# Bio-molecular variability under different environmental regimens and suitable nutrient supplementation to combat the weather related stress under semi-arid dryland region in Deccani females

# PRABHAT KUMAR PANKAJ\*, D.B.V. RAMANA, CH. SRINIVASA RAO, RITA RANI, M. NIKHILA, HEMANTA C. DAS and G. NIRMALA

ICAR-Central Research Institute for Dryland Agriculture, Santoshnagar, Saidabad,
Hyderabad-500 059 (Telangana State)
\*Email: dr.prabhatkumarpankaj@gmail.com

#### **ABSTRACT**

The present investigation was conducted to study the effect of Temperature-Humidity Index (THI) and find suitable nutrient supplement to combat its adverse effect. Twenty four Deccani female sheep were divided randomly into four groups of similar overall body weight (viz., Control (C), T, (3ml of 10% Zinc Sulphate), T<sub>2</sub> (1ml of 0.1% Selenium) and T<sub>3</sub> (60mg Vitamin E daily)) and reared under semi-intensive conditions at Hayathnagar Research Farm of ICAR-Central Research Institute for Dryland Agriculture, Hyderabad, India between December, 2013 to June, 2014. Various physical, physiological, biochemical and bio-molecular parameters were significantly correlated with THI. Superior body weight and lowered physiological response, plasma enzymes (ALP, SGOT and SGPT) and metabolites (Urea and creatinine) was observed in supplemented groups than control. Significantly superior plasma levels of nutrients and electrolytes (Na+, Cl-, K+, Ca and P) in supplemented groups suggested better availability of nutrients in supplemented animals. In THI-wise analysis (group-I: T<28°C and RH<60%; group-II: T>28°C and RH>60%), performance of supplemented group of animals was superior than that of control animals in terms of attaining superior body weight, maintaining physiological limits, plasma minerals, enzymes, cortisol, nutrients and blood metabolites. Study under present circumstances suggests that supplementing sheep with Zn, Se and Vit. E can built up thermo-tolerance in small ruminants and may help in coping the seasonal stress.

*Keywords:* Agro-meteorological variables, grazing sheep, anti-oxidant, heat stress, thermo-tolerance, bio-meteorology

# INTRODUCTION

Sheep rearing play an imperative role in the livelihood of a large proportion of landless, small and marginal farmers of dryland regions of India (Pankaj *et al.*, 2014). Deccani breed of sheep is spread over the semi-arid areas of Maharashtra, Telangana, Andhra Pradesh and Karnataka having the great potential for mutton production under intensive system of management (Venkat *et al.*, 2015).

Ruminants are least tolerant to the heat stress (Mader *et al.*, 2006). Increase in solar radiation due to climate change considerably increases the thermal load on the grazing animals like sheep and goat during the day, particularly in summer (Ramana *et al.*, 2013). The physiological responses of livestock to heat stress have been well described (Pankaj *et al.*, 2013a), and include increased body temperature (Bernabucci *et al.*, 1999),

respiratory rate (Pankaj *et al.*, 2013b), water intake (Mader *et al.*, 2006) and decreased feed intake (West, 1999). The performance (e.g., growth, milk and wool production, reproduction) and well-being of livestock are strongly affected by climate both, directly and indirectly (Pankaj *et al.*, 2013a). A reduced rate of metabolism, decreased nutrient intake, altered water metabolism, decreased average daily gain (Pankaj *et al.*, 2013b), growth rate is observed in response to heat stress.

The dietary Selenium (Se), Vitamin E and Zinc (Zn) have a role in reducing peroxidation and the subsequent self-protective mechanism from repairing oxidized fatty acid of the lipid membrane of various cells (Bray and Bettger, 1990; Tinggi, 2008). Detailed studies on effect of seasonal variation as well as nutritional supplementation to fight this peril have not been studied much. Thus, present investigation has been carried out to

portray bio-molecular variability of Deccani female sheep under different environmental regimens to realize suitable nutrient supplementation (Se, Zn and Vit. E) for adapting the weather related stress in semi-arid region.

