

Variables observed:

- **The percentage of mortality:** The mortality rate was determined by calculating the percentage of chickens died after given the transport treatment
- **Body temperature (rectal):** Rectal temperature measurement was carried out shortly before the chickens were transported and before they were slaughtered
- **Hematological variables:** The blood was mixed with EDTA in a 5 mL tube and the total number of erythrocytes, hemoglobin concentration, hematocrit value, total number of leukocytes, differential count of leukocyte (percentage of heterophils, eosinophils, basophils, monocytes and lymphocytes) and H/L ratio were monitored following the methods of Kolmer *et al.*[15]

Erythrocytes: The erythrocyte test was carried out using counting chambers method[15]. For this, 20 µL of EDTA-blood sample was mixed with 4000 µL Hayem solution using a micropipette. The solution was subsequently rinsed, mixed well and placed in an incubator for 2 min, following which it was inserted into an improved counting chamber. The total number of erythrocytes was calculated by counting erythrocytes appeared on five areas of erythrocyte boxes using an objective lens magnified 40 times. The total number of erythrocyte was obtained from the multiplication result of the number of erythrocyte with 10000 mm^3.

Hemoglobin: The concentration of hemoglobin was determined using the Spectrophotometer method[15]. A total of 20 µL of blood containing EDTA was mixed with Drabkin solution until even in a tube. After that the mixture was rinsed and then placed in an incubator for 3 min. UV Visible at wavelength of 540 nm was used to measure the absorbance of the solution the real hemoglobin level was obtained from the multiplication of the absorbance with a factor (g dL^{-1}).

Hematocrit: Micro-hematocrit method[15] was used to quantify hematocrit values. A micro-hematocrit tube with wax-sealed bottom was used to place blood sample. The tube was then centrifuged for 5 min at 15000 rpm in a hematocrit centrifuge (Hettick). The blood percentage was obtained with the help of a hematocrit measuring instrument.

Leukocytes: Leukocytes was determined using counting chamber method[15]. A total of 20 µL of blood containing EDTA was mixed with 380 µL Turk solution. The mixture was subsequently rinsed, mixed evenly and then placed in an incubator for 2 min. After that, the mixture was put into the Improved Neubauer's counting chamber. The real leukocyte number was obtained from the multiplication of number of leukocytes with 50 mm^3.

Differential count of leukocyte: Rapid method[15] was used to determine differential count of leukocyte. "Blood sample of 5 mL was carefully dropped using a micropipette onto the end of a glass object until the blood stick. The blood was spread to the edge of the glass slider. The blood was removed with an inclination of 35°. After that, the preparation was dried and fixed with methanol. In the next step the preparation was colored using eosin (color 1) for 20-30 sec. The preparation was then colored for the second time for 15-30 sec. The preparation was subsequently rinsed with running water until the smear clean and dried. The preparation was read under a microscope with the help of emersion oil. The percentage of each differential leukocyte cell was calculated with the help of a hand counter.

- **Measurement of meat quality variables**

Meat pH: Tamzil *et al.*[3] method was used to measure meat pH. A total of 10 mL distilled water was poured into a 100 mL beaker containing 10 g of broiler breast meat. The meat and water were stirred and kept at a temperature of 5°C for 2 h. The solution was then equilibrated at a temperature of 23°C. The measurement of pH was performed using a digital pH meter".

Water holding capacity: A method summarized by Soeparno[16] was used to quantify water holding capacity. About 0.3 g of broiler meat on a glass plate was covered with a filter paper. After that, another glass plate was placed on the top of the filter paper. This system was pressed for 5 min using a load weighing 35 kg. The result of the pressed sample was drawn on a transparent plastic. Wet area outside the sample was determined using a graph paper (cm^2). Water holding capacity (mg) was calculated using the following equation:

