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Hematological Response of Chickens with Different Heat Shock Protein 70 Genotypes to Acute Heat Stress

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Abstract: Hematological responses in chicken with different HSP 70 genotypes to acute heat stress were studied using 28 kampong chickens, 24 Arabic chickens and 4 commercial chickens. The experimental chickens were selected randomly from a group of chickens with HSP 70 genotypes identified and were exposed to ambient temperature (40°C) for 0.5, 1.0 and 1.5 h. Results showed that erythrocyte, hematocrit and leukocyte in all chicken lines decreased in response to acute heat stress with the highest decrease in commercial chickens, followed by Arabic and kampong chickens. Regardless of acute heat stress exposure, there was no significant difference in erythrocyte, hemoglobin, leukocyte, heterophil, eosinophil, basophil, lymphocyte, monocyte and heterophil/lymphocyte ratio in all chicken lines studied. Arabic and commercial chickens had lower hematocrit as compared to kampong chickens. However, acute heat stress increased the percentage of heterophil, basophil, lymphocyte and heterophil/lymphocyte ratio without affecting eosinophil and monocyte. It was found that there was no interaction between lines of chicken and acute heat-stress exposure on the hematological parameters measured. The lowest percentage of lymphocytes was found in chickens with DD HSP 70 genotype while the highest was found in chickens with AD genotype. The results indicated that there was a relationship of heat resistance or tolerance to lymphocyte expression. Chickens that were the most tolerant to acute heat stress had the highest lymphocyte percentage (AD genotype) whereas those that were the least tolerant had the lowest lymphocyte percentage (DD genotype).

Key words: HSP 70 gene, acute heat stress, kampong chicken, Arabic chicken, hematology

INTRODUCTION

Kampong chicken is Indonesian native chicken which is raised for meat and egg production locally. This local chicken line survive well in traditional management system and has been a significant contribution to food security in rural areas. The weakness of kampong chicken is its slow growth rate and low egg production potential (Nishida *et al*., 1980; Iskandar *et al*., 2000). To meet the needs and requirement of chicken meat and eggs consumption in Indonesia, Arabic and commercial chicken (broiler and layer) were introduced. The three lines of chicken have different development and domestication backgrounds and have different physiological capacities to respond to various breeding environments. Kampong chicken is a descendant of the red jungle fowl (*Gallus gallus*) which is domesticated in South East Asia including Indonesia (Fumihito *et al*., 1996; Sartika and Iskandar, 2007; Sulandari *et al*., 2007a,b). Arabic chicken is a local layer hen coming from overseas and is a descendant of braekel kriel silver and braekel kriel gold chickens (European local chicken) (Sulandari *et al*., 2007a,b; Sartika and Iskandar, 2007), where as commercial chicken (broiler and layer) is a layer type developed in sub-temperate and cold climate regions.

Chicken is warm-blooded animal (*homeothermic*) with body temperature ranges between 40.5-41.5°C (Etches *et al*., 2008). A characteristic of poultry is it does not have sweat glands and almost all parts of its body are covered by feathers. These biological characteristics make poultry raised in warm areas susceptible to heat stress (Cooper and Washburn, 1998; Lin *et al*., 2005: Al-Fataftah and Abu-Dieyeh, 2007; Al-Ghamdi, 2008; Al-Aqil and Zulkifli, 2009; Zulkifli *et al*., 2009; Ajakaiye *et al*., 2010). Stress in poultry can decrease erythrocyte count (Toghyani *et al*., 2006), hemoglobin concentration (Hilman *et al*., 2000; Puvadolpirod and Thaxton, 2000), hematocrit (Coles, 1982; Altan *et al*., 2000; Zulkifli *et al*., 2009), percentages of leukocytes (Nathan *et al*., 1976; Mashaly *et al*., 2004) and leukocyte differentiation (Altan *et al*., 2000).

When poultry is stress, homeostasis zone in the body is disturbed and the control and integrating systems of the body attempt to restore the conditions to the

