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Diallyl-n-Sulfide of Garlic Inhibits Glycogenolysis in Heat-Stressed Laying Sentul Chicken

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ABSTRACT

Heat stress causes a decrease in metabolic function and immunity, which results in a decrease in production. The provision of natural extracts such as the active compound dyally n-suldifa (Dn-S) is one strategy to overcome the adverse effects of heat stress. One hundred and twenty five female laying native chickens, with an average body weight of 1213.83±15.52 g, 40 weeks old, were used in this experiment, to study the impact of Dn-S administration from garlic on the metabolite profile of the glycogenolysis pathway. laying. Laying hens were distibuted into five treatment groups, each with 25 samples. Dn-S isolation from garlic isolated by distillation technique. The study was carried out with five types of experimental treatments, as follows the group with a comfort zone temperature (24°C) and without the administration of Diallyl n-Sulfide (Dn-S), heat stress (38°C) and without Dn-S, heat stress (38°C) and 100 µL Dn-S, heat stress (38°C) and 125 µL Dn-S, heat stress (38°C) and 150 µL Dn-S/head. Based on the results of the study, it was shown that heat stress causes an increase in the rate of glycogenolysis and intermediate metabolites and their catalyzing enzymes. It appears that the administration of 150 µL Dn-S, effectively reduces the rate of glycogenolysis. It was concluded that heat stress on laying hens could be avoid by administering garlic Dn-S.

Keywords : Diallyl n-Sulfide Glycogenolysis, heat-stressed

INTRODUCTION

Sentul chikens have been developed and cultivated throughout Indonesia for a long time. Based on the category of body temperature adaptation, chickens are classified as homothermic animals. This group of animals, physiologically has a system that is able to maintain the normal range of body temperature, which is 38 – 39°C. However, chickens have a comfortable environmental zone or the so-called thermoneutral zone to be able to express their genetic abilities, which is 21-25°C.

Increasing environmental temperature is an important problem for the development and growth of quail. The environmental temperature above the comfort zone causes the physiological and biochemical processes to be directed more towards homeostasis (Mushawwir et al., 2010, 2011, 2020a.b; Ammer et al., 2018). Homeostasis is increased in order to achieve a balanced degree of organ function and metabolic rate in order to regulate body heat (thermoregulation). This process has an impact on the shift in the function of energy, from the purpose of production to energy to fulfill the achievement of homeostasis. The longer exposure to heat stress, the lower feed intake (Allen et al., 2015; Istvan et al., 2020) so that energy adequacy is reduced.

On the other hand, heat stress also increases the risk of DNA mutation and denaturation of body proteins (enzymes, receptors, cell transporters, hormones). DNA mutations and protein denaturation are triggered by increased production of free radicals (Gehrke et al., 2013; Slimen et. al., 2016; Mushawwir et al., 2019) from mitochondrial oxidation-reduction (cellular stress), and/or direct stress in the form of environmental heat radiation to the body of quail. This condition has a wide impact on metabolism, especially energy synthesis in the matrix in the mitochondria (Gray et al., 2015; Tian et al, 2015; Carrol et al., 2016).

The first step or stage of reshuffling the molecule in these circumstances, in order to meet energy is the reshuffle or catabolism of carbohydrate reserves (polysaccharides) in the cytoplasm, namely glycogen degradation. This catabolic reaction is known as glycogenolysis. The main purpose of this reaction is the formation of glucose into pyruvate, either with the help of oxygen (aerobic glycolysis), or without oxygen (anaerobic glycolysis).

One of the strategies adopted to reduce the impact of heat stress is the provision of natural feed additives, namely diallyn n-sulphide (Dn-S) from garlic (garlik). Dn-S is an volatile component that has reactive oxygen (O) and sulfur (S) atoms to be able to bind to free radicals (Tian et al., 2015, Tanuwiria et al., 2020;

Mushawwir et al., 2021c). It is hoped that with its reaction ability, it is able to prevent the bad effects of free radicals in lowering the metabolic rate. It is also expected to be able to overcome protein denaturation so that protein function can be optimal.

