Organic Diallyl-n-Sulfide (Dn-S) inhibited the glycogenolysis pathway and heart failure of heat-stressed laying hens

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Abstract. Heat stress causes a decrease in metabolic and immune function, thus resulting in decreased production. Natural extracts such as the active compound diallyl-n-sulfide (Dn-S) are one strategy to overcome the adverse effects of heat stress. One hundred and fifty female laying hens, with a mean bodyweight of 1191.38±25.54 g, aged 11 weeks, were used in this experiment to study Dn-S’s impact from garlic on the metabolite profile of the glycogenolysis pathway and heart failure in laying hens. The sample of laying hens was divided into five treatment groups, each with 25 samples and the Dn-S from garlic isolated by distillation technique. The study was carried out with three types of experimental treatment, groups with a zone temperature of heat stress (38°C) and without Dn-S, heat stress (38°C) and 100 µL Dn-S/sample, heat stress (38°C) and 1125 µL Dn-S/sample, respectively. Based on the results of the study, it shows that heat stress causes an increase in the rate of glycogenolysis. It appears that the administration of 200 µL Dn-S effectively reduces the rate of glycogenolysis and can maintain a normal heart condition. It was concluded that heat stress in laying hens could be avoided by administering diallyl n-sulfide (Dn-S) from garlic. Dn-S has an essential role in preventing changes in the osmotic pressure of body fluids. Overall, it can cope with the metabolic and physiological changes associated with heat stress.

1. Introduction

The livestock productivity, especially laying hens, is closely related to physiological stress (including oxidative stress and decreased affinity of membrane proteins as receptors). Stress triggers a decrease in immunity [1,2] and metabolic rate for production [3–5]. This state stimulates homeostatic activity to return to normal physiological conditions. Although in many cases, increased homeostatic activity in response to decreased immunity and metabolism, does not indicate a return to normal physiological states but ends in physiological stress [6,7].

The microclimate environment of the cage, such as temperature and humidity, is a physical component of the environment that also causes physiological stress, especially heat stress. Heat stress is triggered by an increase in temperature and/or an imbalance between the environment’s temperature and humidity. The risk of heat stress is increasing along with the increasing global warming [8,9]. Indonesia as a tropical country has a greater risk compared to subtropical countries.
Based on the physiological molecular perspective, several important effects of heat stress are the occurrence of oxidative stress and decreased cell membrane fluidity, including a decrease in the function of membrane proteins as receptors, transport, ligands [10], signal transmission [11] and intercellular communication [12,13]. Both have an impact on decreasing metabolic rate and overall on poultry performance, immunity and even mortality.

Oxidative stress results in decreased biochemical and physiological functions, gene mutations and cell death. Oxidative stress is triggered by free radicals in cells, namely reactive oxygen or Reactive Oxygen Species (ROS), including Superoxide (O2\(^-\)), hydrogen peroxide (H\(_2\)O\(_2\)) and hydroxyl radical (HO\(^-\)) [5,14]. The increase in ROS is the impact of increasing oxidative phosphorylation activity in the mitochondria for ATP (energy) synthesis, maintaining the basal metabolism of livestock [10,15]. ROS harms lipid and protein metabolism because ROS reduces protein translation and lipid metabolism in the framework of protein biosynthesis for growth. ROS with high levels will destroy essential proteins such as enzymes. On the other hand, excessive heat radiation from the environment causes increased cell proteins' denaturation [16,17].

Previous studies have shown that the physiological system, such as heart rate (HR), respiratory rate and rectal temperature (RT), and egg production in laying can be affected by free radicals. The effect of free radicals on commercial layer hens during growing phase on might have indirect economic losses due to reducing metabolism rate in the liver [11,18], immunity [19], heart failure [13] and decreased growth performance [20]. Also, several reviews have been focused on the effect of heat stress on laying, such as effect on liver metabolic [21], kidney and ileum histologic [22,23]. In addition, physiological and biochemical indicators of stress very related to tissue function [20,24].

Countermeasures are needed so that the negative impact of oxidative stress can be minimized. Nutritional management is expected to overcome the negative effects of this heat stress [1,12]. The results of previous studies show that the use of synthetic diallyl compounds can act as anti-carcinogens [10,18], reduce free radicals and increase membrane protein activity. Provision of organic feed additives (diallyl compounds) is a better effort related to food safety. One source of diallyl is garlic (garlic).

It is hoped that the provision of diallyl compounds in poultry can overcome oxidative stress and increase transferrin activity, and in turn can improve the performance of liver metabolism and prevent decreased heart function. Laying hens are one type of poultry that can be used as experimental animals related to the theme of this research. Apart from having a high metabolic rate, making it easy to respond to heat stress in the environment, it also has a short growth cycle so that its biological activity is expected to play a role in responding to the treatment of this study.

