

# **A Novel Non-Invasive Approach to Detect Cellular Signals of Oxidative Stress due to Extreme Environmental Temperatures in Backyard Broiler from Arid Tracts in India**

## **ABSTRACT**

The present investigation was aimed to investigate use of non invasive methods to detect cellular signals of oxidative stress due to extreme environmental temperature periods (ETPs) in broilers from arid tracts in India. ETPs i.e. moderate, extreme hot and extreme cold were used to sample the birds. Broilers were maintained under natural environment in backyard poultry farms. Non invasive tools determined in the study were serum gamma glutamyl transferase (GGT) and monoamine oxidase (MAO) enzymes. Moderate mean overall values were  $60.00 \pm 2.11 \text{ U L}^{-1}$  and  $156.00 \pm 253 \text{ U L}^{-1}$ , respectively. The mean values of serum GGT and MAO during extreme hot ETP and extreme cold ETP were significantly ( $p \leq 0.05$ ) higher than the respective mean moderate overall value. Maximum percent change in mean value was observed during extreme hot ETP than in extreme cold ETP. It was 214.10% increase for serum MAO. The mean overall value during extreme hot ETP was significantly ( $p \leq 0.05$ ) higher as compared to respective extreme cold ETP mean overall value. Age effect showed a significant ( $p \leq 0.05$ ) increase in the mean values of both the markers being highest in the broilers of 8 weeks of age. Results of present study suggested that the degree of development of oxidative stress was higher during extreme hot ETP in comparison to extreme cold ETP. The results demonstrated that higher activity levels of enzymes may contribute to better tolerance to environmental temperature by increasing the protection capacity against oxidative damage. On the basis of pattern of observations obtained in the present study it can be recommended that broilers must be supplemented with proper antioxidants to protect them from ill effects of adverse environmental temperatures and oxidative stress. Upshot of the endeavor evidently specified the use of GGT and MAO as novel non invasive approach to detect cellular signals of oxidative stress due to extreme environmental temperature periods in broilers.

**Key words:** Environmental temperature period, GGT, MAO, non invasive, novel, oxidative stress

## **INTRODUCTION**

The negative effects of high environmental temperature during some months of the year on poultry production have been of great concern in many countries. Birds in the arid tract encounter with greater ambient variables. This thermal discomfort sometimes goes to the extent that improper expression of genetic potential is observed. Varying ambience may produce detrimental effect if timely and proper measures are not taken in broiler houses (1). Body temperature and metabolic rate of birds are relatively high as compared to mammals, which make birds vulnerable to oxidative stress under high environmental temperature. Thermal ambience can influence growth characteristics of birds. High temperature coupled with humidity is more stressful that results in major economical losses to the poultry industry by reducing growth, egg production, hatchability and increasing mortality. Heat stress not only adversely affects production performance but also inhibits immune function (2) and is one of the prominent environmental factors causing cost effective thrashing to the poultry industry as it negatively impinges on growth and production in broiler chickens (3). Heat stress can cause derangement of oxidative stability in broilers (4). Many diseases of chicken are associated with oxidative cell damage like nutritional muscular dystrophy (5). Most of the studies in broilers associated with ambient stress have been carried out by

exposing them to extreme temperature artificially. There is scarcity of research regarding reproducible quantification of heat stress effects, which can be suitable for the evaluation of dietary intervention strategies to combat heat stress conditions. Broiler production suffers great losses due to effect of thermal stress. Elevate ambient temperature increases the time to reach market weight and mortality. Scientific community is showing serious concern to reveal the underlying mechanisms related to the presumed oxidative cell damage and antioxidant status in broilers during extreme environmental temperature periods for the use in veterinary clinical practice. Now the time has come to think about the impact of ambience stress in the form of oxidative stress which can be originator of various diseases in which mass mortality is observed which could have a great impact on the economical structure of poultry raiser. If development of oxidative stress can be stopped at an earlier step, further losses can be prevented. However, the melancholy is that presence of oxidative stress can only be established by laboratory findings (6). Looking towards the paucity of research work on the use of markers of oxidative stress in broilers reared in natural conditions of extreme environmental temperatures, the present endeavor was launched with the aim to investigate use of non invasive methods to detect cellular signals of oxidative stress due to extreme environmental temperature periods (ETPs) in back yard broilers from arid tracts in India.

