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Effect of Organic Selenium during HS in Broiler Chicken: The Interplay of HSP-70 and PGC-1α

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ABSTRACT

Background: ROS (Reactive Oxygen Species) accumulated during heat stress induced oxidative stress is involved in triggering the activation of transcription factor, heat shock factor-1 (HSF-1) which further results in the up regulation of HSP. A cooperation between PGC1α and HSF1 in the induction of HSPs in response to thermal stress was reported in mice. Thus, the present study was conducted to investigate the effect of HS (Heat Stress) on HSP-70 and PGC-1α expression in broilers.

Method: The study was conducted in two phases, one during autumn and the other during summer with a total of 300 birds. During the first phase, 60 chicks were divided into six replicates with 10 birds in each and during the second phase, 240 chicks were divided into four groups with six replicates containing 10 birds in each. The experimental rations given to different groups were as control (Basal ration), HS I (Basal ration), HS II (Basal ration+0.3 ppm Se, HS III (Basal ration+0.6 ppm Se) and HS IV (Basal ration+0.9 ppm Se). Hepatic tissues were collected for analysis at 21 d and 42 d.

Result: The MDA levels and expression of HSP-70 protein in hepatic tissues were increased due to HS both at 21 and 42d, while the expression of PGC-1α protein increased at 42 d. Se supplementation at 0.3 ppm was effective in decreasing the concentration of MDA and HSP-70 protein at 21d, whereas 0.6 ppm and 0.9 ppm were effective at 42d. Se supplementation decreased the expression of PGC-1α protein at 42 d during HS.

Key words: Heat shock response, Heat stress, Oxidative stress, PGC-1α, Selenium.

INTRODUCTION

Heat shock proteins (HSPs) are a group of highly conserved proteins, synthesized in response to physical, chemical and biological stresses like heat stress (Gan *et al*., 2013) and they play an important role in the stabilization of the internal environment and survival of stressed cells (Leandro *et al*., 2004 and Jaiswal *et al*., 2017). These proteins are reported to play a role in redox regulation, energy metabolism, protein degradation, chaperoning, DNA damage sensing and repair (Gulyas *et al*., 2017). It has been suggested that the ability of HSP to directly sense the changes in the extracellular environment may serve as a biological marker for stress response (Manjari *et al*., 2015).

HSPs are classified based on their molecular weight ranging from 10 to 150 kDa (Benjamin and McMillan, 1998). Among HSPs, HSP-70 has been studied extensively and it appears to be more closely associated with heat tolerance (King *et al*., 2002). Increased HSP70 expression is believed to be an important adaptive mechanism to deal with oxidative stress-related changes in cell proteome under various stressful conditions (Surai *et al*., 2019). Different authors have reported the increased expression of HSP-70 in various tissues during HS in poultry (Xu *et al*., 2018, Zuo *et al*., 2015, Hasheimi *et al*., 2012). A well-known trace mineral, selenium which is also an antioxidant, was found to be responsible for the decrease in the expression of HSPs and MDA levels in hepatic tissues of broilers (Liu *et al*., 2015). Supplementation of selenium was observed to reduce the HSP-70 expression in broilers during HS (Rajkumar *et al*., 2017, Gan *et al*., 2013 and Dukare *et al.,* 2020).

Peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α) is a key metabolic transcriptional co-activator protein that associates with numerous transcription factors and affects the expression of their target genes (Xu *et al*., 2016). It acts as a master regulator of ROS scavenging enzymes, mitochondrial biogenesis, function and it also has a prominent role in the metabolic adaptations to the energetic status (Cantó and Auwerx, 2009; Mastropasqua *et al*., 2018). When cells are exposed to oxidative stress, PGC-1α is positively up regulated resulting in enhanced mitochondrial antioxidant defense and consequently prevents cell death

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associated with mitochondrial failure (Olmos *et al*., 2009). High levels of H_2O_2 promote protein kinase B (Akt) phosphorylation resulting in its activation, which further results in activation of PGC-1α. This up-regulates the mitochondrial antioxidant defense system of the cell by increasing the levels of MnSOD, catalase, UCP-2, thioredoxin reductase.

Xu *et al*. (2016) reported a cooperation between PGC1α and HSF1 in the induction of HSPs in response to stresses like thermal and oxidative stress in mice. This supports the possibility that HSF1 induces PGC1α which is required for HSP induction. Hence, the present study was conducted to reveal the relation between HSP-70 and PGC-1α during HS and upon Se supplementation in broilers.

