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Decrypting bio-molecular variability in small ruminants and antioxidants to combat bio-meteorological stress in semi-arid tropics

PRABHAT KUMAR PANKAJ*, D.B.V. RAMANA, G. NIRMALA, K. RAVI SHANKAR and CH. SRINIVASA RAO

ICAR-Central Research Institute for Dryland Agriculture, Santoshnagar, Saidabad, Hyderabad-500 059

**E-mail: dr.prabhatkumarpankaj@gmail.com*

ABSTRACT

Present investigation was conducted to unwind the effect of heat load index (HLI) and suitable nutrient supplement to combat the adverse effect using 24 Deccani females divided randomly into 4 groups (viz., Control (C), T_1 (3ml of 10% Zinc Sulphate), T_2 (1ml of 0.1% Selenium) and T_3 (60mg Vitamin E daily)) at Hayathnagar Research Farm of ICAR-Central Research Institute for Dryland Agriculture, Hyderabad, India. All environmental variables were recorded at 30 min intervals using automatic weather station and in sheep, physical, physiological, biochemical, hormonal and anti-oxidant profile was determined monthly. HLI was significantly correlated with various physiological, biochemical and bio-molecular parameters, however, catalase and cortisol may be considered as best HLI indicators in the sheep ($r=0.911^{**}$ and 0.864^{**}, respectively). Highly significant correlation between HLI and average daily weight gain ($r=0.817^{**}$), suggest that HLI significantly controls the growth rate thereby affecting final body weight.

Key words: Agro-meteorological variables, grazing sheep, heat load index, heat stress, thermo-tolerance, bio-meteorology.

Sheep rearing is one of the important livelihood activities for the farmers of arid and semi-arid regions of India, where most of the animals are reared under extensive or semi-intensive system and are exposed to severe ambient environmental conditions. Most of the research works for environmental studies in animal science are based on temperature-humidity index (THI) which considers only temperature and humidity, however, under grazing system, animals are exposed to other factors too like solar radiation, wind speed, etc. Thus, an innovative approach have been considered for this study by using Heat Load Index (HLI) which takes care of other environmental variables too (Gaughan *et al*., 2008, Al-Haidary *et al*., 2012 and Mengistu *et al*., 2017).

Deccani breed of sheep is spread over the greater part of the central peninsular region where seasonal productivity losses upto 25-30% in terms of body weight have been reported (Pankaj *et al*., 2013b; Ramana *et al.,* 2013). The dietary Selenium (Se) is important for reducing peroxidation (Tinggi, 2008). Vitamin E (Vit. E) is an important antioxidant (Etsuo *et al*., 1985). Zinc (Zn) is associated with enzymes stabilizing the structures of RNA, DNA, and ribosomes contributes to the antioxidant defence system (Bray and Bettger, 1990). Detailed study on effect of environmental stressors as well as nutritional supplementation of antioxidants to fight this peril has not been done under semi-arid climatic conditions. Thus, in the present study, an attempt have been made to understand the changes observed in Deccani female sheep under different environmental protocols (bio-meteorology) expressed in terms of HLI and suitable supplementation to deal with the losses observed during extreme weather conditions using anti-oxidants like Se, Zn and Vit.E.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at Hayathnagar Research Farm of ICAR-Central Research Institute for Dryland Agriculture, Hyderabad, India (17°27'N latitude and 78°35'E longitude and about 515m above sea level) between December 2014 to June 2015. The climate is semi-arid with hot summers and mild winters. The mean maximum air temperature ranges from 35.5 to 39.0°C, whereas in winter, it ranges from 14.0 to 17°C. The annual long term rainfall for the area is 746 mm.