## MATERIALS AND METHODS

To investigate use of non invasive methods to detect cellular signals of oxidative stress due to extreme environmental temperature periods (ETPs) in back yard broilers from arid tracts in India, blood samples were collected to harvest the serum from three hundred and sixty male poultry birds (White Leghorn) of 6-8 weeks of age groups from private backyard poultry farms in and around Bikaner, Rajasthan, India. Broilers were maintained under natural environment with standard management conditions. Blood samples were collected during moderate (control), extreme hot and extreme cold environmental temperature periods (ETPs) during slaughtering. Sampling was carried out in morning hours during moderate, hot and cold environmental temperature periods. Blood was collected directly into the clean, dry test tubes without any anticoagulant. After collection of the blood, test tubes were kept in the slanting position for 30 minutes and blood was allowed to clot. Then the clot was separated from the walls of the each test tube with the help of sterilised stainless steel wire and then each test tube was centrifuged at 3000 rpm for 30 minutes. Supernatant clear serum (non-haemolysed) was pipetted out into sterilised plastic vials. All the parameters of antioxidant status were analysed in fresh samples. In each environmental temperature period (ETP), 120 blood samples were collected. Broilers were also categorized according to age as 6 weeks old, 7 weeks old and 8 weeks old in each environmental temperature period. Each category included 40 broilers. Average minimum and maximum environmental temperatures °C during moderate, hot and cold environmental temperature periods were  $20.10 \pm 0.02$  and  $28.12 \pm 0.03$ ;  $29.20 \pm 0.03$  and  $45.78 \pm 0.02$ ; and  $4.80 \pm 0.10$  and  $15.30 \pm 0.20$ , respectively. Average relative humidity % at 8.00 am and 5.00 pm during moderate, hot and cold environmental temperature periods were  $53.50 \pm 1.00$  and  $31.10 \pm 1.00$ ;  $27.15 \pm 0.02$  and  $10.93 \pm 0.03$ ; and  $68.02 \pm 2.00$  and  $32.50 \pm 1.00$ , respectively. To assess antioxidant status, serum enzyme indicators of antioxidant status were determined. These included gamma glutamyl transferase (EC 2.3.2.2., GGT) and monoamine oxidase (EC 1.4.3.4., MAO). Gamma glutamyl transferase (GGT) was determined by spectrophotometric method as described by Wolf and Williams (7). GGT hydrolyses the substrate  $\gamma$ -glutamyl-p- nitroanilide to yield p-nitroaniline and  $\gamma$ -glutamyl compounds.  $\gamma$ -glutamyl-p-nitranillide is a chromogenic substrate and glycylglycine is a most commonly used acceptor molecule. Serum is incubated with substrate and the reaction is monitored at 405 m $\mu$ . Change in optical density per minute is recorded to determine the enzyme activity. Monoamine oxidase (MAO) was determined by the

colorimetric method of Green and Haughton (8) and with little modification as per Kataria *et al.* (9). The method is based on the measurement of the aldehyde formed during the enzymic oxidation of tyramine. The unstable aldehyde is prevented from decomposing by the presence of semicarbazide, which is then converted into the corresponding 2, 4 dinitrophenylhydrazone. The colour of this material is intense in alkaline solution which provides a sensitive measure of the enzymic activity.

The main parameters of the present investigation were non invasive tools in the serum of broilers during moderate, hot and cold environmental temperature periods (ETPs). The main effects were classified as environmental temperatures and age. The subsets of environmental temperatures were moderate, hot and cold environmental temperature periods and of age were 6 weeks, 7 weeks and 8 weeks. For each subset data were expressed as mean  $\pm$  SE of mean and statistical significance was assessed (10).