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homeostasis zone. Some of the activities that the poultry does to restore the homeostasis zone are panting, reducing feed intake and increasing water consumption (Tamzil *et al*., 2013b), sprinkling its body with dust and regulating body metabolism to maintain a constant body temperature. When stress cannot be overcome physiologically, genetic method will be used by activating HSP genes including HSP 70 working only under stress conditions. HSP 70 genes contained several polymorphic sites that can be used as chicken's marker that are more tolerant at high temperatures (Mazzi *et al*., 2003; Zhen *et al*., 2006; Gaviol *et al*., 2008; Tamzil *et al*., 2013a). Tamzil *et al*. (2013a) successfully mapped the HSP 70 genotypes in kampong, Arabic and commercial chickens and showed that there were such seven genotypes in kampong chicken (AA, AB, AC, CC, AD, DD and BC genotypes), six genotypes in Arabic chickens (AA, AB, AC, CC, AD and BC genotypes) and one genotype in commercial chicken (DD genotype). Genotype mapping indicate that heat tolerant or resistant parameters of chickens to heat stress are related to the genotype of the chickens (Tamzil *et al*., 2013b). The present study evaluated the interaction between lines of chickens and genotype of HP 70 and heat tolerance of chickens to heat stress with focus on the hematological dynamics and cellular immune systems.

MATERIALS AND METHODS

Birds: Twenty eight kampong, twenty four Arabic and four commercial chickens (laying hen type) were randomly selected from groups which HSP 70 genotypes had been identified by using Polymerase Chain Reaction (PCR)-Single Strand Conformation Polymorphism (SSCP).

Heat stress exposure: This study was designed in a completely randomized design with a 2x4 factorial arrangement. The first factor was chicken line which consisted of 2 levels i.e., kampong chicken and Arabic chicken. The second factor was the duration of acute heat stress exposure (40°C ambient temperature) which consisted of 4 levels i.e., 0 (as a control without heat stress), 0.5, 1.0 and 1.5 h. Twenty eight kampong chickens of genotypes AA, AB, AC, CC, AD, DD and BC and twenty four Arabic chickens of genotypes AA, AB, AC, CC, AD and BC and four commercial chickens of genotype DD were used. One bird of each HSP 70 genotype from kampong, Arabic and commercial chickens was assigned as a control (without exposure to any heat stress) and the others were exposed to acute heat stress test on 40°C for 0.5, 1.0 and 1.5 h in a chamber. The chamber was square shaped, from wooden board and in 33x33x75 cm^3. The chamber was also equipped with heater, thermostat, blower, digital thermometer, ventilation, feed and water spot. At the base of the chamber, a divider wire was placed and aluminum foil was placed on top of it to collect the manure. Commercial chicken was not included in this design due to only one genotype was found (DD and no replicates). Acute heat stress test was done alternately. Two hours prior to heat stress, chickens were fasted but water was available *ad libitum*.

Blood parameter measurements: After heat-stress challenge test was conducted, blood samples were taken through brachial vein using a 1 cc insulin syringe then it was put into 5 mL EDTA tube. The sample from each chicken was used for measurement of erythrocytes, hemoglobin, hematocrit, leukocytes and leukocyte differentiation.

Erythrocytes: The number of erythrocyte was counted using count room method (Kolmer *et al.*, 1959). Twenty microliter of blood in EDTA was put into 4000 µL Hayem solution using micropipette, then it was rinsed and mixed until the solution was homogen, after that it was incubated for two minutes. The result was put into Improved Neubauer count room. The number of erythrocytes was counted on five field boxes with 40 times magnification of objective lens. The number of erythrocytes was determined by multiplying the result of counted erythrocytes with 10000 (mm^3).

Hemoglobin: The percentage of hemoglobin was measured by using Spectrophotometer method (Kolmer *et al.*, 1959). Twenty microliters of blood in EDTA was put into Drabkin solution using micropipette and then the solution was homogenized and incubated for 3 min. Absorbance of the solution was read at the wave length of 540 nm using a spectro photometer (UV-Visible). Hemoglobin value was calculated by multiplying the absorbance with hemoglobin factor (g/dL).

Hematocrit values: Hematocrit value was measured by using microhematocrit method according to method of Kolmer *et al*. (1959). Blood sample was drawn into microhematocrit tube and the bottom part of the tube was blocked by paraffin. The tube was put in a hematocrit centrifuge (Hettick) and was centrifuged at 15.000 rpm for five minutes. The percentage of blood cell was read using hematocrit measurement tools.

Leukocyte: The effect of acute heat stress on leukocyte concentration in various HSP 70 genotypes was measured by using counting chamber method (Kolmer *et al*., 1959). Three hundred and eighty microliters of Turk solution was put into a tube glass using micropipette. Twenty microliters of blood sample in EDTA was added to the Turk solution using micropipette. After that, the reaction mixture was rinsed and mixed until it was homogen and the solution was incubated for two minutes. After incubation, the solution

was poured into counting chamber of Improved Neubauer. Concentration of leukocytes was determined in four areas using 10 times magnification objective of lens. Concentration of leukocyte was determined by multi plying the number of counted leukocyte results with 50 (mm^3).