#### MATERIALS AND METHODS

Twenty four Deccani females (8-9 month age with around 16 Kg body weight) were divided randomly into four groups of similar overall body weight (viz., Control (C), T<sub>1</sub> (3ml of 10% Zn Sulphate), T<sub>2</sub> (1 ml of 0.1% Se) and T<sub>3</sub> (60 mg Vit. E) daily and reared under semi-intensive conditions at Hayathnagar Research Farm of ICAR-Central Research Institute for Dryland Agriculture, Hyderabad, India (17°27'N latitude, 78°35'E longitude and 515m above sea level) between December, 2013 to June, 2014.

All the physiological parameters (respiratory rate (RR), pulse rate (PR), rectal temperature (RT) were measured weekly before feeding at 8:30 AM. 5ml blood was collected from all the 24 animals before feeding at 8:45 AM monthly and suitable analysis was done using various biochemical kits of Biosystem (Glucose, total protein, albumin, creatinine, urea, SGOT, SGPT, ALP, cholesterol, triglycerides, calcium and phosphorus) and hormonal kits from IBL international company.

Environmental variables were recorded using automatic weather station. In sheep, physical, physiological, biochemical, hormonal, enzymatic and antioxidant profiles were determined monthly using suitable techniques. Temperature Humidity Index (THI) was calculated using the following formula.

$$THI = 0.8 \times T_a + (RH \times (T_a - 14.4)/100) + 46.4$$

Where T<sub>a</sub> is temperature in centigrade; RH in %

In THI-wise analysis, all the observations under various treatments were divided into group-I ( $T_{max}$ <28°C, RH<sub>max</sub><60%) and group-II ( $T_{max}$ >28°C, RH<sub>max</sub>>60%). Suitable statistical analysis was carried out using SAS 9.2 to find out mean  $\pm$  SE, Pearson correlation coefficient and statistical significance using General Linear Model and Univariate options.

### RESULTS AND DISCUSSION

THI<sub>max</sub> varied from 79.5 to 92.7 which was significantly (p<0.05) correlated with various physical, physiological, biochemical and bio-molecular parameters

suggesting the major role of these variables on production traits of sheep.

**Body weight**: Initially, there was no significant difference between the groups, however, a gain of 20.9-23.0% in body weight was observed in supplemented animals (table 1) which may be attributed to change in feed intake (Pankaj *et al.*, 2013a; Venkata *et al.*, 2015) and supplementation of antioxidants enabled them to grow more even under adverse THI.

Physiological responses: All the animals under study were able to maintain their RR, PR and RT within physiological limits (table 1). A significantly (p<0.05) high rise in RR (min<sup>-1</sup>) was observed in control groups (12.9%) as compared to the supplemented groups (3.9 to 6.2%) which may be due to role of respiration in evaporation of moisture from respiratory tract, thereby preventing hypothermia under high ambient temperatures (Thompson, 1985). Similarly, a significantly (p<0.05) high PR (min<sup>-1</sup>) was observed in control groups (12.27%) as compared to supplemented groups (3.7 to 7.3%) which may be due to innate reflex of animals upon exposure to high environmental temperature. Thus, supplemented groups had better adaption towards adverse environmental stress (Pourouchottamane et al., 2013; Venkata et al., 2015) as compared to control.

Anti-oxidative enzymes: Serum Catalase level (kUl<sup>-1</sup>) increased with increase in THI, which was significantly (p<0.05) lower in control group than supplemented (table 1) with maximum in Se (63.0%), followed by Zn (50.8%) and Vit. E (44.0%) supplemented animals. Higher serum catalase activities indicate the higher rate of hydrogen peroxide formation (Cam et al., 2009; Kataria et al., 2010), an important marker to assess oxidative stress in animals. Lipid peroxidase (µM-1) level was decreased significantly (p<0.05) with increase in THI level (table 1), where maximal inhibition was observed in Se (30.4%), followed by Zn (16.5%) and Vit. E (15.4%) supplemented animals. These enzymes are used as a biomarker to show the index of oxidative stress (Kim et al., 2005) and a significant change in enzyme levels in the animals supplemented with the anti-oxidants confirmed the protective role of Zn, Se and Vit. E.

**Stress hormone**: Cortisol level (ngml<sup>-1</sup>) was significantly increased (p<0.01) with increase in THI level, (table 1) which was minimally changed in Se (68.5%) followed by Zn (80.5%) and Vit. E (81.4%) supplemented animals.