MATERIALS AND METHODS

The present study was planned during the autumn and summer seasons of 2020-21 in College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati Andhra Pradesh with 300 commercial broiler chicks (Cobb 400) under a deep litter system. Two hundred and forty birds were divided into four groups with six replicates (10 birds in each) during summer months to study the effect of selenium supplementation during heat stress. A group of 60 birds divided into six replicates with 10 birds in each replicate was reared during autumn months to provide a thermo neutral control. The experimental rations given to different groups were as control (Basal ration), HS I (Basal ration), HS II (Basal ration+0.3 ppm L-selenomethionine), HS III (Basal ration+0.6 ppm L-selenomethionine) and HS IV (Basal ration+0.9 ppm L-selenomethionine). All the other managemental conditions were maintained uniform throughout the experiment.

The composition of basal ration was maintained uniform as per ICAR standards (2013) in both the phases of the experiment (Table 1). L-Selenomethionine (*Excential* Se 4000, ORFFA) was mixed in the basal ration as 7.5, 15 and 22.5 g/100 kg to get the concentrations of 0.3 ppm, 0.6 ppm and 0.9 ppm Se respectively.

The hepatic tissues were collected after the slaughter of selected birds from each replicate at 21 and 42 d. The tissues were homogenized in Tris-HCl at 4°C using a tissue homogenizer (Pricelly's, USA).The homogenate was analyzed immediately for oxidative stress by malonaldehyde (MDA) concentration (Niehius and Samuelsson, 1968). The protein content of homogenate was estimated by Lowry *et al*. (1951). HSP-70 protein expression in the liver was estimated by ELISA (*fine* test, China). PGC-1α in the liver was estimated by ELISA (*E*labscience, USA) according to the manufacturer's instructions using a multimode plate reader (Synergy, USA).

The generated data was subjected to statistical analysis using an independent sample t-test to compare control (autumn) and HS control (I) and one-way ANOVA (to compare different treatments of heat stress) followed by Duncan's multiple comparisons test (SPSS version 20.0).

RESULTS AND DISCUSSION

The liver being highly active metabolically and more susceptible to heat stress than other organs (Jastrebski *et al*., 2017), it is chosen to study the expression of HSP-70 in the present study. The physiological changes due to HS are accompanied by damage at the cellular level which results in significant induction of heat shock proteins to maintain cellular homeostasis. They are used as an important and effective biomarker for heat stress management (Murugesan *et al*., 2017 and Archana *et al*., 2017). Thus, the heat-shock response may be used as a tool to better understand the response of the bird to elevated temperatures.

Table 1: Ration formulated for summer and autumn phases of the experimental study.

Feed ingredient	Pre-starter (Kg/100 Kg)	Starter (Kg/100 Kg)	Finisher (Kg/100 Kg)
Deoiled rice bran	4.6	3.0	3.5
Maize	47.5	50.4	55.6
Soyabean meal	41.3	40.0	34.4
Ca	1.3	1.26	1.04
Methionine	0.11	0.08	0.04
Veg oil	3.09	3.26	3.42
Trace mineral-vitamin mix	2.0	2.0	2.0
Di calcium phosphate	0.1	-	-
		Chemical composition	
ME (kcal/kg)	3000	3050	3100
Crude protein (%)	22.00	21.50	19.50
Calcium (%)	1.00	0.95	0.85
Avail phosphorus (%)	0.45	0.40	0.38

Trace mineral-Vitamin premix provided (per kilogram of diet) thiamine, 1; pyridoxine, 2; cyanocobalamine, 0.01; niacin, 15; pantothenic acid, 10; á-tocopherol, 10; riboflavin, 10; biotin, 0.08; menadione, 2; retinol acetate, 2.75; cholecalciferol, 0.03; choline, 650 mg/kg diet.copper,7.5; manganese, 50; zinc, 45 mg/kg diet.

Table 2: Effect of HS on MDA, HSP-70 and PGC-1α concentration in hepatic tissue.

	MDA (nM/g tissue)		HSP-70 (ng/mg protein)		PGC-1α (µg/mg protein)	
	21 d	42 d	21 d	42 d	21 d	42 d
Control (Autumn)	594.16^{a}±13.61	443.04^{a}±10.37	93.08^{a}±2.25	88.80^{a}±1.61	0.24±0.026	0.38^{a}±0.008
HS	1087.01^{b}±21.9	1178.78^{b}±57.57	192.93^{B}±6.47	152.30^{b}±13.69	0.25±0.027	0.78^{b}±0.090

Mean±SE with different superscripts differ significantly in a column (p<0.05).

Table 3: Concentration of MDA, HSP-70 protein and PGC-1α in hepatic tissues of broilers during HS.

