Heat tolerance in goat- A genetic update

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Abstract
Livestock undergo various kinds of stress such as physical, nutritional, chemical, psychological and thermal stress. Among them thermal stress is most concerning now a days in the ever changing climatic scenario. High ambient temperatures, high direct and indirect solar radiations, and humidity are environmental stressing factors, according to the climatic changes. Thermal stress redistributes the body resources including protein and energy at the cost of decreased growth, reproduction, production and health. Goats (Capra hircus) are relatively resistant to harsh environmental conditions. Thermal stress stimulates sort of complex responses which are fundamentals in the preservation of cell survival. Physiological responses to thermal stress are change in rectal temperature, respiration rate, heart rate and skin temperature. Heat tolerance in goat is controlled by heat tolerance genes such as HSP32, HSP40, HSP60, HSP70, HSP90, HSP90AB1, HSP110, CRP, VEGF, cNOS, iNOS etc. At molecular level changes in gene expression of proteins ensure protections. Altogether these physiological, biochemical and molecular responses make the goats to survive in harsh environment.

Keywords: Gene, Goat, Stress, Temperature, Response

Introduction
Due to climatic variations and global warming, threats are become major to affect the sustainability of livestock production system. Climate change poses formidable challenge to the development of the livestock sector [1]. International panel for climate change (IPCC) indicated an increase in temperature by 0.2°C per decade and predicted that the surface temperature of the earth may increase between 1.8 °C to 4°C by the end of this century [2]. Stress has been conceived as a reflex reaction that occurs ineluctably when animals are exposed to adverse environmental conditions and which is the cause behind many unfavorable consequences, ranging from discomfort to death of the animal [3]. The rise in temperature along with increased precipitation resulting from climate change is likely to aggravate the heat stress in animals affecting their productive and reproductive performance [4,5]. Ruminants do not maintain strict homeothermy under stress despite having well developed mechanism of thermoregulation. The environmental stressing factors that improve strain on animals are high ambient temperature, solar radiation and humidity [6]. High environmental temperature challenges animals’ ability to maintain energy, thermal, water, hormonal and mineral balance [7].

Stress is reaction of the body to stimuli that disturb homeostasis often with detrimental effects. Among all the stress factors, thermal stress is most concerning now a days in the ever changing climatic scenario. In tropical and sub tropical regions, high ambient temperature is the major constraint on animal production, whereas extreme low temperature in temperate regions is also detrimental to livestock. Thermal stress includes both heat stress, during extreme summer season as well as cold stress, during extreme winter season. High environmental temperature is the major concern in tropical and arid areas whereas at the same time very low environmental temperature in temperate areas is also lethal. Temperature determines metabolic rates, heart rates and other important factors within the bodies of animals, so an extreme temperature change can easily distress the animal body. The effect of high temperature is further aggravated when heat stress is accompanied by high ambient humidity.

Since 9000 BC, goat is the oldest domesticated species of the Indian subcontinent [8]. Globally goat plays an important role in the economy of thousands poor livestock owners who earn their livelihood by rearing them in different terrain and climatic conditions. Goat rearing is a traditional occupation of small, marginal farmers and landless laborers in semiarid, arid and...
hilly and mountain regions of developing countries, inhospitable to conventional crop cultivation. Goat farming has several advantages over the husbandry of other livestock species. Goat is known as the poor man’s cow because of its nourishing milk and as the principal meat producing animal in India [9]. Perhaps, it is the only livestock that fits well for effective utilization in the diverse socio-economic situations of the developing countries. Goats perform better in combating adverse climatic conditions than its domestic counterparts [10]. This ability to combat climatic change is multifactoral [11]. Low body mass and small body size, low metabolic requirements, ability to reduce metabolism, digestive efficiency in relation to feeding strategies, efficiency of utilization of high fiber forage, ability to economize the nitrogen requirement and efficient use of water are the various attributes of goats that aid in surviving in adverse climatic conditions. Although goats are resistant to thermal stress at a greater extent but they suffer from heat and cold stress beyond their comfort zone, which is environmental temperature 13-27 °C for Indian goats. Browsing of goats to open fields during most of the day hours makes them susceptible to environmental stress. The purpose of the present review is to provide an integrative explanation of thermoregulatory responses of goat at cellular and molecular level during thermal stress.

