



Strategies for Reducing the Effect of Heat Stress on Buffalo Bull Reproduction

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Recent data from the Department of Animal Husbandry, Dairying & Fisheries, Government of India (2019-2020) reveals that India boasts a staggering 109.8 million buffaloes, constituting approximately 57.7 percent of the global buffalo population. Moreover, nearly 45% of the nation's milk production is attributed to buffaloes. However, despite their significant contribution, efforts to maximize buffalo productivity face obstacles due to ongoing climate change-induced heat stress. Climate change, acknowledged universally, poses a critical challenge. Heat stress adversely impacts the genetic potential, management practices, and environmental conditions affecting bull reproductive performance. Buffaloes are especially susceptible to heat stress due to inherent limitations such as fewer sweat glands and darker skin pigmentation. Therefore, scientific management strategies are essential to optimize production efficiency in the face of heat stress.

Heat stress arises from exposure to high temperatures and humidity, disrupting oxidative glucose metabolism and mitochondrial function, and triggering excessive reactive oxygen species (ROS) formation in sperm cells, leading to heightened lipid peroxidation and abnormalities in spermatozoa. In India, particularly during the summer, temperatures range from 30°C to 46°C, with annual rainfall averaging between 760 to 960 mm, peaking in July and August, and relative humidity ranging from 45% to 99% (Bhakat *et al.*, 2015). Assessment of bull fertility typically involves metrics like conception rate (CR) achieved through insemination with viable spermatozoa in females. However, reports indicate that heat stress significantly impairs spermatozoa production in buffalo bulls. Hence, this article underscores the importance of employing various management techniques and semen additives as strategies to mitigate the adverse effects of heat stress.

Reproductive Biology of Buffalo

Buffaloes are recognized as seasonal breeders, exhibiting greater reproductive efficiency during the winter and autumn seasons compared to other times of the year. However, inherent biological limitations constrain the breeding capacity of buffalo bulls relative to cattle bulls. These limitations include smaller testicular size, lower sperm output rates, and reduced epididymal reserves. Additionally, buffalo spermatozoa are more vulnerable to heat stress (HS) and oxidative damage due to their inadequate cytoplasmic antioxidant granule content and high levels of polyunsaturated fatty acids in their membrane. These factors are attributed to the lower cholesterol levels and larger head size of buffalo spermatozoa compared to those of other domestic animals (Kumar *et al.*, 2019).

Temperature humidity Index (THI)

The Temperature-Humidity Index (THI) serves as a prominent indicator of heat stress in animals, particularly those raised in tropical and subtropical climates. Environmental factors,

notably temperature and humidity, significantly influence both the quality and quantity of sperm in animals. Optimal conditions for spermatogenesis typically require air temperatures ranging from 13-18°C and relative humidity levels of 55-65%, along with moderate levels of sunshine (see Table 1). Disruptions in spermatogenesis can occur due to elevated testicular temperatures resulting from increased environmental temperatures, elevated body temperatures, or a combination of both factors (Sharma *et al.*, 2017). Various environmental elements, including temperature, relative humidity (RH), solar radiation, air movement, and precipitation, collectively contribute to heat stress. Temperature and relative humidity are key components utilized in THI models to gauge the extent of heat stress on an animal's reproductive functions.

Table 1: Classification of zone based on THI value (Arunpandian *et al.*, 2021)

S.No.	THI (%)	Stress level	Symptoms in buffalo
1	<72	None	Normal in production
2	72-78	Mild	Respiratory and rectal temperature increased
3	79-88	Moderate	Water intake increased, food intake decreased, and rate of respiration significantly increased
4	89-98	Severe	Panting and restlessness Ruminantion and urination decreased
5	>98	Danger	Buffalo may die

Strategies for overcoming heat stress

Heat stress in animals poses a significant challenge to production, leading to substantial economic losses for farmers. Fortunately, there exist several methods to mitigate this heat stress effectively. These include modifying the animal's environment physically, developing heat-tolerant breeds through genetic means, managing nutrition effectively, and utilizing semen additives to mitigate damage retroactively. The effectiveness of management measures to alleviate heat stress can vary depending on environmental conditions. For instance, evaporative cooling is found to be more effective in mitigating heat stress in dry conditions compared to humid ones (Bohmanova *et al.*, 2007).

Environmental changes: Common methods for adjusting environmental temperatures typically involve the installation of shade structures and evaporative cooling systems. Shades, which mitigate heat stress by blocking solar radiation, can directly or indirectly alleviate heat stress. It's recommended that shade structures in corrals be constructed between 3.6 and 4.2 meters high to effectively reduce solar radiation. Studies have shown that shading can decrease the incoming radiant heat load by 30% or more, benefiting animal productivity by providing shade for feed and water (Slimen *et al.*, 2014). Cooling systems, on the other hand, help reduce heat stress by utilizing water misting and forced ventilation. These systems, which often incorporate sprayers and fans, are commonly installed in free-stall barns or beneath shade structures in open-area corrals. Through fast evaporation, fine water droplets are dispersed into the air stream, cooling the surrounding environment in fogging and misting systems. Combining dairy fans with mist cooling and implementing rubber mat flooring can be effective strategies for mitigating heat stress in buffalo bulls (Chikkagoudara *et al.*, 2020).