Leukocyte differentiation: The value of leukocyte differentiation was calculated by using the Rapid methods (Kolmer *et al*., 1959). Five microliters of blood using a micropipette was dropped at the end of the glass object and the drop was allowed to ad here and spread on the edge of the glass slider. Blood was spreaded in the object glass with 35°C tilt. The preparation was dried and fixated with methanol. The next step was staining by dipping the preparation in eosin for 20-30 sec. After that it was moved and dipped in the second staining for 15-30 sec. Then it was rinsed by using running water and then dried. The preparation was read under a microscope using emersion oil. The number of each leukocyte cell was counted.

Data analysis: The effects of chicken lines and acute heat stress on all observed variables were analyzed using analysis of variance and when there was a significant difference, further test was done using least square mean. The effect of heat stress in commercial chicken and the effect of genotype HSP 70 against all observed variables were analyzed descriptively, whereas to determine the relationship among variables, the correlation analysis was conducted.

RESULTS AND DISCUSSION

The effects of acute heat stress on erythrocytes, hemoglobin, hematocrit and leukocyte in kampong and Arabic chickens were presented in Table 1. The data indicated that there was no interaction effect between chicken lines and acute heat stress on the value of erythrocytes, hemoglobin, hematocrit and blood leukocytes ($p>0.05$). The values of erythrocytes, hemoglobin and leukocytes measurements in kampong and Arabic chickens were similar ($p>0.05$) but the hematocrit values of kampong chickens were higher than those of Arabic chickens ($p<0.05$). Acute heat stress exposure dramatically decreased the erythrocytes, hemoglobin, hematocrit and blood leukocyte parameters ($p<0.01$).

The data in Table 1 provided information that the values of erythrocytes, hemoglobin and leukocytes of kampong and Arabic chickens were similar. If the concentrations of erythrocytes, hemoglobin, hematocrit and leukocyte of the two chicken lines (kampong and Arabic chickens) were compared to those of commercial chickens, it appeared that the concentrations of erythrocytes and hemoglobin in the blood of commercial chickens were relatively higher while the values of hematocrit and leukocytes were lower. Erythrocytes and hemoglobin concentrations in the blood of commercial chicken were $2.615\pm0.642x10^6/mm^3$ and 9.97±2.62 g/dL while the hematocrit and leukocyte concentration were $28.75\pm0.80\%$ and $5.95\pm2.22x10^3/mm^3$, respectively.

Effect of acute heat stress in the poultry lowers the values of erythrocytes, hemoglobin, hematocrit and leukocyte. Acute heat stress affects the synthesis, stability and activity of enzymes in the body. Exposure to high temperatures also contributes to volatility of some biochemical compounds (Noor and Seminar, 2009) including erythropoietin hormone. Erythropoietin is a hormone produced in the kidneys by triggering the production of pro-erythroblast from hematopoietic cells in the bone marrow. Erythropoietin is a primary hormone regulating the process of production, promotion, differentiation and development of red blood cells in the body (Guyton and Hall, 2007). High ambient temperature exposure inhibits the production of erythrocyte in the bone marrow. Erythropoietin stimulates erythropoies is followed by the decrease of hematocrit and hemoglobin due to hemolysis. The effects of acute heat stress on erythropoies is process and hemoglobin concentration were widely studied. Acute heat stress in poultry decreases the number of erythrocyte (Puvadolpirod and Thaxton, 2000; Toghyani *et al*., 2006) and hemoglobin (Hilman *et al*., 2000). Concentration of erythrocyte in commercial chickens kept at 33°C at the age of 21 days is $2.31x10^8$ (Toghyani *et al*., 2006). Artificial stress using ACTH treatment decreases the level of erythrocyte from $2.01\pm0.08x10^6$ to $1.92\pm0.05x10^6$ cell/mm^3 (Puvadolpirod and Thaxton, 2000).