Significantly (P<0.05) higher Cortisol concentration in the hot months may be due to the elevation of glucocorticoids in response to the elevated ambient temperature, which results in better adaptation of the animal to the aberrant conditions (Silva *et al.*, 2003).

**Serum enzymes**: Alkaline phosphatase level (Ul<sup>-1</sup>) was significantly (p<0.05) decreased with increase in THI level (table 2), but such differences were absent in supplemented groups. Serum SGOT and SGPT levels (Ul<sup>-1</sup>) were significantly (p<0.05) increased with increase in THI level (table 2), however, this increase was significantly

(p<0.05) high in control group (33.9%) as compared to supplemented groups which may be due to the increase in stimulation of gluconeogenesis by corticoids (Habeeb, 1987).

**Serum nutrients**: Serum glucose level (mgdl<sup>-1</sup>) decreased significantly (p<0.05) with increase in THI, however control animals had least level of glucose (table 2). The utilization of glucose was significantly (p<0.05) more in control (35.7%) animals than supplemented (13.8 to 17.2%). Glucose concentration in blood was exhausted

**Table 1:** Levels (Mean±SE) of B.wt., physiological responses, anti-oxidative enzymes and stress hormone under different THI classes in various groups of Deccani female sheep (n=24)

|        |           |                      | Physiological responses |                              | Anti-oxidative enzymes       |                                  | Stress Hormone                          |                                |
|--------|-----------|----------------------|-------------------------|------------------------------|------------------------------|----------------------------------|---|--------------------------------|
| Groups | THI<br>gr | B. wt.<br>(kg)       | R.T.<br>(°F)            | R.R.<br>(min <sup>-1</sup> ) | P.R.<br>(min <sup>-1</sup> ) | Catalase<br>(kUl <sup>-1</sup> ) | Lipid peroxidase<br>(μM <sup>-1</sup> ) | Cortisol (ngml <sup>-1</sup> ) |
| C      | I         | 18.41±0.51a          | 103.17±0.15             | 32.18±0.87 <sup>b</sup>      | 92.28±4.38°                  | 17.78±1.48a                      | $2.38 \pm 0.08^{cd}$                    | 46.38±2.27a                    |
|        | II        | 21.34±0.59b          | 102.72±0.17             | 36.33±1.01°                  | $103.60\pm5.06^{d}$          | 23.95±1.70°                      | 2.24±0.09°                              | 94.89±2.62°                    |
| Τ,     | I         | 18.31±0.51a          | 102.74±0.15             | $25.71 \pm 0.87^a$           | 73.70±4.38a                  | 21.26±1.48b                      | 2.24±0.08°                              | 45.54±2.27a                    |
| 1      | II        | 22.17±0.59b          | 102.51±0.17             | 27.31±1.01a                  | 79.10±5.06 <sup>b</sup>      | 32.06±1.70e                      | 1.87±0.09b                              | 82.22±2.62b                    |
| Т,     | I         | $18.80 \pm 0.51^{a}$ | 102.57±0.15             | $25.89 \pm 0.87^a$           | 75.56±4.38a                  | $27.65\pm1.48^{d}$               | $2.04\pm0.08^{bc}$                      | 44.75±2.27a                    |
| 2      | II        | 23.13±0.59b          | 102.01±0.17             | 26.91±1.01a                  | 78.33±5.06 <sup>b</sup>      | $45.06\pm1.70^{\rm f}$           | 1.42±0.09a                              | 75.39±2.62b                    |
| Т,     | I         | 18.80±0.51ª          | 102.78±0.15             | $27.05 \pm 0.87^a$           | 77.19±4.38b                  | 21.98±1.48b                      | 2.28±0.08°                              | 44.25±2.27a                    |
| 3      | II        | 22.72±0.59b          | 102.24±0.17             | 25.44±1.01a                  | 73.69±5.06a                  | 31.67±1.70e                      | 1.93±0.09b                              | 80.28±2.62b                    |

<sup>&</sup>lt;sup>abcdef</sup>Different superscripts in a column vary significantly at 5% level of significance