Selection of animals

Twenty four Deccani females of 6-7 months age and

13.25±0.43kg body weight were divided randomly into four groups of similar overall body weight (viz., Control (C), T_1 (3ml of 10% Zn Sulphate daily), T_2 (1ml of 0.1% Se daily) and T_3 (60mg Vit. E daily)) and reared under semi-intensive conditions. All the animals were maintained as per standard management and feeding practices and let loose for grazing from 09:00 to 15:00 hrs during the study period. One kg of chopped Hybrid Napier (Co-4 variety) green fodder was offered daily alongwith *ad libidum* chopped sorghum stover. Apart from roughages, animals were provided with 250 gms of concentrate mixture formulated at farm by using 40% crushed maize, 35% wheat bran, 22% groundnut cake, 2% salt and 1% mineral mixture. Body weights of animals were recorded fortnightly for three consecutive days before feeding.

Recording of data

All the physiological parameters (respiratory rate in number per minute, pulse rate in number per minute, rectal temperature in ºC 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 (Creatinine, urea, calcium and phosphorus) and hormonal kits from IBL international company. In sheep, physical, physiological, biochemical, hormonal, enzymatic and anti-oxidant profiles were determined monthly using suitable techniques.

Development of HLI

Air temperature (T_a, °C), solar radiation (SR, Wm^{-2}), wind speed (WS, ms^{-1}) and relative humidity (RH, %) was used to calculate black globe temperature (BGT) following Hahn *et al*., (2009) and thereby heat load index (HLI) following Gaughan *et al.,* (2008) as give below;

BGT $= (1.33 \times T_a) - (2.65 \times (\text{sqrt}T_a)) + (3.21 \times \text{Log}(SR+1)) + 3.5$

Where, T_a is air temperature in ºC

Solar radiation (SR) recorded in $MJm^{-2}day^{-1}$ was converted to Wm^{-2} as follow

SR $(Wm^{-2}) = SR\ (MJ\ m^{-2}\ day^{-1})/0.0864$

Daily wind speed (WS) in kmph was converted to ms^{-1} as

WS $(ms^{-1}) = WS\ (kmph) \times 0.277778$

HLI was calculated from December 2014 to June 2015 using the following equation

HLI $= (8.62) + 0.38 \times RH + (1.55 \times BGT) - (0.5 \times WS) + e^{(-WS + 2.4)}$

Where e = the base of the natural logarithm (approximate value of e = 2.71828).

Total monthly sunshine in hours was calculated by adding the sunshine durations and was compared with HLI.

The statistical analysis was carried out using SAS 9.2 to find out mean±SE, Pearson correlation coefficient and statistical significance using general linear model and univariate options.

RESULTS AND DISCUSSION

The maximum and minimum values of heat load index (HLI_{max} and HLI_{min}) along with total sunshine duration for December 2014 to June 2015 presented in Table 1 showed that HLI_{max} increased from 79.54 in December 2014 to 92.69 in June 2015, while HLI_{min} varied between 54.66 (December 2014) and 68.0 (April 2015) with the average HLI between 67 and 80. Since the average HLI more than 75 is considered as the detrimental for growth and development, the most of period during April, May and June was stressful while the rest of the period (December to March) was comfortable (Table 1).

Body weight

The average daily gain (ADG) was very low in control group under severe LHI conditions (5 g per day), but when the sheep were supplemented with antioxidants, comfortable ADG was observed even during extreme LHI conditions (40, 36 and 38 g d^{-1} in T_1, T_2 and T_3, respectively) (Table 2) which may be attributed to change in feed intake (Pankaj *et al*., 2013a; Venkata *et al*., 2015). This suggests that the supplementation with Zn, Se and Vit. E can help the animals in acquiring better growth even under adverse HLI conditions.

Physiological responses

All the animals under study were able to maintain their rectal temperature within physiological limits. An increase in the ambient air temperature could be the reason for slight increase in rectal temperature which is most correlated trait (r=0.312) in the experimental sheep which was less pronounced in supplemented groups (Table 3). A significantly high rise in respiratory rate was observed in control group as compared to the supplemented groups with progressive increase in LHI with significant correlations (r=0.235). Increase in respiration rate in the present study could be due to heat stress as respiration helps in evaporation of moisture from the respiratory tract and prevention of hypothermia under high ambient temperatures.

