J I T A A
Journal of the Indonesian Tropical Animal Agriculture
Accredited by Ditjen Penguatan Risbang No. 60/E/KPT/2016

J. Indonesian Trop. Anim. Agric.
pISSN 2087-8273 eISSN 2460-6278
http://ejournal.undip.ac.id/index.php/jitaa
42(4):279-287, December 2017
DOI: 10.14710/jitaa.42.4.279-287

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

R. Amizar[1,2], S. Suharti[1], Jakaria[1] and R. Mutia[1,*]

[1]Faculty of Animal Science, Bogor Agricultural University ,
Jl. Agatis, Kampus IPB Dramaga, Bogor 16680 - Indonesia
[2]Permanent address: Faculty of Animal Science, Andalas University,
Kampus Unand Limau Manis, Padang 25163 – Indonesia
**Corresponding E-mail: rmutia.1@gmail.com*

Received July 15, 2017; Accepted September 20, 2017

ABSTRAK

Tujuan dari penelitian ini adalah untuk mengkaji pengaruh suplementasi selenium (Se) organik terhadap ekspresi gen *heat shock protein 70* (HSP70), kadar malondialdehid (MDA) dan produktivitas ayam broiler yang dipelihara di lingkungan tropis. Tiga jenis kandang didesain dengan lingkungan yang berbeda dalam percobaan ini: kandang dengan lingkungan suhu yang nyaman menggunakan AC suhu 22^{o}C (R0), kandang lingkungan tropis (± 30^{o}C) tanpa Se organik (R1), dan kandang lingkungan tropis dilengkapi dengan 0.30 ppm Se organik (R2). Penelitian ini menggunakan 120 ekor broiler (*unisex*). Ada 40 ekor ayam/kandang setiap perlakuan. Rancangan penelitian menggunakan rancangan acak lengkap dengan empat ulangan untuk setiap perlakuan. Data dianalisis secara statistik menggunakan model linier umum program SAS. Hasil penelitian menunjukkan bahwa kelompok R0 dan R2 signifikan meningkatkan ($P<0.05$) konsumsi ransum, bobot badan, pertambahan bobot badan dan menurunkan konversi ransum dibandingkan dengan kelompok R1. Ekspresi gen HSP70, aktivitas enzim GSH-Px dan kadar MDA pada kelompok R2 dan R0 signifikan menurun ($P<0.05$) dibandingkan dengan kelompok R1. Kesimpulan penelitian adalah broiler yang diberikan 0.30 ppm Se organik di lingkungan tropis memiliki produktivitas dan ekspresi gen HSP 70 yang sama dengan broiler dipelihara di lingkungan yang nyaman.

Kata Kunci: broiler, gen HSP70, lingkungan tropis, produktivitas, selenium organik.

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^{o}C (R0), tropical environment pens (±30^{o}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-Zhgoul *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 m^2); each pen contained 10 chicks. The treatments in this experiment were: 1) comfortable environment pens with temperature of Air Conditioner (AC) adjusted at 22^0C (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

Table 1. Ingredient and Nutrient Content of the Basal Diets for Broiler

	Age (week)	
	0-3 (Starter)	4-5 (Finisher)
Ingredients		
Maize (%)	51.00	59.02
Soybean meal (%)	26.00	20.50
Fish meal (%)	8.00	7.00
Corn gluten meal (%)	9.65	9.08
Oil (%)	2.50	2.50
Dicalcium phosphate (%)	1.25	-
Salt (%)	0.50	0.50
Premix[a] (%)	0.30	0.30
$CaCO_3$ (%)	0.80	1.10
Nutrient content [b] (as feed basis)		
Crude protein (%)	22.43	20.44
Crude fiber (%)	2.37	2.39
Crude fat (%)	4.92	5.16
Metabolizable Energy (kkal kg^{-1})	3128	3204
Methionine (%)	0.57	0.52
Lysine (%)	1.44	1.22
Methionine + Cystein (%)	0.96	0.86
Calcium (%)	1.17	0.93
P available (%)	0.73	0.45
Natrium (%)	0.28	0.27
Selenium (ppm)	0.23	0.20

[a] Premix provided (in mg/kg premix): vit A 1200000 IU; vit D3 200000 IU; vit E 800; vit K 200; vit B1 200; vit B2 500; vit B6 50; vit B121200µg; vit C 2500; Ca-D pantothenate 600; niacin 4000; choline chloride 1000; methionine 3000; lysine 3000; manganese 12000; iron 2000; iodine 20; zinc 10000; cobalt 20; copper 400; santoquin 1000; zinc bacitracin 2100.

