Correlation Between Blood Parameters, Physiological and Liver Gene Expression Levels in Native Laying Hens Under Heat Stress

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Abstract. The heat stress plays an important role in feed efficiency, blood profile, rate metabolism. Growth performance, health and its influence economic loss of laying hens can be strongly affected by climate. The study to investigate the effect of the heat stress on blood parameters (White Blood Cells/WBC, lymphocytes, neutrophils, glucose, creatinine, creatinine kinase/CK), physiological status (rectal temperature/RT, heart rate/HR), and liver gene expression (Glutamate pyruvate transaminase/GPT, Glutamate oxaloacetic transaminase/ GOT, Threonine dehydrogenase/TDH, heat shock protein 70/HSP70) in laying hens. Two hundred native laying hens were randomly assigned to 10 groups of temperature humidity Index (THI), with 20 animals per group. They were kept in environmental condition with temperature humidity index (THI) ranging from 65.50 to 85.72 in temperature humidity. Blood plasma samples were used to determine the concentration of blood biochemical parameters, using a commercial kit by an automatic biochemical analyser. Liver gene expression levels were analyzed by Real-time PCR. The results showed that their RT, HR, WBC, neutrophils, creatinine, CK, liver gene expression levels were increased in high THI compared to at low THI. In contrast, glucose and lymphocytes levels was decreased in high THI. In addition, HR, RT, WBC, neutrophils, creatinine, CK, liver gene expression were positively correlated with THI. On the other hand, negatively correlated with THI were showed by glucose and lymphocite. As conclusion, based on these results, it can be concluded that all parameters in this study are closely associated with heat stress. These parameters can be consolidated to heat stress biomarkers in native laying hens.

1. Introduction
An animal is never independent of the environment in which it lives. The heat stressed native laying hens causes economic losses because it decreases egg production and growth performance. Since the climate of West Java is gradually changing to more heat, economic due to heat stress loss is also
increasing. Therefore, promoting optimal environmental factors (temperature and humidity) can be used to avoid heat stress and decrease economic and metabolic losses. Under most environmental condition heat is being lost from the body and when appropriate the rate of heat loss is modified by activities of heat exchange affectors or modulator in the physiological system in the body [1]. Humidity also plays a main role in the metabolism regulation in animal.

An indicator of heat stress is the temperature humidity index (THI). This indicator can be used to estimate the level heat stress of native laying hens in order to improve feed management and efficiency, also housing management [1, 2].

Previous studies have shown that thermoregulation system for example heart rate (HR), respiratory rate and rectal temperature (RT), and egg production in laying can be affected by heat stress [3]. The effect of heat stress on commercial layer hens during growing fase on might have indirect economic losses due to reduce metabolism rate, immunity and decreased growth performance [4]. Also, several reviews have been focused on the effect of heat stress on laying, such as effect on liver metabolic, kidney and ileum histologic [5]. In addition, physiological and biochemical indicators of stress very related to environment, especially housing temperature and humidity, have not been studied in native laying hens. Therefore, the objective of the present study was to investigate the effect of the heat stress on blood parameters (White Blood Cells/WBC, lymphocites, neutrophil, glucose, creatinine, creatinine kinase/CK), physiological status (rectal temperature/RT, heart rate/HR), and liver gene expression (Glutamate pyruvate transaminase/GPT, Glutamate oxaloacetate transaminase/GOT, Threonine dehydrogenase/TDH, heat shock protein 70/HSP70) in native laying hens.

2. Materials and Methods

2.1. Animal

Two hundred native laying hens (12 months age, 1325.32 ± 10.64 g) were used in this experiment, with twenty hens for each experiment. The twenty hens housed in an environmentally controlled and located in three region were Kuningan, Purwakarta, Sukabumi, during 1 month.

2.2. Experimental condition and treatment

This study was conducted in ten different experiments. All experiments were designed to have six levels of dry bulb temperature (25°C to 37°C) and five levels of humidity (65% to 89%). During heat stress treatment period, all animal were housed in constant heat and humidity condition, for Sukabumi, THI = 68, 70, 72; Purwakarta, THI = 73, 75, 77, 78 and Kuningan, THI = 79, 81, 83.

2.3. Physiological and Biochemical Determination of Plasma and Liver

Rectal temperature and heart rate were measured weekly, at 01.00-02.00 p.m. Blood samples were collected from each laying hens, selected at random from each treatment group on week 4, from the wing vein (vena cava superior), using a sterilized syringe and vacum tube contain K3EDTA. Hematological parameters (WBC, lymphocytes and neutrophils) were analyzed by a hematology analyzer. The blood sampel collected were also centrifuged to separated the plasma. The plasma was using to determination of concentration of biochemical parameters (glucose, creatinine, CK) by an automatic biochemical analyzer, using commercial kit.