Therefore, it is important to study the impact of Dn-S administration in relation to the use of catalyzed glycogen reserves for energy fulfillment (glycogenolysis) under heat stress conditions.

Through this study, a hypothesis was established that administration of Dn-S was able to suppress the enzymes involved in the breakdown of glycogen or glycogenolysis in heat-stressed laying hens.

MATERIAL AND METHODE

Animal and Experiment Design

One hundred and twenty five (125) Sentul Chickens, female, with an average body weight of 1213.83±15.52 g, 40 weeks old, were used in this experiment. Sentul chicken samples were divided into five treatment groups, 25 each. Each treatment consisted of five replications, so that each replication consisted of 5 samples of Sentul Chicken.

The study was conducted with five types of experimental treatment, as follows:

A : Comfort zone temperature (24°C) and without giving Diallyl n-Sulfide (Dn-S)
B : Heat stress (38°C) and without Dn-S
C : Heat stress (38°C) and 100 µL Dn-S/sample
D : Heat stress (38°C) and 125 µL Dn-S/sample
E : Heat stress (38°C) and 150 µL Dn-S/sample

The administration of Dn-S was carried out every morning before being given drinking water and rations, orally, fed directly into the cranial part of the oesophagus, using a micro pipette with a tip. Sentul chicken samples were placed in a single-level colony cage which was insulated based on the experimental unit. The experimental cage was made from a combination of wooden blocks and wire rang. The experimental cages used were 5 units. One drum unit consists of Sentul Chicken with a cage temperature according to the comfort zone, with a temperature range of 23-25°C or an average of 24°C. The other four cages are equipped with incandescent lamps as a heat source and a thermostat, with a drum temperature range of 37-39°C or an average of 38°C. Heat treatment is given at 07.00 in the morning until 20.00 in the evening.

Sentul chicken rations during the experiment were administered ad-libitum. The ration given was a commercial ration obtained from a poultry shop in Bandung. The composition of metabolic energy and nutrient rations are presented in Table 1.

Table 1. Content of Metabolic Energy and Nutrient in Experiment Ration

Metabolic Energy and Nutrient	composition
Metabolic Energy (Kkal/kg)	2741,52
Crude Protein (%)	16,73
Calsium (%)	3,31
Phospor (%)	2,09
Lysin (%)	1,85
Methionine (%)	1,17
Crude Fiber (%)	4,81
Crude Fat (%)	6,25

Preparation of Diallyl n-Sulfide of Garlic

The separation of diallyl compounds was carried out according to Block (1985) and Amin et al. (2014). The expected characteristics of diallyl isolates are physically clear with a slight yellow, specific odor of garlic, boiling point of 180°C and molecular weight ranging from 141.21 to 146.28 g.mol-1 (Mushawwir et al., 2020c, 2021a).

The sliced garlic sample was put into a round bottom flask then added aquadest until the entire sample was completely submerged in a long necked round bottom flask that had been assembled in a water distillation device.

Distillation is carried out in the first step at a temperature of 75-80°C and the next step for 4-5 hours at a temperature of 100°C (Block, 1985). The dially compound isolates obtained were analyzed using the GS/MS technique. The content of Dn-S isolated from garlic present in Table 2, as follow,

Table 2. Contents of Dn-S Isolated from Garlic*

Contents Dn-S	%
Diallyl sulfide	13.63
Diallyl disulfide	42.63
Diallyl trisulfide	19.53
Diallyl tetrasulfide	6.36
Methyl allyl disulfide	9.74
Methyl allyl trisulfide	2.84
UNK	1.60
UNK	0.52
UNK	1.11

UNK: Unknnown, *analyzed in Indosain Lab., Bandung.

