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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Acute Heat Stress Responses of Three Lines of Chickens with Different Heat Shock Protein (HSP)-70 Genotypes

M.H. Tamzil[1], R.R. Noor[2], P.S. Hardjosworo[2], W. Manalu[3] and C. Sumantri[2]
[1]Faculty of Animal Sciences, Mataram University, Jl. Majapahit Mataram, Lombok, West Nusa Tenggara-83125, Indonesia
[2]Faculty of Animal Sciences, [3]Faculty of Veterinary Medicine, Bogor Agricultural University, Jl. Agatis IPB Campus, Darmaga, Bogor, West Java-16680, Indonesia

Abstract: An experiment was conducted to study the response of three chicken lines with different HSP 70 genotypes to acute heat stress. Twenty eight kampong chicken (native chicken, with seven genotypes i.e., AA, AB, AC, CC, AD, DD and BC) and twenty four Arabic chicken (with six genotypes i.e., AA, AB, AC, CC, AD and BC) and four commercial chickens (with one genotype i.e., DD) were used and randomly allocated in a factorial arrangement into groups which their HSP 70 genotypes had been identified. Acute heat stress was exposed at 40°C for 0.5, 1.0 and 1.5 h, respectively, using chamber in 33 x 33 x 75 cm^3. Parameters measured were the onset of panting (minute), panting frequency (times/minute), feed consumption (g/minute), water consumption (mL/minute), manure water content (%), rectal temperature (°C), serum corticosterone concentration (μg/dL) and HSP 70 expression (copy mRNA). The result of this study showed that there was an interaction between chicken lines and acute heat stress exposure on water consumption and manure water content. Chicken lines affected panting frequencies and manure water content but it did not affect the onset of panting, feed and water consumptions, rectal temperature, serum corticosterone concentration and expression of HSP 70. Acute heat stress increased panting frequency, drinking water consumption, manure water content, rectal temperature, serum corticosterone concentration, HSP 70 expression and it decreased feed consumption. The highest response on panting frequency, rectal temperature, serum corticosterone concentration and expression of HSP 70 was found in the DD genotype and the lowest in AD genotype. The most rapid onset of panting occurred in DD genotype and the slowest in AD genotype. The study revealed interaction between chicken lines and HSP 70 genotypes in heat resistance. Kampong chicken had the highest heat resistance as compared to Arabic and commercial chickens but HSP 70 genotypes that was the most tolerant to high ambient temperature was AD genotype where as the lowest tolerant was DD genotype.

Key words: Native chicken, HSP 70, panting, rectal temperature, corticosterone hormone

INTRODUCTION

Kampong chicken is the Indonesian native chicken which originally came from red jungle fowl (Gallus gallus) domesticated in South-East Asia, including in Indonesia (Nishida *et al*., 1980; Fumihito *et al*., 1996; Sartika and Iskandar, 2007; Sulandari *et al*., 2007ab). Arabic chicken is a local layer hen coming from overseas which were braekel kriel silver and braekel kriel gold (Europe local chicken) which came to Indonesia in the 1990s (Sulandari *et al*., 2007ab; Sartika and Iskandar, 2007) while commercial chicken is laying hens imported from cold climate region.

Poultry is warm-blooded animal (body temperature is about 40.5-41.5°C) (Etches *et al*., 2008), almost all parts of its body are covered by feather and it does not have sweat glands thus it is vulnerable to the danger of heat stress because of difficulties in releasing their body heat to environment (Cooper and Washburn, 1998; Lin *et al*., 2005; Al-Fataftah and Abu-Dieyeh, 2007; Al-Ghamdi, 2008; Zulkifli *et al*., 2009; Al-Aqil and Zulkifli, 2009; Ajakaiye *et al*., 2010). Stressed poultry can be identified by panting frequency (Hilman *et al*., 2000; Etches *et al*., 2008; Gaviol *et al*., 2008), a decrease in feed consumption, an increase in water consumption and manure water content (Altan *et al*., 2000; Garriga *et al*., 2006), an increase in serum corticosterone concentration (Sigel, 1980; Puvadolpirod and Thaxton, 2000ab; Zulkifli *et al*., 2009) and HSP 70 expression (Gabriel *et al*., 1996; Mahmoud *et al*., 2004; Zhen *et al*., 2006; Yu and Bao, 2008).