Total RNA of GPT, GOT, TDH and HSP70 were isolated from the frozen liver samples, before reverse transcription into complementary DNA using standard procedures. All procedures were conducted by specific commercial kit (Rneasy Kit Qiagen, 2014).
2.4. Statistical Analysis

The data were statistically analyzed by one way analysis of variance (ANOVA) using the GLM procedure of SAS Version 8.2\textsuperscript{16} for a correlation degree (SAS Institute, 2001).

3. Result and Discussion

Person correlation test for the relationship between all parameters in this study is presented in Table 1. In Table 1, showed that correlation of all parameters were presented a positive association. In contrast, showed a negative association by lymphocytes with neutrophils.

The highest correlation showed by RT with HSP70 gene expression in all parameters. Correlation all parameters to HSP 70 showed higher than the other association combination.

Table 1. The relationship (r index) between physiological, biochemistry and liver gene expression parameters related to heat stress in native laying hens

|        | WBC  | Lym  | Neut | Glu  | Cre  | CK   | RT   | HR   | GPT  | GOT  | TDH  | HSP70 |
|--------|------|------|------|------|------|------|------|------|------|------|------|-------|
| WBC    | -    | 0.35 | 0.23 | 0.37 | 0.45 | 0.67 | 0.52 | 0.67 | 0.63 | 0.58 | 0.73 |       |
| Lym    | -    | -0.34| 0.38 | 0.48 | 0.53 | 0.64 | 0.75 | 0.51 | 0.42 | 0.35 | 0.62 |       |
| Neut   | -    | 0.31 | 0.51 | 0.48 | 0.68 | 0.69 | 0.61 | 0.41 | 0.53 | 0.69 |      |       |
| Glu    | -    | 0.21 | 0.23 | 0.23 | 0.35 | 0.53 | 0.45 | 0.63 | 0.33 |      |      |       |
| Cre    | -    | 0.92 | 0.63 | 0.35 | 0.77 | 0.65 | 0.72 | 0.58 |      |      |      |       |
| CK     | -    | 0.43 | 0.38 | 0.64 | 0.64 | 0.76 | 0.78 |      |      |      |      |       |
| RT     | -    | 0.78 | 0.23 | 0.51 | 0.39 | 0.84 |    |      |      |      |      |       |
| HR     | -    | 0.34 | 0.68 | 0.27 | 0.76 |      |    |      |      |      |      |       |
| GPT    | -    | 0.45 | 0.69 | 0.78 |    |      |    |      |      |      |      |       |
| GOT    | -    | 0.75 | 0.73 |      |    |      |    |      |      |      |      |       |
| TDH    | -    | 0.68 |      |      |    |      |    |      |      |      |      |       |
| HSP70  | -    |      |      |      |    |      |    |      |      |      |      |       |

WBC : White blood cells; Lym : lymphocytes; Neut : Neutrophils; Glu : glucose; Cre : creatinine; CK : creatine kinase; RT : rectal temperature; HR : heart rate; GPT : glutamate pyruvic transaminase; GOT : glutamate oxaloacetate transaminase; TDH : Threonine dehydrogenase; HSP 70 : Heat shock protein 70

In Table 2 can be seen that all parameters included physiological, biochemistry and liver gene expression showed a positive correlation (r) with THI. Conversely, a negative correlation (-0.12) showed by lymphocytes on THI. The heart rate and HSP 70 gene expression, both indicated highest association with THI in all parameters in this research.

Based on the results of this study, RT was higher correlation with HSP 70 at levels THI as than that at the other parameters (Table 1). This result is in accordance with the results of previous studies on many different breeds of hens [6] and also many animal [7]. This our results showed also that RT was showed high correlation with THI (Table 2). In Table 1 can be seen also that all parameters indicated high association with HSP 70. In the same way also showed a high positive correlation degree (r) of HSP with THI.
Table 2. The relationship between physiological, biochemistry and liver gene expression parameters in native laying hens with THI

| Parameter                          | Degree Correlation (r) with THI |
|------------------------------------|---------------------------------|
| Whole Blood Cells                  | 0.47                            |
| Lymphocytes                        | -0.12                           |
| Neutrophils                        | 0.78                            |
| Glucose                            | 0.41                            |
| Creatinine                         | 0.58                            |
| Creatine Kinase                    | 0.69                            |
| Rectal Temperature                 | 0.78                            |
| Heart Rate                         | 0.83                            |
| Glutamate Pyruvate Transaminase    | 0.47                            |
| Glutamate Oxaloacetate Transaminase| 0.56                            |
| Thrionne Dehydrogenase             | 0.54                            |
| Heat Shock Protein 70              | 0.86                            |

