Indian Journal of Animal Sciences **92** (12): 1440–1444, December 2022/Article
https://doi.org/10.56093/ijans.v92i12.114280

Role of organic selenium in resisting oxidative stress during tropical summer in broiler chicken

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Received: 23 August 2021; Accepted: 9 October 2022

ABSTRACT

The present study was conducted to investigate the effect of selenium supplementation on the oxidative status of hepatic tissue during heat stress in broilers. 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 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 collected at 21 and 42d were analyzed for oxidative status. HS-induced oxidative stress was revealed by the significant elevation in lipid hydroperoxides, MDA and protein carbonyl levels which in turn triggered the activities of anti-oxidant enzymes, i.e GPx, GST, SOD and G6PD in the hepatic cytosol. The total antioxidant capacity and glutathione concentration were significantly altered due to HS. Selenium at 0.3 ppm was effective in counteracting the oxidative stress at 21d while 0.6 ppm of Se was more effective at 42d. Se supplementation at 0.6 ppm improved the activities of antioxidant enzymes and G6PD. The total antioxidant capacity and GSH concentrations were better improved with 0.6 ppm of Se compared to other treatments.

Keywords: Antioxidant enzymes, GPx, Heat stress, Oxidative stress, Selenomethionine, Total antioxidant capacity

Heat stress is characterized by increased oxidative stress associated with an imbalance between the production of free radicals and the antioxidants, damaging all the components of cells including proteins, lipids and DNA leading to harmful effects in cell metabolism (Trostchansky *et al*. 2016). Physiologically, chicken responds to increased oxidative stress by enhancing the synthesis of antioxidant enzymes which were reported to be effective only when the required cofactors are available in adequate quantity (Rama Rao *et al*. 2015).

Selenium (Se) is one of the important trace elements, involved in cellular antioxidative mechanism and redox maintenance. Among different forms of selenium supplements, organic selenium is reported to be superior in terms of absorption efficiency and incorporation into different selenoproteins (Surai *et al*. 2018). However, selenium is an essential trace element with a narrow margin of safety (Yang *et al*. 2012). The selenium recommendations for broiler ration as per United States National Research Council (NRC 1994), is 0.15 mg Se/kg of diet which may be sufficient to meet needs for the maintenance of health and production during thermoneutral conditions. Surai *et al*. (2018) suggested that the need for selenium supplementation may depend on the type and level of oxidative stress in the birds. During tropical summer, HS-induced oxidative stress is much anticipated against which higher levels of dietary selenium may provide significant protection in broilers.

Thus, the present investigation was carried out to study HS-induced oxidative stress in broilers compared to those under thermoneutral conditions of autumn and to evaluate the oxidative status with selenium supplementation above the recommendations during HS in broilers.

MATERIALS AND METHODS

Housing and management: The present study was planned during the autumn and summer seasons under tropical conditions with 300 commercial broiler chicks (Cobb 400). A group of 60 birds divided into six replicates with 10 birds in each replicate was reared during autumn months to provide a thermoneutral control. 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

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stress. The composition of basal ration was maintained uniform as per ICAR standards (2013) in both the phases of the experiment (Table 1).

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
Soybean 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
*Vitamin-mineral mix	2.0	2.0	2.0
P Dical 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

L-Selenomethionine (*Excential* Se 4000*,* ORFFA) was mixed in the basal ration as 7.5, 15 and 22.5g / 100 kg to get the concentrations of 0.3 ppm, 0.6 ppm and 0.9 ppm of Se respectively. 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). All the other managemental conditions were maintained uniform throughout the experiment.

Thermohumidity Index (THI): The temperature of microclimate in the poultry shed was recorded using a digital thermometer. THI index was calculated with the following formula.

THI = 0.85**Tmax* + 0.15**Tmin* (Lallo *et al*. 2018).

where *Tmax* is the maximum daily temperature, *Tmin* is the minimum daily temperature.