Table 2: Levels (Mean±SE) of serum enzymes and serum nutrients under different THI classes in various groups of Deccani female sheep (n=24)

| Groups         | THI gr | Serum enzymes (Ul-1)  |                          |            |               | Serum nutrient     |                    |                           |                     |
|----------------|--------|-----------------------|--------------------------|------------|---------------|--------------------|--------------------|---------------------------|---------------------|
|                |        | Alk. Phos.            | SGOT                     | SGPT       | Glucose (mgdl | 1)Chol. (mmoll-1)  | Trigly. (mmoll-1   | ) TP (gdl <sup>-1</sup> ) | Albumin (gdl-1)     |
| C              | I      | 201.33±8.89b          | 85.21±2.98a              | 16.47±0.96 | a 66.15±2.12° | 59.93±2.18°        | 31.96±1.76d        | 7.70±0.32°                | 3.72±0.15°          |
|                | II     | $130.39 \pm 10.27^a$  | 114.11±3.44°             | 25.95±1.11 | c 42.51±2.44a | 37.63±2.52ª        | 15.39±2.03ª        | $5.33\pm0.36^{a}$         | $2.34 \pm 0.17^a$   |
| T <sub>1</sub> | I      | 198.72±8.89b          | $84.04\pm2.98^a$         | 14.09±0.96 | a 66.07±2.12° | 59.92±2.18°        | $32.86 \pm 1.76^d$ | 8.10±0.32°                | $3.85\pm0.15^{c}$   |
| •              | II     | $137.52\pm10.27^a$    | 108.19±3.44 <sup>b</sup> | 20.94±1.11 | b 54.68±2.44b | 47.06±2.52b        | $22.00\pm2.03^{b}$ | $6.67 \pm 0.36^{b}$       | $3.21 \pm 0.17^{b}$ |
| Τ,             | I      | $204.25\pm8.89^{b}$   | $83.78\pm2.98^a$         | 14.69±0.96 | a 66.35±2.12° | $61.50\pm2.18^{c}$ | $32.81 \pm 1.76^d$ | $8.08\pm0.32^{c}$         | $3.95\pm0.15^{c}$   |
| -              | II     | $140.44 \pm 10.27^a$  | 96.69±3.44 <sup>b</sup>  | 18.97±1.11 | b 57.22±2.44b | 51.39±2.52b        | 26.22±2.03°        | $6.89 \pm 0.36^{b}$       | $3.36 \pm 0.17^{b}$ |
| T,             | I      | $202.54 \pm 8.89^{b}$ | $85.36\pm2.98^a$         | 13.92±0.96 | a 66.58±2.12° | 59.63±2.18°        | $32.86 \pm 1.76^d$ | $8.05\pm0.32^{c}$         | $3.83\pm0.15^{c}$   |
| -              | II     | $135.19\pm10.27^a$    | 104.39±3.44 <sup>b</sup> | 18.62±1.11 | b 55.82±2.44b | $52.11\pm2.52^{b}$ | 25.39±2.03°        | $6.75\pm0.36^{b}$         | $3.13\pm0.17^{b}$   |

<sup>&</sup>lt;sup>abcd</sup>Different superscripts in a column vary significantly at 5% level of significance

Table 3: Levels (Mean±SE) of serum electrolytes and metabolite under different THI classes in various groups of Deccani female sheep (n=24)

