

*Original Research***Effect of Melatonin on Blood Electrolytes, Biochemical Profiles and SOD Activity of Beetal and Assam Hill Goat Exposed to Direct Sunshine during Summer in Assam****Dilruba Hasin^{1*}, Arundhati Bora², Jitendra Goswami³, Abdul Saleque⁴, Anubha Baruah⁵, Isfaqul Hussain⁶, Birendra Kr. Sarmah⁷ and Ranjit Kr. Bora⁸**¹Division of Veterinary Physiology, FVSc & A.H, SKUAST-K, Shuhama, Srinagar-190006, J&K, INDIA^{2,3,5&7}Department of Veterinary Physiology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-22, Assam, INDIA⁴Goat Research Station, AAU, Burnihat, Assam, INDIA⁶Division of Veterinary Microbiology & Immunology, SKUAST-K, Shuhama, Srinagar-190006, J&K, INDIA⁸Department of Livestock Production and Management, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-22, Assam, INDIA***Corresponding author: dhasin7@gmail.com**

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Abstract

The present study was carried out to evaluate the heat-stress ameliorating effect of melatonin. A total of 18 animals (5-6 months old) of each healthy Beetal and Assam Hill Goat (AHG) was divided into three groups containing 6 animals in each. One group was kept indoor (T-1), while other two groups (T-2 and T-3) of each breed were exposed to direct sunshine @6 hours/day for 30 days. Melatonin was fed @6 mg/animal to one of the two exposed groups (T-3). Blood samples were collected at weekly interval before and after exposure. Blood Na⁺ and K⁺ decreased significantly ($P<0.01$) in all the exposed groups and their level were significantly lower ($P<0.01$) in T-2 group than in T-3 group. The Na⁺ and K⁺ level were significantly higher in all the groups of AHG than Beetal goats. The superoxide dismutase activity decreased in all the exposed groups, but the decrease was only significant ($P<0.01$) in the T-2 group. The SOD activity were significantly ($P<0.01$) higher in AHG than the Beetal. Serum total protein and albumin decreased significantly ($P<0.01$), but their level was significantly ($P<0.01$) higher in T-3 group of Beetal goat. In AHG, total protein was significantly higher in T-3 group, but no significant ($P>0.05$) difference was observed for albumin. The blood glucose decreased insignificantly after the exposure in both the breeds, but it was significantly ($P<0.01$) higher in the T-3 group of both the breeds. Melatonin plays a positive role in the different blood electrolytes, blood biochemicals and anti-oxidative enzyme activity in AHG and Beetal along with stress ameliorating effects and AHG are more adapted to heat stress due to solar radiation under agro-climatic conditions of Assam.

Key words: Assam Hill Goat, Beetal, Biochemicals, Electrolytes, Direct Sunshine, Melatonin, SOD



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Introduction

Goat plays an important role in the economy of livestock owners who earn their livelihood by rearing them in different terrain and climatic condition. They are primarily raised on grazing resources under extensive management system in different parts of the country. India, being a tropical country has wide range of climatic conditions, and the animals such as goat, which graze and browse for many hours a day, is regularly exposed to extreme weather conditions like wide variation in temperature, humidity, rainfall etc. In Assam being the sub-tropical region of the country, they are regularly exposed to hot and humid weather conditions. This causes extreme stresses to animals leading to various physiological, biochemical, blood electrolytes changes as well as alteration in the enzymatic activity and lead to decreased growth and loss of production.

Biochemical homeostasis plays an important role in the attempt of animals to counteract heat stress. Blood biochemistry is not only the important marker for the general health and basic metabolic pattern of an animal but also throw light on the adaptive modification of different climatic conditions. Moreover, heat stress stimulates production of free radicals and reactive oxygen species detrimental to the animals (Tanaka *et al.*, 2008). Melatonin, a hormone secreted by the pineal gland act as a direct free radical scavenger and as an antioxidant indirectly. It increases the levels of antioxidant and inhibits the pro-oxidative enzymes, having effects on physiological rhythms, reproduction and immune system (Tan *et al.*, 2000). It helps in reduction of cortisol secretion and mineral excretion under stress and during thermal stress, the level of melatonin increases significantly (Sejian and Srivastava, 2010).