The data in Table 1 also provided information that the acute heat stress exposure for only 0.5 hour did not affect the value of hematocrit and leukocytes ($p>0.05$), but when the heat stress exposure was extended to 1 h it decreased hematocrit and leukocytes values significantly. The data indicated that the 0.5 h of exposure to heat stress was still on the alarm phase. However, when heat stress exposure was extended to 1 and 1.5 h, stress status increased to resistant phase or even to exhaustion phase (dead). The decrease of hematocrit value was caused by the high ambient temperature exposure that lead to the decreased number of erythrocytes (erythropoiesis) which in turn affected the hematocrit value. The decrease of hematocrit value might be due to the damage of erythrocytes, the decrease of erythrocyte production or the decrease of erythrocytes number and size (Coles, 1982; Hilman *et al*., 2000; Altan *et al*., 2000) or due to the increase in water consumption during the exposure to heat stress (Tamzil *et al*., 2013b) that eventually diluted blood cell concentrations, included leukocyte. The decrease number of leukocytes during acute exposure to heat stress was caused by the increased concentrations of glucocorticoid hormones in plasma

Table 1: Concentration of erythrocytes, hemoglobin, hematocrit and blood leukocytes in kampong and Arabic chickens exposed to acute heat stress

	Variables			
Treatment	Erythrocytes ($x10^6/mm^3$)	Hemoglobin (g/dL)	Hematocrit value (%)	Leukocytes ($x10^3/mm^3$)
Chicken lines (CL)				
Kampong chicken	2.46±0.04	9.62±0.13	32.46±0.55	5.89±0.09
Arabic chicken	2.42±0.04	9.43±0.14	29.79±0.56	6.07±0.09
Acute heat stress (AHS)				
Control	3.06±0.06	11.77±0.20	37.30±0.82	7.31±0.14
0.5 h	2.58±0.05	10.49±0.18	33.52±0.76	6.56±0.13
1 h	2.26±0.05	8.89±0.19	28.75±0.79	5.67±0.13
1.5 h	1.87±0.05	6.96±0.18	24.94±0.76	4.38±0.12
Effect				
Chicken line (CL)	NS	NS	**	NS
Acute heat stress (AHS)	**	**	**	**
CLxAHS	NS	NS	NS	NS

**: Highly significant (p<0.01), NS: Not significant (p>0.05)

(Davis *et al*., 2008; Tamzil *et al*., 2013b). The phenomenon of decrease number of leukocytes as the effect of high temperature exposure was also reported by Nathan *et al*. (1976) and Mashaly *et al*. (2004).

There is a close relationship between leukocyte profile and glucocorticoid levels in the blood. The increased secretion of glucocorticoid during heat-stress exposure could depress leukocyte and lymphocyte cell number and the increase in heterophil percentage. This phenomenon occurs in all vertebrates, both in the natural-stress condition and artificially-simulated stress condition by glucocorticoid hormone supplementation. Acute-heat stress exposure to 40°C ambient temperature for 1.5 hours increased the concentrations of corticosterone hormone in the plasma (Tamzil *et al*., 2013b) which was also associated with the decreased number of leukocytes. The correlation value (r) between the level of the corticosterone hormone and leukocyte concentration was -0.8. This correlation indicated that an increase in the corticosterone hormone concentration in the plasma would decrease the leukocyte concentration in the blood. However, the correlation between the expression of HSP 70 gene and serum leukocyte concentration was low (r = -0.3). This correlation indicated that the increased expression of HSP 70 caused a small effect on the decrease of blood leukocyte number.

Previous study showed that acute heat stress exposure increased expression of HSP 70 and corticosterone hormone (Tamzil *et al*., 2013b) but lowered concentration of erythrocytes. HSP 70 expression was negatively correlated with the concentration of erythrocytes with r = -0.1. The low value of r indicated that the increasing expression of HSP 70 gave a small effect on the decreasing number of erythrocyte, while the levels of the corticosterone hormone were negatively correlated with the concentration of erythrocytes, with r = -0.8. These results indicated that an increase in the corticosterone hormone in the blood would be followed by a decreasing concentration of erythrocytes. It was also found that the expression of HSP 70 was negatively correlated with hemoglobin concentrations, with r = -0.2. The low value of r indicated that the increased expression of HSP 70 gave a small contribution to the decrease of hemoglobin levels, while the increasing level of the corticosterone hormone was negatively correlated with hemoglobin concentration with r = -0.9. It meant that the increase in corticosterone hormone concentration in the blood would be followed by a decrease in hemoglobin concentration.

Acute heat stress increased the expression of HSP 70 and the levels of the corticosterone hormone (Tamzil *et al*., 2013b) but decreased erythrocytes concentration. HSP 70 expression and corticosterone hormone levels were negatively correlated with hematocrit value (r = -0.3 and -0.8, respectively). This meant that the increased expression of HSP 70 gave small effect on the decrease of hematocrit values but the increased levels of the corticosterone hormone would be followed by a decrease in hematocrit value. On the other hand, erythrocyte concentration in blood were positively correlated with hematocrit values(r = 0.8). This meant that the decrease in erythrocyte concentration would be followed by a decrease in hematocrit value.