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

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

Cooking loss: Cooking loss was determined using the method of Tamzil *et al.*[3] which was extracted from Soeparno[16]. Five grams sample (x) in a plastic bag was cooked at a temperature of 80°C for 1 h. After being taken out of from the bag, the

sample was separated from its broth. Tissues were then used to swab the sample gently. Weight of the sample (Y) was determined using a scale. Cooking loss was measured with the following equation:

$$\frac{x-y}{x}\times 100$$

Tenderness: Tenderness test was determined using penetrometer method as used by Tamzil *et al.*[3]. 1 cm^3 of meat sample was placed under the end of a penetrometer needle. This set the needle to 0 position. At the same time, 50 g load (a) was released with a stopwatch for 10 sec. The depth of the needle was shown by penetrometer scale (b). The meat tenderness was determined using the following equation:

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

Statistical analysis: Analysis of Variance and LSMEAN with General Linear Model procedures of SAS software[17] were used to tabulate and analyze data obtained during the study.

RESULTS AND DISCUSSIONS

A comparison of the data in Table 1 shows that transportation treatment significantly altered the hematological variables and meat quality parameters of broiler chickens. Transportation increased body temperatures of the broiler chickens by about 4°C. The rectal temperature of the broilers before transportation ranged between 40.85 and 40.96°C, which increased to 45.12°C, post-transport (above normal body temperature which ranged from 40.5-41.5°C)[6]. This is because of the acute stress experienced during the transport[4] triggered by heat exposure from the sun as well as from other stressors. Being homeothermic animals, broiler chickens are sensitive to acute stress, especially when the ambient temperature is above the thermo-neutral temperature range of 20-25°C[18,19].

Erythrocytes levels also increased after transport ($p<0.01$). The increase of erythrocytes level is because during the transportation, the birds experience hypoxia (lack of oxygen), which leads to the release of erythropoietin hormone that is responsible for the formation of erythrocytes[20]. Erythropoietin stimulates erythropoiesis by stimulating pro-erythroblast production of hemopoietic cells in the bone marrow[21]. Through this mechanism, hypoxia 2α and β (HIF-2α and β) stimulate erythropoietin production[22].

Table 1: The effect of the transportation process and resting time treatments on rectal temperature, mortality rate, homeostasis condition and quality of broiler meat

	Treatments			
Variables	I	II	III	p-value
Rectal temperature				
• Before transportation (°C)	40.96	40.85	40.94	
• After transportation (°C)	-	45.12	40.94	<0.0001
Mortality rate (%)	0.00	40.00	33.00	
Erythrocyte ($\times 10^6$/mm^3)	4.24^a	2.67^b	2.82^b	<0.0010
Hemoglobin (Hb) (g dL^{-1})	8.30	8.55	8.24	0.5210
Hematocrit value (%)	43.84	47.85	48.40	0.0634
Leukocyte ($\times 10^3$/mm^3)	16.40^a	22.50^b	19.60^b	0.0001
Leukocyte differentiation				
• Heterophils (%)	30.60^a	48.40^b	36.85^a	<0.0040
• Lymphocyte (%)	65.51^a	45.56^b	50.655^a	<0.0020
• Monocyte (%)	5.58	1.67	1.430	0.0530
• Eosinophils (%)	2.41	1.21	1.850	0.0410
• Basophiles (%)	0.00	0.00	0.000	-
• H/L ratio	0.47^a	1.06^b	0.86^a	<0.0030
Meat quality				
• pH	4.62	4.72	4.98	0.0570
• Water holding capacity	14.89^a	7.89^b	7.88^b	<0.0001
• Tenderness	2.40	2.25	2.80	0.4700
• Cooking loss	38.26^a	30.60^b	38.07^a	<0.0010

I: Control group (no treatment), II: Given transportation treatment and immediately slaughtered, III: Given transportation treatment and slaughtered after resting for 12 h, Different superscripts on the same line show a significant difference ($p<0.01$)