Measurement of heat stress
THI: Estimating the severity of heat stress in livestock and poultry is being formulated using both ambient temperature and relative humidity, termed as the temperature–humidity index (THI) [12]. THI can be used as a tool for formulation of thermal comfort zone for dairy goats [13]. When temperature is measured (°F), the equation to determine THI is as follows [12]:

\[ \text{THI} = db \cdot F - \left\{ (0.55 - 0.55 \text{ RH}) \cdot (db \cdot F - 58) \right\}, \]

where \(db \cdot F\) is the dry bulb temperature and \(\text{RH}\) is the relative humidity (RH%/100, for goats. The obtained values indicate the following: values <82 = absence of heat stress; 82 to <84 = moderate heat stress; 84 to <86 = severe heat stress and over 86 = extreme severe heat stress [12].

When temperature is expressed in °C, the equation changes as follows [14]:

\[ \text{THI} = 0.8 \times \text{AT} + \left( \text{RH} \% / 100 \right) \times \left\{ (\text{AT} - 14.4) + 46.4 \right\} \]

Where AT= is the air temperature (°C) and RH is the relative humidity (RH%).

GTHI: The Globe Temperature and Humidity Index (GTHI) is a physical index used for characterization of thermal comfort rate for the most diverse animal species. Higher value of the index indicates thermal discomfort. This condition results in imbalance in the thermo neutral zone of the animals [14].

\[ \text{GTHI} = \text{TBG} + 0.36 \times \text{Tdp}-330 \]

TBG = thermometer temperature of black globe (°C); TDP = temperature of the dew point (°C); 330 = constant.

Response to heat stress in goats
Thermal stress includes both heat stress during extreme summer and cold stress in extreme winter [15]. These changes demand adaptation of animals to harsh environmental conditions [16]. Response to heat stress in goats can be broadly classified into physiological, biochemical and molecular responses (Figure 1).

![Fig 1. Physiological, biochemical and molecular responses to heat stress in goats.](image)

**Physiological responses**
Stress is the common factor for physiological and hematological alterations in the body [17]. The exposure of Aradi goat to heat stress resulted in the significant increase in
temperature (coat, skin, vaginal and rectal), heart rate and respiration rate. However, a significant decrease in T3 and T4 concentration and significant increase in cortisol level in serum was observed. Similar results were also recorded due to heat stress on behavior and physiological parameters in Osmanabadi goats. The study identified significantly higher respiration rate and pulse rate, decreased rumination, lying time, frequency of urination and frequency of defecation. Blood parameters such as RBC count, WBC count, PCV% and Hemoglobin% were significantly higher in severe heat stressed Black Bengal goats. The effect of thermal stress also increases ESR as recorded in Toggenberg and Beetal goats. Increased quantity of red blood cells hemoglobin concentration during heat stress could be a result of reduced oxygen tension leading to an increased production of erythropoietin as an adaptive mechanism to low oxygen level. Coat coloration is a qualitative trait and has a role in heat tolerance. Long haired goats tolerate radiant heat better than short haired goats and that white or light brown goats do better than dark brown or black goats. Highest mean value of heat stress index was recorded in black coat coloured goats as compared to their white, brown and grey coloured counterparts. This could be as a result of the absorption of solar radiation by the dark pigmentation there by making the animal to be thermally stressed. The effect of coat pigmentation and Wattle genes on some hematological characteristics of heat stressed African dwarf goats concluded that coat pigmentation is an important determinant of blood characteristics under high environmental temperature which may be due to high heat absorbance rate of coat pigmentation. Similar results were found in West African dwarf goats in which significantly higher rectal temperature and respiration rate, non significantly higher pulse rate, decreased body weight and daily weight gain was recorded. The decrease in body weight and daily weight gain may be due lower feed intake in high temperature and humidity. Adverse effect of heat stress also includes reduced libido, reduced sperm output and sperm motility in males and lower fertility, conception rate in females with lower embryonic survival. Genetic studies on reproductive performance of indigenous goats recorded a higher age of sexual maturity, age at first calving and kidding interval in summer season as compared to winter season. The birth weight of kids born in summer season was significantly lower than those born on other months in Tellicherry goats. Sex, reproductive status and foetal number exert significant effects on some physiological parameters in goats during hot dry season. Change in behavioral responses like defecation, urination, standing time, lying time, shade seeking behavior, water intake and drinking frequency were observed during heat stress. Exposure to heat stress affects neuroendocrine response by inducing secretion of adrenalin, noradrenalin, thyroxine, corticosteroids and renin.