[b] Analyzed value.

[c] Calculated based on analyzed ingredient composition.

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^{o}C, followed by a reverse transcriptase reaction. RNA was reversed- transcribed to a complementary DNA in a reaction mixture using Transcriptor Synthesis 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 DNA/RNA copy number calculator (http://endmemo.com/bio/dnacopynum.php).

The complementary DNA was then used for quantitative real time PCR (qRT-PCR) (Analytic Jena, AG qTower 4 kanal, Germany). RT-PCR was performed using the SYBR Green Select Master Mix Kit (Applied Biosystem, USA). The primer was used to analyze the expression of HSP70 gene examined by cGAPDH: Forward-5'GTG TTA TCA TCT CAG CTC CCT CAG-3', Reverse-5'GGT CAT AAG ACC CTC CAC AAT G-3', cHSP70: Forward-5'GAC AAG AGT ACA GGG AAG GAG AAC-3', Reverse-5'CTG GTC ACT GAT CTT TCC CTT CAG-3' [8]. In summary, 10 µL of the reaction mixture contained 5 µL master mix, 0.25 µL forward primer (10 pmol), 0.25 µL reverse primer (10 pmol), 1 µL cDNA of the sample and 3.5 µL of nuclease free water. Cycling parameters respectively were, 95^{o}C for 5 min, 39 cycles of 95^{o}C for 10 s, followed by 60^{o}C for 20 s and 72^{o}C for 30 s.

All samples were analyzed using mean of the threshold cycle (Ct) values. Relative mRNA expression profiling was further used with formula $2^{-\Delta CT}$. The delta Ct (ΔCt) values were calculated as the difference between target gene (HSP70) and the reference gene (GAPDH): ($\Delta Ct = Ct_{\text{gene of interest}} - Ct_{\text{internal control}}$) (Schmittgen and Livak, 2008).

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^{o}C 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).

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 ^{0}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

Table 2. Temperature and Humidity during 35 Days of Experiment

Weeks	Control Pens (R0)						Tropical Pens (R1 and R2)					
	Temperature (^oC)			Humidity (%)			Temperature (^oC)			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
		26.4			78.4			27.6			81.0	

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.

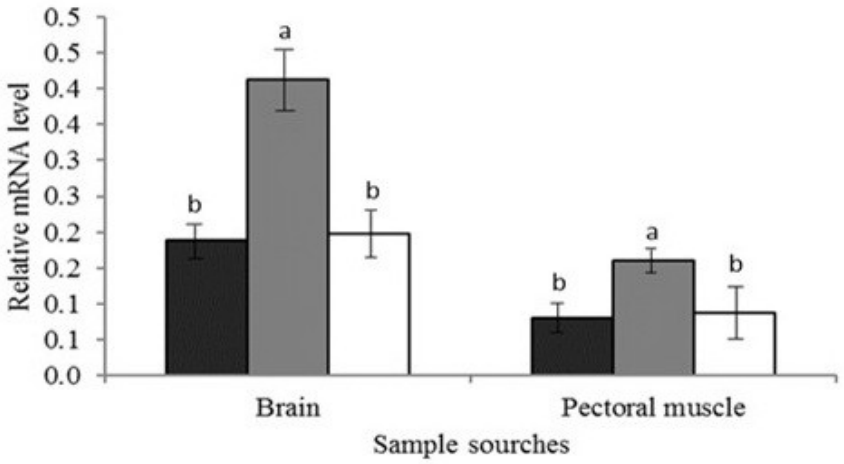

Figure 1. qRT-PCR Validation for HSP70 Gene Expression from Broiler in Brain and Pectoral Muscle Samples.