Transportation also increased the leukocyte concentration (Table 1; $p<0.05$). During the transport process, the birds are exposed to direct sunlight and the corresponding temperature range of 25.9-33.6°C is generally above the optimum temperature range for broiler chicken (20-25°C)[10,18,19]. The heat stress which is exacerbated by the other stressors happening during the transportation process, in turn, triggers changes in leukocyte level and the percentage of lymphocytes and heterophils[2,8]. Stress in chickens is characterized by changes in the leukocyte's differentiation[18,19]. This happens because stress increases the levels of glucocorticoid hormones, especially corticosterone[22,19]. An earlier study of acute stress on three types of chickens (Kampong, Arabic and Commercial chickens) showed an increase of corticosterone levels and H/L ratio[10,23]. When the stress continues, broilers may experience exhaustion, which is the most dangerous phase of stress[24]. This is the reason why transportation caused high mortality in groups II (40%) and III (33%). In view of this, it is recommended not to transport broilers using conventional methods when the sun is hot (i.e., between 9 am and 12 noon).

The data in Table 1 also show that body temperature of the broilers that were transported, when given rest period of 12 h, were back to normal (40.5-41.5°C)[6]. It can also be seen that the erythrocyte, leukocyte, heterophile and lymphocyte

counts after the resting treatment of 12 h after transportation were comparable to that of the broilers from the control group. Apparently, the 12 h resting period is enough to restore the homeostatic conditions characterized by the decrease of plasma CORT levels and glycolysis muscle, which in the end can improve meat quality[11].

The transportation treatment also increased the meat pH and decreased the water holding capacity and cooking loss of meat (Table 1; $p<0.01$). This is because during the transportation process, the birds are captured, loaded and unloaded into the trucks and handled when moved from cages to boxes, which are major stressors. In addition, the high density of the livestock in the boxes, motion restrictions, social disturbances, winds, vehicular motion, collisions, heat radiation, shocks, lack of feed and drinking water and noise levels further exacerbate the stress level of the birds[2-5,25]. These conditions cause disrupted homeostasis[24], which directly affects the metabolic conditions of the broilers and when they are slaughtered, affects muscle postmortem metabolism and meat quality[26].

In stressed chicken bodies, glycolytic processes and ATP hydrolysis will set in[11,12]. After slaughtering, the muscle glycogen of chickens will experience glycolysis enzymatically and produce lactic acid, which triggers changes in the pH of the meat, causing meat to become pale, soft and exudative (PSE)[12]. According to Fanatico *et al.*[27], the increase in meat pH value has a negative effect on meat storability. High pH provides a good environment for meat-destroying bacteria[22,26]. It follows from the above that the transport treatment causes broiler chickens to suffer from stress leading to poor quality of the meat.

However, the broilers that were rested for 12 h after transportation were able to recover the pre-transport hematological conditions and meat quality, as evident from the lower water holding capacity, cooking loss and meat pH (Table 1; $p<0.01$). This is because rest causes normalization of homeostasis in the body[24], by activating the neurogenic system and nerves[13], which reduces the accumulation of lactic acid in the muscles. As a result, the meat obtained when the broilers are slaughtered after an appropriate post-transport period is of good quality with low pH, pale color, soft and exudative (PSE)[12]. Thus giving a post-transport rest for 12 h for the broilers minimizes the transportation stress effect on meat quality.

CONCLUSION

The transport treatment caused stress to the broilers. Indeed, a 3 h long transportation process increased the erythrocyte levels, leukocyte level, rectal temperature, heterophile percentage, mortality, H/L ratio and meat pH and decreased the percentage of lymphocytes, the value of water holding capacity and cooking loss. Giving the broilers a resting time of 12 h after transportation, however, restored the hematological conditions and improved the quality of broiler meat. The results of this study thus clearly highlight that giving a 12 h rest period post-transportation represents an appropriate low cost technology to maintain the quality of broiler chicken meat.

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SIGNIFICANCE STATEMENT

The results of this study found that giving a 12 h of resting period after transportation could improve the quality of broiler meat which decreased due to the effects of transportation stress. Therefore the results of this study can act as appropriate technology to maintain the quality of broiler chicken which decreases due to transportation stress.

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