Table1: Heat load Index (HLI) and total monthly sunshine during the experiment

Particulars	December 2014	January 2015	February 2015	March 2015	April 2015	May 2015	June 2015
HLI_{max}	79.54	80.47	84.21	87.56	92.36	90.98	92.69
HLI_{min}	54.66	58.8	60.02	64.73	68.0	67.55	65.49
Total sunshine (hrs)	156.3	145.6	184.6	218.8	207.6	241.0	165.7

Table 2: Body weight gain as affected by the treatments

Treatments	Body weight gain (kg animal^{-1})	Average daily gain (g day^{-1})
Control	5.95^{a}	5.0^{a}
T_1(Zinc sulfate)	10.92^{b}	40.0^{b}
T_2(Selenium)	10.50^{b}	36.0^{b}
T_3(Vit. E)	11.34^{b}	38.0^{b}

^{ab} Significant at the 0.01 level (2-tailed)

Table 3: Correlation of weight gain, physiological responses, serum anti-oxidative enzymes,stress hormone, electrolytes and metabolite levels with heat load index (HLI_{max})

Parameters	Correlation coefficients
Average daily gain (g d^{-1})	-0.817**
Physiological responses	
Rectal temperature (°C)	0.312**
Respiratory rate (min^{-1})	0.235**
Pulse rate (min^{-1})	0.294*
Anti-oxidative enzymes	
Catalase (kUl^{-1})	0.911**
Lipid peroxidase (µM)	-0.512**
Stress hormone, Cortisol (ngml^{-1})	0.864**
Serum electrolytes	
Sodium (mmoll^{-1})	-0.067
Potassium (mmoll^{-1})	-0.491**
Chlorine (mmoll^{-1})	-0.413**
Phosphorus (mgdl^{-1})	-0.284**
Calcium (mgdl^{-1})	-0.454**
Plasma metabolites (mgdl^{-1})	
Creatinine	0.512**
Urea	0.624**

**Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed).

Similarly a significant (r=0.294*) rise in pulse rate (min^{-1}) with increase in HLI indicates higher pulse rate under stress condition.Higher pulse rate in the month of May-June could be due to the exposure of the animals to high environmental temperature (Aboul-Naga, 1987). This increases blood flow from the core to the surface to give a chance for more heat to be lost by sensible (loss by conduction, convention and radiation) and insensible (loss by diffusion water from the skin) means from the animal.Physiological responses are immediate indication of body reflexes towards any stressor which suggested that supplemented groups had better adaption towards adverse environmental stress (Venkata *et al.*, 2015; Pourouchottamane *et al.*, 2013).

Anti-oxidative enzymes

Catalase level (kUl^{-1}) in the serum of all the experimental animals increased with increase in HLI levels (r=0.911**) (Table 3). Higher catalase activities showed activation of defense system, whereas, its lowered activities could have a negative impact on cellular resistance against the oxidant induced damage of the cell (Cam *et al.*, 2009). Thus, results suggest increased heat load tolerance in supplemented groups of animals.

Lipid peroxidase (µM) level decreased significantly (r=-0.512**) with increase in HLI level in all the supplemented groups, but this change was little in control group of animals. Significant decrease in LPO levels in the animals supplemented with the anti-oxidants confirmed the protective role of Zn, Se and Vit. E.

Stress hormone

Cortisol level (ngml^{-1}) significantly increased with increase in HLI level as evident by highly significant correlations (r=0.864**) (Table 3). Significantly higher cortisol concentration in the hot months may primarily 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 electrolytes

Serum sodium (Na) level decreased with increase in

HLI but it was non-significant. Serum potassium (r=-0.491), phosphorus (r=-0.284), calcium (r=-0.454) and chloride (r=-284) level significantly decreased with increasing levels of HLI in all groups of Deccani female sheep (Table 3). Decrease in electrolytes (Na, K, Cl, Ca, and P) levels with increase in atmospheric temperature may be due to increase in urinary excretion of these electrolytes and loss of electrolytes output in sweat and also through panting (EI Nouty *et al*., 1980).