In the heat stressed poultry, homeostasis zone in the body is disturbed and thermoregulation center tries to bring back body temperature to normal ranges before heat stress occurred. If heat stress keeps increasing and thermoregulation center cannot handle it by using metabolic pathway, genetic pathway will be used by

Corresponding Author: M.H. Tamzil, Faculty of Animal Sciences, Mataram University, Jl. Majapahit Mataram, Lombok, West Nusa Tenggara-83125, Indonesia

activating HSP genes, including HSP 70 which works only during stress condition (Noor and Seminar, 2009). Some workers report that there are some polymorphism sites which can be used to mark chicken which is more tolerable towards high ambient temperature (Mazzi *et al*., 2003; Zhen *et al*., 2006; Gaviol *et al*., 2008). A study reported by Tamzil *et al*. (2013) showed a successfully mapping of HSP 70 genotypes in kampong chickens and Arabic chickens. Seven genotypes in kampong chicken observed are AA, AB, AC, CC, AD, DD and BC and six genotypes in Arabic chicken are AA, AB, AC, CC, AD and BC.

The tolerance level of HSP 70 genotype towards heat stress had been tested with a focus of study on panting behavior, feed and water consumption and manure water content. The present study was therefore designed to study the association between the genotypes of HSP 70 in chickens, Arabic and commercial chickens with the level of heat resistance, through behavioral variables approach, feed and water consumption and water content of manure.

MATERIALS AND METHODS

Bird: Twenty eight of kampong, twenty four Arabic and four commercial chickens (laying hen type) were randomly taken from their 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 2 x 4 factorial arrangement. The first factor was chicken strain 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 having DD genotype 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 imposed 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 33 x 33 x 75 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*.

Panting onset was the time of first panting response after exposure to acute heat stress, while panting frequency was counted in the last five minutes of acute heat stress test using hand counter. Rectal temperature was measured by entering digital thermometer into the rectum. Blood samples were taken from the wing vein (brachial vein) using 1 mL insulin syringes and they were stored at ±3°C for 16 hours and then they were centrifuged for 20 min at 2500 rpm to obtain serum. Serum corticosterone concentrations were analyzed using ELISA kit (Enzyme Linked Assay Immonosorbent, Diagnostic Automation. Inc. USA). The amount of feed and water remaining were measured, whereas manure was collected and dried in an oven at 70°C for 48 h to determine water content of manure. At the end of the acute heat stress exposure, the experimental chickens were slaughtered and their brains were taken using sterile equipment and were stored in liquid nitrogen and stored at -80°C to measure HSP 70 expression.

Total RNA extraction: Total RNA extraction was performed using Total RNA Mini Kit 50 preps. Twenty-five milligrams of brain samples were dissolved in 1.5 µL tubes using 400 µL RB buffer and 4 µL mercaptoethanol which was then incubated for 3 min at room temperature. After that, the solution was moved to filter column and centrifuged at 1000 g for 30 seconds.

Filter column part was removed and sediment formed was added with 400 µL ethanol 70% then the solution was homogenized by vortexing. After it was moved to RB, the column was centrifuged at 14 000 g for 1 minute. After the sediment was discarded, RB column was transferred to a 2 mL new tube and was added with WI buffer and then centrifuged at 14000 g for 30 seconds. After the precipitate was discarded, 600 µL of wash buffer was added then it was centrifuged at 14000 g for 30 seconds. After the precipitate was discarded, it was centrifuged at 14000 g for 2 minutes and then the column was transferred to 1.5 mL tube. After addition of 50 µL of RNA-se free water, samples were left for 2 minutes and then centrifuged at 14000 g for 1 minute. After that, RNA was stored for ±45 minutes, followed by a reverse transcriptase reaction.

Reverse transcriptase: Single strand cDNA synthesis was performed using total RNA through reverse transcriptase reaction using Transcriptor Synthesis First Strand cDNA Kit. Solution consisted of 5 µL sample of total RNA, 1 µL oligo (dT) and 15 µL H_2O incorporated into 0.2 mL tubes. Solution was heated at 65°C for 10 minutes and then it was immediately put in the ice bath. Next, 6 µL 5 X buffer was added, 0.5 µL RNase inhibitor, 0.5 µL dNTP and 0.5 µL reverse transcriptase. After that

the solution was incubated at 55°C for 30 minutes and 85°C for 5 minutes. Once this process was completed, cDNA was ready to use.

