The expression of heat shock protein 70 gene with organic selenium supplementation and its effect on productivity of broilers in tropical environment

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ABSTRACT

The purpose of this experiment is to study the effect of organic selenium (Se) supplementation on the expression of heat shock protein 70 gene (HSP70), glutathione peroxidase (GSH-Px) enzyme activity, malondialdehyde (MDA) and productivity of broilers in tropical environment. Three kinds of environmental pens were designed in this experiment: comfortable environment pens with temperature of air conditioner adjusted at 22°C (R0), tropical environment pens (±30°C) without organic Se (R1), and tropical environment pens supplemented with 0.30 ppm organic Se (R2). One hundred and twenty broiler chickens (unisex) were used in this study. There were 40 chicks per pen for each treatment. The experimental design was completely randomized with four replications for each treatment. The data were statistically analyzed using the general linear model of SAS program. Results showed that R0 and
R2 groups had significantly increased (P<0.05) feed intake, body weight, body weight gain, and decreased feed conversion ratio compared to R1 groups. Meanwhile, the expression of HSP70, GSH-Px enzyme activity and MDA of R2 groups and R0 groups were significantly lower (P<0.05) than that of R1 groups. It was concluded that the broilers given 0.30 ppm organic Se in tropical environment had similar productivity and expression of HSP 70 with broilers kept in comfortable environment.

Keywords: broiler, HSP70 gene, organic selenium, productivity, tropical environment

INTRODUCTION

In tropical environment, such as Indonesia its temperature and humidity have an impact on the performance of broiler chickens. Heat stress is triggered by the changes in ambient temperature. Heat stress can affect the productivity of broiler chickens (Dai et al., 2009; Virden and Kidd, 2009; Xie et al., 2015). The newest strains of broiler chickens are the result of intensive selection with rapid growth ability which must be maintained at comfortable temperature. Al-fataftah and Abu-Dieyeh (2007) reported that broiler chickens reared in comfortable temperature improved feed conversion value and higher average body weight than the chickens kept in hot temperatures. Genetically, heat stress not only can activate but also deactivate genes related to metabolic signals received from internal factors, such as nutrition and external factors such as environment. These factors may alter the genetic process, performance and affect the health of chickens. One of the genes that directly respond to heat stress is the heat shock protein 70 (HSP70) (Al-Aqil and Zulkifili, 2009; Noor and Seminar, 2009).

HSP 70 gene has an important role not only in the biology and biochemistry of cell but also plays a role in response to temperature stress related to its functional activity as chaperons (Bukau and Horwich, 1998). HSP 70 can be an advanced tool or method which is required especially for detecting genes and genomics markers that have been tested for its accuracy in response to heat stress in broiler chickens (Al-Zghoul et al., 2013).

Heat stress also leads to oxidative stress associated with a reduced antioxidant status in the bird in vivo, as reflected by increased oxidative damage and lowered plasma concentrations of antioxidant vitamins and minerals (Sahin et al., 2009). Therefore, the addition of antioxidant into diet is to increase the activity of antioxidant enzyme which acts to protect the body from oxidative damage caused by free radical (Rusli et al., 2015).

Selenium (Se) is an essential trace mineral known to possess antioxidant and has a profound impact on several physiological functions and productivity. Selenium, as a part of an antioxidant enzyme (Upton et al., 2009; Surai and Fisinin, 2014), detoxifies oxygen radicals and peroxides (Oliveira et al., 2014), and can reduce heat stress caused by tropical environment climate (Ibrahim et al., 2011; Yuan et al., 2012). Organic Se commonly used in the diets of broiler chickens can be derived from yeast. Organic Se is a constituent of selenoproteins and contains mainly selenomethionine which possesses antioxidant properties (Delezie et al., 2014).

Therefore, this study is very important because it provides information about the effect of organic Se supplement on the expression of heat shock protein 70 (HSP70) genes, glutathione peroxidase (GSH-Px) enzyme activity, malondialdehyde (MDA), and productivity of broilers in tropical environment.