## RESULTS

The present investigation was aimed to investigate use of non invasive methods to detect cellular signals due to ambience stress in broilers from arid tracts in India. Male broilers were grouped according to age as 6 weeks, 7 weeks and 8 weeks groups in each ETP. The mean values of serum GGT and MAO during extreme hot ETP and extreme cold ETP were significantly ( $p \leq 0.05$ ) higher than the respective mean moderate overall value (Table 1). Maximum percent change in mean value was observed during extreme hot ETP than in extreme cold ETP (Table 2). It was 214.10% increase for serum MAO. The mean overall value during extreme hot ETP was significantly ( $p \leq 0.05$ ) higher as compared to respective extreme cold ETP mean overall value.

Age effect showed a significant ( $p \leq 0.05$ ) increase in the mean values of both the markers being highest in the broilers of 8 weeks of age. **Per cent** changes showed greater increase during extreme hot ETP in comparison to extreme cold ETP in all the mean values of broilers according to age for GGT and MAO both. Magnitudes of age related variations were 1.70, 1.15 and 1.41 times, respectively for serum GGT and 1.27, 1.21 and 1.42 times, respectively for serum MAO in moderate, extreme hot and extreme cold ETPs, being highest in extreme hot ETP. It was observed that maximum increase of GGT value on percent basis was observed in the broilers of 6 weeks of age during extreme hot and cold ETPs and MAO value in the broilers of 6 weeks of age during extreme hot and in the broilers of 8 weeks of age during cold ETPs. Magnitude of increase was less during extreme cold ETP for GGT. Results of present study suggested that the degree of development of oxidative stress was higher during extreme hot ETP in comparison to extreme cold ETP.

## DISCUSSION

### Gamma glutamyl transferase

Elevated serum GGT activity reflected enhanced oxidative stress (11) due to environmental temperature as a potent stressor (12). Serum GGT is a commonly used indicator of hepatotoxicity in poultry (13), however, research pertaining to serum GGT in broilers is scanty in the literature. Gamma glutamyl transferase transfers gamma-glutamyl functional groups and is found in many tissues, the most notable one being the liver and has a significance as a diagnostic signal marker. It plays a key role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione and detoxification of many drugs. Other lines of evidence indicate that GGT can also exert a prooxidant role, with regulatory effects at various levels in cellular signal transduction and cellular pathophysiology. Hence researchers (14) have put forward that increased levels of serum GGT should also be looked in terms of oxidative stress along with liver problems. Though countable efforts, but scientists have started paying attention towards the role of GGT as a possible marker of oxidative stress in chickens (15,16).

There is scarcity of literature on the age associated studies in serum GGT in broilers. Earlier reports in poultry suggested that the activity of GGT was higher at 35 days of age in broilers (17). Perhaps in this period there is greater body development with a concurrent significant increase of liver metabolism (17). To finish off, it can be surmised that increased activity of the serum GGT in present study was due to oxidative stress in the broilers and it can be proposed that serum GGT can be used as a novel marker of hepatic involvement as well as a cell signal marker of oxidative stress in broilers.

#### **Mono amine oxidase**

Activity of serum MAO is now increasingly gaining importance as a biomarker of oxidative stress as it catalyses the breakdown of dopamine in brain, however, paucity of work is there in broilers. Many earlier researchers have emphasized upon the association of variation in activity of MAO to stress conditions in mammals (18). Oxidative deamination of several monoamines catalysed by MAO can result in significant reactive oxygen species production contributing to oxidative stress (19). Increased oxidation of dopamine by monoamine oxidase is associated with an oxidant stress, expressed as a rise in the level of oxidised glutathione. Researchers (20) have studied the developmental changes in monoamine oxidase activities towards 5-hydroxytryptamine in chicks. Monoamine oxidase is considered to be a potent source of reactive oxygen species in mitochondria. They are flavoproteins and located in the outer mitochondrial membrane and catalyze the oxidative deamination of neurotransmitters and dietary amines, generating hydrogen peroxide (21). The monoamine catabolism generates aldehydes, ammonia and hydrogen peroxide. MAO have been extensively studied at the level of the central nervous system but its role as a novel marker to detect cellular signals of oxidative stress has been scarcely investigated. The observations of variation in serum MAO levels triggered the assumption that extreme ETPs were able to produce oxidative stress in broilers and serum MAO activities can be used to detect oxidative stress in broilers.