Real-time quantitative PCR (RT-PCR): Testing was done using real-time PCR quantitative using DNA samples obtained from the reverse transcriptase reaction of total RNA genes HSP 70 using Kappa Probe Fast Universal 2 x qPCR Master Mix (1 mL). PCR primers used were forward 5'-GGCACCATCACTGGGCTTA-3' primer, Reverse 5''-TCCAAGCCATAGGCAATAGCA-3' and probe 50-FAM-CGTGATGCGTATTATCAATGAGCCCACA-Tamra-3' (Zhen *et al*., 2006). Real-time quantitative PCR was performed using 20 µL solution consisted of 0.4 µM probe, 0.5 µM primer, Master Mix and 2 µL of cDNA template which were designed based on guidelines issued by the ABI 7000 Sequence Detection System. PCR cycle program was performed for 2 minutes at 50°C, 10 minutes at 95°C followed by 40 cycles for 15 seconds at 95°C and one minute at 60°C. Standard curve was drawn by plotting natural log with cycle threshold (C_T) towards natural log number of molecules used in the standard DNA samples (dilutions ranging from 9.65 x 10^5-9.65 x10^9 copies/µL), in which C_T is the cycle where statistic increased significantly towards the amount of signal generated by PCR reaction when it was first detected. C_T was calculated based on default setting of sequence detection results of real-time software. The equation of the graph is used to calculate the number of cDNA molecules per microgram from oligo-dT premed total cDNA. Then it was tested using the same reaction of plat as a standard.

Statistical analysis: The main effects of chicken line and acute heat stress (40°C) on the all variables observed were analyzed using analysis of variance. When the result was significantly different, it tested further using LSMEAN, while data of acute heat stress effect on commercial chickens and HSP 70 genotypes effect on all observed variables were analyzed descriptively.

RESULTS AND DISCUSSION

There were interactions effect between chicken line and the duration of acute heat stress on water consumption and manure water content (P<0.01) but there was no interaction effect between chicken line and acute heat stress on the onset of panting, panting frequency, feed consumption, rectal temperature, serum corticosterone concentration and HSP 70 expressions (P>0.05). Chicken lines (kampong and Arabic chickens) affected the onset of panting, water consumption and manure water content (P<0.01) but there was no effect on panting frequency, feed consumption, rectal temperature, serum corticosterone concentration and HSP 70 expression (P>0.05). Acute heat stress affected panting frequency, feed and water consumption, manure water content, rectal temperature, serum corticosterone concentration and HSP 70 expression (P<0.01) but there was no effect of acute heat stress on the onset of panting (P>0.05).

Kampong and Arabic chickens responded differently to acute heat stress. Arabic chickens exposed to acute heat stress had faster onset of panting as compared to kampong chicken (Table 1) and the onset of panting in commercial chicken was faster than in Arabic chicken.

Table 1: The effect of heat stress acute on kampong and Arabic chickens

		Acute heat stress period (h)				
Parameters	Chicken line	Control	0.5	1	1.5	Average±SE
	Kampong	-	4.58±0.27	4.79±0.29	4.66±0.28	4.68±0.20^a
Panting onset (minute)	Arabic	-	4.00±0.29	4.19±0.29	3.95±0.29	4.05±0.20^b
	Average±SE	-	4.29±0.20	4.49±0.20	4.30±0.20	
Panting frequency	Kampong	-	813.78±47±86	940.55±51.70	1057.28±47.86	953.42±29.85
(times/minutes)	Arabic	-	831.18±51.70	946.40±51.70	1082.67±51.70	937.21±28.39
	Average±SE	-	822.48±35.23^a	943.47±36.56^a	1069.98±35.23^b	
Feed consumption	Kampong	0.47±0.02	0.42±0.01	0.15±0.01	0.05±0.01	0.27±0.01
(gram/minute)	Arabic	0.48±0.01	0.38±0.01	0.17±0.01	0.06±0.01	0.27±0.01
	Average±SE	0.48±0.01^a	0.40±0.01^b	0.16±0.01^c	0.06±0.01^d	
Water consumption	Kampong	0.11±0.02	0.38±0.01	0.44±0.02	0.59±0.01	0.38±0.01^a
(mL/minute)	Arabic	0.07±0.02	0.33±0.02	0.49±0.02	0.71±0.02	0.40±0.01^b
	Average±SE	0.09±0.01^a	0.35±0.01^b	0.47±0.01^c	0.65±0.01^d	
Manure water content (%)	Kampong	59.24±1.23	64.04±1.04	74.54±1.13	80.61±1.04	69.66±0.56^a
	Arabic	62.41±1.13	63.32±1.13	79.25±1.13	80.50±1.13	71.37±0.49^b
	Average±SE	60.82±0.84^a	63.68±0.77^b	76.89±0.80^c	80.65±0.77^d	
Rectal temperature (°C)	Kampong	41.18±0.27	43.26±0.23	43.77±0.25	45.01±0.23	43.30±0.12
	Arabic	41.17±0.25	43.02±0.25	43.95±0.25	44.97±0.25	43.28±0.13
	Average±SE	41.17±0.19^a	43.14±0.17^b	43.86±0.18^b	44.99±0.17^b	
Corticosteron hormone	Kampong	1.30±0.58	3.66±0.49	4.97±0.53	8.54±0.49	4.62±0.26
concentration (µ/dL)	Arabic	1.68±0.53	3.27±0.53	6.37±0.53	9.13±0.53	5.11±0.26
	Average±SE	1.49±0.39^a	3.46±0.36^b	5.67±0.37^c	8.84±0.36^d	
HSP 70 expression	Kampong	0.15±127.83	4.81±108.04	19.95±116.69	35.06±108.04	14.35±1.51
(x10^7) (mRNA)	Arabic	0.15±116.69	4.50±116.69	19.95±116.69	44.28±116.69	17.23±1.82
	Average±SE	0.15±86.5^a	4.66±79.5^a	19.95±82.51^b	184.67±79.51^c	