[a,b] 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.

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

can stimulate adrenal glands to release corticosterone hormones in poultry (Etches *et al.,* 2008). If the stress condition still continues and the body cannot cope through metabolic pathway, the genetic pathway is thus used to activate HSP70 gene function. Tamzil *et al*. (2013) reported that acute heat stress can increase HSP 70 expression and concentration of serum corticosterone.

Supplementation of organic Se in the broilers diets at the tropical environment can reduce HSP70 gene expression. Organic Se has an important role in the regulation of mRNA levels (Sunde *et al*., 1997); it is a constituent of selenoproteins and contains mainly selenomethionine (SeMet) which possesses antioxidant properties (Surai and Fisinin, 2014). Mahmoud and Edens (2005) reported that expression of HSP70 was decreased by organic Se supplement. Moreover, the regulation of mRNA levels on the expression of HSP70 gene is important to better understand the mechanism underlying regulation by Se status.

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 H_2O_2 and organic hydroperoxide into non-toxic component. Selenium is an integral part of the enzyme system glutathione peroxidase (Glutathione: H_2O_2 oxidoreductase) which functions in detoxification of H_2O_2 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.

The Content of Malondialdehyde (MDA)

Increasing MDA level ($P<0.05$) was discovered in broilers kept 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.

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 ± 0.07^{b}	148.27 ± 23.4^{b}
R1	1.19 ± 0.36^{a}	277.33 ± 81.6^{a}
R2	0.56 ± 0.20^{b}	155.26 ± 30.4^{b}

[a,b] 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.

Broiler Performance

Heat stress in broiler chickens occurred at daytime due to the average temperature of 32.87oC 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 significantly had increased ($P< 0.05$) feed intake, body weight,

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

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

[a,b] 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.

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.

ACKNOWLEDGMENTS

The authors would like to thank the Directorate General of Higher Education of Indonesia for Beasiswa Unggulan (superior scholarship) and supporting this research through Funding Research Program-BOPTN (Project ID No.238/IT3.41.2/L2/SPK/2013).

REFERENCES

Al-Aqil, A. and I. Zulkifli. 2009. Changes in heat shock protein 70 expression and blood characteristics in transported broiler chickens as affected by housing and early age feed restriction. Poult. Sci. 88:1358-1364.

Al-Fataftah, A.R.A and Z.H.M. Abu-Dieyeh. 2007. Effect of chronic heat stress on broiler performance in Jordan. Int. J. Poult. Sci. 6(1):64-70.

Al-Zhgoul, M.B., A.E.S. Dalab, M.M. Ababneh, K.I. Jawasreh, K.A.A. Busadah and Z.B. Ismail. 2013. Thermal manipulation during chicken embryo genesis results in enhanced HSP70 gene expression and the acquisition of the thermotolerance. Res. Vet. Sci. 95:502-507.

Aljuobori, A., I Zulkifli, A.F. A.F. Soleimani, Abdullah, J.B. Liang and A. Mujahid. 2016. Higher inclusion rate of canola meal under high ambient temperature for broiler chickens. Poult. Sci. 95:1326-1331

Assenmacher, I. 1973. The peripheral endocrine glands. Avian Biology. Vol 3. Farmer, D.S. and J.R. King (Eds). Academic Press. New York. P.186-286.

Ayala, A., M.F. Mu-oz and S. Argüelles. 2014. Lipid peroxidation: production, metabolism and signaling mechanisms of malo-ndialdehyde and 4-Hydroxy-2-nonenal. Oxid. Med. Cell Longev. P. 1-31.