2. Materials and methods

2.1. Animal
One hundred and fifty laying hens (15 months age, 1758.89±25.73 g) were used in this experiment, with fifty hens for each experiment groups. The animal samples housed in an environmentally controlled and located in Sukamulya Animal Fams, Sukabumi, during 2 month.

2.2. Experimental condition and treatment
The study was conducted with five types of experimental treatment, as follows:
A: Heat stress (38\(^\circ\)C) and without Dn-S
B: Heat stress (38\(^\circ\)C) and 100 µL Dn-S/sample
C: Heat stress (38\(^\circ\)C) and 1025 µL Dn-S/sample
Administration of Dn-S is carried out every morning before being given drinking water and rations, by means of orally, by feeding it directly into the cranial part of the esophagus, using a micro-pipette with a tip. The experimental layer chicken samples were placed in a one-tier colony cage which was insulated based on the experimental unit. The experimental cage is made from a combination of wooden blocks and wire rang. The experimental cages used were 5 units. Each cage is equipped with an
incandescent lamp as a heat source and a thermostat, with a drum temperature range of 37–39°C or an average of 38°C. Heat treatment was given at from 06.00 a.m. to 08.00 p.m.

2.3. Plasma biochemical determination of liver glycogenolysis and heart failure
Blood sample collection in each sample of experimental chickens was carried out at the end of each month. Blood samples were collected from the pectoralis externa vein as much as 3 mL of each sample, using a 22 needle-sized spuit. Blood samples were collected into a 3 mL venojette tube containing EDTA. The venojette tube containing the blood sample is placed in a cool box containing ice gel as a coolant.

The venojette tube filled with blood is then centrifuged at the Physiology and Biochemistry Laboratory, Faculty of Animal Husbandry, Padjadjaran University, to separate the blood plasma. The blood plasma that has been obtained is inserted into a sample tube to be analyzed for metabolites related to glycogenolysis and heart function.

Analyzes of samples of glycogenic metabolites and heart function have been carried out using a spectrophotometer technique. Standards and reagents used, reaction methods and the number of samples and reagents thereof follow the instructions in the kit procedure based on Biolabo KIT, France and Mybiosource KIT, MyBiosource Inc. USA, as well as Randox, UK.

2.4. Statistical analysis
The data were statistically analyzed by technique of one way analysis of variance (ANOVA) using the GLM procedure of SAS Version 8.2 for a Completed Randomly Design [7,10].

3. Result and discussion
Current research has shown the ability of organic Dn-S to inhibit glycogenolysis in laying hens. The results of statistical analysis showed a significant effect (P<0.05) of the level of Dn-S administration on the rate of glycogenolysis (table 1).

| Metabolic | Levels | Dn-S 0 µL | Dn-S 100 µL | Dn-S 125 µL |
|-----------|--------|-----------|-------------|-------------|
| Glikogen (mg/g) | 0.37±0.02a | 0.38±0.02b | 0.46±0.02c |
| Glikogen Phosforilasi (IU/dL) | 0.33±0.01a | 0.33±0.01a | 0.30±0.02b |
| Glukosa 1-Phospat (IU/dL) | 0.39±0.03a | 0.36±0.02b | 0.33±0.03c |
| Phosfoglukomutase (IU/dL) | 0.41±0.02a | 0.42±0.03a | 0.38±0.02c |
| Glukosa 6-Phospat (mg/dL) | 0.29±0.02a | 0.30±0.01a | 0.26±0.02a |
| Glukosa 6-Phosphatase (IU/dL) | 0.31±0.03a | 0.28±0.01b | 0.28±0.03b |
| Glukosa (mg/dL) | 75.95±2.66a | 74.35±3.84a | 65.67±3.07b |

a,b,cMeans in the same row with a different letter of superscripts are significantly different (P<0.05); Values are given in Mean±SD.

The results of this study indicate that Dn-S appears to be effective in reducing the rate of glycogenolysis under heat stress. The results (table 1) show that administration of Dn-S appears to reduce (P<0.05) the metabolite compounds in the conversion of glycogen to glucose, as well as the enzymes that catalyze the reshuffle reactions. The effectiveness of Dn-S utilization appeared to be optimum by giving 125 µL. The optimization of the role of Dn-S in reducing the rate of glycogenolysis was seen with quail glycogen levels in non-heat stress conditions, not significantly different (P>0.05) with glycogen levels in laying hens experiencing heat stress but given Dn-S 125 µL. This optimization was also seen with the levels of catalytic enzymes and metabolite compounds as a result of enzyme hydrolysis, which did not show any difference with the group of laying hens without heat stress. Although, one of the metabolite compounds still shows high levels, namely glucose 1-phosphate.
Based on these results (table 1), it can be explained that heat stress increases the metabolic rate, especially to provide energy-related to the homeostasis process. Homeostasis increases in heat stress [20,25], aims to maintain biochemical and physiological processes for survival [26] and reproduction [27]. Heat radiation to the internal milio environment of livestock causes the livestock to instinctively reduce its feed intake or ration consumption. This reduction aims to prevent heat from digestion of food (heat increment) in the intestine [28,29] and also metabolic heat [30] and an increase in free radicals [3,21]. As compensation for the decrease in feed intake, livestock activate the glycogenolysis mechanism, triggered by an increase in nerve stimulants through neurotransmitters [24,31], thereby increasing levels of the hormone epinephrine [29].

