



## HUMID HEAT STRESS RESPONSES IN DOG FROM ARID TRACTS

Sunita Pareek, Satynendra Budania, Mamta Saini, S.S.Singhal, Ashish Joshi, B.S.Saini and Nalini Kataria\*

Department of Veterinary Physiology, College of Veterinary and Animal Science, Bikaner, Rajasthan University of Veterinary and Animal Sciences, Bikaner-334 001, Rajasthan, India.

Corresponding Author:- **Nalini Kataria**  
E-mail: [nalinikataria@rediffmail.com](mailto:nalinikataria@rediffmail.com)

### Article Info

Received 22/09/2016  
Revised 27/09/2016  
Accepted 09/10/2016

**Keywords:** Antioxidants, arid tract, Humid-hot, Labrador, Oxidative stress.

### ABSTRACT

The present investigation was conducted to weigh up humid-heat stress responses in Labrador dogs. Sera were obtained to determine endogenous antioxidants like vitamin E, A, C and glutathione in healthy adult male and female dogs. During moderate ambience sampling, temperature humidity index varied (THI) as 70-72 and during humid-hot, THI varied as 82-84. Upshot displayed significant ( $p \leq 0.05$ ) shrinking in the values of serum vitamin E, A, C and glutathione during humid-hot ambience as compared to moderate ambience. It indicated depletion of antioxidant status of dogs and signified the development of oxidative stress during humid-hot ambience. Greatest per cent change was observed in the mean value of serum vitamin A. Per cent changes for each parameter were higher respectively in female than male. The present investigation has tried to evaluate the relationship of antioxidants with the oxidative stress. It can be assumed that humid-hot stress yielded the oxidative stress in the dogs. Female animals were affected with greater magnitude than male animals. Interpretation of these variations will help to provide perceptive approach of various hidden mechanisms in the development of oxidative stress. Results undoubtedly demonstrated that besides pathologies, abiotic stressors can also be the factors in the promotion and expansion of oxidative stress. Pets must be supplemented with antioxidants to enhance endogenous antioxidant status to safeguard them from the likelihood of oxidative stress.

### INTRODUCTION

An increasing concern toward oxidative stress has been recorded in order to find their direct or indirect involvement in various mechanisms regarding pathologies. A choice of antioxidant agents used to monitor physiological functions emerges to bestow gains, but with growing facts of the defectiveness of a unidimensional antioxidant tactic, there is a necessity for an integrated methodology using a blend of antioxidant compounds with balancing or interdependent effects. Specific data on the effects of natural antioxidant substances in dogs are scanty. Rising trend in the scientific community to give a new insight to physiological changes associated with abiotic stressors has given enough evidences to reveal hidden mechanisms regarding humid heat stress-induced lowering

of the normal functions. Exposure to humid heat produces stress causing instigation of severe physiological dysfunctions resulting in heat-associated pathologies embracing heat stroke, heat cramp, heat exhaustion and even death [1]. It has become imperative to find out the precise mechanisms governing stress reactions. Oxidative stress is a condition in which free radicals are generated which can exert toxic effects on the cells. Cells possess well equipped antioxidant defense mechanisms to detoxify the free radicals. Antioxidant defense mechanism can be enzymatic or non enzymatic. Non enzymatic mechanisms include endogenous antioxidants like vitamin A, C, E and glutathione. Environmental variation is appreciated as a chief peril to the existence of animals and ecosystems, and



the upholding of production.

The existing inclination for the knowledge about requirement of animal is enhancing. For animals, heat stress is the most taxing amongst all the abiotic stressors. Tumbling the bang of abiotic stress on livestock necessitates a multidisciplinary tactic with importance on health and immune-nutrients. It is important to understand the livestock responses to environment and to analyse them carefully in order to alter environment-related management practices. Future research needs for ameliorating abiotic stress in livestock are to identify strategies for developing and monitoring appropriate measures of heat stress; to assess the genetic components, including the genomics and proteomics of heat stress in livestock; and to develop alternative management practices for reducing abiotic stress and improving animal well-being and performance.

The most important antioxidants include vitamins A, C and E, which work by slowing or stopping the development of reactive oxygen in cells. Recent studies have focused on the role of oxidative stress in animal life [2]. Dogs of Labrador breed are kept as companions by large number of families. Hence it becomes crucial to keep the pets stress free and well-timed uncovering of stress can strengthen the measures which help in preventing the incidence of diseases. Rareness of literature on this viewpoint in the dogs was adequate to embark on the initiation of a project with comprehensible directions to assess oxidative stress in dogs.