In Assam, goats are primarily reared under extensive system and it becomes necessary to find novel ways to counteract the adverse effect of solar radiation. Therefore, the present investigation was undertaken with the objective-to evaluate the effect of melatonin on blood electrolytes, biochemical profiles and SOD activity in newly introduced Beetal goats in Assam with reference to native breed of goat i.e., Assam hill goat (AHG) under direct sunshine exposure during peak summer for their adaptability with the climate of Assam.

Materials and Methods

Experimental Design

Thirty six healthy goats consisting of 18 Beetal goats and 18 AHG of 5-6 months old, which were maintained in the Goat Research Station, Burnihat, CVSc, AAU, Guwahati-22, was selected. Each breed of goat were subdivided into three groups (T-1, T-2 and T-3) containing 6 animals in each. One group was



kept indoor (T-1) as control while other two groups of each breed were exposed to direct sunshine for a period of six hours from 8:00 hrs to 14:00 hrs in a fenced open paddock. Melatonin (Meloset Tablet 3mg, Aristo Pharmaceuticals Pvt. Ltd., Mumbai) was fed @ 6 mg/goat/day (Daramola *et al.*, 2007) to one of the two sunshine exposed groups (T-3) before the exposure. The study was conducted for a period of 30 days in the month of June considering the peak summer heat. The animals were maintained under standard feeding and managemental system. The experiment was duly approved by the Institutional Animal Ethics Committee. Blood samples with and without anticoagulant were collected from the animals before and after the exposure at weekly interval. The serum was separated and used immediately or stored at -20°C for further use. Serum glucose, total protein and albumin were estimated in “Vatscan” (Abaxis inc., Union City, CA94587, USA). Blood sodium, potassium and chloride were estimated using RAPIDLab^(R) 348 Blood Gas Analyzer (Siemens, USA). The superoxide dismutase (SOD) was measured using SOD assay kit (Cayman Chemical Company, USA) with the help of an ELISA reader (Dynamica, Microplate Readers, Australia).

Temperature -Humidity Index (THI)

Temperature-Humidity Index (THI) was calculated using the data as per Mader *et al.* (2006) with the following formula-

$$THI = (0.8 \times Tdb) + [(RH/100) \times (Tdb - 14.4)] + 46.4$$

Statistical Analysis

All the data were analysed with the help of SAS Software Release 9.3. The difference between treatments were analysed using a two way analysis of variance (ANOVA). Difference with a confidence level of 0.05 was considered to be significant.

Result and Discussion

Temperature Humidity Index

The month wise mean THI ranged from 81.46 in May to 83.44 in August with an overall mean of 83.0 during summer. The THI has been widely used as an indicator of thermal stress in livestock (Vaidya *et al.*, 2010). The value of 70 or less are considered comfortable, 75-78 stressful and values greater than 78 cause extreme distress. Therefore, in the present study the calculated value of THI in summer was found to be very stressful for the animals.

Blood Electrolytes

The Na⁺ concentrations in pre- and post-sunshine-exposed Beetal goats (T-2) were 143.38 ± 0.26 and 133.66 ± 0.88 mE./L and in AHG, they were 147.27 ± 0.37 and 134.26 ± 0.91mEq/L, respectively (Table 1).

Table 1: Blood sodium, potassium concentration (mEq/L, Mean ± SE) of Beetal and AHG in different treatment groups at pre- and post-exposure period and blood chloride concentration (mEq/L, Mean ± SE) of Beetal and AHG.

