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The Effects of Ginger Volatile Oil (GVO) on The Metabolic Profile of Glycolytic Pathway, Free Radical and Antioxidant Activities of Heat-Stressed Cihateup Duck

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***Abstract*— One hundred twenty-five female ducks, with an average body weight of 1485.51±14.72 g, aged eight weeks, were used in this experiment to examine the effect of ginger volatile oil (GVO) on the metabolic profile of the glycolytic pathways, free radical and antioxidant activity in heat-stressed Cihateup ducks. GVO isolation was carried out by the distillation technique. Cihateup duck samples were divided into five treatment groups, each group with 25 animals. Each treatment consisted of five replications so that each repetition consisted of 5 duck samples. The treatment in this study was A: temperature of the comfort zone (24°C) and without administration of GVO; B: heat stress (38°C) and without GVO; C: heat stress (38°C) and 150 µL GVO/tail; D: hot stress (38°C) and 200 µL GVO/tail, E: Hot stress (38°C) and 250 µL GVO/tail. GVO was given every morning orally (force-fed). The data then statistically analyzed with ANOVA procedure to determine the treatment effect. Duncan's multiple range test was used to compare the treatment effect. The results showed that the metabolic profile of the glycolytic pathway appeared to be in the normal range by administering 250 µL GVO. The reduction of free radicals activity and increased endogenous antioxidants (Glutathione Peroxidase) activity were also found in GVE treated ducks. In conclusion, the heat stress of Cihateup duck was reduced by administering the GVO.**

***Keywords*— GVO; glycolytic pathway; free radical; antioxidant; duck.**

I. INTRODUCTION

Based on the thermoregulatory mechanism, duck is categorized as a homoiothermic animal. This group of animals physiologically has a system that can maintain a normal range of body temperature, which is 38-39°C. Nevertheless, ducks have a comfort zone or thermoneutral zone to be able to carry out normal metabolism, which is 21-25°C. Changes in environmental temperature cause heat dissipation, which requires biochemical and physiological changes. At present, increasing environmental temperatures is an essential issue for the development and growth of ducks. The ambient temperature above its comfort zone causes the physiological and biochemical processes to be more focused on achieving homeostasis [1]. Homeostasis increases in order to achieve a degree of equilibrium of organ function and metabolic rate in the framework of regulating body heat (thermoregulation) [2]. This process has an impact by shifting the energy allocation, from production to homeostasis maintenance. The more prolonged exposure to this heat stress, the decreased feed intake [3,4], so that energy sufficiency is reduced.

An imbalance between feed intake and energy requirements for heat-stressed animals shifts the metabolism state by adjusting biochemistry and physiological signals, mainly due to hormone [5] and neurotransmitters activity [6]. This shift is characterized by increased nutrient catabolism through the glycogenolytic pathway, instead of glycolysis in the normal state. The rate of glycogenolysis continues to stay in a high state during stress [7]; on the contrary, it will decrease with decreasing levels of stress. Simultaneously, this decrease is characterized by reactivation of glycolysis [8]. Therefore, an increase in the rate of glycolysis can be an indicator of stress reduction in ducks.

Furthermore, heat stress also increases the risk of DNA mutation and denaturation of body proteins (enzymes, receptors, cell transporters, hormones). DNA mutation and protein denaturation are triggered by increased free radical production [6,9] from the oxidation of mitochondrial reduction (cellular stress), and direct stress in the form of environmental heat radiation to ducks. This condition has a broad impact on metabolism, specifically the synthesis of energy in the matrix in the mitochondria [10]. This phenomenon directly affects glycolytic activity [11] and free

radical production [8,12] and antioxidant activity [4,7]. Free radicals stimulate the oxidation of long-chain fatty acid-equal fatty acids to malondialdehyde (MDA). The results of previous studies show that the higher production of free radicals, the higher MDA production, therefore, MDA is able to reflect the number of radical compounds as a result of cellular stress from heat stress.