| Groups         | THI gr | . Serum electrolytes      |                      |                           |                         |                         | Plasma metabolites (mgdl <sup>-1</sup> ) |                      |
|----------------|--------|---------------------------|----------------------|---------------------------|-------------------------|-------------------------|--|----------------------|
|                |        | Na (mmoll <sup>-1</sup> ) | K (mmoll-1)          | Cl (mmoll <sup>-1</sup> ) | P (mgdl <sup>-1</sup> ) | Ca (mgdl-1)             | Creatinine                               | Urea                 |
| C              | I      | 140.07±1.24a              | 4.13±0.06bc          | 93.34±0.94ª               | 7.95±0.32°              | 11.76±0.35 <sup>b</sup> | 0.96±0.06a                               | 34.89±1.46a          |
|                | II     | 145.26±1.44 <sup>b</sup>  | $3.36 \pm 0.06^a$    | $103.06 \pm 1.08^{c}$     | $5.49 \pm 0.37^{a}$     | 9.70±0.41a              | $1.54\pm0.06^{b}$                        | 47.67±1.68°          |
| T <sub>1</sub> | I      | $138.74 \pm 1.24^{a}$     | $4.13\pm0.06^{bc}$   | $93.23 \pm 0.94^a$        | $8.02 \pm 0.32^{c}$     | 11.99±0.35 <sup>b</sup> | $0.89 \pm 0.06^a$                        | $32.53 \pm 1.46^a$   |
| •              | II     | $138.35 \pm 1.44^a$       | $3.76 \pm 0.06^{b}$  | $97.99 \pm 1.08^{b}$      | $7.06 \pm 0.37^{b}$     | 9.96±0.41a              | $1.27 \pm 0.06^{b}$                      | $43.22 \pm 1.68^{b}$ |
| Τ,             | I      | $138.76\pm1.24^{a}$       | $4.13\pm0.06^{bc}$   | $93.41 \pm 0.94^a$        | $8.06\pm0.32^{c}$       | 11.97±0.35 <sup>b</sup> | $0.88 \pm 0.06^a$                        | $33.40{\pm}1.46^a$   |
| -              | II     | $138.04 \pm 1.44^a$       | $3.96 \pm 0.06^{b}$  | $95.55 \pm 1.08^{b}$      | $6.89 \pm 0.37^{b}$     | 10.98±0.41a             | $1.16\pm0.06^{b}$                        | $41.17 \pm 1.68^{b}$ |
| $T_3$          | I      | $139.44 \pm 1.24^a$       | $4.15\pm0.06^{bc}$   | $93.44 \pm 0.94^a$        | $7.99\pm0.32^{c}$       | 12.06±0.35 <sup>b</sup> | $0.90\pm0.06^{a}$                        | $33.54 \pm 1.46^a$   |
| ŭ              | II     | $138.18{\pm}1.44^a$       | $3.66{\pm}0.06^{ab}$ | $97.23 \pm 1.08^{b}$      | $6.93 \pm 0.37^{b}$     | $10.04 \pm 0.41^a$      | $1.25\pm0.06^{b}$                        | $42.83 \pm 1.68^{b}$ |

<sup>&</sup>lt;sup>abc</sup>Different superscripts in a column vary significantly at 5% level of significance

by hot climate due to decrease in insulin concentration and decrease in energy metabolism (Herbein *et al.*, 1985; Habeeb, 1987). Similar trend in other serum nutrients (cholesterol, triglycerides, total protein and albumin) were observed (table 2) due to decreased metabolism (Habeeb, 1987).

**Serum electrolytes**: Serum sodium (Na) level was increased (3.7%) in control groups, however, it remained unchanged in all the supplemented groups. Serum chloride (Cl) level was significantly (p<0.05) increased, whereas potassium (K), phosphorus (P) and calcium (Ca) level was significantly decreased with increasing levels of THI in all groups of Deccani female sheep (table 3). Changes in serum electrolyte levels may be due increased urinary excretion of these electrolytes and loss through sweat and panting (El-Nouty *et al.*, 1980).

**Plasma metabolites**: Serum creatinine and urea level was increased significantly (p<0.05) with increase in THI in all the groups of animals, however, this increase was more in control animals (60.4%) than the supplemented group (31.8 to 42.7%). This may be due to loss of fluids through panting and concentration of blood as a result of heat stress.

#### **CONCLUSION**

Temperature and humidity in the tropics considerably increases thermal load on the animal grazing during the summer months. The physiological responses of animals to environmental stress during peak summer and their blood nutrient and electrolyte balance showed that seasonal heat stress have profound effects on productivity of sheep. Antioxidant (Zn, Se and Vitamin E) supplementation in Deccani sheep helped in development of weather resilience as exhibited by favourable blood picture and achievement of higher body weight.

# **ACKNOWLEDGEMENT**

Special thanks to Dr. V.U.M. Rao, Project Coordinator, AICRPAM for providing weather data and Director, CRIDA for providing necessary facilities from NICRA budget.