MATERIALS AND METHODS

Birds and Diet

In this experiment, one hundred and twenty day-old CP707 broiler chicks were used. They were individually weighed and then randomly distributed into 12-floor pens (1.5x1.5 m²); each pen contained 10 chicks. The treatments in this experiment were: 1) comfortable environment pens with temperature of Air Conditioner (AC) adjusted at 22°C (Efendi, 2010) without organic Se (R0); 2) tropical environment pens without organic Se (R1), and 3) tropical environment pens supplemented with 0.30 ppm organic Se (R3). An air conditioner was used for 3-week-old broiler chickens until the end of the experiment (5 weeks). They were exposed to natural variation in environmental temperature without any human control. Experimental began in the growers’ period. The experimental diets were prepared as crumbles and formulated to contain equal amounts of energy and crude protein (Table 1), which are necessary to meet the minimum nutrients requirements of broiler chickens, based
on Lesson and Summer (2005) recommendation. Further, the diets were supplemented with organic Se (Optimin®SeY, Selko, Netherland). In this experiment, water and feed were provided ad libitum. Feed intake and body weight were determined weekly. Feed conversion ratio was calculated as g food intake per g live body weight. Mortality was recorded as it occurred. The experiment lasted for 35 days. Considering the animals’ health, whole blood was drawn from the wing (branchial) veins from two chicks per treatment group and placed in EDTA-containing tubes. Then the chicks were humanely euthanatized in the daytime at 12.00-14.00 pm. Samples from the brain and pectoral muscle were collected for total RNA extraction and real time PCR (RT-PCR).

**RNA Extraction and RT-PCR**

The samples from the brain and the pectoral...
muscle were collected from 24 chicks (two chicks from each treatment group) and stored separately in 1.5 µL tubes which contained 500 µL RNA. Total RNA was extracted using Gene JET RNA Purification Kit (Thermo Scientific, Lithuania, EU). After that, RNA was stored at -20°C, followed by a reverse transcriptase reaction. RNA was reversed-transcribed to a complementary DNA in a reaction mixture using Transcriptor First Strand cDNA kit (Thermo Scientific, Lithuania, EU) using PCR (GeneAmp PCR system 9700, AB Applied Biosystem, Singapore). The quantification of complementary DNA was checked (260:280 nm absorbency) using spectrophotometer (Agilent 8453, USA). Furthermore, based on the cDNA quantification standard concentration was checked using spectrophotometer (Hitachi U-2001, China).

Statistical Analysis
Statistical analysis was performed as a complete randomized design using the general linear model of SAS program (SAS, 2011). Significant differences among treatments were determined by Duncan’s multiple range test at a level of P<0.05 (Steel and Torrie, 1980).

RESULTS AND DISCUSSIONS
Temperature and Humidity
Table 2 shows the average temperature and relative humidity of pens during 5 weeks of the experiment at Dramaga, Bogor District, West Java Province, Indonesia. Average temperature in tropical environment in this experiment was higher than the optimum temperature for broilers’ growth at 4-5 weeks. In grower period, the broilers were sensitive to high temperature and humidity, thus broilers need comfortable temperature for optimum production. Comfortable temperature for optimal production in broiler chickens is 18-27 °C (Kuczynski, 2002; Olanrewaju et al., 2010; Sohail et al., 2011; Aljuobori et al., 2016).

High temperature in tropical environment has negative effects on broilers’ performance. Heat stress of experimental pens (R1 and R2) changes the normal air circulation in broilers and improves the metabolism activity rapidly in the cell. Heat stress caused by long period of exposure to heat at day time can increase mortality (Mushtaq et al., 2007; Noor and Seminar, 2009; Roussan et al., 2008).