Berne, R.M and M.N. Levy. 1990. Principles of

Physiology. The C.V Mosby Company, St. Louis, MO.
Bukau, B and A.L. Horwich. 1998. The Hsp70 and Hsp60 chaperone machines. Cell. 92:351-366.
Chia-Yi, Y., L. Yih-Jing and W.H. Guoo-Shyng. 2012. Aluminium overload increases oxidative stress in four functional brain areas of neonatal rats. J. Biomed. Sci. 19(51):1-9.
Clarkson, P.M. and H.S. Thomson. 2000. Antioxidants: What role do they play in physical activity and health. Am. J. Clin. Nutr. 729:637-346.
Dai, S.F., L.K. Wang., A.Y. Wen, L.X. Wang and G.M. Jin. 2009. Dietary glutamine supplementation improves growth performance, meat quality and colour stability of broilers under heat stress. Br. Poult. Sci. 50(3):333-340.
Delezie, E., M. Rovers, A. Van der Aa, A. Ruttens, S. Wittocx and L. Segers. 2014. Comparing responses to different selenium sources and dosages in laying hens. Poult. Sci. 93:3083–3090.
Efendi, D. 2010. Performa dan Respon Fisiologi Ayam Broiler yang diberi Ransum Mengandung 1,5% Ampas Buah Merah (*Pandanus conoideus*) pada Waktu Pemberian dan Suhu Kandang Berbeda. Tesis. Institut Pertanian Bogor, Bogor, Indonesia.
Etches, R.J., T.M. John and G.A.M. Verrinder. 2008. Behavioral, Physiological, Neuroendocrine and Molecular Response to Heat Stress. In: N.J. Daghir. Poultry production in Hot Climates. P. 49-69.
Flohe, L and W.W. Gunzler. 1984. Assays of glutathione peroxidase. Methods Enzymol. 105:114 -121.
Hilman, P.E., N.R. Scot and A. Van Tienhoven. 2000. Physiological, responses and adaption to hot and cold environment. In: Stress physiology in livestock. Vol.3, Poultry (Yousef M. K, Ed.). CRC press. Florida P. 1-71.
Ibrahim, M.T., B.H. Eljack and I.M.T. Fadlalla. 2011. Selenium supplementation to broiler diets. J. Anim. Sci. 2(1):12-17.
Kuczynski, T. 2002. The application of poultry behaviour responses on heat stress to improve heating and ventilation system efficiency. Electr. J. Pol. Agric. Univ. 5:1-11.
Leeson, S and J.D. Summers. 2005. Commercial Poultry Nutri¬tion. 3rd ed. University Books. Guelph, Ontario.
Mahmoud, K.Z and F.W. Edens. 2005. Influence of organic selenium on hsp70 response of heat-stressed and enteropathogenic Escherichia coli-challenged broiler chickens (Gallus gallus). Comp. Biochem. Phys. C. 141:69-75.
Mushtaq, T., M.A. Mirza, M. Athar, D.M. Hooge., T. Ahmad, G. Ahmad, M.M.H. Mushtaq and U. Noreen. 2007. Dietary sodium and chloride for twenty-nineto forty-two-day-old broiler chickens at constant electrolyte balance under subtropical summer conditions. J. Appl. Poult. Res. 16:161-170.
Noor, R.R. And K.B. Seminar. 2009. Rahasia dan Hikmah Pewarisan Sifat (Ilmu Genetika dalam Al -Qur'an). IPB Pr. Bogor.
Olanrewaju, H.A., J.L. Purswell, S.D. Collier and S.L. Branton. 2010. Effect of ambient temperature and light intensity on physiological reactions of heavy broiler chickens. Poult. Sci. 89:2668–2677.
Oliveira, T.F.B., D.F.R. Rivera, F.R. Mesquita, H. Braga, E.M. Ramos and A.G. Bertechini. 2014. Effect of different sources and levels of selenium on performance, meat quality, and tissue characteristics of broilers. J. Appl. Poult. Res. 23:15-22.
Paglia, D.E and W.N. Valentine 1967. Studies on the quantitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70:158-169.
Pamok, S., W. Aengwanich and T. Komunitrin. 2009. Adaption to oxidative stress and impact of chronic oxidative stress on immunity in heat stresses broiler. J. Therm. Biol. 34:353-357.
Rao, S.V.R., B. Prakash., M.V.L.N. Raju, A.K. Panda, S. Poonan and O.K. Murthy. 2013. Effect of supplementing organic selenium on performance, carcass traits, oxidative parameters and immune responses in commercial broiler chickens. Asian-Aust. J. Anim. Sci. 26(2):247-252.
Rice-Evans, C. and T.D. Anthony. 1991. Techniques in Free Radical Research. Elsevier. P. 146-202.
Roussan, D.A., G.Y. Khwaldeh, R.R. Haddad, I.A. Shaheen, G. Salameh and R. Al Rifai. 2008. Effect of ascorbic acid, acetylsalicylic acid, sodium bicarbonate, and potassium chloride supplementation in water on the performance of broiler chickens exposed to heat stress. J. Appl. Poult. Res. 17:141-144.