The utilization of natural ingredients is one of the strategies adopted to reduce the impact of this heat stress. The use of natural ingredients has been extensively studied [13,15] related to free radicals [8,16,17]. Ginger has the potential as an anti-stress material because it contains volatile oil [16,18]. However, to our knowledge, there is a lack in the study of ginger volatile oil (GVO) on heat-stressed local ducks.

II. Materials and Methods

A. Livestock, Experimental Design, and Ration

One hundred twenty-five (125) female ducks, with an average body weight of 1485.51±14.72 g, aged eight weeks, were used in this experiment. Cihateup duck samples were divided into five treatment groups, each with 25 animals. Each treatment consisted of five replications so that each repetition consisted of 5 duck samples.

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

- A: Temperature comfort zone (24°C) and without GVO administration
- B: Heat stress (38°C) and without GVO
- C:Heat stress (38°C) and 150 µL GVO/tail
- D: Heat stress (38°C) and 200 µL GVO/tail
- E: Heat stress (38°C) and 250 µL GVO/tail

GVO administration was done every morning before being given drinking water and ration orally (force-fed) directly into the cranial esophagus cavity, using a micropipette with its tip. Experimental animals were placed in a colony housing that is partitioned according to the experimental unit (pen). The pen was made of a combination of wooden beams and ram wire—animal cages used as many as five units. One cage unit consisted of twenty-five ducks with a cage temperature following the comfort zone, with a temperature range of 23-25°C or an average of 24°C. The other four cages were equipped with incandescent lamps as heat sources and thermostats, with a drum temperature range of 37-39°C or an average of 38°C. The heat treatment was given at 07.00 AM till 8.00 PM.

Drinking water was given *ad-libitum*, given 15 minutes after giving GVO treatment. Experimental duck rations during the study were administered *ad-libitum*. The ration given was commercial ration, with nutrient content is as shown in Table 1.

B. Ginger Volatile Oil Isolation

Separation of GVO sequential compounds was carried out based on the procedure [19]. The characteristics of volatile oil isolates were physically clear with slightly yellow, specific odor, boiling point 180°C, and a molecular weight ranging from 141.21-146.28 $g.mol^{-1}$ [19,21]. The isolated GVO was stored in a closed container to prevent the evaporation of the active compounds.

TABLE I
Ration Nutrient Content

Item	Content
Metabolisable energy (Kkal/kg)	3161.26
Crude Protein (%)	22.10
Calcium (%)	1.86
Phospor (%)	1.33
Lysine (%)	1.74
Methionine (%)	0.83
Crude fiber (%)	5.18
Crude fat (%)	6.72

Ginger samples were obtained from the traditional market, the outer skin of the ginger was cleaned first using flowing water (tap water), then dried in the sun, until all the remaining water on the surface of the skin evaporates. Ginger that has been thinly sliced, then mashed using a blander.

The distillation was carried out the first step at a temperature of 75-80°C. The volatile oil obtained in the first stage was collected in a separating funnel and then separated between volatile and water. Then the essential oil was collected in a closed container.

C. Sample Collection and Analysis

This experiment has been carried out for two months at Al Musthofa Duck Farm, Sukabumi, West Java, Indonesia. Blood samples were taken at each duck experiment every month. Blood samples were collected from the jugular vein as much as 3 mL from each animal using a syringe with a needle size of 22. Blood samples were collected into 3 mL blood sampling tubes containing EDTA. Blood samples were placed in a cool box containing ice gel as a cooler (4°C).

Whole blood was then centrifuged at 1500 g, 4°C for 10 minutes in the Laboratory of Physiology and Biochemistry, Faculty of Animal Husbandry, Padjadjaran University, to separate the blood plasma. The obtained blood plasma was inserted into the sample cuvette for analysis of glycolic and antioxidant-related metabolites. In contrast, the deposition of blood cells from the centrifuge results was collected in a separate cuvette for analysis of free radical activity (Malondyaldehide/MDA).