Note: Means in the same row and column with different superscripts differ significantly (P<0.01)

However, the onset of panting in commercial chickens was faster than in the Arabic and kampong chickens. Commercial chicken showed panting after 3.77 minutes of acute heat stress exposure, whereas Arabic and kampong chickens showed panting 4.05 and 4.68 minutes, respectively, after exposure to acute heat stress. These different responses were probably due to the body weight differences in each genotype. Commercial chicken had heavier body weight and had smaller ratio of body surface area to body weight as compared to Arabic and kampong chickens (Table 2). These conditions affect the rate of sensible heat loss. Chicken having smaller ratio of body surface area to body weight has slower rate of sensible heat loss as compared to chicken having greater ratio of body surface area to body weight. This is the main reason why the onset of panting (insensible heat loss) in commercial chicken was faster than in Arabic and kampong chicken and also the onset of panting in Arabic chicken was faster than in kampong chicken.

The present study can be used as one of parameters that kampong chicken has a better heat stress resistance or tolerance as compared to Arabic and commercial chickens and Arabic chicken has a better heat stress resistance as compared to commercial chicken. Panting is a physiological response taken by the poultry suffering from heat stress to release the excess of body heat to the environment (Etches *et al*., 2008). Through the panting process, poultry trying to release the excess of body heat through evaporation through the respiratory tract (Gaviol *et al*., 2008). Poultry suffering from heat stress release excess of body heat through panting process. In the panting process, heat can be released in a greater amount together with water evaporation. Poultry experiencing panting is a sign that the poultry is already under heat stress and vice versa. Kampong chicken was the last showing panting response that indicated that it suffered from heat stress was slower than the other genotypes of chicken.

The data in Table 1 show that acute heat stress did not affect the onset of panting ($P>0.05$). Panting in poultry is a physiological response to release the excess of body heat caused by the increase in environmental temperature. In this study, the temperature of the environment (stress temperature) was similar to 40°C, therefore each chicken showed the onset of panting at the same time.

Panting frequencies of kampong and Arabic chickens exposed to acute heat stress were similar (Table 1). When comparing to panting frequency, it appeared that commercial chicken had higher panting frequency as compared to Arabic and kampong chickens. Panting frequency of commercial chicken in this study was about 961.89 times per minute which means that within a minute panting frequency of commercial chicken was 8.47 times more often than that of Arabic chicken and 24.68 times more often than that of kampong chicken. The differences of panting frequency of commercial chicken, Arabic chicken and kampong chicken were due to the differences in body weight where commercial chicken had higher body weight than kampong and Arabic chickens (Table 2). Heavier chicken has smaller ratio of body surface to body weight as compared to their counterpart. These conditions affect the rate of sensible heat loss. Smaller ratio of body surface area to body weight has slower rate of sensible heat loss as compared to the greater one. That is why commercial chicken experienced the onset of panting (insensible heat loss) more often than did Arabic and kampong chickens.

Table 2: Average of body weight and body surface relative experiment chicken

Chicken line	Body weight (g)	Body surface relative
Kampong	1.161±0.022	0.95
Arabic	1.209±0.023	0.94
Commercial	1.644±0.029	0.85

Panting frequency data (Table 1) revealed that chicken exposed to acute heat stress for 0.5 and 1.0 hour did not show a significant increase in panting frequency ($P>0.05$). However, panting frequency increased after 1.5 hours exposure to acute heat stress. This means that stress occurred in the first 0.5 and 1.0 hour still in the alarm phase and after exposing to heat for 1.5 hours, stress levels increased to resistant phase, even to exhaustion phase (death).