Biochemical responses
Heat stress affects normal biochemical parameters and affect overall metabolism in goats. Liver function is altered due to variation in the ambient temperature affecting goats of both the sexes in various age groups. An increased mean value of serum urea and creatinine in Marwadi goats in ambient temperature was reported. Higher serum creatinine in heat stress could be due to higher metabolic activity in liver and muscles. Increased serum urea, creatinine, potassium, plasma lactate concentration, aspartate aminotransferase and lactate dehydrogenase activity was recorded in Saanen goat kids with naturally occurring heat stroke. However, a significant decrease in serum total proteins, albumin, glucose, urea and creatinine levels in female non-pregnant adult Zarabi goats was recorded. Change in anti oxidant levels, plasma or serum enzymes and metabolites like blood glucose and total cholesterol levels was also found. Plasma lactate concentration seems to be a reliable indicator for the prognosis of heat stroke in goat kids. A significantly higher levels of plasma Na+ concentration and no change or decreased plasma K+ concentration after heat stress in Balady and Damascus breeds of goats exposed to heat stress in desert of Sinai was recorded. However, a significant increase in serum K+ and decrease in the serum Ca2+ in dwarf goats exposed to heat stress was recorded. The study also observed the elevation of serum glucose which could be due to the stress induced activation of cortical secretion and the stimulation of gluconeogenesis. The increase of electrolyte concentration in the body fluid exposed to heat will reduce their thermoregulatory evaporation. There is significant decrease in plasma vitamin E, vitamin C and total plasma antioxidant activity during heat stress in goats. A study carried out on Beetal and Toggenberg breeds of goats concluded that there is a significant higher values of serum AST (Aspartate aminotransferase) and ALP (Alkaline phosphatase) and lower values of serum ALT (Alanine aminotransferase) during thermal stress in summer season as compared to winter season. Blood non esterified fatty acids did not change in heat stressed goats. It was further reported that heat stress exerts some important changes in the metabolic functions, inflammatory status and productivity in dairy goats. Dairy goats under heat stress produce a 3-10% decrease in milk yield with reduced fat, protein and lactose content.

Molecular response: Response to heat stress can be attributed by various changes in the gene expression. A list of various genes that may aid in heat tolerance in goats along with chromosome number and number of exons and base pairs are listed. Some of these genes are affected by a wide variety of different stressors and probably represents a non specific cellular response to stress, others may eventually found to be specific to certain types of cells. The genes control response to heat stress either by altering heat production and loss balance or by exerting cytoprotective actions. Approximately there are fifty different genes that change expression during thermal stress.

- HSF- Heat Shock Factor 1, IL-6 – Interleukin 6, MAPK 14-Mitogen Activated Protein Kinase 14, NOS 1- Nitric Oxide Synthase 1, NOS 2- Nitric Oxide Synthase 2, NOS 3- Nitric Oxide Synthase 3, UCP 3- Uncoupling Protein 3, VEGF- Vascular Endothelium Growth Factor, CRP- C Reactive Protein, ATP1A1 – Na+/K+ transporting subunit alpha A1, ATP1B1 – Na+/K+ transporting subunit beta1, HSP90AA1 (Heat Shock Protein, 90kDa alpha, class A member 1) and HSP90AB1 (Heat Shock protein, 90kDa alpha, class B member 1) and HSP90B1 (Heat Shock protein, 90kDa beta, member 1), BI-1 (Bax Inhibitor-1)
Table 1: Genes expressed in response to heat stress

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Gene</th>
<th>Chromosomal Position</th>
<th>No. of exons</th>
<th>Base pairs</th>
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<td>14</td>
<td>18670</td>
</tr>
<tr>
<td>2</td>
<td>IL-6</td>
<td>04/05</td>
<td>3884</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>MAPK 14</td>
<td>23/12/13</td>
<td>72969</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>NOS 1</td>
<td>17/30</td>
<td>191160</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>NOS 2</td>
<td>19/27</td>
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<td></td>
</tr>
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<td>6</td>
<td>NOS 3</td>
<td>04/26</td>
<td>18449</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>VEGF</td>
<td>23/6/8</td>
<td>16070</td>
<td></td>
</tr>
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<td>02/12</td>
<td>17837</td>
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</table>

