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Altered serum vitamin E and glutathione levels in broilers during hot humid ambience

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Abstract
An experiment was conducted to examine the effect of hot humid ambience on serum antioxidant level of non-descript broiler chicken. Two hundred forty apparently healthy non-descript broilers were sampled during moderate (control) and hot humid ambiences to determine serum antioxidants level. Antioxidants determined in this study were serum vitamin E and glutathione. In each ambience one hundred twenty non-descript broilers were sampled. Each group of 120 birds was further subdivided into 3 categories according to age namely 2 week, 4-6 weeks and >8 weeks having 40 non-descript broilers in each category. Highly significant effect of hot humid ambience on serum antioxidant levels was observed. Moderate overall mean value of serum vitamin E and glutathione were 13.86 ± 0.19 µmol L^{-1} and 7.40 ± 0.12 µmol L^{-1}, respectively. The decrease in overall mean values of serum vitamin E and glutathione determined in the present investigation was highly significant ($p \leq 0.01$) during hot humid ambience as compared to overall moderate mean value. Age effects showed a highly significant ($p \leq 0.01$) decrease in the mean value being highest in the broilers of 2 weeks of age for both parameters. The findings of present study in respect with to both the parameters indicated that supplementation of antioxidants can be recommended during extreme ambiences to reduce the effect of oxidative stress.

Keywords: hot-humid ambience, non-descript broilers, antioxidants, oxidative stress

Introduction
Poultry raised in tropical countries are exposed to the deleterious effects of high ambient temperature during few months of the year. Broiler production suffers great losses due to adverse environmental temperature particularly in terms of productivity. Miscellaneous stressors incorporating environmental, technological, nutritional and biological/internal are responsible for decreased productive and reproductive performance as well as compromised health of birds (Surai and Fisinin, 2016) [17]. Heat stress can affect the overall performance as it is responsible for derangement of blood parameters and oxidative stability in broilers (Ghazi *et al*., 2012) [6]. Overproduction of free radicals, along with imbalance of antioxidant defense and oxidative stress are the major causes of the deleterious consequences of stress in poultry. Low level of antioxidants and high level of free radicals lead to the development of oxidative stress in the body (Bangyuan *et al*., 2013) [1]. There are antioxidant systems in birds which combat free radical production and maintain redox (antioxidant/prooxidant) balance. Environmental stress causes evident changes in the levels of biomarkers of oxidative stress (Pareek and Kataria, 2020) [16]. Earlier researchers have documented that the presence of stress can be depicted by the depletion of antioxidants due to extreme ambiences in the animals (Maan, 2020 and Pareek, 2020) [10, 15]. The insufficient quantity of antioxidants in the feed may cause deficiency. Among various levels of the antioxidant defense there are free radical scavenging antioxidants (vitamin E, ascorbic acid, glutathione), with vitamin E being the key biological antioxidant in the cell membranes. Antioxidant vitamins have corroborated to protect the biological membranes against the damage of reactive oxygen species and the role of vitamin E as an inhibitor –"chain blocker"- of lipid peroxidation has been well recognized. Therefore, Vitamin E defends cells and tissues from oxidative injury provoked by free radicals. Glutathione is a major intracellular thiol-disulfide redox buffer that functions as a cofactor for many antioxidant enzymes. Reduced glutathione is a tripeptide made up of glutamic acid, cysteine, and glycine. glutathione has an easily oxidizable sulfhydryl group that defends against oxidant injury by both enzymatic and nonenzymatic mechanism. To understand the real worth of these birds, to explore the productive potential and to establish the reference values of antioxidant parameters is of immense importance in the field of veterinary physiology and related spheres

Therefore, the present investigation aims to discover the fundamental mechanism associated with oxidative cell damage and antioxidant status in broiler during moderate and hot humid ambience in poultry birds and ultimately enhance their productive performance.

Materials and Methods

To achieve the objectives of the proposed plan, 240 apparently healthy birds belonging to the slaughter house located in and around Bikaner district, Rajasthan was screened during moderate ambience and humid hot ambiences. Broilers were maintained under natural environment with standard management conditions. Sampling was carried out in morning hours during both ambiences. Blood was collected directly into a clean, dry test tubes without any anticoagulant in duplicate. Both serum antioxidants were evaluated in fresh samples in each ambience, 120 blood samples were collected from each ambience. Broilers were also categorized according to age as 2 weeks old, 4-6 weeks old and > 8 weeks old in each ambience. Each category included 40 broilers. To assess antioxidant status, serum non-enzyme indicators of antioxidant status were determined. These included serum vitamin E and glutathione. Serum vitamin E was determined by spectrophotometric method of Nair and Magar (1955) [12] and modification as per Kataria *et al*. (2010a) [9]. Serum glutathione was recorded by the rapid colorimetric micro method of Owens and Belcher (1965) [13] with modifications (Wilson, 1968) [19]. The main effects were classified as ambience and age. The subsets of ambience were moderate and hot humid ambience and of age were 2 weeks, 4-6 weeks and > 8 weeks. For each subset data were expressed as mean ± SE of mean and statistical significance was assessed. IBM SPSS software (version 20.0) was used for statistical analysis.