There was no difference in feed consumption between kampong and Arabic chickens in the control group without acute heat stress exposure and during acute heat stress exposure (Table 1). It means that kampong and Arabic chickens have the same ability to consume feed in the same amount in the non-stressed and stressed conditions. The lowered feed consumption in acute heat stress in both kampong and Arabic chickens was caused by thermoregulation center to reduce feed intake to reduce endogenous or metabolic heat production or heat increment as an additional heat load in the body (Table 1). Poultry suffering from heat stress always try to reduce feed consumption in order to reduce heat increment production which is a result of the digestion activity and nutrient metabolism. Garrga *et al*. (2006) reported that chickens kept in cages with temperature set on 30°C and 70% relative humidity had lower feed consumption (163 g/bird/day) as compared to those kept in control environment (205 g/bird/day), with temperature 20°C and 50% relative humidity.

Effect of acute heat stress on water consumption in kampong and Arabic chickens is also presented in Table 1. In general, acute exposure of kampong and Arabic chickens to heat stress increased water consumption and Arabic chicken consumed more water during heat stress as compared to kampong chickens. However, acute heat stress for 0.5 h, the water

consumption of kampong chicken was higher than the water consumption of Arabic chickens. After one hour on acute heat stress, Arabic chicken water consumption was higher than that of kampong chicken. Consumption of water showed a more striking difference after receiving acute heat stress treatment for 1.5 h. Effect of acute heat stress treatment on water consumption showed a different pattern in commercial chicken. In the control group, commercial chicken had the lowest water consumption as compared to Arabic and kampong chickens. After being exposed to acute heat stress, water consumption of commercial chicken was higher than that of kampong and Arabic chickens. Water consumption of commercial chicken in the control environment was 0.05 mL/bird/minute. After receiving acute heat stress for 0.5, 1.0 and 1.5 h, water consumption of commercial chicken increased to 0.48, 0.57 and 0.89 mL/bird/minute respectively. The high water consumption in commercial chicken is parallel with panting frequency as compared to Arabic and kampong chicken. Water loss due to evaporation during panting should be replaced by drinking water. When the high water consumption is used as a measure of chickens response to heat stress, then the kampong chicken can be classified as chicken that relatively resistant to heat stress as compared to Arabic and commercial chickens.

The difference between Arabic and kampong chicken in water consumption is a sign that the two lines of chickens have different abilities to consume drinking water with different volumes and to cope with heat stress. Kampong chicken consumed less water than did Arabic chicken, the same thing happened for kampong and Arabic chicken that consumed less water than commercial chicken.

The water content of feces indicated that acute heat stress increased manure water content in Arabic and kampong chicken in different manners. During exposure to heat stress for 0.5 h, kampong chicken and Arabic chicken had similar manure water content. After 1.0 h, manure water content of Arabic chicken was much higher than that of kampong chicken. After 1.5 h, kampong and Arabic chicken manure water contents were relatively similar. Effect of acute heat stress on commercial chicken fecal water content also showed the same pattern with kampong and Arabic feces water content. Manure water content in control commercial chicken was 60.82%. After being exposure to acute heat stress at 40°C for 0.5, 1 and 1.5 h, manure water content increased to 62.06, 79.09 and 80.32% respectively. After 1 hour heat stress, kampong and Arabic chickens had different feces water contents. This difference is associated with the higher water consumption in Arabic chicken than kampong chicken.

Arabic chicken manure water content was higher than kampong and commercial chickens (Table 1). In this study, manure water content of commercial chicken was 70.57%. The high manure water content in the Arabic chicken is due to higher water consumption in Arabic as compared to kampong chicken. An interesting point in this study is that Arabic chicken manure water content was higher than that of commercial chicken. In fact, commercial chicken had higher water consumption as compared to Arabic chicken. The difference in manure water content is more likely due to the differences in metabolic rate. Arabic chicken produced higher carbon dioxide (CO_2) and water (H_2O) as a result of this metabolic rate than that of commercial chicken. Metabolic water produced during metabolism was discharged as urine with feces. This is the reason that Arabic chicken had higher fecal water content as compared to commercial chicken.

The results showed that there was no interaction between the line of chicken and acute heat stress on rectal temperature ($P>0.05$). Line of chicken did not affect rectal temperature response to acute heat stress ($P>0.05$) but acute heat stress increased rectal temperature in both lines of chickens ($P<0.01$). This study showed that kampong and Arabic chicken have relatively the same rectal temperature. Rectal temperatures of these two lines of chickens were also relatively the same with commercial chicken rectal temperature (43.71°C). This means all chicken lines have relatively the same rectal temperature and when they were exposed to acute heat stress their rectal temperatures will increase significantly. Chicken exposed to acute heat stress experienced disorders in releasing body heat (Altan *et al*., 2000) due to inequillibrium between heat loss and heat gain from endogenous heat production and heat gain from the environment. Cooper and Wasburn (1998) stated that the increase in cage temperature of more than 31°C caused the thermoregulation center could not maintain chicken body temperature within normal range. Thus, increasing environmental temperature resulted in increasing heat gain from the environment and decreased heat loss to the environment that finally increased body temperature. The excess of heat gain cannot be removed through the sweat glands in the skin because poultry are classified as vertebrates that do not have sweat glands and their entire bodies are covered with feathers.