Quantification of Gene Expression of Heat Shock Protein 70 (HSP70)
The results of the quantification of gene expression of heat shock protein 70 (HSP70) can be seen in Figure 1. Broiler chickens kept in tropical environment pens supplemented with for further analysis of GSH-Px activity. The total activities of glutathione peroxidase (GSH-Px) in plasma were determined by Paglia and Valentine (1967) method, which was modified by Flohe and Gunzler (1984). The level of Malondialdehyde (MDA) in pectoral muscle was determined by Rice-Evans and Anthony (1991) modified Thiobarbituric Acid Reactive Substances (TBARS) method. The activities of GSH-Px and level of MDA were checked using spectrophotometer (Hitachi U-2001, China).

Evaluation of Biochemical Parameters
Blood samples were collected in tubes containing heparin as anti-coagulant; their plasma was isolated by centrifuging at 1200 rpm for 4 minutes. The plasma obtained was stored at -80°C
organic Se compared to those kept in comfortable environment pens were not significantly different (P>0.05) in terms of HSP70 gene expression in the brain and pectoral muscle. Gene expression of HSP70 mRNAs in the brain was higher than that in the pectoral muscle. When poultry is exposed to heat stress, neurogenic system is activated to release catecholamine, epinephrine, and norepinephrine (Siegel, 1980). Epinephrine has the most dominant role in altering metabolism (Assenmacher, 1973); it is related to changing body signal to impose kinase enzyme protein activation especially glycogenolysis and gluconeogenesis (Berne and Levy, 1990). Stress

Table 2. Temperature and Humidity during 35 Days of Experiment

| Weeks | Control Pens (R0) | Tropical Pens (R1 and R2) |
|-------|-------------------|---------------------------|
|       | Temperature (°C)  | Humidity (%)              | Temperature (°C) | Humidity (%) |
|       | Morn Day Eve      | Morn Day Eve              | Morn Day Eve    | Morn Day Eve |
| 1     | 26.6 27.6 29.1    | 77.6 78.9 69.7            | 24.0 32.8 27.7  | 96.7 60.0 81.9 |
| 2     | 26.4 27.6 28.0    | 78.0 75.0 73.6            | 25.0 33.1 28.0  | 93.7 61.4 83.9 |
| 3     | 25.2 28.1 27.2    | 83.6 73.7 73.6            | 24.9 32.8 28.9  | 91.3 66.0 78.0 |
| 4     | 23.4 27.4 27.7    | 84.7 78.0 78.3            | 24.9 32.9 29.2  | 88.6 63.6 80.0 |
| 5     | 23.3 27.4 27.4    | 84.0 71.9 79.3            | 24.1 32.7 27.8  | 82.4 61.6 78.0 |
|       | 25.0 27.6 27.9    | 81.6 75.5 74.9            | 24.6 32.9 28.3  | 90.5 62.5 80.3 |

R0 = broiler reared in comfortable environment pens without supplementation of organic Se, R1 = broiler reared in tropical environment pens without supplementation of organic Se, R2 = broiler reared in tropical environment pens with supplementation of 0.30 ppm organic Se, Morn = morning, Eve = evening.

Figure 1. qRT-PCR Validation for HSP70 Gene Expression from Broiler in Brain and Pectoral Muscle Samples.

*Means the different superscript lowercase letters indicates significantly different (P<0.05).*

■ R0 = broiler reared in comfortable environment pens without supplementation of organic Se,
■ R1 = broiler reared in tropical environment pens without supplementation of organic Se,
□ R2 = broiler reared in tropical environment pens with supplementation of 0.30 ppm organic Se.
The Activity of Glutathione Peroxidase (GSH-Px) Enzyme

The activity of GSH-Px enzyme in tropical environment pens without organic Se addition was significantly higher (P<0.05) than other treatments due to heat stressed. The activities of GSH-Px enzyme of broilers kept in comfort environment pens and tropical environment pens with dietary organic Se were not significantly different (Table 3). GSH-Px enzyme would increase as a result of heat stress (Pamok et al., 2009). GSH-Px is an antioxidant enzyme that reduces the negative impact of free radicals in cell, due to detoxification of H₂O₂ and organic hydroperoxide into non-toxic component. Selenium is an integral part of the enzyme system glutathione peroxidase (Glutathione: H₂O₂ oxidoreductase) which functions in detoxification of H₂O₂ and organic hydroperoxide (Surai, 2003). The effects of organic Se on activity of GSH-Px enzyme were lower in the present study in broilers reared under heat stress at tropical environment pens. We could only speculate that in tropical environment pens, the activity of organic Se mechanisms is associated with scavenged free radical in cell during exposure to heat stress.