Effect of acute heat stress in kampong and Arabic chicken on leukocyte differentiation (heterophil, eosinophil, basophils, lymphocytes and monocytes percentage) and ratio of H/L were presented in Table 2. It could be seen that there was no interaction between lines of chicken and percentage of heterophil, basophils, lymphocytes, monocytes and ratio of H/L (p>0.05). Kampong and Arabic chickens had the same levels of percentage of leukocyte differentiation (p>0.05). Acute heat-stress exposure increased the percentage of heterophil, basophils and the ratio of H/L and decreased the percentage of lymphocytes and monocytes (p<0.01) but did not affect the concentration of eosinophil (p>0.05).

The data in Table 2 showed that kampong and Arabic chickens had relatively the same leukocyte differentiation values, but when it compared to the leukocyte differentiation of commercial chicken, the percentages of heterophil, basophil and the ratio of H/L in commercial chicken were relatively higher and the percentages of

Table 2: Value of leukocyte differentiation in kampong and Arabic chickens exposed to acute heat stress

	Variables					
Treatment	Heterophil (%)	Eosinophil (%)	Basophil (%)	Lymphocytes (%)	Monocytes (%)	H/L ratio
Chicken lines (CL)						
Kampong chicken	13.33±0.35	0.17±0.14	0.52±0.42	85.99±0.35	1.00±2.00	0.16±0.04
Arabic chicken	13.58±0.33	0.25±0.21	0.42±0.35	85.67±0.35	0.08±0.17	0.16±0.05
Acute heat stress (AHS)						
Control	9.35±0.49	0.10±0.14	0.00±0.00	90.18±0.52	0.36±0.20	0.10±0.07
0.5 h	12.24±0.45	0.25±0.17	0.38±0.20	87.21±0.48	0.00±0.00	0.14±0.06
1 h	14.75±0.46	0.33±0.33	0.58±0.12	84.33±0.50	0.00±0.00	0.17±0.06
1.5 h	17.488±0.45	0.15±0.02	0.92±0.12	81.58±0.48	0.00±0.00	0.21±0.06
Effect						
Chicken line (CL)	NS	NS	NS	NS	NS	NS
Acute heat stress (AHS)	**	NS	NS	**	NS	**
CLxAHS	NS	NS	NS	NS	NS	NS

**: Highly significant (p<0.01), NS: Not significant (p>0.05)

eosinophil, lymphocytes and monocytes were lower. The average percentages of heterophil, eosinophil, basophils, lymphocytes, monocytes and the ratio of H/L in commercial chickens were 16.50±7.72, 0.00, 0.75±0.50, 82.75±8.06, 0.00 and 0.21±0.12, respectively. The high percentages of heterophil, basophils and ratio of H/L and the decreasing percentages of eosinophil, lymphocytes and monocytes in commercial chicken as compared to kampong and Arabic chickens were due to the higher concentrations of glucocorticoid hormone, especially corticosterone hormone. Commercial chickens exposed to acute heat stress had higher corticosterone concentration as compared to Arabic and kampong chickens (Tamzil *et al*., 2013b). The concentrations of the corticosterone hormone in kampong and Arabic chicken were 4.617±0.261 and 5.112±0.264 µg/dL, respectively, where as in commercial chicken it was much higher, 6.325±3.571 µg/dL (Tamzil *et al*., 2013b). The increasing percentage of heterophil, basophils, the ratio of H/L and the decreasing percentage of lymphocyte and monocytes as the effect of acute heat stress were associated with the increased level of glucocorticoid hormone which in turn affected the components of blood leukocytes (heterophil, basophils, lymphocytes and monocytes) and ratio of H/L (Puvadolpirod and Thaxton, 2000). This study obtained a positive correlation between the level of the corticosterone hormone and heterophil percentage and value of ratio H/L, with r = 0.9. However, a negative correlation was found between the corticosterone hormone concentration and the percentage of lymphocytes, with r = -0.9. It meant that an increase in the corticosterone hormone percentage would be followed by an increase in heterophil percentage and ratio of H/L and a decreasing percentage of lymphocytes. The expression of HSP 70 expression during acute heat stress was assumed to contribute to these phenomena. Positive correlations between HSP 70 expression and heterophil percentage and ratio H/L were found, with r = 0.5 and r = 0.6, respectively. However, a negative correlation between the expression of HSP 70 and the percentage of lymphocytes was found (r = 0.5). It meant that the increase in HSP 70 expression would result in an increase in heterophil percentage and ratio of H/L and a decrease in lymphocytes percentage in the blood. When r values between corticosterone hormone level and leukocyte components and r value between HSP 70 expression and leukocyte components were observed carefully, it appeared that corticosterone hormone levels in the blood had higher contribution to the percentage of leukocyte components as compared to HSP 70 expression. Davis *et al*. (2008) suggested that stressed poultries higher corticosterone hormone percentage and ratio of H/L. This is the reason why the ratio of H/L can be used as an indicator of stress and higher glucocorticoid level in the blood (Davis *et al*., 2008). Corticosteroids can inhibit the immune system functions in the body, including the proliferation of lymphocytes, immunoglobulin production, cytokine production, cytotoxicity and anti-inflammatory agents (Munck *et al*., 1984).