There was no interaction between the line of chicken and the acute exposure to heat stress on serum corticosterone concentration ($P>0.05$) was also observed. The serum corticosterone concentrations of both kampong and Arabic chickens showed no significant differences ($P>0.05$). It means that serum concentrations of corticosterone in kampong and Arabic chickens were relatively the same. However, serum

corticosterone concentrations of commercial chicken (6.32 µg/dL) were lower than those of kampong and Arabic chickens. The high serum corticosterone concentration in commercial chicken is an indicator of different susceptibility to stress. In this study, commercial chicken was more sensitive to heat stress as compared to kampong and Arabic chickens.

Acute heat stress increased serum concentrations of the corticosterone ($P<0.01$). The serum corticosterone concentrations of kampong and Arabic chickens were relatively similar. Acute heat stress (40°C) for 0.5 h in the poultry significantly increased serum corticosterone concentration. The serum corticosterone concentrations increased significantly with the increased duration of heat stress. This concurrence explained that during suffering from heat stress, particularly in the resistant phase, an additional energy is needed due to the increased activity of some organs, such as increased in respiratory rate, heart rate and rate of peripheral blood circulation. On the other hand, the decrease in feed consumption stimulated synthesis of corticosterone and cortisol hormones to produce energy substrate, glucose, from non-glucose substance (gluconeogenesis). Corticosterone and cortisol are the major adrenal cortical hormones that belong to glucocorticoid that function in glycolysis process, gluconeogenesis and lipolysis (Ewing *et al*., 1999). In vitro studies found that corticosteroids works by binding specific receptors on lymphoid cells, resulting a change in enzyme function and nucleic acid formation and decreased in protein synthesis (Sullivan and Wira, 1979).

When poultry suffering from heat stress, neurogenic system is activated to release catecholamine, epinephrine and norepinephrine (Sigel, 1980). Of all these compounds, epinephrine seems to have the most dominant role in altering metabolism (Assenmacher, 1973), especially in changing kinase enzyme protein activation as a body signal to impose glycogenolysis and gluconeogenesis (Berne and Levy, 1990). Stress can stimulate adrenal glands to secrete corticosterone hormones in poultries, turkeys, pigeons, ducks and quails (Etches *et al*., 2008). This is a clear indication that increased serum corticosterone concentrations can be used as an indicator stress in poultry (Puvadolpirod and Thaxton, 2000; Spasojevi *et al*., 2007; Etches *et al*., 2008; Vahdatpour *et al*., 2009; Sohail *et al*., 2010).

The increased serum corticosterone concentration in poultry exposed to heat stress has been reported by previous researchers. Serum corticosterone concentrations of poultry reared in conventional cages after being exposed to heat stress for 6 h increased from 3.5-4.8 µg/mL, whereas broilers raised in a chamber with controlled temperature after being exposed to heat stress for 6 h, their blood corticosterone concentrations increased from 2.8-15.4 µg/mL (Al-Aqil and Zulkifli, 2009). Crating and heat exposure at 32°C for 3 h caused an increased in plasma corticosterone content from 3.15 µg/mL (control treatment) to 4.49 µg/mL, after being exposed to heat at 32°C for 3 h (Zulkifli *et al*., 2009).

Blood corticosterone concentration is positively correlated with rectal temperature, with $r = 0.85$, meaning an increase in the corticosterone hormone concentration will be followed by an increase in rectal temperature. It is also found that the blood corticosterone concentration is negatively correlated with feed intake, with $-r = 0.85$. This means that an increase in blood corticosterone concentrations will be accompanied by an increase in rectal temperature and a reduction in feed consumption. The decrease in feed consumption is a thermoregulatory response to reduce body heat load by decreasing heat production from the activity of digestion and metabolism of nutrients.

The results showed that there was no interaction between chicken line and the duration of acute heat stress on the expression of HSP 70 ($P>0.05$). There was no difference in the HSP 70 expression between kampong and Arabic chickens ($P>0.05$) but when it was compared to HSP 70 expression in commercial chicken ($30.61\pm54.21 \times 10^7$mRNA), HSP 70 expression of kampong and Arabic chickens were relatively lower. If HSP 70 expression data were used as the main indicator of heat resistance, then kampong and Arabic chicken were two lines of chickens having relatively similar resistance to heat stress that was higher than commercial chicken.