All parameters of metabolites and enzymes involved in the glycolytic pathway, as well as free radical and antioxidant activity, have been analyzed using spectrophotometer techniques. The standards and reagents used, the reaction method, and the number of samples and reactants were based on the instructions in the kit procedure (Biolabo, France).

D. Data Analysis

Data on glycolysis metabolite levels, free radical and antioxidant activity obtained were analyzed using an analysis of variance (ANOVA), using IBM SPSS 21 program. Duncan's multiple range tests were carried out on significant differences.

III. Results and Discussions

A. Impact of GVO on Glycolysis

The effect of GVO administration on the levels of plasma glycolytic metabolites of heat-stressed ducks is shown in Table 2. In this experiment, the A group was placed in an ideal temperature (comfort zone=themoneutral zone) and without GVO administration. The average glucose level in this duck group was 0.85 mg/g (Table 2). Significant reduction in glucose levels ($P<0.05$) in duck groups treated with heat stress without GVO and with GVO up to 200 µL, i.e. 236.43 mg/dL; 207.31 mg/dL; 219.42 mg/dL and 227.75 mg/dL, showing that during heat stress there was a decrease in glycolic as a result of degradation, although given a GVO of up to 200 µL.

The effectiveness of GVO was shown in the group of ducks that were given GVO as much as 250 µL. This effectiveness was indicated by the glucose level of this duck group (231.72 mg/g), not significantly different ($P>0.05$) from the duck group without heat treatment. The results of this study indicated that heat stress increased the rate of energy supply from alternative pathways, such as glycogenolysis [22].

TABLE II
Ration Nutrient Content

Metabolites	Temperature 24°C and without GVO	Heat-stress treatments (38°C)[1]			
		without GVO	GVO 150 µL	GVO 200 µL	GVO 250 µL
Fructose 1,6-biphospate (mg.dL-1)	0.76[a]	0.47[b]	0.49[b]	0.52[c]	0.73[ad]
D-Glyceraldehyde 3-Phosphate (mg.dL-1)	0.43[a]	0.45[b]	0.41[bc]	0.40[bc]	0.39[ac]
Glucose 6-Phospate (mg.dL-1)	0.52[a]	0.41[b]	0.42[b]	0.48[c]	0.51[a]
Lactate dehydrogenase (IU.dL-1)	0.12[a]	0.76[b]	0.95[b]	0.71[c]	0.22[ad]
Glucose (mg.dL-1)	236.43[a]	207.31b	219.42[b]	227.75[c]	231.72[a]d
Lactate (mg.dL-1)	1.05[a]	1.48b	1.72[b]	0.83[c]	0.97[a]
Pyruvate (mg.dL-1)	62.41[a]	45.95b	56.82[b]	77.64[b]	73.77[c]

1Different superscripts within a row indicate a significant difference (0.05)

Intermediate metabolites (fructose 1,6-biphosphate, D-glyceraldehyde 3-phosphate, glucose 6-phosphate) appear to decrease, as a result of a decreased biochemical function, while the enzyme (lactate dehydrogenase) appeared to increase.

The results of this study indicated that during heat stress exposure in experimental ducks caused aerobic glycolytic activity to decrease, and should increase anaerobic glycolysis. The same research results have been shown by several previous researchers on poultry [23,24], and on polygastrics [22,25,26]. Increased lactate dehydrogenase enzyme shows pyruvate catabolism activity to increase lactate. In this experiment, there was an increase in lactate levels up to 1.72 mg/dL ($P<0.05$) compared to the duck group without heat stress. Lactate reduction by the administration of VGO appeared capable of a minimum administration of 200 mg/dL. These results indicated that essential oils were able to inhibit anaerobic glycolysis [21,27].

Based on these results (Table 2), it can be explained that heat stress increased the rate of alternative metabolism, primarily to provide energy to the process of homeostasis maintenance. Homeostasis is increasing in a state of heat stress [7], aimed at maintaining biochemical and physiological processes for survival [5] and reproduction [13].