Table 3. Effect of Supplementation Organic Se on Glutathione Peroxidase (GSH-Px) Activity and Content of Malondialdehyde (MDA) in Broiler (1-5 Weeks of Age)

| Treatment | MDA (mg/100g) | GSH-Px (mU/mg protein) |
|-----------|---------------|------------------------|
| R0        | 0.37±0.07b    | 148.27±23.4b           |
| R1        | 1.19±0.36a    | 277.33±81.6a           |
| R2        | 0.56±0.20b    | 155.26±30.4b           |

a,b Means within the same column with different superscripts are significantly different (P<0.05).

Broiler Performance

Heat stress in broiler chickens occurred at daytime due to the average temperature of 32.87°C in tropical environment pens. Heat stress in chickens was characterized not only by panting activity but also by spreading their wings to reduce body heat. Chickens do not have sweat glands which make it difficult for them to dispose their body heat to the environment (Hilman et al., 2000; Etches et al., 2008). The ambient temperature during day exceeded the comfortable temperature so that the chickens in the tropical pens experienced heat stress. Organic Se supplementation in chickens reared in tropical environment pens without organic Se addition (Table 3). Similar results have been reported that supplementation of organic Se significantly reduces MDA value in broiler chickens reared in tropical temperature (Rao et al., 2013). MDA, which is a free radical product, was formed by lipid peroxide (Clarkson and Thomson, 2000; Ayala et al. 2014). The MDA value is an indicator of damage cell or tissue which is caused by free radicals. Organic Se is an antioxidant that could prevent heat stress by triggering production of free radicals in the metabolism. Organic Se changed free radicals into stable product, so lipid peroxidase will be stopped.
body weight gain and feed conversion ratio compared to the chickens reared in tropical environment pens without organic Se supplementation (Table 4). Mortality occurred from the highest to lowest, respectively, at the R0, R1, and R2. Performance of broilers supplemented with organic Se reared in tropical condition as good as broiler chickens reared in comfortable environment pens. This suggests that organic Se supplementation is quite effective to overcome heat stress in broilers reared in tropical environment pens which have varied temperature and humidity.

CONCLUSION

Addition of 0.30 ppm organic Se in broiler diets reared in tropical environment decreased expression of HSP70 gene, reduced activities of GSH-Px, MDA level and improved the productivity of broilers as good as those reared in comfortable environment, as indicated by the improved feed intake, weight gain and feed conversion ratio.

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Table 4. Effect of supplementation organic Se on performance in broiler (1-5 weeks of age)¹

| Items² | Treatments |
|--------|------------|
|        | R0         | R1         | R2         |
| Feed intake (g/bird) | 2215 ± 34ᵃ | 1929 ± 182ᵇ | 2153 ± 136ᵃ |
| Body weight (g/bird)  | 1351 ± 51ᵃ | 1078 ± 42ᵇ  | 1308 ± 53ᵃ  |
| BWG (g/bird)          | 1310 ± 51ᵃ | 1035 ± 42ᵇ  | 1266 ± 54ᵃ  |
| FCR                 | 1.69 ± 0.04ᵇ | 1.86 ± 0.11ᵃ | 1.70 ± 0.12ᵇ |
| Mortality (bird)     | 2          | 6          | 3          |

¹ Each value represents the mean of 10 replicates for each treatment. ² BWG = Body weight gain, FCR = Feed conversion ratio. ᵃᵇ Means within the same column with different superscripts are significantly different (P<0.05).
R0 = broiler reared in comfortable environment pens without supplementation of organic Se, R1 = broiler reared in tropical environment pens without supplementation of organic Se, R2 = broiler reared in tropical environment pens with supplementation of 0.30 ppm organic Se.
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