# REFERENCES

Bernabucci, U., Bani, P., Roochi, B., Lacetera, N. and Nardone, A. (1999). Influence of short-term and long-term exposure to hot environment on rumen

- passage rate and diet digestibility by Friesian heifers. *J. Dairy Sci.*, 82: 967-973.
- Bray, T.M. and Bettger, W.J. (1990). The physiological role of zinc as an antioxidant. *Free Radic. Biol. Med.*, 8(3): 281-291.
- Cam, Y., Atalay O. and Atasever, A. (2009). Plasma malondialdehyde level and erythrocyte catalase activity in rams and ewes infected with sheep pox. *Indian Vet. J.*, 86:525-526.
- El-Nouty F.D., Elbanna I.M., Davis T.P. and Johnson H.D. (1980). Aldosterone and ADH response to heat and dehydration in cattle. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.*, 48: 249.
- Habeeb, A.A. (1987). The role of insulin in improving productivity of heat-stressed farm animals with different techniques. Ph.D. Thesis, Faculty of Agriculture, Zagazig University, Zagazig, Egypt.
- Herbein, J.H., Aiello, R.J., Eckler, L.I., Pearson, R.E. and Akers, R.M. (1985). Glucagon, insulin, growth hormone and glucose concentration in blood plasma of lactating dairy cows. *J. Dairy Sci.*, 68: 320-325.
- Kataria, N., Kataria, A.K., Maan, R. (2010). Evaluation of oxidative stress due to hot environmental condition in healthy Marwari goats from arid tract in India. *Philippine J. Vet. & Anim. Sci.*, 36(2): 175-184.
- Kim, S.F., Huri, D.A. and Snyder, S.H. (2005). Inducible nitric oxide synthase binds, S-nitrosylates and activates cyclooxygenase-2. *Sci.*, 310: 1966-1970.
- Mader, T.L., Davis, M.S. and Brown-Brandl, T. (2006). Environmental factors influencing heat stress in feedlot cattle. *J. Anim. Sci.*, 84: 712–719.
- Pankaj, P.K., Ramana, D.B.V., Nirmala, G., Dupdal, R., Nikhila, M., Rani, R. and Sudheer, D. (2014). Strategies for livestock management in rainfed agro-ecosystem. In National seminar on New dimensional approaches for livestock productivity and profitability enhancement under era of climate change, Anand, Gujarat, India.
- Pankaj, P.K., Ramana, D.B.V., Pourouchottamane, R. and Naskar, S.. (2013a). Livestock Management under Changing Climate Scenario in India. *World*

- J. Vet. Sci., 1: 25-32.
- Pankaj, P.K., Ramana, D.B.V., Rani, R., Nikhila, M., Sudheer, D. and Chowdary, K.P. (2013b). Thermotolerance of sheep in relation to coat color. *J. Agrometeorol.*, 15: 163-169.
- Pourouchottamane, R., Pankaj, P.K, Banik, S., Naskar, S., Venkatsubramanian, V. and Ramana, D.B.V. (2013). Effect of micro-environmental variations on biomolecular profile and performance of pig. *J. Agrometeorol.*, 15: 138-143.
- Ramana, D.B.V., Pankaj, P.K., Nikhila, M., Rani, R. and Sudheer, D. (2013). Productivity and physiological responses of sheep exposed to heat stress. *J. Agrometeorol*, 15: 71-76.
- Silva, R.G., La Scala Junior, N., Tonhati, H. (2003). Radiative properties of skin and hair coat of cattle

- and other animals. *Transactions of the ASAE*, 46: 913-918.
- Thompson, G.E. (1985). Respiratory system. *In*: Young, M.K. (Ed.), Stress Physiology in Livestock. CRC Press, Inc., Boca Raton, Horida, USA, pp.155-162.
- Tinggi, U. (2008). Selenium: its role as antioxidant in human health. *Env. Hlth. & Prev. Med.*, 13(2): 102-108.
- Venkata, R.N., Pankaj, P.K., Ramana, D.B.V., Rani, R., Nikhila, M. and Sudheer, D. (2015). Physiological responses to intensification in Deccani sheep. *Int. J. Pl. Anim. & Env. Sci.*, 5(1): 50-55.
- West, J.W. (1999). Nutritional strategies for managing the heat-stressed dairy cow. *J. Dairy Sci.*, 2: 21-35.