## MATERIALS AND METHODS

The present investigation was commenced to determine humid-hot stress responses in dogs of Labrador breed from arid tracts. Thirty healthy adult (between 3 and 5 years) Labrador male (15) and female (15) dogs kept by the private owners with almost similar type of management conditions were screened. These animals were free from endo- and ecto-parasites as gauged by customary faecal and skin examination, respectively. Blood sample were collected in sterile tubes to harvest sera in morning hours during moderate and humid-hot ambiances. Sampling during October-November months were carried out when average temperature humidity index varied as 70-72 considering the period as moderate. Sampling during July-August months were carried out when average temperature humidity index varied as 82-84 considering the period as humid-hot. Serum vitamin E, vitamin A, vitamin C and glutathione were determined in each ambience.

Serum vitamin E was determined by the spectrophotometric method of Nair and Magar[3] with little modification [4]. This is a highly sensitive method based upon the colour reaction between phosphomolybdic acid and vitamin E. Determination of serum vitamin A was carried out by Varley [5] with little modification [4]. In the test proteins were precipitated by using alcohol and the retinol and carotenes were extracted into light petroleum. After recording the concentration of yellow colour due to

carotenes, the light petroleum was evaporated and the residue was dissolved in chloroform before carrying out colour reaction. Determination of vitamin C was carried out as described by Varley [5]. This method is based upon the titration of serum ascorbate by 2,6-dichlorophenolindophenol dye. Serum glutathione was determined by the rapid colorimetric micro method of Owens and Belcher [6] with modifications for serum samples [4]. Mean value of each parameter obtained during moderate ambience was considered as control.

## RESULTS AND DISCUSSION

The mean  $\pm$  SEM values of serum vitamin E, vitamin A, vitamin C and glutathione during moderate and humid-hot ambiances are presented in table 1 and per cent changes are depicted in figure 1 and 2 for male and female, respectively. Results designated significant ( $p \leq 0.05$ ) diminution in the concentrations of serum vitamin E, vitamin A, vitamin C and glutathione during humid-hot ambience in contrast to respective moderate ambience mean value. Per cent variation was calculated for mean value of each parameter during humid-hot ambience as compared to respective moderate ambience mean value. Enormity of per cent variation was found to be peaked in the mean value of serum vitamin A. Per cent changes for each parameter were higher respectively in female than male. Female animals were affected with greater magnitude than male animals. Availability of information concerning antioxidant status in the dogs of Labrador breed during moderate and humid-hot ambience is pitiable.

### Vitamin E

Vitamin E assists to safeguard cell membranes in addition to enhance the immunity. The vitamin E deficiency in puppies begins to happen with muscle paralysis and muscle weakness in their limbs. A diminished muscle mass and enhanced sensitivity to pain is observed. High atmospheric temperature and humidity of extreme hot months amplify neuroendocrine mechanisms and lipid peroxidation which in turn add to the reduced antioxidant response. Vitamin E is also imperative in the supervision of stressed animals [7]. Its use is encouraged to fracos oxidative stress [8]. A powerful antioxidant like vitamin E is crucial in enlivening and sustaining immune system health because it reduces the number of free radicals formed and prevents much of the damage that could lead to serious health problems. Vitamin E supplements included in dog's diet can reinforce its immune system to help keep it healthy and strong for longer. Vitamin E is a neuroprotectant and a powerful antioxidant. Decreased serum vitamin E levels during hot-humid ambience revealed its depletion to combat the free radicals and marked the development of oxidative stress.

### Vitamin A



Decreased concentration of vitamin A during hot-humid ambience could be due to its enhanced mobilization for a mixture of metabolic functions [4]. It is illustrious that vitamin A is an indispensable nutrient for cellular function together with other aspects like reproduction and development. Dearth of vitamin A causes its deficiency affecting growth, proliferation and differentiation of epithelial tissues. Mechanisms involved in visual and reproductive functions are also affected [9]. In humans, vitamin A stores are identified to be completely linked with numerous gauges of innate immune action across a wide range of stress, insinuating that vitamin A is a proven armor against various pathogens at concentrations higher than those required to keep up normal vision [10]. Besides well defined role, vitamin A is now widely accepted as an important antioxidant by shielding the body from the peril of oxidative stress. Many a times it has been observed that animals supplemented with vitamin A also show lower plasma level of vitamin A. Such cases clearly indicate towards the oxidative stress in which vitamin A is used to neutralize the free radicals [4]. It is believed that requirements for immunity are higher than for growth or reproduction. Low vitamin A during hot-humid ambience denoted the development of oxidative stress.