In the control group in this study, the expression of HSP 70 was detected with 0.15±86.54 x 107mRNA value. This means that poultry was experiencing heat stress at 29.3°C (ambient temperature = chamber temperature in this experiment). This is understandable because at 29.3°C (ambient temperature), body temperature had reached 40.50-41.18°C which in this range of temperature (41-42°C), poultry experienced heat stress (Hilman *et al*., 2000). HSP 70 expression is strongly influenced by heat stress (Zhen *et al*., 2006). On organisms growing on a wide range of temperatures, HSP 70 will give maximum response at 10-15°C above the comfortable temperature zone (Ewing *et al*., 1999). Comfortable temperature of adult chickens is around 18-23.9°C (Czaririck and Fairchild, 2008), meaning that the expression of HSP 70 in adult chickens will appear at temperatures 5.4°C above comfortable temperature zone.

Acute heat stress (40°C) for 0.5 h did not increase HSP 70 expression and the expression increased dramatically after 1 hour exposure to acute heat stress. These data imply that in kampong and Arabic chickens, HSP 70 will be synthesized maximally after they were exposed to acute heat stress (at 40°C chamber temperature) for 1 h. Collier *et al*. (2008) stated that the stress on resistance phase lead to changes in gene expression in the form of an increased HSP expression

and the decreased expression of other proteins. Yu and Bao (2008) reported that mRNA HSP 70 expression in some organs of chickens increased significantly and reached its peak after being exposed to heat stress for 2 h. Mahmoud *et al*. (2004) obtained that expression of HSP 70 mRNA of chicken reached a peak after the chicken was exposed for 1 h to heat stress as compared to control, while Gabriel *et al*. (1996) found that the expression of HSP 70 mRNA had begun to emerge since the first 1 h of heat stress exposure and reached its peak after 3 h being exposed to heat stress. The function of HSP 70 produced during heat stress is to protect proteins that are sensitive to high temperature from degradation, or prevent the damage of proteins which can lead to permanently damage of cells functions and survival which will finally affect poultry survival (Noor and Seminar, 2009; Etches *et al*., 2008).

When the results of this study were compared to the results of Zhen *et al*. (2006) who observed HSP 70 expression in brain tissue was 1.82 x 10^9 mRNA, then the expression of HSP 70 in this study was much lower which is 14.35±1.51 x 10^7 mRNA in kampong chicken, 17.23±1.82 x 10^7 mRNA in Arabic chickens and 30.61±54.21 x 10^7 mRNA in commercial chicken. The results illustrate the differences in responses to heat exposure of different 30.61±54.21 x 10^7 mRNA lines of chickens. Zhen *et al*. (2006) used crossed lines between Xinghua x Taihe chicken and silkies and White recessive Rock chicken which were exposed to heat stress for 4 h at 44°C, whereas this study used Arabic, kampong and commercial chicken which were exposed to heat at 40°C for 0.5 up to 1.5 h.

Acute heat stress in this study increased HSP 70 expression and concentration of serum corticosterone. Nevertheless, there was no strong correlation between the expression of HSP 70 with corticosterone hormone concentration. The value of r was obtained only 0.28 which means that the increased expression of HSP 70 had little effect on the increasing of corticosterone hormone concentration.

Data in Table 3 show that HSP 70 genotypes affect panting onset, panting frequency, feed consumption, water consumption, manure water content, rectal temperature, concentration of corticosterone hormone and HSP 70 expression. AD genotypes was the last in HSP 70 genotypes that experienced panting followed by AC, BC, CC, AB and AA. DD genotypes was the most responsive (the least tolerant) to acute heat stress exposure that showed the fastest panting response. The average difference in panting onset between AD and DD genotypes was 1.34 minutes meaning the DD started panting 1.34 minutes faster than the AD genotype. This indicates that AD genotype is a candidate of HSP 70 genotypes that relatively more heat resistant. The effect of HSP 70 genotypes on panting frequency showed that HSP 70 genotypes experienced panting with the highest frequency was CC and DD genotypes and, on the contrary, the AD genotype experienced the lowest frequency. Panting frequency in all HSP 70 genotypes in this study ranged between 808.72-1041.1 times per minute.

The effect of HSP 70 genotypes on feed consumption is presented in Table 3. It shows that the differences of HSP 70 genotypes caused the differences in feed consumption of chicken. Chickens with BC HSP 70 genotypes had the highest feed consumption, followed by AA, AC, AB, DD, AD and CC genotypes. HSP 70 genotype also influences water consumption of kampong and Arabic chickens tested. The highest water consumption was showed by chicken having CC genotypes, followed by DD, AB, AA, BC, AC and AD genotypes. HSP 70 genotypes also affects manure water content. AC genotypes had the highest manure water content followed by DD, AB, CC, AA, BC and AD genotype.