Sample collection and analysis: The hepatic tissues were collected after the slaughter of one bird from each replicate at 21 and 42 d. The tissues were homogenized in 25 mM Tris-HCl buffer using a tissue homogenizer (Pricelly's, USA). The homogenate was analyzed immediately for oxidative stress by the estimation of malondialdehydes (MDA) (Niehius and Samuelsson 1968), lipid hydroperoxides (Jiang *et al*. 1992), and protein carbonyl content (Reznick and Packer 1994) using a multimode plate reader (Synergy, USA). The tissue homogenate prepared in 50 mM phosphate buffer (*p*H 7.2) and centrifuged at 13,000 rpm for 25 min at 4°C using a refrigerated centrifuge (Remi, India). The protein content of homogenate was estimated by Lowry *et al*. (1951). The cytosol was analyzed for the activities of antioxidant enzymes i.e superoxide dismutase (SOD) (Misra and Fridovich 1972), catalase (Beer and Sizer 1952), glutathione peroxidase (GPx) (Rotruck *et al*. 1973) and Glutathione S- transferase (GST) (Habig *et al*. 1974) using a UV-Visible spectrophotometer (Schimadzu, USA). The total antioxidant capacity (TAC) (Citui and Linguri 2017), glutathione concentration (Ellman 1959) and the activity of G6PD (Worthington enzyme manual 1993) of cytosol was analyzed using a multimode plate reader.

Statistical analysis: The generated data was subjected to statistical analysis using an independent sample t-test, and one-way ANOVA followed by Duncan's multiple comparisons test (SPSS version 20.0)

RESULTS AND DISCUSSION

The weekly THI indices recorded show a significant ($P<0.01$) difference between the two phases of the study. The increased THI revealed the heat stress during the summer season (Table 2) as supported by the previously reported THI based stress ranges for poultry as normal <27.8, moderate 27.8–28.8, severe 28.9–29.9 and very severe ≥30.0 (Lallo *et al.* 2018).

Table 2. THI of broiler house during the study

S. No	Control (Autumn)	HS
Week-1	$28.72^{a}\pm0.56$	$35.57^{b}\pm0.30$
Week-2	$28.43^{a}\pm0.12$	$35.76^{b}\pm0.05$
Week-3	$28.43^{a}\pm0.26$	$36.15^{b}\pm0.33$
Week-4	$26.80^{a}\pm0.70$	$35.48^{b}\pm0.31$
Week-5	$26.39^{a}\pm0.29$	$36.20^{b}\pm0.53$
Week-6	$26.83^{a}\pm0.28$	$38.63^{b}\pm0.17$

[a, b] Mean±SE with different superscripts in a row differ significantly ($P<0.01$)

In the present study, HS triggered oxidative stress was evidenced by a significant ($P<0.01$) increase in MDA levels (2.5 fold), lipid hydroperoxides (6 fold) and protein carbonyl content (4 fold) when compared to that of control at 42 d (Supplementary Table 1). The results are in concurrence with earlier reports of increased oxidative stress at ambient temperatures above the thermoneutral zone in birds (Zhang *et al*. 2018, Bai *et al*. 2019, Kerrihard *et al*. 2015). Malondialdehydes are the end products of lipid peroxidation while lipid hydroperoxides are formed as intermediates during the propagative phase of lipid peroxidation. Elevated levels of both MDA and lipid hydroperoxides indicate ongoing oxidative damage during the summer study (Sener *et al*. 2004). Increased protein carbonyl content indicates oxidative stress-induced damage proteins (Zhang *et al*. 2017).

A significant ($P<0.01$) reduction observed in MDA and lipid hydroperoxides (Table 3) with selenium supplementation during HS could be due to the enhanced activity of antioxidant defense system (Enzymes and TAC) and the decrease in the generation of free radicals by the inhibition of NADPH oxidase (Habibian *et al*. 2015).

A dose-dependent decrease ($P<0.01$) in protein carbonyl content was observed with selenium supplementation at 42 d during HS. A similar protective effect of selenium supplementation at 0.3 ppm was reported earlier during

Table 3. Effect of Se on oxidative stress in hepatic tissues of broilers during heat stress