#### **Conclusion**

The fullness of above discussion helped in concluding that in present study elevated serum GGT levels pointed towards oxidative stress in broilers. Serum GGT values will have important implications both clinically and epidemiologically because measurement of serum GGT is easy, reliable and not expensive. Upshot of the study clearly indicated towards use of GGT and MAO as novel non invasive approach to detect cellular signals of oxidative stress due to variations in environmental temperatures in broilers.

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245 Table 1: Mean  $\pm$  SEM values of gamma glutamyl transferase and monoamine oxidase (U  
 246 L<sup>-1</sup>) in the serum of broilers during varying environmental temperature periods (ETP<sub>s</sub>).

| Age groups   | Gamma glutamyl transferase     |                                 |                                 |                                 | Monoamine oxidase              |                                 |                                 |                                 |
|--------------|--------------------------------|---------------------------------|---------------------------------|---------------------------------|--------------------------------|---------------------------------|---------------------------------|---------------------------------|
|              | Over all                       | 6 weeks                         | 7 weeks                         | 8 weeks                         | Over all                       | 6 weeks                         | 7 weeks                         | 8 weeks                         |
| Moderate ETP | 60.00 <sup>b</sup> $\pm$ 2.11  | 44.00 <sup>b</sup> $\pm$ 0.10   | 61.00 <sup>bd</sup> $\pm$ 0.11  | 75.00 <sup>bd</sup> $\pm$ 0.10  | 156.00 <sup>b</sup> $\pm$ 2.53 | 138.00 <sup>bd</sup> $\pm$ 0.12 | 154.00 <sup>bd</sup> $\pm$ 0.12 | 176.00 <sup>bd</sup> $\pm$ 0.13 |
| Hot ETP      | 156.00 <sup>b</sup> $\pm$ 2.45 | 146.00 <sup>bd</sup> $\pm$ 0.10 | 154.00 <sup>bd</sup> $\pm$ 0.10 | 168.00 <sup>bd</sup> $\pm$ 0.11 | 490.00 <sup>b</sup> $\pm$ 2.67 | 443.00 <sup>bd</sup> $\pm$ 0.11 | 487.00 <sup>bd</sup> $\pm$ 0.10 | 540.00 <sup>bd</sup> $\pm$ 0.11 |
| Cold ETP     | 103.00 <sup>b</sup> $\pm$ 2.33 | 86.00 <sup>bd</sup> $\pm$ 0.11  | 101.00 <sup>bd</sup> $\pm$ 0.10 | 122.00 <sup>bd</sup> $\pm$ 0.12 | 290.00 <sup>b</sup> $\pm$ 2.22 | 240.00 <sup>bd</sup> $\pm$ 0.11 | 287.00 <sup>bd</sup> $\pm$ 0.13 | 343.00 <sup>bd</sup> $\pm$ 0.12 |

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 248 i. Figures in the parenthesis indicate number of broilers.  
 249 ii. Overall value is the mean value obtained from broilers in an ETP irrespective of  
 250 age.  
 251 iii. <sup>b</sup>, marks significant (p $\leq$ 0.05) differences among moderate, hot and cold ETP mean  
 252 values for a row.  
 253 iv. <sup>d</sup>, marks significant (p $\leq$ 0.05) differences among mean values of age group within an  
 254 ETP.  
 255

256 Table 2: **Per cent** change in the mean values of Gamma glutamyl transferase and  
 257 monoamine oxidase (U L<sup>-1</sup>) in the serum of broilers during varying environmental  
 258 temperature periods (ETP<sub>s</sub>).

| Parameters |          | Gamma glutamyl transferase |         |         |         | Monoamine oxidase |         |         |         |
|------------|----------|----------------------------|---------|---------|---------|-------------------|---------|---------|---------|
| Age groups |          | Over all                   | 6 weeks | 7 weeks | 8 weeks | Over all          | 6 weeks | 7 weeks | 8 weeks |
| % change   | Hot ETP  | 160.00                     | 231.81  | 152.45  | 124.00  | 214.10            | 221.01  | 216.23  | 206.81  |
|            | Cold ETP | 71.66                      | 95.45   | 65.57   | 62.66   | 85.89             | 73.91   | 86.36   | 94.88   |

259 **Per cent** changes have been calculated from respective moderate mean values