NOS: Nitric oxide is a gaseous lipophilic free radical. It has three isoforms such as NOS type 1 or neuronal (nNOS), NOS type 2 or inducible (iNOS) and NOS type 3 or endothelial (eNOS) [42]. Nitric oxide regulates vascular hemostasis, hematopoiesis and peripheral immune response. Even in a small concentration, physiological and cellular activities are affected. Nitric oxide is required for full expression of active vasodilatation of the skin during hyperthermia. The effects of thermal stress on mRNA and protein expression of iNOS, eNOS and cNOS of goats in vivo during winter, moderate and summer season revealed that there is a higher relative mRNA expression of iNOS, eNOS and cNOS during summer [43]. The interaction between nitric oxide and HSPs plays an important role in the adaptive enhancement of resistance to thermal stress. iNOS protects the heart from myocardial ischaemia occurring due to heat stress [44].

Leptins and interleukins: Leptins are a kind of circulating adipocytokines whose concentration increases rapidly when the animal is exposed to hot environment [45]. The studies showed that expression of leptin mRNA is higher during winter season as compared to summer season [46]. The leptin is a pleiotropic hormone in caprine and changed leptin expressions in peripheral blood mononuclear cells (PBMCs) in vivo may represent an adaptive mechanism to environmental temperatures in goats suffering from severe thermal stress [46]. Under stress conditions Hypothalamus-Pituitary Axis gets stimulated which stimulates the production of catecholamine and glucocorticoids. Cytokine production and modulation of stress occur due to production of interleukins. Interleukins can act locally and systemically. Their action locally is to modulate cellular immune response. Systemically they change behavior, metabolism and neuroendocrine secretion. IL-6 is considered as the primary mediator of metabolic response to inflammation by inducing production of a broad array of acute phase proteins. IL-1 and IL-6 inherently link muscle protein degradation with production of hepatic acute phase proteins as a response to inflammatory stimuli [47]. Production of these proinflammatory cytokines directly affects bone growth and found to modulate the mechanism of proteins, fats and carbohydrates [48].

Heat Shock Proteins (HSP): HSPs are evolutionary conserved family of proteins induced in response to biological stress in living cells [49]. HSPs form a primary system for intracellular cell defence [50,51]. Cellular tolerance to heat stress is regulated by the HSPs and their synthesis at cellular level is temperature dependant. HSPs are released in response to environmental and oxidative stress. Production of HSPs activates proteins that block apoptosis. The principal HSPs range in molecular mass from 15 to 110 kDa and are divided into groups based on both size and function [52]. HSPs family consists of many proteins of which HSP 110, HSP 100, HSP 90, HSP 70, HSP 60, HSP 40, HSP 10 and other smaller families [53] are extensively studied in thermal stress. A significantly higher expression of HSPs was recorded in goats during summer season as compared to winter season in tropical regions [54]. HSPs act as molecular chaperons and prevent abnormal protein folding and aggregation [55]. They take part in the assembly of proteins without being a part of the final structure of the protein. This enhances the ability of
the cell to sustain injury, oxidative stress and enhance the tolerance of the cell towards high temperature thus bringing in thermo stability with the cells [56]. Expression of HSPs is regulated by heat shock transcription factor (HSF1) and they have a short term and quick effect on the process of the heat stress of cell survival mechanism [57].

i. HSP 70- Restriction Fragment Length Polymorphism (RFLP) analysis in goats revealed the location of HSP 70 genes with Major Histocompatibility Complex Class I [58]. The goat HSP 70-1 cDNA encoding HSP 70 protein of 641 amino acids residues was found highly conserved among the domestic animals [59]. In a study among four different breeds of goats Jakhrana, Barbari, Jamunapari and Sirohi, it was found that Sirohi goats exhibited higher HSP mRNA level expression than the other breeds and it can be inferred that Sirohi breeds have higher heat tolerant activity as compared to others [60]. In a similar study, to assess the impact of heat and nutritional stress simultaneously on the adaptive capability in Osmanabadi goats, higher expression of HSP 70 mRNA in PBMCs (Peripheral Blood Mononuclear Cells) was recorded as indicated by behavioural and physiological response [61]. Therefore, plasma HSP 70, PBMC HSP 70 and adrenal HSP 70 gene expression may act as an ideal biological marker for assessing the impact of heat stress in goats [61, 62].