Radiation of heat into the internal milieu environment of livestock causes the livestock to reduce feed intake or feed consumption instinctively. This decrease aims to prevent heat from digesting food (heat increment) in the intestine [7,10] and also metabolic heat [10,28] and increased free radicals [10,16]. To compensate for the reduction in feed intake, animal activates the glycogenolysis mechanism, triggered by an increase in nerve stimulants through neurotransmitters [29,30], thereby increasing epinephrine hormone levels [6,31].

Overall, the effectiveness of GVO can create normal energy metabolism through glycolysis and shows that GVO can improve metabolic balance, as well as the effectiveness of GVO in heat regulation and homeostasis [28]. Continuous heat stress in high conditions causes necessary behavior to evaporate body heat. This evaporation has an impact on increasing blood pH [1,3] compared to ducks not exposed to excessive heat.

One crucial factor that plays a role related to blood acidity is the ambient temperature. The influence of high ambient temperature causes the appearance of panting, which is the behavior of releasing heat through breathing by panting (fast and short breathing) or hyperventilation. Through this behavior, the release of H2O and CO2 compounds becomes excessive [12,28], causing the formation of bicarbonate (H2CO3) to decrease [29]. Bicarbonate is a proton donor of H+ and forms carbonic acid (HCO3-).

The ability of GVO with a level of 250 µL to overcome the effects of heat on the metabolic system of ducks, illustrates that sulfides in essential oils can bind to proteins mainly on protein H atoms, causing reduced protein denaturation [9,18,32]. This means reducing cell death and maintaining protein function [28,33]. Both positive effects can simultaneously maintain the proteins in the erythropoietic system and the protein of blood cells (erythrocytes or leukocytes). The results of the study showed the role of GVO in maintaining blood precursor proteins

from damage caused by reactive compounds (ROS) [16,17,34].

The role of diallyl n-sulfide contained in GVO can control and overcome heat stress related to its ability to increase reaction kinetics with H_2O. The diallyl 2,3n-Sulfide binding energy contained in GVO with high H_2O causes difficulty in being evaporated and excreted through the kidneys resulting in decreased body fluid loss.

This GVO is also effective by increasing the reaction kinetics with H2O as well as by its electrostatic interaction patterns, both of which have an impact on controlling heat stress through increased adaptation of body fluids [23]. It directly affects the increase in cation retention of body fluids, especially Na+ so that it can maintain the osmotic pressure of body fluids.

Adaptation of body fluids also maintains water retention so that extracellular fluid volume is maintained so that cell membrane fluidity can be maintained [10]. As a result of maintaining membrane fluidity, glucose transportation into cells is not obstructed [35].

B. Free Radical Activity and Antioxidants

The impact of GVO administration on the activity of free radicals and antioxidants of heat-stressed ducks are shown in Table 3. The results showed that free radical activity (MDA) appeared to be increased in the group of ducks which received heat stress (P<0.05), i.e., 2.85 µMol.mL-1 compared to without heat stress (1.03 µMol.mL-1). Neither to the antioxidant activity of glutathione peroxidase.

TABLE III
IMPACT OF GINGER VOLATILE OIL ON FREE RADICALS AND ANTIOXIDANT LEVELS OF CIHATEUP DUCKS WITHOUT AND WITH HEAT STRESS

Parameters	Temp. 24°C and without GVO	Heat-stressed treatments (38°C)			
		without GVO	GVO 150 µL	GVO 200 µL	GVO 250 µL
Malondyaldehyde/MDA (µMol.mL-1)	1.03[a]	2.85[b]	2.25[b]	1.73[c]	1.36[d]
Glutatione Peroxidase (nMol.mL-1)	2.02[a]	2.34[b]	2.31[b]	2.21[c]	2.11[d]

[1]Different superscripts within a row indicate a significant difference (0.05)

High heat stress has an impact on increasing the production of reactive oxygen species (ROS). ROS are oxygen-derived compounds that are more reactive than oxygen in primary conditions. This ROS can enter the bloodstream, over this compound will cause oxidative stress. ROS gives rise to the peroxidation of fatty acids with proteins, cellular nucleic acids, fats, especially PUFAs, resulting in lipid peroxidation.