Group	Blood Sodium (mEq/L, Mean ± SE)				Blood Potassium (mEq/L, Mean ± SE)				Blood Chloride (mEq/L, Mean ± SE)	
	Beetal		AHG		Beetal		AHG		Beetal	AHG
	Pre-Exposure	Post exposure	Pre-Exposure	Post-Exposure	Pre-Exposure	Post-Exposure	Pre-Exposure	Post-exposure		
T-1	143.48 ^A ± 0.27	143.01 ^A ± 0.30	147.40 ^A ± 0.21	146.84 ^{Ab} ± 0.26	4.17 ^A ± 0.09	4.05 ^A ^b ± 0.13	4.26 ^A ± 0.07	4.23 ^A ^b ± 0.13	105.42 ^B ± 0.12	106.36 ^A ± 0.10
T-2	143.38 ^A ± 0.26	133.66 ^B ^c ± 0.88	147.27 ^A ± 0.37	134.26 ^B ^c ± 0.91	4.01 ^B ± 0.10	2.43 ^A ^c ± 0.06	4.10 ^B ± 0.09	2.65 ^A ^c ± 0.15	105.56 ^B ± 0.13	106.70 ^A ± 0.11
T-3	143.17 ^A ± 0.26	138.44 ^B ^b ± 0.96	147.24 ^A ± 0.37	140.10 ^B ^a ± 0.73	4.09 ^B ± 0.12	3.10 ^A ± 0.12	4.18 ^B ± 0.16	3.30 ^A ± 0.07	105.67 ^B ± 0.13	106.76 ^A ± 0.11

Mean values with different superscripts in a column and subscripts in a row differ significantly

The corresponding values for melatonin fed group (T-3) were 143.17 ± 0.26 and 138.44 ± 0.96 mEq/L in Beetal and 147.24 ± 0.37 and 140.10 ± 0.73 mEq/L in AHG, respectively. Though the level was significantly lower in all the exposed groups, the post-exposure Na⁺ concentration was also significantly (P<0.01) lower in T-2 group than the T-3 group of both the breeds. Similar results were also observed in the K⁺ concentrations in both the breed of goats as presented in the Table 1. The Cl⁻ concentration in T-1, T-2 and T-3 groups were found to be 103.80 ± 0.17, 103.69 ± 0.16 and 104.09 ± 0.17 mEq/L in Beetal and in AHG they were 104.17 ± 0.13, 104.01 ± 0.13 and 104.43 ± 0.13 mEq/L, respectively (Table 1). The Cl⁻ concentrations were higher in all the treatment groups in AHG than that of Beetal, but they did not differ significantly (P>0.05). The present findings, corroborates, with the recent findings of Hooda and Upadhyay (2014), who recorded significant decrease in blood Na⁺, K⁺ and Cl⁻ concentrations in Alpine x Beetal cross male kids upon exposure to thermal stress in a climatic chamber along with feed restriction. They also recorded higher level of cortisol in this group of animals. However, they did not find any difference in Na⁺, K⁺ and Cl⁻ including cortisol concentrations in the group of thermal stressed kids without any feed restriction. El-Khalek *et al.* (2002) found decreased plasma Na⁺ and K⁺ concentrations in crossbred goats (Damascus x Baladi) under Egyptian conditions when they were exposed to direct solar radiation for 3 hrs during the hottest month of summer season. On the contrary, Abdel-Fattah (2014) found significant increase in plasma Na⁺ concentration after 4-days of heat stress in two breeds of goats namely, Balady and Damascus goats in Egypt.

This difference may be attributed to variation in the sweating rates in different breed. In the present study, melatonin fed groups of goat (T-3) had significantly higher level blood Na⁺ and K⁺ at post-exposure indicating role of melatonin in preventing the depletion of these electrolytes, thereby minimized the loss of electrolytes balances in the animal body. Melatonin may do this by inhibiting responsiveness of adrenal glands to ACTH leading to reduced cortisol secretion (Torres-Farfan *et al.*, 2003). Furthermore, we found significantly higher level of serum Na⁺, K⁺ and Cl⁻ concentrations in AHG in comparison to Beetal goats,

but there were no any significant differences in the rate of decreases of the electrolyte concentrations (data not shown). As such, no breed differences were recorded in terms of loss of electrolytes due to heat stress, although their normal concentrations varied between the two breeds during summer.