The effect of HSP 70 genotype on the value of erythrocytes, hemoglobin, hematocrit, leukocytes and leukocyte differentiation were presented in Table 3. It can be seen that the percentage of blood erythrocytes in 7 HSP 70 genotypes studied, erythrocyte value was around 2.354-2.540x10^6/mm^3. The highest erythrocyte concentration was found in BC genotype, followed by AB, DD, AC, CC, AA and AD genotypes. The data in Table 3 also showed that all HSP 70 genotypes in this study had relatively the same hemoglobin concentrations, even though there was a tendency that the hemoglobin concentration was the highest in chickens with AA genotype and the lowest was in chicken with DD genotype. Table 3 also showed that each chicken HSP 70 genotype had different hematocrit values. The highest hematocrit percentage was found in BC genotype followed by AA, AC, AD, AB, DD genotypes and the lowest was found in CC genotype. The data in Table 3 also indicated that each HSP 70 genotype had different levels of leukocytes. The highest value of leukocytes was found in AD genotype, followed by AC, AB, BC, AA, CC genotypes and the lowest was in DD genotype.

Table 3: Hematological parameters in different HSP 70 genotypes in kampong and Arabic chickens

	HSP 70 genotype						
Parameters	AA	AB	AC	AD	BC	CC	DD
Erythrocyte (x10^6/mm^3)	2.40±0.06	2.48±0.09	2.43±0.15	2.35±0.03	2.54±0.17	2.40±0.21	2.47±0.12
Hemoglobin (g/dL)	9.87±0.24	9.44±0.33	9.33±0.39	9.2±0.33	9.6±0.39	9.25±0.29	9.65±0.29
Hematocrit value (%)	32.75±1.91	29.75±1.88	31.6±1.93	31.5±1.95	33.5±2.09	28.5±1.98	28.91±2.09
Leukocyte (x10^3/mm^3)	5.93±0.21	5.96±0.22	6.13±0.23	6.16±0.21	5.94±0.25	5.89±0.19	5.53±0.24
Heterophil (%)	13.50±1.50	14.12±1.42	12.75±1.47	11.62±4.44	13.71±4.43	15.00±4.40	16.25±4.32
Eosinophil (%)	0.12±0.119	0.00±0.00	0.37±0.112	0.25±0.037	0.25±0.097	0.17±0.012	0.12±0.124
Basophil (%)	0.62±0.214	0.12±0.08	0.50±0.34	0.25±0.15	0.50±0.28	0.50±0.31	0.75±0.37
Lymphocyte (%)	85.87±1.53	85.75±1.54	86.00±1.49	87.75±1.46	85.42±1.493	84.50±1.47	82.75±1.46
Monocyte (%)	0.12±0.08	0.00±0.00	0.25±0.06	0.00±0.00	0.00±0.00	0.00±0.00	0.12±0.05
H/L ratio	0.16±0.02	0.16±0.13	0.15±0.03	0.13±0.04	0.16±0.04	0.18±0.15	0.20±0.02

The data in Table 3 also showed that the percentage of heterophil in this study was around 11.62-16.25%. The highest percentage was found in chickens with DD genotype followed by CC, AB, BC, AA, AC genotypes and the lowest percentage was found in AD genotype. It meant that HSP 70 genotypes that most susceptible to heat stress was the DD genotype and on the other hand AD genotype was a genotype candidate of HSP 70 chickens that was relatively resistant to the dangers of heat stress. From the data in Table 3 it was clear that the highest percentage of basophils was found in chicken with DD HSP 70 genotype, followed by AA, AC, CC and BC (with the same percentage) AD genotype and the lowest percentage of basophils was found in chickens with AB genotype. It was also clear that HSP 70 genotype containing the highest percentage of lymphocytes was AD genotype and the lowest percentage was found in chickens with DD genotype. These data provided information that HSP 70 genotype that was most vulnerable to the dangers of heat stress was the DD genotype while AD was the one that was the most resistant. Thus AD genotype was a candidate of HSP 70 genotypes that was resistant to heat stress.