These results of this study showed closely associated with body temperature control of laying hens [8]. Environmental conditions, such as temperature, can alter weak bonds and disrupt threedimensional protein structure. Increasing temperature weakens the hydrogen bonds that stabilize α-helices and β-sheets [9]. High temperature can cause the protein to unfold, or denature. Previous studies have shown that the HR and RT are increased by heat stress in commercial laying [10]. Our result showed that HR and RT were closely associated indicators with heat stress. They were most sensitive to heat stress.

Once denatured, a protein can no longer perform its proper function and may even damage cells. Therefore, once a protein is even partially denatured, the cell must either refold it into the proper conformation, or destroy it before it can do any cellular damage. Molecular chaperones bind to denatured proteins, folding them into the proper configuration [12]. During heat stress, cells increase the levels of molecular chaperones called heat shock proteins to cope with the increased number of denatured proteins.

Heat shock proteins (HSP’s) are molecular chaperones that use the energy of ATP to catalyze protein folding after translation. Chaperones can also help refold proteins that have become denatured as a result of thermal stress. Many cells exposed to extreme temperatures undergo a “heat shock response”, which leads to a dramatic increase in the levels of specific proteins that help repair damaged proteins [12]. During a heat shock, the cell undertakes a rapid in cease in the synthesis of several critical Hsp’s. The cell can halt the transcription and translation of other genes, sparing biosynthesis. It stimulates the expression of the HSP genes by activating a heat shock factor (HSF), a transcription factor that binds to the heat shock elements in the promoters of genes for heat shock protein [13,14].

Although, there is still some uncertainty about the exact mechanism of activation of HSF, the trigger for the process is thought to involve damaged protein. In the absence of thermal stress, most of the cellular HSF is bound to HSP70 as inactive monomers. When the cell is stressed, the chaperones are lured away from HSF by damaged proteins [15]. The released HSF can then form trimers, which in turn bind the heat shock element on the HSP genes, activating them. Once the damaged proteins are repaired, HSP70 is free to bind HSF monomers and reverse the transcriptional activation [16,17].

In general, avian carry out body temperature control according to the discharge of body heat through evaporation, in particular by panting. For this reason, when animals are exposed to high temperature and humidity, physiological heat regulation is increased to control their body temperature. In this study, we
confirmed that this phenomenon showed by positive association between all parameter and positive correlation all parameter with THI (Table 1 and 2).

In Table 1 and 2 have been reported that stress in laying hens might caused an impairment of liver function [18]. In the present study, GOT, GPT and TDH levels were analyzed as liver damage markers [19] were associated at THI. The low correlation in plasma glucose level with THI can be explained by different factors, including reduced energy intake as a consequence of increased activities for thermoregulation, and negative effect of heat on gluconeogenesis as an endocrine acclimation to hot condition [20].

On the other hand, white blood cells, and it differensiasion (lymphocites and neutrofils) plays a main role in the heat stress condition. Several studies [21,22] have reported the relationships between heat stress and immune cell function in bovine, also in avian [23]. Regarding lymphocyte function in hens exposed to hot environments, some authors have reported an improvement [24] while others have described an impairment [22]. The reduction of lymphocyte during high THI means that exposure to heat and humidity during treatment can decrease the number of viable cells and reduce their responsiveness to mitogens. The acute phase response with its changes in blood plasma composition that react to disturbances of their homeostasis with a set of physiological changes [25]. This responses is associated with changes in lipid [26], creatinine and CK [27] and carbohydrate metabolism [24] and in some proteins such as HSP [27] and transaminase [28].

HSP are rewarding compounds chaperoning damaged molecules of cellular by heat stress [27]. The HSP work cognitively in cellular and tissue homeostasis [29] and are released intracelullaraly and extracellulary in an inducible form in response to stress [6,12, 29]. HSP70 level was increased in high THI. Exposure to stressors will stimulate a response of heat shock protein resulting in intra cellular HSP70 concentrations [25,26, 30].

4. Conclusion
Analysis among blood (hematologic, biochemistry), physiological and liver gene expression parameters in native laying hens heat stressed. In conclusion, Physiological parameter (HR, RT), also liver gene expression are closely correlated.

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