Treatment	MDA (nM/g tissue)		Lipid hydroperoxides (mM/g tissue)		Protein carbonyl content (nM/mg protein)	
	21d	42d	21d	42d	21d	42d
I (HS)	1087.01^{a}±21.9	1178.78^{a}±57.57	82.76^{a}±4.94	223.72^{a}±6.21	9.16^{a}±0.22	10.05^{a}±0.34
II (HS +0.3 ppm Se)	996.18^{b}±10.05	889.52^{b}±17.00	63.80^{bc}±3.14	169.59^{b}±1.65	7.08^{bc}±0.62	3.49^{b}±0.22
III (HS +0. 6ppm Se)	1089.54^{a}±28.71	592.79^{c}±25.86	70.32^{b}±1.74	99.70^{c}±5.54	6.31^{c}±0.40	4.09 ^{b}±0.18
IV (HS +0.9 ppm Se)	1082.57^{a}±31.54	581.12^{c}±18.31	71.79^{ab}±3.04	80.95^{c}±0.96	7.96^{ab}±0.14	2.11^{c}±0.16

$^{a, b, c, d}$ Mean±SE differ significantly in a column at P<0.01.

HS in broilers where the highest dose understudy was 0.3 ppm (Rama Rao *et al*. 2015, Liu *et al*. 2015). However, in the present study, higher selenium levels showed more amelioration against HS-induced oxidative stress in broilers.

HS resulted in a significant increase in the activity of antioxidant enzymes, SOD and GPx (P<0.05) by 2.5 fold at 42 d (Supplemntary Table 2). The findings are in agreement with the previous reports of Lin e*t al*. (2006) and Del Vesco *et al*. (2017), where heat stress triggered the activity of SOD and GPx to protect the cells from the negative consequences of excessive free radicals. The major role of glutathione peroxidase in removing the H_2O_2 radicals produced by the SOD in hepatic tissues of broilers was exposed by its high rate of increase than that of catalase and GST. The lower activity of antioxidant enzymes at 42 d compared to that at 21 d might be due to increased utilization of these enzymes to detoxify the free radicals produced and exhaustion of cofactors required for antioxidant enzyme activity.

The activity of SOD (Table 4) and GPx (Fig. 1) was better improved at 42 d with 0.6 ppm of selenium compared to other levels of selenium supplementation. Similar to the present findings, Rama Rao *et al*. (2013) and Xu *et al*. (2014) reported an increase of SOD and GPx activities in the spleen with 0.3 ppm organic selenium supplementation during cyclic HS. Increased activity of catalase at 42d with 0.6 ppm selenium might be attributed to increased NADPH production due to increased activity of G6PD (Nobrega-Pereira *et al*. 2016).

A significant increase (P<0.05) in the activity of G6PD (Supplementary Table 2) during HS confirms its role as a major source of the reductant NADPH, on which the entire antioxidant system relies and thus, essential in preventing ROS-mediated cell death (Stanton *et al*. 2012).

TAC assay measures numerous compounds with the antioxidant activity which includes urate, ascorbate, bilirubin, thiols, α-tocopherol, carotenoids, and flavonoids (Young 2001). The decrease (P<0.05) in TAC and GSH

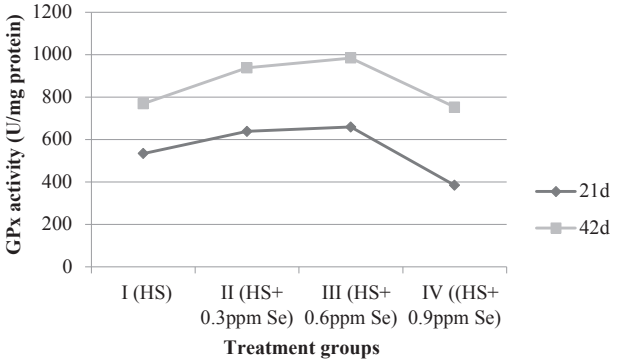

Fig. 1. Effect of selenium supplementation on GPx activity of hepatic tissues during HS in broilers (P<0.05).

concentration (Supplementary Table 2) observed at 42 d despite an increase in the activity of G6PD might be due to excessive utilization of antioxidants by the antioxidant enzymes in response to HS induced oxidative stress. Our results are in agreement with the earlier reports where TAC and GSH concentration decreased in response to HS in broilers (Zhang *et al*. 2018a and Bai *et al*. 2019).