ii. HSP 90 - HSP 90 family consists of HSP90AA1 (Heat Shock protein, 90kDa alpha, class A member 1) and HSP90AB1 (Heat Shock protein, 90kDa alpha, class B member 1), HSP90B1 (Heat Shock protein, 90kDa beta, member 1) and Trap1 (TNF receptor associated protein 1) [63]. HSP90AB1 and HSP90AA1 are localized in cytoplasm. Higher induction of HSP90AA1 occurs as compared to HSP90AB1 [64]. The first level of regulation is during gene transcription in which HSF1 (Heat Shock Factor 1) binds to HSEs (Heat Shock Elements). HSEs are thought be the major transcription factors for HSPs. Along with HSP70, HSP90 proteins are also predominantly anti-apoptotic [65]. Since HSP90s are involved in stabilization and transport of all types of proteins so its contributory role in the stress modulation in the goats cannot be ignored.

Bax Inhibitor 1: Bax Inhibitor 1 is an evolutionary conserved endoplasmic reticulum protein and is a common cell death suppressor in eukaryotes [66]. BI-1 mediates stress induced apoptosis by linking to endoplasmic reticulum stress signaling. Unfavorable environmental conditions, nutrients and ATP deprivation disrupts the endoplasmic reticulum functioning inducing stress [67] and lead to the accumulation of unfolded proteins, which are detected by transmembrane sensors initiating the unfolded protein response (UPR) to restore ER proteostasis [68]. The UPR is a signaling cascade enabling the cell to adapt different stresses, if prolonged, this will result in Ca²⁺ release from the ER promoting apoptosis and pro apoptotic ER signaling [67]. During stress normal cells upregulate the UPR resulting in adaptation [68]. BI-1 over expression has a protective effect on ER stress by inhibiting the reactive oxygen species accumulation [69]. BI-1 regulates the ER stress by modulating three tansmembrane proteins viz. ATF 6 (Activating Transcription Factor 6), PERK (PKR like ER kinase) and IRE 1 (inositol-requiring-1) [70].

ATPase family: The ATP1A1 and ATP1B2 proteins are preferentially expressed under restrictive conditions due to environmental stress [71] and is equally sensitive to oxidative stress [72]. Na⁺/K⁺ATPase is a member of ATPase family, a group of integral transmembrane carrier proteins [73]. This membrane protein consists of a large catalytic subunit (alpha), a smaller glycoprotein subunit (beta) as two major polypeptides [74] and a gamma subunit [75]. The beta subunit is essential for ion recognition and maintenance of membrane integrity. The sensitivity of Na⁺/K⁺ATPase to oxidative stress may imply some correlation with heat stress [76]. Na⁺/K⁺ATPase protein maintains the electrochemical gradient of Na⁺ and K⁺ ions across the cytomembrane. This electrochemical gradient provides energy for the membrane transport of metabolites, nutrients and ions [77]. A significant alteration in the Na⁺/K⁺ATPase activity was observed when animals are subjected to thermal stress [78]. Therefore, Na⁺/K⁺ATPase enzyme represents a plausible candidate for heat tolerance traits [79].

C-Reactive protein (CRP): CRP is regarded as a positive acute phage reactant in mammals and birds [48] and their expression are affected by heat stress [41]. CRPs increase in blood in response to inflammation and tissue damage [80]. CRP can be used as a useful marker for assessing the stress levels in a dairy herd [81]. CRP levels can increase quickly and dramatically during inflammation [82] and is also believed to play an important role in innate immunity, as an early defense system against infections. CRP activates complement system, bind Fc receptors [83] and can function as an opsonin [84], enhancing phagocytosis with certain infections.