Sample Collection and Analysis

This experiment was conducted for two months at the Jaya Sentul Chicken Farm, Subang. Blood samples were taken from each experimental Sentul Chicken at the end of every month. Blood samples were collected from the jugular vein as much as 3 mL from each Sentul Chicken, using a syringe with a needle size of 22. The blood sample was collected into a 3 mL venojette tube containing EDTA. The venojette tube containing the blood sample was placed in a cool box containing ice gel as a cooler.

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

Analysis of samples of glycogenolytic metabolites has been carried out using spectrophotometer techniques. Standards and reagents used, reaction methods and quantities of samples and reagents followed the instructions in the procedure kit based on Biolabo KIT, France and Mybiosource KIT, MyBiosource Inc. USA.

Data Analysis

Data on the levels of glycogenolysis metabolites obtained were analyzed using an analysis based on a completely randomized design mathematical formula (Gomez and Gomez, 1995). Furthermore, a comparison test has been carried out to determine the difference in the average levels of metabolites in different treatments, using Duncan's different test

RESULT AND DISCUSSION

The effect of administering dialyl n-sulphide (Dn-S) on Sentul chickens of heat stress, based on the results of the study is presented in Table 3.

In this experiment, the Sentul Chicken group was placed in a cage with an ideal temperature (comfort zone = thermoneutral zone) and without Dn-S administration. The average glycogen content in the Sentul chicken group was 0.93 mg/g (Table 3). There was a significant decrease in glycogen levels ($P<0.05$) in groups of Sentul chickens treated with heat stress without Dn-S and with Dn-S 100 µL to 125 µL, which were 0.37 mg/g respectively; 0.39 mg/g and 0.76 mg/g, indicating that during heat stress there was a decrease in glycogen as a result of glycogen degradation or catabolism (glycogenolysis), even though Dn-S was given up to 125 µL.

The effectiveness of Dn-S administration was seen in the group of chickens that were given 150 µL of Dn-S, this was indicated by the glycogen content of the Sentul Chicken group (0.92 mg/g), not significantly different ($P>0.05$) with the Sentul chicken group without heat treatment. The results of this study indicate that heat stress increases the rate of energy supply from alternative pathways (Xu et al., 2015; Nurmalia et al., 2020), such as glycogenolysis (Gray et al., 2015; Ammer et al., 2018; Jiwandidi et al., 2020), there is a decrease in glycogen (Kamil et al., 2020; Mushawwir et al., 2021a, c), an increase in glycogen synthesis. glucose (Renaudeau et al., 2012; Tian et al., 2015; Mushawwir at al, 2020d,e).

Table 3. Glycogenolysis Pathway Metabolites Of Sentul Chicken Under And Without Heat Stress and Administration of Dn-S

Metabolites	Temperature of 24°C and Without Dn-S (A)	Heat Stres (38°C) and Dn-S Levels			
		Without Dn-S (B)	Dn-S 100 µL (C)	Dn-S 125 µL (D)	Dn-S 150 µL (E)
Glycogen (mg/g)	0.93[a]	0.47[b]	0.39[b]	0.76[c]	0.92[ad]
Glucose (mg/dL)	71.66[a]	87.34[b]	84.46[b]	73.32[c]	70.78[c]
Glucose 1-Phospat (IU/dL)	0.40[a]	0.54[b]	0.55[b]	0.49	0.44[d]
Phosphoglucomutase (IU/dL)	0.32[a]	0.52[b]	0.57[c]	0.55[c]	0.31[ad]
Glycogen Phosphorylation (IU/dL)	0.34[a]	0.44[b]	0.42[bc]	0.45[bc]	0.37[ac]
Glucose 6-Phospat (mg/dL)	0.14[a]	0.26[b]	0.33[b]	0.28[c]	0.18[ad]
Glucose 6-Phospatase (IU/dL)	0.21[a]	0.36[b]	0.35[b]	0.31[c]	0.23[a]

[a,b] Different letter superscripts on each average parameter, on the same row show a significant difference ($P<0.05$)

The data in Table 3, clearly shows that the activity of the enzyme that catalyzes glycogen breakdown was very active in Sentul chickens exposed to heat stress without Dn-S administration.