Serum Super Oxide Dismutase (SOD) activity

The activity of serum SOD in pre and post-exposed Beetal goats (T-2) were 1.81 ± 0.01 , and 1.72 ± 0.01 U/ml and in AHG, they were 1.88 ± 0.01 and 1.80 ± 0.01 U/ml, respectively (Table 2). The corresponding activity in the T-3 group were 1.83 ± 0.01 and 1.77 ± 0.01 U/ml in Beetal and 1.88 ± 0.01 and 1.85 ± 0.01 U/ml in AHG, respectively. The post-exposure activity was significantly ($P < 0.01$) lower in the T-2 group in both the breed of goats. Although the post-exposure activity of SOD were lower in T-3 group, but did not differ significantly ($P > 0.05$) from pre-exposure activity in both the breed of goats.

Table 2: Serum SOD activity (U/mL, Mean \pm SE) of Beetal and AHG in different treatment groups at pre- and post-exposure period

Group	Serum SOD activity (U/ml, Mean \pm SE)			
	Beetal		AHG	
	Pre-exposure	Post-exposure	Pre-exposure	Post-exposure
T-1	$1.85_A^a \pm 0.01$	$1.83_A^b \pm 0.01$	$1.93_A^a \pm 0.01$	$1.92_A^b \pm 0.01$
T-2	$1.81_A^a \pm 0.01$	$1.72_B^a \pm 0.01$	$1.88_A^a \pm 0.01$	$1.80_B^a \pm 0.01$
T-3	$1.83 \pm 0.01_A^a$	$1.77_A^{ab} \pm 0.01$	$1.88_A^a \pm 0.01$	$1.85_A^{ab} \pm 0.01$

Mean values with different superscripts in a column and subscripts in a row differ significantly

In the present investigation, serum SOD activity were significantly low in the post exposure T-2 group in comparison T-3 and these observation suggested that the sunshine or solar radiation caused significant oxidative stress in T-2 groups of goats of Beetal as well as AHG. In addition, melatonin had a significant role in ameliorating the oxidative stress due to solar radiation as the post-exposure activity of SOD in T-3 did not differ significantly from the pre-exposure values. There are no reports on the SOD activity in serum after exposure to sunshine to goat. However, our observations were in consonance with Kumar *et al* (2010), who found decreased activity of serum in heat-stressed buffaloes. Furthermore, Al-Badwi *et al* (2013) reported significant decrease in serum SOD activity in Aardi goats exposed to transportation-stress. They suggested that the decrease activity was due to increase consumption of SOD to overcome the oxidative stress. Furthermore, the serum SOD activity was found to be significantly higher in all the treatment groups of AHG in comparison to Beetal goats during summer. These findings might indicate that AHG are more adapted to oxidative stress due to solar radiation.

Blood Biochemicals

The serum total protein concentrations in pre and post-exposed Beetal (T-2) were 5.17 ± 0.05 g/dl and 4.73 ± 0.08 g/dl and in AHG they were 5.40 ± 0.06 and 5.00 ± 0.07 , respectively (Table 3).

Table 3: Serum total protein and albumin concentration (gm/dl, Mean \pm SE) of Beetal and AHG in different treatment groups at pre- and post-exposure period