Results and Discussion

The decrease in overall mean value of serum vitamin E was highly significant ($p \leq 0.01$) during hot humid ambience as compared to overall moderate mean value. A decrease of 38.31% was observed in overall mean value of hot humid ambience as compared to overall mean value of moderate ambience. Age effects showed a highly significant ($p \leq 0.01$) decrease in the mean value being highest in the broilers of 2 weeks of age. On % basis maximum decrease in the mean value of serum vitamin E was found in broilers of 2 weeks of age (42.44%). The interaction between age X ambiences was highly significant ($p \leq 0.01$) for serum vitamin E which showed the effect of ambience on the birds of all age groups. Vitamin E has a potent antioxidant effect and alleviates the ill effects of heat stress (Dalolio *et al.*, 2015) [4]. Surai *et al.* (2019) [18] found increase vitamin E levels in poultry. Vitamin E prevents oxidative damage of thiol rich protein constituents of cellular membranes, polyunsaturated fatty acids and the cytoskeleton and nucleic acid so maintains the structural and functional integrity of sub cellular organelles (Panda and Cherian, 2013) [14].

The decrease in overall mean value of serum glutathione was highly significant ($p \leq 0.01$) during hot humid ambience as compared to overall moderate mean value. A decrease of 35.94% was observed in overall mean value of hot humid ambience as compared to overall mean value of moderate ambience. Age effects showed a highly significant ($p \leq 0.01$) decrease in the mean value being highest in the broilers of 2 weeks of age. On % basis maximum decrease in the means value of serum glutathione was found in broilers of 2 weeks of age (42.34%). Hassan and Asim (2020) [7] observed no significant change in glutathione level in heat stress treatment groups. Chen *et al.* (2020) [3] and Hu *et al.* (2021) [8] found increased glutathione levels in chicken and heat stressed broilers, respectively. Glutathione protects cells from oxidative stress directly by scavenging reactive oxygen species as free radicals and peroxides that are produced in metabolism and by using glutathione-dependent enzymes such as glutathione peroxidase and glutathione S-transferase (Meister 1983; Cappiello *et al.* 2013) [11, 2]. Therefore, it is a very important endogenous antioxidant and plays a key role in the antioxidant defense system (Enkvetchakul *et al*. 1995) [5].

Table 1: Mean ± SEM values of serum vitamin E (µmol L^{-1}) in non -descript broilers

Key effects	Subgroups	Mean ± SEM values	
		Moderate	Humid hot
Age	2 weeks (40)	$11.52^{a, x} \pm 0.20$	$6.63^{b, x} \pm 0.07$
	4-5 weeks (40)	$13.94^{a, y} \pm 0.10$	$8.65^{b, y} \pm 0.16$
	>8 weeks (40)	$16.13^{a, z} \pm 0.11$	$10.36^{b, z} \pm 0.06$
Overall mean values		$13.86^{A} \pm 0.19$	$8.55^{B} \pm 0.15$

$^{A, B}$ marks highly significant ($p \leq 0.01$) differences between overall mean values of both ambience

$^{a, b}$ marks highly significant differences ($p \leq 0.01$) between mean values of different age groups in a row

$^{x, y, z}$ marks highly significant differences ($p \leq 0.01$) between mean values of different age groups in a column

Table 2: Analysis of variance of serum vitamin E (µmol L^{-1}) in non -descript broilers

Source of variation	DF	MSS	p-Value
Ambience	1	1696.919	0.000
Age	2	347.556	0.000
Ambience X Age	5	479.946	0.000
Error	236	0.704	

DF Marks degree of freedom MSS Marks mean sum of squares

Table 3: Mean ± SEM values of serum glutathione (µmol L^{-1}) in non -descript broilers

Key effects	Subgroups	Mean ± SEM values	
		Moderate	Humid hot
Age	2 weeks (40)	$6.47^{a, x} \pm 0.16$	$3.73^{b, x} \pm 0.12$
	4-5 weeks (40)	$7.32^{a, y} \pm 0.18$	$4.70^{b, y} \pm 0.10$
	>8 weeks (40)	$8.42^{a, z} \pm 0.21$	$5.79^{b, z} \pm 0.08$
Overall mean values		$7.40^{A} \pm 0.12$	$4.74^{B} \pm 0.09$

$^{A, B}$ marks highly significant ($p \leq 0.01$) differences between overall mean values of both ambience

$^{a, b}$ marks highly significant differences ($p \leq 0.01$) between mean values of different age groups in a row

$^{x, y, z}$ marks highly significant differences ($p \leq 0.01$) between mean values of different age groups in a column

Table 4: Analysis of variance of serum glutathione (µmol L^{-1}) in non -descript broilers

Source of variation	DF	MSS	p-Value
Ambience	1	425.283	0.000
Age	2	80.622	0.000
Ambience X Age	5	117.346	0.000
Error	236	0.926	

DF Marks degree of freedom MSS Marks mean sum of squares

Conclusion

It can be concluded that there was a decline in both vitamin E

and glutathione levels which marked the presence of oxidative stress and decrease levels ensure the depletion of antioxidants in counteracting the free radicals produced in broilers during hot humid ambience, Hence, it is recommended that inclusion of antioxidants in diet of poultry can decrease the ill effects of extremes of temperature on poultry birds.

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