Data of HSP 70 genotype effect on rectal temperature are presented in Table 3. The data show that the highest rectal temperature was found in chicken with DD HSP 70 genotype, followed by CC, AA, BC, AB, AC genotypes and the lowest rectal temperature was found in AD genotype. It means that AD genotype is a candidate of HSP 70 genotype that is resistant to heat stress.

Data presented in Table 3 also show the effect of HSP 70 gen genotype on serum corticosterone concentrations in which HSP 70 genotype of each chicken produced different concentration of

Table 3: The effect of HSP 70 genotype on kampong and Arabic chickens

	Genotype of HSP 70						
Parameter	AA	AB	AC	AD	BC	CC	DD
Panting onset (minute)	3.90±0.279	4.02±0.268	4.90±0.298	4.94±0.267	4.54±0.289	4.43±0.288	3.60±0.293
Panting frequency (time/minute)	988.13±51.69	932.25±51.27	991.32±50.39	808.72±47.86	855.65±51.61	1041.10±49.49	1021.00±59.87
Feed consumption (g/minute)	0.28±0.16	0.26±0.14	0.27±0.17	0.25±0.15	0.28±0.18	0.25±0.15	0.26±0.13
Water consumption (mL/minute)	0.43±0.018	0.45±0.017	0.39±0.016	0.37±0.015	0.41±0.017	0.51±0.016	0.46±0.018
Manure water content (%)	70.69±1.23	71.11±1.03	71.60±1.13	70.18±1.04	70.33±1.18	71.06±1.09	71.39±1.12
Rectal temperature (°C)	43.65±0.24	43.17±0.23	43.12±0.24	42.72±0.23	43.45±0.25	43.69±0.24	43.75±0.23
Corticosterone hormone (µg/dL)	5.30±0.57	5.9±0.57	5.9±0.58	3.5±0.60	4.70±0.49	4.50±0.53	6.30±0.54
Expression of HSP 70 (x10^7) (mRNA)	0.14±0.035	0.15±0.034	0.20±0.031	0.11±0.031	0.15±0.032	0.02±0.033	5.13±0.039

corticosterone. The highest serum corticosterone concentration was found in DD genotype while the lowest was found in AD genotype. It means that chicken with DD genotype was the most intolerant to heat stress and experienced stress faster as compared to other genotypes. On the other hand, AD genotype showed the opposite response. Chicken with AD genotype is the most tolerant to heat stress as compared to other genotypes. This result implies that DD genotype is a type of chicken that cannot stand or intolerant to heat stress while AD genotype is HSP 70 genotype candidate that tolerant to heat stress.

Data of HSP 70 expression in different HSP 70 genotypes are presented in Table 3. The data indicate that each HSP 70 genotype gives different expressions of HSP 70. The highest expression of HSP 70 was found in DD genotype while the rest of the genotypes gave no meaningful difference. The DD HSP 70 genotype was found in two different individual chickens from 2 lines namely commercial chicken and kampoeng chicken. DD genotype in these two chicken lines produces different HSP 70 expression which is 30.61 x 10^7 mRNA on commercial chicken and 5.127 x 10^7 mRNA on kampong chicken. The high number of HSP 70 expression in DD genotype means DD genotype is the type that the most intolerant to heat stress, while the other genotypes give same response. Thus, all HSP 70 genotypes except DD genotype are HSP 70 genotype candidate that resistant to heat stress. When value of HSP 70 is counted numerically, AD genotype is the genotype showed the lowest HSP 70 expression. Level of HSP 70 expression is an indicator of stress level experienced by an organism. High level of expression means high level of stress and vice versa. It can be concluded that DD genotype is HSP 70 genotype of chicken that the most susceptible and the most intolerant to heat stress danger and AD genotype is the genotype that the most tolerant and less susceptible to high ambient temperature.

Taking into account the effect of HSP 70 genotype on the observed variables, it appears that each genotype of HSP 70 showed a different response to acute exposure to heat stress. AD genotype, the HSP 70 genotype is the most resistant to acute heat stress and on the opposite is DD genotype. The difference in the resistance of both genotype HSP 70 is due to the influence of D allele. Under condition of homozygous (DD), this allele will be transformed into a genotype that cannot stand the heat but in heterozygous state (AD), it would show the opposite response.

Conclusion: This study revealed that there is a strong relation between chicken genotypes and HSP 70 genotypes in heat resistance. Kampong chicken had a better heat resistance as compared to Arabic and commercial chickens, whereas HSP 70 genotypes of chickens that most tolerant to high ambient temperatures is AD and DD genotype was the least tolerant to high ambient temperature.

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