The TAC was significantly improved (P<0.01) with 0.6 ppm of selenium supplementation. Increase in (P<0.01) GSH concentration with 0.3 ppm and 0.6 ppm of Se at 42 d was associated with enhanced activity of G6PD (Table 5). These findings are following Liu *et al*. (2015) who reported lower GSH levels in Se deficient compared to Se sufficient birds. Le Boeuf *et al*. (1985) reported that selenium supplementation strengthens the antioxidant system through the enhanced activity of G6PD.

Selenium at 0.9 ppm was associated with a decrease in the concentration of TAC, GSH and the activities of G6PD, SOD, GPx, and Catalase. A similar finding of the decrease in TAC and GSH with supra nutritional selenium was reported in broilers (Huang *et al*. 2016 and Balogh *et al.* 2004). Further, the growth performance of birds supplemented with 0.9 ppm of Se was decreased in comparison to the lower Se supplemented groups in the

Table 4. Effect of Se on antioxidant enzymes in hepatic tissues of broilers during heat stress

Treatment	GST (U/mg protein)		SOD (U/mg protein)		Catalase (U/mg protein)	
	21d	42d	21d	42d	21d	42d
I (HS)	18.27^{a}±0.16	8.34^{a}±0.07	8.31^{b}±0.075	8.19^{c}±0.26	8.59^{a}±0.20	7.84^{c}±0.13
II (HS + 0.3 ppm Se)	13.65^{c}±0.35	8.39^{a}±0.14	15.68^{a}±0.21	12.32^{a}±0.68	7.88^{b}±0.16	8.78^{b}±0.07
III (HS + 0.6 ppm Se)	15.94^{b}±0.22	7.37^{b}±0.11	7.65^{c}±0.12	13.45^{a}±0.15	9.10^{a}±0.17	11.99^{a}±0.42
IV (HS +0.9 ppm Se)	13.56^{c}±0.24	6.28^{c}±0.21	7.74^{c}±0.13	10.59^{b}±0.39	8.95^{a}±0.18	5.07^{d}±0.21

$^{a, b, c, d}$ Mean±SE differ significantly in a column at P<0.05.

Table 5. Effect of Se on antioxidants in hepatic tissues of broilers during heat stress

Treatment	TAC ($\times10^{-5}$ mM GSH eq/mg protein)		Glutathione (µg/mg protein)		*Glucose-6-Phosphate dehydrogenase ($\times10^{-3}$ U/mg protein)	
	21d	42d	21d	42d	21d	42d
I (HS)	$0.54^{b}\pm0.013$	$0.12^{c}\pm0.004$	$47.94^{b}\pm1.30$	$35.29^{b}\pm0.48$	$3.71^{c}\pm0.11$	$1.76^{b}\pm0.04$
II (HS + 0.3 ppm Se)	$0.52^{b}\pm0.010$	$0.27^{b}\pm0.007$	$45.28^{b}\pm0.48$	$50.77^{a}\pm2.03$	$5.62^{a}\pm0.21$	$2.39^{a}\pm0.20$
III (HS + 0.6 ppm Se)	$0.60^{a}\pm0.010$	$0.35^{a}\pm0.020$	$46.20^{b}\pm1.15$	$51.51^{a}\pm0.87$	$4.73^{b}\pm0.09$	$2.46^{a}\pm0.14$
IV (HS +0.9 ppm Se)	$0.56^{ab}\pm0.008$	$0.16^{c}\pm0.003$	$52.92^{a}\pm1.10$	$36.61^{b}\pm0.77$	$4.50^{b}\pm0.06$	$2.01^{b}\pm0.14$

[a, b, c, d] Mean±SE differ significantly in a column at P<0.01 and *at P<0.05.

present study (Jayasri 2020).

Finally, it is suggested that the broilers could better counteract the HS-induced oxidative stress with Se supplementation by enhancing the antioxidant defense system. Though 0.3 ppm of selenium was protective during early growth, it is more effective at 0.6 ppm in activating the antioxidant system during 21-42 d. Based on the present findings, we conclude that selenium supplementation at 0.6 ppm improved the antioxidant status of hepatic tissues when compared to 0.3 ppm and 0.9 ppm during HS in broilers.

ACKNOWLEDGEMENTS

We thank Sri Venkateswara Veterinary University, Andhra Pradesh, India for providing all the necessary infrastructure and fund for the research. We also thank ORFFA, India for providing organic selenium (L-Selenomethionine) in the form of *Excential* Se 4000.

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