Rusli, R.K., K.G. Wiryawan, T. Toharmat, Jakaria and R. Mutia. 2015. Effect of mangosteen pericarp meal and vitamin e supplements on the performance, blood profiles, antioxidant enzyme and HSP 70 gene expression of laying hens in tropical environment. Int. J. Poult. Sci. 14:570-576.

Sahin, K., N. Sahin, O. Kucuk, A. Hayirli and A.S. Prasad. 2009. Role of dietary zinc in heat-stressed poultry: A review. Poult. Sci. 88:2176–2183.

SAS Institute Inc. 2011. SAS/OR® 9.3 User's Guide: Mathematical Programming. SAS Institute, Inc, Cary, NC, USA.

Schmittgen, T.D. and K.J. Livak. 2008. Analyzing real-time PCR data by the comparative CT method. Nat. Protoc. 3(6):1101-1108.

Siegel, H.S. 1980. Physiological stress in birds. Bioscience. 30:529-533.

Sohail, M.U., Z.U. Rahman, A. Ijaz., M.S. Yousaf, K. Ashraf, T. Yaqub, H. Zaneb, H. Anwar and H. Rehman. 2011. Single or combined effects of mannan-oligosaccharides and probiotic supplements on the total oxidants, total antioxidants, enzymatic antioxidants, liver enzymes, and serum trace minerals in cyclic heat-stressed broilers. Poult. Sci. 90:2573-2577.

Steel, R.G.D. and J.H. Torrie. 1980. Principles and Procedures of Statistics. McGraw-Hill Book Co. Inc. New York.

Sunde, R.A., B.M. Thompson, M.D. Palm, S.L. Weiss, K.M. Thompson and J.K. Evenson. 1997. Selenium regulation of selenium dependent glutathione peroxidases in animals and transfected CHO cells. Biomed. Environ. Sci. 10:346-355.

Surai, P.F and V.I. Fisinin. 2014. Selenium in poultry breeder nutrition: An update. Anim. Feed. Sci. Technol. 191:1–15.

Surai, P.F. 2003. Natural antioxidants in Avian Nutrition and Reproduction. Nottingham Univ Pr. England (UK).

Tamzil, M.H., R.R. Noor, P.S. Hardjosworo, W. Manalu and C. Sumantri. 2013. Acute heat stress responses of three lines of chickens with different heat shock protein (HSP)-70 Genotypes. Int. J. Poult. Sci. 12(5):264-272.

Upton, J.R., F.W. Edens and P.R. Ferket. 2009. The effects of dietary oxidized fat and selenium source on performance, glutathione peroxidase, and glutathione reductase activity in broiler chickens. J. Appl. Poult. Res. 18:193–202.

Virden, W.S and M.T. Kidd. 2009. Physiological stress in broilers: Ramifications on nutrient digestibility and responses. J. Appl. Poult. Res. 18:338–347.

Xie, J., L. Tang., L. Lu, L. Zhang, X. Lin, H.C. Liu, J. Odle and X. Luo. 2015. Effects of acute and chronic heat stress on plasma metabolites, hormones and oxidant status in restrictedly fed broiler breeders. Poult. Sci. 94:1635–1644.