Overall, the effectiveness of Dn-S in reducing the rate of glycogenolysis shows that Dn-S is able to improve metabolic balance [25] related to heat regulation and homeostasis [28]. Continuous heat stress in high conditions causes important behavior to evaporate body heat, this has an impact on increasing blood pH [27] compared to quails that are not exposed to excess heat. One of the important factors that play a role in the acidity of blood is the temperature of the environment. The effect of high environmental temperature causes important behavior, which is the behavior of releasing heat through exhalation by gasping (quick and short breathing) or hyperventilation. Through this behavior, the release of H2O and CO2 compounds becomes excessive [10,25], which causes the formation of bicarbonate (H2CO3) to decrease. Bicarbonate is a proton donor H+ and forms carbonic acid (HCO3-). The ability of Dn-S with a given level of 125 µL to overcome the impact of heat on the quail’s metabolic system, illustrates that Dn-S has the ability to bind to proteins, especially at protein H atoms, causing reduced protein denaturation. This means reducing cell death and maintaining protein function [25,29]. The two positive impacts are simultaneously able to maintain the proteins in the erythropoiesis system and the proteins in the blood cells (erythrocytes and leukocytes). The results of research by previous study [25,30], showed the role of Dn-S in maintaining blood precursor proteins from damage due to reactive compounds (ROS).

In the other hand, in table 2 presented the response of laying hens to biomarkers of heart failure with Dn-S levels in ration. The results showed a significant reduction (P<0.05) of cardiovascular biomarkers circulating in the blood plasma. The results of this study reveal the effectiveness of Dn-S in avoiding damage to heart cells.

The role of Dn-S which is able to control and overcome heat stress is related to its ability to improve reaction kinetics with H2O. High Dn-S bond energy with H2O makes it difficult to evaporate and excreted through the kidneys, resulting in decreased body fluid loss. In addition, the amphipathic biomolecules present in blood plasma cause a very beneficial interaction with Dn-S, which carries charged S and O atoms, thereby increasing the electrostatic intrusion pattern. An investigation previously [6,28,32] stated that this electrostatic interaction can maintain the structure of proteins, carbohydrates and lipids that bind to it. Indirectly, reducing the risk of damage to biomolecules due to heat and increasing body fluids’ capability to retain heat.

**Table 2.** The response of laying hens to biomarkers of heart failure with Dn-S levels in the ration.

| Cardiovascular Biomarkers          | Levels               |
|-----------------------------------|----------------------|
|                                   | Dn-S 0 µL            | Dn-S 100 µL       | Dn-S 125 µL       |
| CRP High Sensitivity (mg/L)       | 9.5±±0.74a           | 6.85±±0.11b       | 5.83±±0.84c       |
| H-TFABP (ng/mL)                   | 7.24±±1.01a          | 5.52±±0.44b       | 4.94±±0.23b       |
| Homocysteine (µmol/L)             | 11.33±±2.85a         | 9.76±±1.32a       | 8.75±±0.22b       |
| γ-Glutamin Transpeptidase (IU)    | 25.45±±3.64a         | 16.27±±2.34b      | 14.43±±2.03b      |
| sPLA2-IIA (ng/dL)                 | 52.81±±5.15a         | 32.75±±2.04b      | 32.34±±3.45b      |

CRP High Sensitivity = C-Reactive Protein; H-TFABP= Heart-Type Fatty acid-binding Protein; sPLA2-IIA = Secretory Phospholipase-A2-IIA.

Means in the same row with a different letter of superscripts are significantly different (P<0.05); Values are given in Mean±SD.
The reduced risk of molecular damage in tissues is primarily a manifestation of the ability of Dn-S to act as an antioxidant. This ability causes tissue resistance (especially the heart) to prevent cell death, resulting in lower tissue damage [14,29]. Cells' ability to maintain and maintain themselves causes a decrease in the migration of molecules contained in heart cells to the blood vessel system [20–22,24,33,34].

4. Conclusion
Organic Dn-S is very useful for preventing the increase of glycogen breakdown of heat-stressed laying. Dn-S has also shown the ability to effectively reduce the damage to heart cells.

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