Data on HSP 70 chicken genotype and monocyte percentage in Table 3 also showed that HSP 70 chicken genotype affects the percentage of monocytes in the blood. AC genotype had the highest percentage of blood monocytes followed by AA and DD genotypes with the same percentage of monocytes, whereas others genotypes did not have monocytes. Heterophil and lymphocyte ratio in various HSP 70 genotypes showed that H/L ratio were different in different HSP 70 genotypes. The highest value of H/L ratio was found in chicken with DD HSP 70 genotype while the lowest was found in chickens with AD genotype. These data provided information that the DD HSP 70 genotype was the most susceptible to heat stress, while the most heat-resistant genotype was the AD genotype. Thus it can be concluded that AD genotype is a candidate of HSP 70 genotypes chickens that is resistant to heat stress.

Conclusion: It is concluded that there was a relationship between chicken lines and HSP 70 genotypes of chicken to the level of heat resistance. Kampong and Arabic chickens have better heat resistance than commercial chicken. AD HSP 70 genotype is a heat-resistant genotype candidate while DD genotype is the opposite, is a heat intolerant genotype.

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REFERENCES

Ajakaiye, J.J., J.O. Ayo and S.A. Ojo, 2010. Effects of heat stress on some blood parameters and egg production of Shica Brown layer chickens transported by road. Biol. Res., 43: 183-189.

Al-Aqil, A. and I. Zulkifli, 2009. The changes in heat shock protein 70 expression and blood characteristics in transported broiler chickens as affected by housing and early age feed restriction. Poult. Sci., 88: 1358-1364.

Al-Fataftah, A.A. and Z.H.M. Abu-Dieyeh, 2007. Effect of chronic heat stress on broiler performance in Jordan. Int. J. Poult. Sci., 6: 64-70.

Al-Ghamdi, Z.H., 2008. Effects of commutative heat stress on immune responses in broiler chickens reared in closed system. Int. J. Poult. Sci., 7: 964-968.

Altan, O., A. Altan, M. Cabuk and H. Bayraktar, 2000. Effects of heat stress on some blood parameter in broilers. Turk. J. Vet. Anim. Sci., 24: 145-148.

Coles, E.H.V., 1982. Veterinary Clinical Pathology. 2nd. Ed. W.B. Saunders Company, Philadelphia.

Cooper, M.A. and K.W. Washburn, 1998. The relationships of body temperature to weight gain, feed consumption and feed utilization in broiler under heat stress. Poult. Sci., 77: 237-242.

Davis, A.K., D.L. Maney and J.C. Maerz, 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Func. Ecol., 22: 760-772.

Etches, R.J., T.M. John and A.M. Verrinder Gibbins, 2008. Behavioural, physiological, neuroendocrine and molecular responses to heat stress. In: Nuhad J. Daghir (ed.). Poultry Production in Hot Climates. Trowbridge. Cromwell Press, 49-69.

Fumihito, A.F., T. Miyake, M. Takada, R. Shingu, T. Endo, T. Gojobori, N. Kondo and S. Ohno, 1996. Monophyletic origin and unique dispersal patterns of domestic fowl. Proc. Natl. Acad. Sci. USA., 93: 6792-6795.

Gaviol, H.C.T., E. Gasparino, A.J. Prioli and M.A.M. Soares, 2008. Genetic evaluation of the HSP70 protein in the japanese quail (*Coturnix japonica*). Genet. Mol. Res., 7: 133-139.

Guyton, A.C. and J.E. Hall, 2007. Fisiologi Kedokteraan. Edisi 11. Penerbit Buku Kedokteran, Jakarta.

Hilman, P.E., N.R. Scot and A. Van Tienhoven, 2000. Physiological, responses and adaption to hot and cold environments. in: Yousef MK (Ed.). Stres Physiology in Livestock. Poult. CRC Press, Florida, 1-71.

Iskandar, S., H. Resnawati and T. Pasaribu, 2000. Growth and carcass responses of three lines of local chickens and its crossing to dietary lysine and methionine. In the proc. of the 3rd International Seminar on Tropical Animal Production and Total Management of Local Resources. Faculty of Animal Science, Gajah Mada University.

Kolmer, J.A., E.H. Spaulding and H.W. Robinson, 1959. Approved Laboratory Technic. Fifth edition. Appleton-Century-Crofts, Inc. New York.