The main target in lipid peroxidation by ROS is a polyunsaturated fatty acid (PUFA) in the lipid membrane. PUFAs that are degraded by ROS will result in alkane formation, such as MDA [36].

High ROS concentrations cause ROS to react with fats, proteins, cellular nucleic acids, resulting in local damage and specific organ dysfunction. Fat is a biomolecule that is vulnerable to free radical attack. Components of animal cell membranes contain many sources of PUFA. PUFA is a biomolecule that is easily damaged by oxidizing compounds. The breakdown of fat hydroperoxides often involves catalysis of transition metal ions [31], resulting in short-chain carbonyl compounds such as cytotoxic aldehydes and ketones [37]. The breakdown of carbon bonds during lipid peroxidation led to alkane formation, such as malondialdehyde (MDA) [20,38].

The decrease in MDA seems to begin to decrease with the administration of 200 µL GVO up to the level of 250 µL, although this decrease in MDA is still higher at 1.36 µMol.mL-1 (P<0.05) compared to the duck group without heat stress (1.03 µMol. mL-1). This reduction shows that GVO is able to act as an antioxidant. The ability of GVO as an antioxidant is also seen by decreasing the activity of endogenous antioxidants (Glutathione Peroxidase), along with increasing levels of GVO administration.

Increased levels of glutathione peroxidase (P<0.05) from 2.02 nMol.mL-1 in the group of ducks kept at a comfortable temperature to 2.34 nMol.mL-1 in the group of ducks that received heat stress up to 38°C. shows one of the mechanisms of homeostasis in maintaining the normal biochemical condition of cells [39]. Heat stress triggers an increase in free radicals resulting from oxidation-reduction in the mitochondria [40], as a consequence of increased energy requirements in heat regulation [33].

The results of previous studies have reported that chemical signals in the form of neurotransmitters increase in heat stress to simulate antioxidant activity [15], an increase in catalase and glutathione peroxidase levels under stress [41]. These antioxidants play a role in preventing free radical activity [42], as well as preventing cell death and inflammation [43].

Table 3 also shows that the levels of glutathione peroxidase began to decrease with the level of administration of 200 µL GVO. Decreased glutathione peroxidase results of this study were able to emphasize that GVO can act as an antioxidant in preventing free radical activity [44,45] so that the oxidation of fatty acids to MDA decreases [46,47]. Some previous research results show the same symptoms that the administration of volatile oil from garlic can reduce the role of antioxidants and endogenous in fish cells [17], reduce catalase activity in Petetan chickens [48], and reduce glutathione peroxidase levels in pigs [49] and poultry [27,35,50].

The ability of GVO to reduce the antioxidant levels of glutathione peroxidase shows that the chemical compounds in GVO are able to act as effective antioxidants so that the role or activity of endogenous antioxidants (glutathione peroxidase) can be replaced. The effectiveness of volatile oil as an antioxidant has been reported by previous researchers, partly because it has a compound structure with free oxygen atoms [16,21]. Other researchers have shown that OH groups in volatile oil compounds can bind with stable oxygen radicals [25].

High heat stress has an impact on increasing the production of reactive oxygen species (ROS). ROS are oxygen-derived compounds that are more reactive than oxygen in primary conditions. This ROS can enter the bloodstream, in excess of this compound will cause oxidative stress. ROS gives rise to peroxidation of fatty acids with proteins, cellular nucleic acids, fats, especially PUFAs, resulting in lipid peroxidation.

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IV. Conclusions

In conclusion, the 250 µL of ginger's volatile oil level reduces the heat stress level by up to about 50%. The volatile ginger oil has an essential role in preventing excessive changes in the normal metabolic rate. Thus GVO can cope with metabolic changes related to heat stress, as indicated by increased levels of the metabolites of the glycolytic pathway and high antioxidant activity.

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