Treatment	MDA(nM/g tissue)		HSP-70 (ng/mg protein)		PGC1-α (µg/mg protein)	
	21 d	42 d	21 d	42 d	21 d	42 d
I (HS)	1087.01^{a}±21.9	1178.78^{a}±57.57	192.93^{a}±6.47	152.30^{a}±13.69	0.25^{b}±0.027	0.78^{a}±0.090
II (HS+0.3 ppm Se)	996.18^{b}±10.05	889.52^{b}±17.00	119.13^{b}±2.67	71.21^{b}±2.89	0.35^{b}±0.021	0.38^{b}±0.030
III (HS+0.6 ppm Se)	1089.54^{a}±28.71	592.79^{c}±25.86	189.21^{a}±10.53	45.57^{c}±2.22	0.52^{a}±0.060	0.31^{b}±0.040
IV (HS+0.9 ppm Se)	1082.57^{a}±31.54	581.12^{c}±18.31	190.86^{a}±13.32	47.01^{c}±1.10	0.54^{a}±0.015	0.31^{b}±0.040

Mean±SE with different superscripts differ significantly within a column (p<0.05).

The present study showed significant (P<0.05) induction of HSP-70 protein both at 21 d (100%) and 42 d (72%) during HS compared to control (Table 2). The present findings are in par with previous studies, where a significant increase ((P<0.05) of HSP-70 mRNA level was observed in chicken liver under HS (Zuo *et al.,* 2015; Rajkumar *et al*., 2017, Xu *et al.,* 2018). The lower induction of HSP-70 at 42d is in concurrence with wang and Edens (1993) who reported that longer preconditioning time was associated with lower expression of HSP70.

Oxidative stress has been proposed as a key mechanism to induce HSP synthesis. Enhanced HSP-70 concentration observed during HS was accompanied by an increase in oxidative stress in the present study, which is as per Mahmoud *et al*. (2004). As reported by Gosslau *et al*. (2000), ROS which accumulates during oxidative stress is generally involved in triggering the activation of transcription factor, heat shock factor-1 (HSF-1) by redox modifications. The activation of HSF-1 was found to be involved in the heat stress-induced upregulation of HSP (Jaiswal *et al*., 2017). According to Gan *et al*. (2013), HSP*s* produced during stress response protect against the free radical damage during oxidative stress. Hence, increased free radical production may be responsible for increased HSP expression during HS.

A significant (P<0.01) increase in PGC-1α expression was observed at 42 d during HS (Table 2). Increased oxidative stress at 42 d might be involved in the activation of PGC-1α through *Akt* activation, which further results in the activation of the antioxidant defense system (Olmos *et al*., 2009). An increase observed in the expression of both PGC-1α and HSP-70 at 42d is following the findings of Xu *et al*. (2016) in mice, who reported the possibility of HSF-1 role in inducing PGC-1α, which further increases HSP expression. However, there was no significant change in the expression of PGC-1α at 21 d during HS.

Selenium supplementation at 0.3 ppm significantly (P<0.05) decreased the expression of HSP-70 (38%) during HS at 21 d whereas a 70% reduction in HSP-70 expression was observed at 42 d with 0.6 ppm and 0.9 ppm Se compared to HS control (Table 3). The results are as per Rajkumar *et al*. (2017) and Dukare *et al*. (2020) who reported a decrease in HSP-70 mRNA expression with selenium supplementation in broilers at 0.3 ppm and 0.25 ppm (nano-Se during summer) levels respectively. A report of 2 to 5 fold increase in the expression of HSPs in the Se deficient broilers is in support of the present findings (Liu *et al*., 2015). Thus, as suggested by Mahmoud and Edens (2005), selenium supplemented chicken appears to be better prepared to withstand the heat stress as shown by the higher threshold for HSP-70 induction.

A decrease in expression of PGC-1α and HSP-70 observed with selenium supplementation during HS supports the interplay between PGC-1α and HSP-70. Decreased oxidative stress observed due to selenium supplementation might be responsible for reduced expression of PGC-1α and HSP-70 both at 21 d and 42 d during HS in broilers. Similar to the present findings at 21 d, increased expression of PGC-1α was observed with supra nutritional selenium supplementation in rats (Stahel *et al*., 2017).

CONCLUSION

We conclude that increased oxidative stress during heat stress was associated with increase in the expression of HSP-70 and PGC-1α proteins in hepatic tissues. The protective effect of organic selenium was evident at 0.6 ppm and 0.9 ppm level of Se supplementation. However, the supplementation of selenium at 0.6 ppm and 0.9 ppm level up to 21d during tropical summer, resulted in higher oxidative stress and the increased expression of HSP-70 and PGC-1α.

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Declaration of no competing interests

We declare that there are no relevant financial or non-financial competing interests to report.

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