Vascular endothelial growth factor (VEGF): VEGF is a glycoprotein that has mitogenic activity for endothelial cells [85] and also induces vascular permeability [86]. Expression of VEGF is affected by heat stress [41]. VEGF induces the proliferation, differentiation, and migration of vascular endothelial cells, increases capillary permeability, and enhances endothelial cell survival by preventing apoptosis [87]. VEGF plays a significant role in embryo vasculogenesis and inactivation of a single VEGF results in embryonic lethality [88]. Exposure to environmental heat stress early in placental development could impair normal placental vascular development due to alterations of VEGF expression during the period of maximal placental growth resulting in placental insufficiency [89]. There is a relationship between VEGF and hypoxia-inducible factor-1 alpha dependent angiogenesis during the periovulatory periods in which hypoxia during ovulation is crucial for establishing the thecal new vasculature [90].

Mitogen activated protein kinase (MAPK): MAPK is also known as Extracellular signal-Regulated Kinase is a member of MAPK subfamily called Stress Activated Protein Kinase (SAPKs) that regulate the transcriptional response to various environmental stress [91]. Cell cycle progression and cell survival or death responses are regulated by MAPK signaling network following a variety of stresses [92]. The translocation of components of the MAPK cascades into the nucleus plays a role in the regulation of a variety of nuclear processes that are essential for the induction of many stimulated cellular functions like proliferation, differentiation, apoptosis and stress response [93]. Heat shock also triggers MAPK activation and MKP-1 induction in Leydig testicular cells [94].

Strategies to combat heat stress
Strategies to combat heat stress in goats can be done by
modification of the environment, nutritional management as a short term measures and genetic selection of better adaptive goats as parents for future generations as a long term measure. The provision of the shade (both natural and artificial) is the simple and cost effective methods to minimize the heat from solar radiation [95,96]. Modification of the microenvironment in the shed can be done to enhance heat dissipation mechanisms by cooling systems that couple evaporative cooling with tunnel ventilation and sprinklers [97, 98]. In extreme heat, grazing time must be decreased and animals may be left for grazing in the early morning and evening hours. Animals suspected for heat stress should be moved to cool place with shed area and ample air circulation. Heat stress causes reduction of dry matter intake (DMI) and nutrient utilization in animals [100]. So, more nutrients are needed to be consumed into smaller volume of feed. Feeding high concentrate diets during hot periods not only results greater consumption but also reduce heat production inside the ruminant body. Antioxidants both enzymatic and non-enzymatic can be supplemented to the diet as heat stress causes oxidative damage and also enhance immunity [101]. Long term strategies include genetic selection. Improvement of adaptability by simultaneous heterosis and crossbreeding is a better option in the hand of goat breeders for producing better offspring capable of withstanding heat stress [102]. Genetic variability for the response to climatic change can be used to select the most tolerant and robust animal to cope up with future climatic changes [103].

Conclusion
The maintenance of thermoneutrality is an important aspect in efficient productivity of the goats. In Indian conditions goats are mainly reared for chevon production. As a result, for genetic improvement in the meat production it is essential to increase their feed intake with greater feed utilization. This higher feed intake often results with higher metabolic heat increment which requires highly efficient thermoregulatory mechanism to maintain physiological and biochemical homeostasis. This high metabolic heat increment accompanied by varied adaptive responses results in heat stress. Varied physiological, biochemical and molecular responses hinders the measurement of the exact magnitude of the heat stress level in goats. Heat stress not only alters energy metabolism but also by behavioral responses by sweating, altered urination and defecation, enhanced water intake and drinking frequency creates an imbalance in the water and mineral (Na⁺, K⁺, Cl⁻) metabolism. The present scenario demands formulation of strategies to enhance adaptability of goats to heat stress for enabling them to express their full genetic potential.

Exposure to high temperature is a major constraint in the productivity of the goats. This condition is more prominent in tropical and subtropical areas which receive direct sun rays. High ambient temperature accompanied with high humidity results in decrease in feed efficiency and utilization, imbalanced energy homeostasis, hormones secretions and production of blood metabolites etc. This causes direct and indirect losses to the livestock owners as it reduces chevon quality, impairs milk production and reproductive performance. A better understanding of the adaptations of the goats to heat stress is necessary to develop suitable strategies. It is the peak time for the new strategies to be formulated for enhancing the heat tolerance capacity of goats without compromising with the productivity. As per the authors a comprehensive research in this area is the need of the time.

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