Group	Serum total protein(gm/dl, Mean \pm SE)				Serum albumin (gm/dl, Mean \pm SE)			
	Beetal		AHG		Beetal		AHG	
	Pre-exposure	Post-exposure	Pre-exposure	Post-exposure	Pre-exposure	Post-exposure	Pre-exposure	Post-exposure
T-1	5.38 ^A \pm 0.05	5.26 ^A \pm 0.08	5.59 ^A \pm 0.06	5.54 ^A \pm 0.07	2.73 ^A \pm 0.05	2.68 ^A \pm 0.07	2.93 ^A \pm 0.08	2.85 ^A \pm 0.06
T-2	5.17 ^B \pm 0.05	4.73 ^A \pm 0.08	5.40 ^A \pm 0.06	5.00 ^B \pm 0.07	2.70 ^A \pm 0.05	2.44 \pm 0.08 ^B	2.82 ^B \pm 0.08	2.55 ^A \pm 0.06
T-3	5.31 ^B \pm 0.07	4.93 ^A \pm 0.12	5.45 ^A \pm 0.08	5.20 ^B \pm 0.14	2.72 ^A \pm 0.07	2.50 ^B \pm 0.09	2.84 ^B \pm 0.10	2.64 ^A \pm 0.08

Mean values with different superscripts in a column and subscripts in a row differ significantly

Corresponding values in the melatonin fed group (T-3) were 5.31 ± 0.07 g/dl and 4.93 ± 0.12 g/dl in Beetal and 5.45 ± 0.08 and 5.20 ± 0.14 in AHG, respectively. The post-exposure value was significantly ($P < 0.01$) lower in both the groups in both the breed of goats. The post-exposure value of T-2 group was also significantly ($P < 0.01$) lower than the post-exposure value of T-3 group in both the breed of goats. Similar results were also observed in the serum albumin concentrations as depicted in Table 3. But the post-exposure albumin concentrations in both T-2 and T-3 groups did not vary significantly ($P > 0.05$).

Serum proteins play an important role in different types of stress in animals and serum total proteins and albumin concentrations have been used as physiological markers of stress in animals (Aggarwal and Singh, 2010). In the present study, serum total protein and albumin were found to decrease significantly ($P < 0.01$) in T-2 groups of both the breeds. The reduced feed intake in heat-stressed animals might lead to decrease in nitrogen concentration in the body, which led to low protein concentration (Sejian, 2013). Mohamad (2012) also observed significantly lower total protein and albumin in desert rams consuming low amount of Lucerne hay. The decrease in plasma protein could also be due to decrease in protein synthesis as a result of decrease in anabolic hormone secretion (El-Masry and Habeeb, 1989) or due to protein catabolism to divert amino acids towards gluconeogenesis (Sejian *et al.*, 2010). The lower level response could also be associated with an increase in water consumption and development of haemodilution (Bernabucci *et al.*, 2010). Habeeb (1987) reported that serum proteins, particularly, albumin concentration usually decrease in heat exposed cattle.

On the other hand, Sejian *et al.* (2010) recorded significant increase of serum total protein when Marwari goats were exposed to 40°C and 60% relative humidity for four hrs in a psychrometric chamber for a period of 10 consecutive days. Kumar *et al.* (2012) recorded high level of plasma total protein (7.4 ± 0.33 g/dl) in summer stressed Beetal goats as compared to pre-summer values (6.7 ± 0.33 g/dl). However, the total protein, albumin and globulin were found to decrease from 6.56 ± 0.19 , 2.53 ± 0.08 , 4.03 ± 0.17 to 5.88 ± 0.21 , 2.08 ± 0.04 , 3.80 ± 0.21 g/dl, respectively in Balady goats subjected to short term heat stress for two days (Helal *et al.*, 2010). Recently, Hooda and Upadhyay (2014) documented decrease in total protein from 5.93 ± 0.13 to 5.32 ± 0.08 gm/dl, albumin from 3.07 ± 0.09 to 2.62 ± 0.09 gm/dl and globulin 2.86 ± 0.08

to 2.50 ± 0.10 gm/dl in Alpine x Beetle cross kids when they were exposed to high temperature with feed restriction as mentioned above. In the present investigation, the serum proteins also decreased in T-3 group, however, the decrease was less in comparison to T-2 group suggesting role of melatonin in relieving the stress due to direct sunshine through its various effect on metabolic pathways.