The activities of the enzymes Phosphoglucomutase and glucose 6-phospathase, were significantly increased by 0.52 and 0.36 IU/dL, respectively, compared to the activity of these two enzymes in chickens without heat stress. Consequences of increased activity of enzymes that play a role in the breakdown of glycogen, resulting in an increase in intermediate compounds, including glucose 1-phosphate and glucose 6-phosphate.

The activity of the two enzymes, as well as the concentration of intermediate compounds, seemed to still be found at high levels, even though the acid group exposed to heat had been given 100 µL of Dn-S. These results confirm that the administration of DN-S 100 µL has not been able to prevent glycogen breakdown under heat stress stress. Glycogen is needed as an alternative energy supply (Carrol et al., 2016; Fabris et al., 2017). The results of previous studies have been reported with consistent results that cows exposed to heat will reduce feed consumption (Allen et al., 2015; Adriani and Mushawwir, 2020); as well as to small ruminants (Khan et al., 2015; Slimen et al., 2016), and the same results were also shown in laying hens and broilers (Mushawwir et al., 2020c, d; Mushawwir et al., 2021a, b,c).

Related the importance of glycogen breakdown, it has also been published by previous researchers that glycogen as an energy reserve (Mushawwir et al., 2010, 2018; Loyau et al., 2014; Jiwandini et al., 2020; Mushawwir et al., 2020d), in the form of polysaccharides (Siskos et al., 2017; ǀTanuwiria et al., 2020), is broken down through the phosphorylation mechanism (Xu et al., 2015; Tian et al., 2015; Slimen et al., 2016) by activating chemical signals through the AMP cycle (Gray et al., 2015; Roland et al., 2016; Sang-Ho et al., 2018), guanylyl cyclase as a fist messenger (Pickler et al., 2013; Carrol et al., 2016; Nurmalia et al., 2020; Mushawwir et al., 2021b). This overhaul mechanism is needed to be able to meet the energy requirements for the homeostasis process in a state of reduced feed consumption (Renaudeau et al., 2012; Ammer et al., 2018; Suwarno et al., 2019; Mushawwir et al., 2020c).

The other side of the results of this study, shows that although glycogen is the main and first pathway that is activated in heat stress conditions, Dn-S administration seems to be able to prevent this glycogen breakdown. This Dn-S capability can be viewed from two aspects. Firstly, Dn-S is able to prevent the activity of enzymes associated with glycogen breakdown (hhh, 8484), possibly through a mechanism of blocking the active site of the enzyme (Adriani et al., 2015, 2018, 2020; Burdick et al., 2011), also by preventing signaling to enzymes (Cai et al., 2017; Dinana et al., 2019), as well as preventing the receptor for extracellular signaling (Gehrke et al., 2013; Ippolito et al., 2014; István et al., 2020, Mushawwir et al. 2021a). Secondly, Dn-S appears to be able to completely prevent thermoreceptor and osmoreceptor activation.

The effectiveness of this Dn-S to prevent glycogenolysis, was seen with the administration of 150 µL. The content of Dn-S which belongs to the essential oil group plays an important role in inhibiting the breakdown of glycogen. The same result seems to have been reported by Suwarno et al. (2019); Mushawwir et al. (2020a,b,c,d,e); Na et al. (2020), Mushawwir et al. (2021a,b), although with different oil extract (Hermawan et al., 2017; Mushawwir et al, 2010, 2018; Kamil et al., 2020)..

CONCLUSION

The results of the present study indicate that oral administration of 150 µL of Dn-S to Sentul chickens exposed to heat suppresses glycogen breakdown or reduces the rate of glycogenolysis.

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