**Vitamin C**

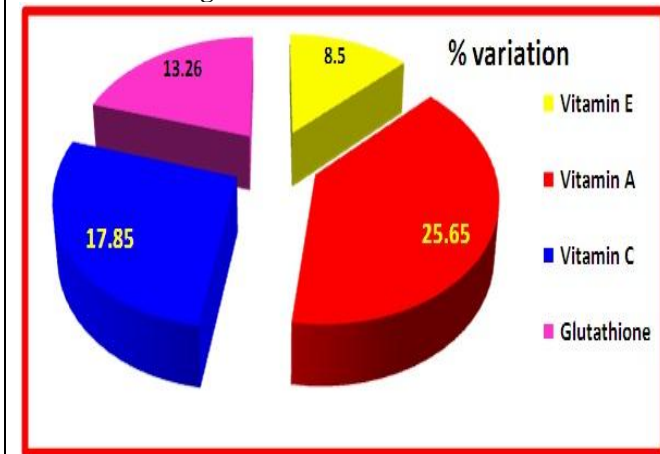
All dogs can derive advantages from antioxidants supplementation, however, dogs with skin, immune system or eye problems, respiratory or cardiovascular illness can draw larger gains. Liver of dog is able to synthesize vitamin C from glucose. However, stress of any kind may produce depletion hence supplementation becomes imperative to benefit the dog. The task of vitamin C is well established as an anti-oxidant shielding the body against oxidative stress [11]. Vitamin C is vital in the synthesis of collagen which is imperative for growth and development. Vitamin C is also important for the proper function of immune system.

Commercial dog foods contain vitamin C because of its antioxidant property. Reversible oxidation-reduction of ascorbic acid with dehydro ascorbic acid is the largely focal chemical property of vitamin C and the foundation for its known physiological activities and stabilities [12]. Ascorbic acid is also stabilized by the antioxidant enzymes superoxide dismutase and catalase [13], which need zinc, copper, manganese and iron. The antioxidant part of vitamin C seems to be a usual linkage in its role in the function and integrity of several cell classes, in detoxification and in the typical functioning of the immune system, adrenal glands, lungs, brain, and eye. Scientists have investigated the ability of vitamin C to increase the anti-oxidative and immune-modulating potential in healthy dogs [14].

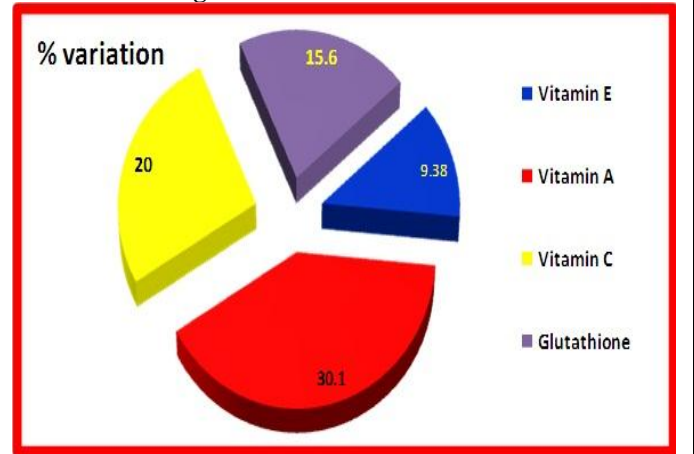
**Glutathione**

Oxidation is a natural process that occurs in living things. Glutathione is overt sovereign of antioxidants and is a tripeptide composed of amino acids cysteine, glutamic acid and glycine. Glutathione prevents cellular damage caused by free radicals and peroxides. Glutathione antioxidant is a minuscule but authoritative nutrient because it performs important functions which include neutralization of free radicals, maintenance of cellular health and functions of vital organs and immunity and detoxification of liver. More renowned antioxidants like vitamins C and E, have short lifespans of function, but glutathione has the power to bring these antioxidants back to functional state. Glutathione recharges itself. Other antioxidants depend on this antioxidant to function properly. It improves antioxidant capacity of blood [15]. Stress is known to reduce its levels. Glutathione deficiency is well associated with neurological disorders and cancers. Lower serum levels of glutathione in dogs during humid-hot implied the existence of oxidative stress.

**Fig 1. Per cent changes in serum antioxidants of male Labrador during humid- hot ambiances**



**Fig 2. Per cent changes in serum antioxidants of female Labrador during humid- hot ambiances**



**Table 1. Serum levels of antioxidants in Labrador during moderate and humid-hot conditions (n=15, mean ± SEM values )**