Lin, H., H.F. Zhang, R. Du, X.H. Gu, Z.Y. Zhang, J. Buyse and E. Decupere, 2005. Thermoregulation responses of broiler chickens to humidity at different ambient temperatures. II. Four weeks of age. Poult. Sci., 84: 1173-1178.

Mashaly, M., M.G.L. Hendricks, M.A. Kalama, A.E. Gehad, A.O. Abbas and P.H. Pattersin, 2004. Effectof heat stress on production parameters and immune responses of commercial laying hen. Poult. Sci., 83: 889-894.

Mazzi, C.M., J.A. Ferro, M.I.T. Ferro, V.J.M. Savino, A.A.D. Coelho and M. Macari, 2003. Polymorphism analysis of the hsp70 stress gene in Broiler chickens (*Gallus gallus*) of different breeds. Genet. Mol. Biol., 26: 275-281.

Munck, A.P., M. Guyre and N.J. Holbrook, 1984. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocrinol. Rev., 5: 25-44.

Nathan, D.B., E.D. Heller and M. Perek, 1976. The effect of short heat stress upon leukocyte count, plasma corticosterone level, plasma and leukocyte ascorbic acid content. Br. Poult. Sci., 17: 481-485.

Nishida, T., K. Nozawa, K. Kondo, S.S. Mansyur and H. Martojo, 1980. Morphological and genetical studies on the Indonesian native fowl. The original and philogeny of Indonesian native livestock investigation on the catle, fowl and their wild form, pp: 47-70.

Noor, R.R. and K.B. Seminar, 2009. Rahasia dan Hikmah Pewarisan Sifat (Ilmu Genetika dalam Al-Qur'an). Bogor. Penerbit IPB. Press, 109.

Puvadolpirod, S. and J.P. Thaxton, 2000. Model of physiological stress in chickens 3. Temporal patterns of response. Poult. Sci., 79: 377-382.

Sartika, T. and S. Iskandar, 2007. Mengenal plasma Nutfah Ayam Indonesia dan Pemanfaatannya. Balai Penelitian Ternak. Pusat Penelitian dan Pengembangan Peternakan, Badan Penelitian dan Pengembangan Pertanian Bogor.

Sulandari, S., M.S.A. Zein, S. Paryanti and T. Sartika, 2007a. Taksonomi dan Asal Usul Ayam Domestikasi. Keanekaragaman Sumber Daya Hayati Ayam Lokal Indonesia. Manfaat dan Potensi. Pusat Penelitian Biologi. Lembaga Ilmu Pengetahuan Indonesia. Bogor, 5-23.

Sulandari, S., M.S.A. Zein, S. Priyanti, T. Sartika, M. Astuti, T. Wijastuti, E. Sujana, S. Darana, I. Setiawan dan and G. Garnida, 2007b. Sumber daya genetik ayam lokal Indonesia. Keanekaragaman sumber daya hayati ayam lokal Indonesia. Manfaat dan Potensi. Pusat penelitian biologi. Lembaga Ilmu Pengetahuan Indonesia. Bogor, 45-104.

Tamzil, M.H., R.R. Noor, P.S. Hardjosworo, W. Manalu and C. Sumantri, 2013a. Polymorphism of the heat shock protein 70 gene in kampong, arabic and commercial chickens. J. Vet., 14: 317-326.

Tamzil, M.H., R.R. Noor, P.S. Hardjosworo, W. Manalu and C. Sumantri, 2013b. Acute heat stress responses of three lines of chickens with different Heat Shock Protein (HSP) 70 genotypes. Int. J. Poult. Sci., 12: 264-272.

Toghyani, M., M. Shivazad, A.A. Gheisari and S.H. Zarkesh, 2006. Performance, carcass traits and hematological parameters of heat-stressed broilers chicks in response to dietary levels of chromium picolinate. Int. J. Poult. Sci., 5: 65-69.

Zhen, F.S., H.L. Du, H.P. Xu, Q.B. Luo and X.Q. Zhang, 2006. Tissue and allelic-specific expression of HSP 70 gene in chickens: basal and heat-stress-induced mRNA level quantified with real time reverse transcriptase polymerase chain reaction. Br. Poult. Sci., 47: 449-455.

Zulkifli, I., A. Al Aqil, A.R. Omar, A.Q. Sazili and A. Rajion, 2009. Crating and heat stress influence blood parameters and heat shock protein 70 expression in broiler chickens showing short or long tonic immobility reactions. Poult. Sci., 88: 471-476.