Plasma metabolites

Serum creatinine level increased significantly (r=0.512**) with increase in HLI in all the groups of animals, however, this increase was quantitatively more in control animals than the supplemented group of Deccani females. Similar trend was observed in the level of serum urea which increased significantly (r=624**) with increase in HLI in all groups of animals. This could be due to loss of more fluids through panting, salivation, urination and concentration of blood as a result of heat stress.

CONCLUSION

Environmental factors like temperature, humidity, wind velocity, solar radiationin the tropics considerably increases thermal load on the animal grazing during the day as most of the time sheep graze in the open fields/paddock. The physiological responses of animals to environmental stress during peak summer and their nutrient balance, showed that seasonal heat stress have profound effects on blood biochemical, enzymatic, hormonal and bio-molecular parameters. Role of anti-oxidant in curbing heat stress was confirmed in the present context, by supplementation of Deccani sheep with Zn, Se and Vitamin E.Catalase and cortisol have been emerged as important bio-markers for expressing heat load in sheep.

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REFERENCES

Aboul-Naga, A.I. (1987). The role of aldosterone in improving productivity of heat-stressed farm animals with different techniques. Ph.D. thesis. Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

Al-Haidary A.A., Aljumaah R.S., Alshaikh M.A., Abdoun K.A., Samara E.M., Okah A.B., Aluraiji M.M. (2012). Thermoregulatory and physiological responses of Najdi sheep exposed to environmental heat load prevailing in Saudi Arabia. *Pak. Vet. J.,* 32:515-519.

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. *Ind. 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.

Etsuo Niki, S., Akira, K., Mitsuo, S., Yorihiro, Y., Tsuchiya, J. and Kamiy, Y. (1985). Effect of Phytyl Side Chain of Vitamin E on Its Antioxidant Activity. *J. Biol. Che.,* 260(4):2191-2196.

Gaughan, J.B., Mader, Terry L., Holt, S.M. and Lisle, A. (2008). A New Heat Load Index for Feedlot Cattle. *J. Anim. Sci.,* 86:226–234.

Hahn, G.L., Gaughan, J.B., Mader, T.L. and Eigenberg, R.A. (2009). Thermal indices and their applications for livestock environments In: J.A. DeShazer (ed.) Livestock energetics and thermal environmental management. American Society of Agricultural and Biological Engineers, St. Joseph, MI, USA. pp 113-130.

Mengistu, U.L., Puchala, R., Sahlu, T., Gipson, T.A., Dawson, L.J. and Goetsch, A.L. (2017). Conditions to evaluate differences among individual sheep and goats in resilience to high heat load index. *Small Rum. Res.,* 147:89-95.

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., Rita, R., Nikhila, M., Sudheer, D. and Chowdary, K.P. (2013b). Thermotolerance of sheep in relation to coat color. *J. Agrometeorol.,* 15 (Special Issue-II):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(Special Issue-I):138-143.

Ramana, D.B.V., Pankaj, P.K., Nikhila, M., Rita, R. and Sudheer, D. (2013). Productivity and physiological responses of sheep exposed to heat stress. *J. Agrometeorol.,* 15(Special Issue-I):71-76.

Silva, R.G., La Scala Junior, N. and Tonhati, H. (2003). Radiative properties of skin and hair coat of cattle and other animals. *Trans ASAE.,* 46:913-918.

Tinggi, U. (2008). Selenium: its role as antioxidant in human health. *Env. Hlth. Prev. Med.,* 13(2):102-108.

Venkata Raju, 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. Plant Anim. & Env. Sci.,* 5(1):50-55.

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