Serum antioxidants	Moderate ambience		Humid-hot ambience			
	Male	Female	Male	% change	Female	% change
Vitamin E μmol L <sup>-1</sup>	6.80 ± 0.04	6.50 <sup>d</sup> ± 0.03	6.22 <sup>b</sup> ± 0.01	8.50	5.89 <sup>bd</sup> ± 0.01	9.38
Vitamin A μmol L <sup>-1</sup>	1.91 ± 0.005	1.86 <sup>d</sup> ± 0.004	1.42 <sup>b</sup> ± 0.009	25.65	1.30 <sup>bd</sup> ± 0.007	30.10
Vitamin C μmol L <sup>-1</sup>	28.00 ± 0.03	25.00 <sup>d</sup> ± 0.04	23.00 <sup>b</sup> ± 0.02	17.85	20.00 <sup>bd</sup> ± 0.03	20.00
Glutathione μmol L <sup>-1</sup>	5.20 ± 0.005	5.00 <sup>d</sup> ± 0.006	4.51 <sup>b</sup> ± 0.01	13.26	4.22 <sup>bd</sup> ± 0.01	15.60

Superscript 'b' indicates that a given parameter differs significantly ( $p \leq 0.05$ ) from respective moderate mean value. Superscript 'd' indicates that a given parameter differs significantly ( $p \leq 0.05$ ) from respective male mean value.

## CONCLUSION

The results of the present study substantiated the occurrence of increased oxidative stress in the humid-hot environment in Labrador dogs. Lower serum levels of endogenous antioxidants validated their depletion in a process to combat the excessive free radicals. Layout of variations in serum antioxidants propped up the potential physiological changes induced by plausible boost in

reactive oxygen species. It can be construed that callous environment harvested the oxidative stress in the dogs.

## ACKNOWLEDGEMENT

The authors are thankful to the pet owners for allowing collecting blood samples to be used in this study.

## CONFLICT OF INTEREST

No conflict of Interest.

## REFERENCES

- Wang X, Yuan B, Dong W, Yang B, Yang Y, Lin X, Gong G. (2015). Humid heat exposure induced oxidative stress and apoptosis in cardiomyocytes through the angiotensin II signaling pathway. *Heart Vessels*, 30(3), 396-405.
- Agarwal S, Satyendra S, Saini BS, Saini M, Singhal SS, Joshi A, Kataria N. (2016). Evaluation of endogenous antioxidant status of pregnant dog, *European Journal of Molecular Biology and Biochemistry*, 3(2), 83-87.
- Nair PP, Magar NG. (1955). Determination of vitamin E in blood. *J Biol Chem*, 220(1), 157-159.
- Joshi A. (2012). Ambience associated variations in the serum biomarkers of oxidative stress in buffaloes of arid tract, Department of Veterinary Physiology, College of Veterinary and Animal Science, Bikaner, Submitted to Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India.
- Varley H. (1988). Tests in liver and biliary tract disease In: *Practical Clinical Biochemistry*, 4<sup>th</sup> edn, CBS publishers New Delhi, 158-467.
- Owens CWI, Belcher RV. (1965). A colorimetric micro method for the determination of glutathione. *Biochem J*, 94(3), 705-711.
- Chirase NK, Greene LW, Purdy CW, Loan RW, Briggs RE, McDowell LR. (2001). Effect of environmental stressors on ADG, serum retinal and alpha-tocopherol concentrations, and incidence of bovine respiratory disease of feeder steers. *J Anim Sci*, 79, 188.
- Bourdel MI, Christine M, Beauvieux D, Peuchant E, Richard HS, Decamps A, Reignier B, Paul Emeriau J, Rainfray M. (2001). Antioxidant defenses and oxidative stress markers in erythrocytes and plasma from normally nourished elderly alzheimer patients. *Age and Ageing*, 30, 235-241.
- Goodman DS. (1984). Vitamin A and retinoids in health and disease. *N Engl J Med*, 310, 1023-1031.
- Ahmad SM, Haskell MJ, Raqib R, Stephensen CB. (2009). Markers of innate immune function are associated with vitamin A stores in men. *J Nutr*, 139, 377-385.
- Padayatty S, Katz A, Wang Y, Eck P, Kwon O, Lee J, Chen S, Corpe C, Dutta A, Dutta S, Levine M. (2003). Vitamin C as an Antioxidant, evaluation of its role in disease prevention. *J Am Coll Nutr*, 22 (1), 18-35.
- Jaffe GM. (1984). Vitamin C. In *Handbook of Vitamins*, (L.J. Machlin, ed.), Marcel Dekker, Inc., New York.
- Miyake N, Kim M, Kurata T. (1999). Stabilization of L-ascorbic acid by superoxide dismutase and catalase. *Biosci Biotechnol Biochem*, 63, 54-57.
- Hesta M, Ottermans C, Krammer-Lukas S, Zentek J, Hellweg P, Buyse J and Janssens GP. (2009). The effect of vitamin C supplementation in healthy dogs on antioxidative capacity and immune parameters. *J Anim Physiol Anim Nutr*, 93(1), 26-34.



Gropper SS, Smith JL, Grodd JL. (2004). *Advanced Nutrition and Human Metabolism, Fourth Edition*, Thomson Wadsworth, Belmont, CA. USA, 260-275.