Blood Glucose

The glucose concentration in pre and post-exposure Beetal goats (T-2) were 53.63 ± 0.05 mg/dl and 52.29 ± 0.31 mg/dl and in AHG they were 55.82 ± 0.48 mg/dl and 54.55 ± 0.48 mg/dl, respectively (Table 4). The corresponding values in the melatonin fed group (T-3) were 53.80 ± 0.30 mg/dl and 54.80 ± 0.33 mg/dl in Beetal and 55.98 ± 0.50 mg/dl and 57.02 ± 0.54 mg/dl in AHG respectively. Although, post-exposure glucose level was lower in T-2 than the T-3 group in Beetal, but it differed significantly ($P < 0.01$) only in the melatonin fed group (T-3).

Table 4: Blood glucose concentration (mg/dl, Mean \pm SE) of Beetal and AHG in different treatment groups at pre and post exposure period during summer

Group	Blood glucose (mg/dl, Mean \pm SE)			
	Beetal		AHG	
	Pre-exposure	Post-exposure	Pre-exposure	Post-exposure
T-1	$53.46 \text{ A}^a \pm 0.30$	$52.96 \text{ A}^b \pm 0.36$	$55.64 \text{ A}^a \pm 0.48$	$55.14 \text{ A}^b \pm 0.4$
T-2	$53.63 \text{ A}^a \pm 0.05$	$52.29 \text{ A}^b \pm 0.31$	$55.82 \text{ A}^a \pm 0.48$	$54.55 \text{ A}^b \pm 0.48$
T-3	$53.80 \text{ A}^a \pm 0.30$	$54.80 \text{ A}^a \pm 0.33$	$55.98 \text{ A}^a \pm 0.50$	$57.02 \text{ A}^a \pm 0.54$

Mean values with different superscripts in a column and subscripts in a row differ significantly

In AHG, though the post-exposure glucose level was lower in T-2 than the T-3 group, but it did not differ significantly ($P > 0.05$). In the present study, there was little variation in blood glucose concentrations after exposure to direct sunshine in both T-2 and T-3 groups of goats of both the breed. Our findings corroborates with the observations of Sano *et al.* (1985) and Soufiane *et al.* (2012), who reported that heat-stressed (HS) lactating goats were able to maintain similar blood glucose levels to thermo neutral (TN) animals with no change in blood insulin concentration.

There are conflicting reports about the level of blood glucose in hot climatic conditions i.e. under thermal stress in cattle, some researchers reported decrease blood glucose (Marai *et al.*, 1995), while others reported increased level of blood glucose (Webster, 1976). They speculated that increase in plasma glucose during hot condition may be due to the decrease in the glucose utilization, depression of both catabolic and anabolic enzyme secretions and subsequent reduction of metabolic rate. Similarly, Hassanin *et al.* (1996) found increased blood glucose in Egyptian Zaraibi goats under heat stress. But, Kumar *et al.* (2012) found significantly lower level of glucose (46.15 ± 1.51 g/dl) in summer-stressed Beetal goats as compared to pre-summer values (63.60 ± 2.14 g/dl). Recently, Hooda and Upadhyay (2014) found significant decrease of blood glucose from 56.83 ± 1.26 to 47.35 ± 1.63 mg/dl along with other bio-chemicals in Alpine x Beetle

cross kids when they were exposed to high temperature of 40-44°C along with feed restriction. However, the values of the above bio-chemicals did not differ significantly in the heat treated group without feed restriction although the values decreased as compared to before exposure values. On the other hand, we observed marginal increase of blood glucose level in the T-3 groups of goats of both the breed. This may be due to direct action of melatonin on hepatocytes to modulate the blood glucose level (Poon *et al.*, 2001). Furthermore, Ocak and Guey (2010) reported that blood glucose level decreases during summer season in goats, which was similar to our present findings.

Conclusion

The study reveals how melatonin plays a positive role in the different blood electrolytes, blood biochemicals and anti-oxidative enzyme activity in AHG and Beetal. At the same time, it also demonstrates the stress ameliorating effects of the melatonin and AHG are more adapted to heat stress due to solar radiation under agro-climatic conditions of Assam.

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