Selection of genetically heat-tolerant breeds: Selecting heat-resistant animals can be beneficial if the chosen breeds can maintain their productivity and survivability when exposed to stressful summer conditions. Cattle with longer hair and darker skin color may be less suited to hot environments, whereas those with thicker hair diameter, shorter hair, and lighter coat color are better adapted to high temperatures (Bernabucci *et al.*, 2010). Heat shock protein (HSP) genes have been identified as potential markers for selection due to their association with thermo-tolerance. Various breeds possess heat-resistant genes, such as

HEAT STRESSF1, HEAT STRESSP70 A1, and HEAT STRESSBP1 in Chinese Holstein cattle and HEAT STRESSP90AB1 in Thai native cattle (Deb *et al.*, 2014). However, before considering HSP as a marker for thermo-tolerance traits, further experiments are needed to fully understand their implications.

Management in terms of Nutrition: In arid and semi-arid regions, where feed resources are limited in both quantity and quality, environmental temperatures tend to be at their highest. This unfavorable condition negatively impacts the reproductive success of livestock. Managing the metabolic effects of heat stress becomes crucial as animals experiencing mild to severe heat stress require an additional 7–25% maintenance requirement. Supplementation of selenium and vitamin E plays a vital role in mitigating the adverse effects of free radicals due to their antioxidant properties. Studies have shown that vitamin E injection positively affects semen quality in bulls under testicular heat stress conditions (Losano *et al.*, 2018). Furthermore, supplementing feed with fatty acids has been found to improve the percentage of progressive motility in semen. This improvement is attributed to the potential role of fatty acids in meeting energy requirements during hot and humid conditions.

Semen extender additive addition: Heat stress disrupts the normal functioning of the reproductive system, affecting various semen parameters such as volume, motility, concentration, and the presence of abnormal spermatozoa, ultimately leading to infertility. Elevated testicular temperatures during summer increase metabolic rates and oxygen requirements. If this heightened metabolism is not accompanied by increased blood flow, the testicular tissue becomes hypoxic, resulting in excessive production of reactive oxygen species (ROS) and lipid peroxidation. Methods to reduce ROS can be categorized into two approaches: neutralization of ROS and reduction in ROS production. Neutralization methods involve the use of antioxidants, reductants, and hydrogen (H₂) gas to counteract ROS. Alternatively, strategies to reduce ROS production focus on minimizing oxygen tension, purifying semen to remove immature or damaged spermatozoa, and reducing mechanical stressors, white blood cell counts, and radiation exposure. Techniques such as Glass wool Sephadex (GSW), magnetic bead separation, filtration, Ficoll wash, and nanoparticle separation are utilized for semen purification (Bisla *et al.*, 2022). Specific scavengers are also employed to target ROS production.

Antioxidants supplementation: Antioxidants play a crucial role in neutralizing reactive oxygen species (ROS) and are commonly utilized for their scavenging properties, which help suppress the formation of ROS. Elevated levels of dead and abnormal spermatozoa, often observed during high Temperature and Humidity Index (THI) conditions, contribute significantly to oxidative stress and serve as a primary source of ROS. Antioxidants function by disrupting the oxidative chain, thereby reducing oxidative stress. They can be categorized into two main groups: enzymatic antioxidants and non-enzymatic antioxidants. Enzymatic antioxidants include superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and glutathione reductase (GR). SOD is particularly effective in mitigating spontaneous oxygen toxicity and lipid peroxidation (LPO).

Non-enzymatic antioxidants encompass various compounds that play critical roles in protecting cells against oxidative damage. Vitamin E, for instance, is a potent chain-breaking lipophilic antioxidant present in cell membranes. It acts by disrupting covalent bonds formed by reactive oxygen species between fatty acid side chains in membrane lipids. This function makes it a vital protective agent against lipid peroxidation and oxidative stress, without interfering with the formation of reactive oxygen species. Both *in vivo* and *in vitro* studies have demonstrated that alpha-tocopherol, a form of vitamin E, effectively shields cells from oxidative damage. Another important non-enzymatic antioxidant is glutathione peroxidase (GSH-Px), which converts hydrogen peroxide into water and lipo-peroxides into alkyl alcohols by utilizing glutathione (GSH). Additionally, glutathione reductase (GSR), whose

activity is induced by oxidative stress, helps regenerate GSH from its oxidized state (GSSG). Vitamin C, on the other hand, is a water-soluble antioxidant with potent chain-breaking properties and minimal toxicity. It scavenges oxygen radicals and reduces lipid peroxidation by neutralizing the effects of hydrogen peroxide (H₂O₂) on DNA (Srivastava and Pande, 2017). Furthermore, vitamin C plays a crucial role in recycling inactive vitamin E, thereby enhancing its antioxidant function.

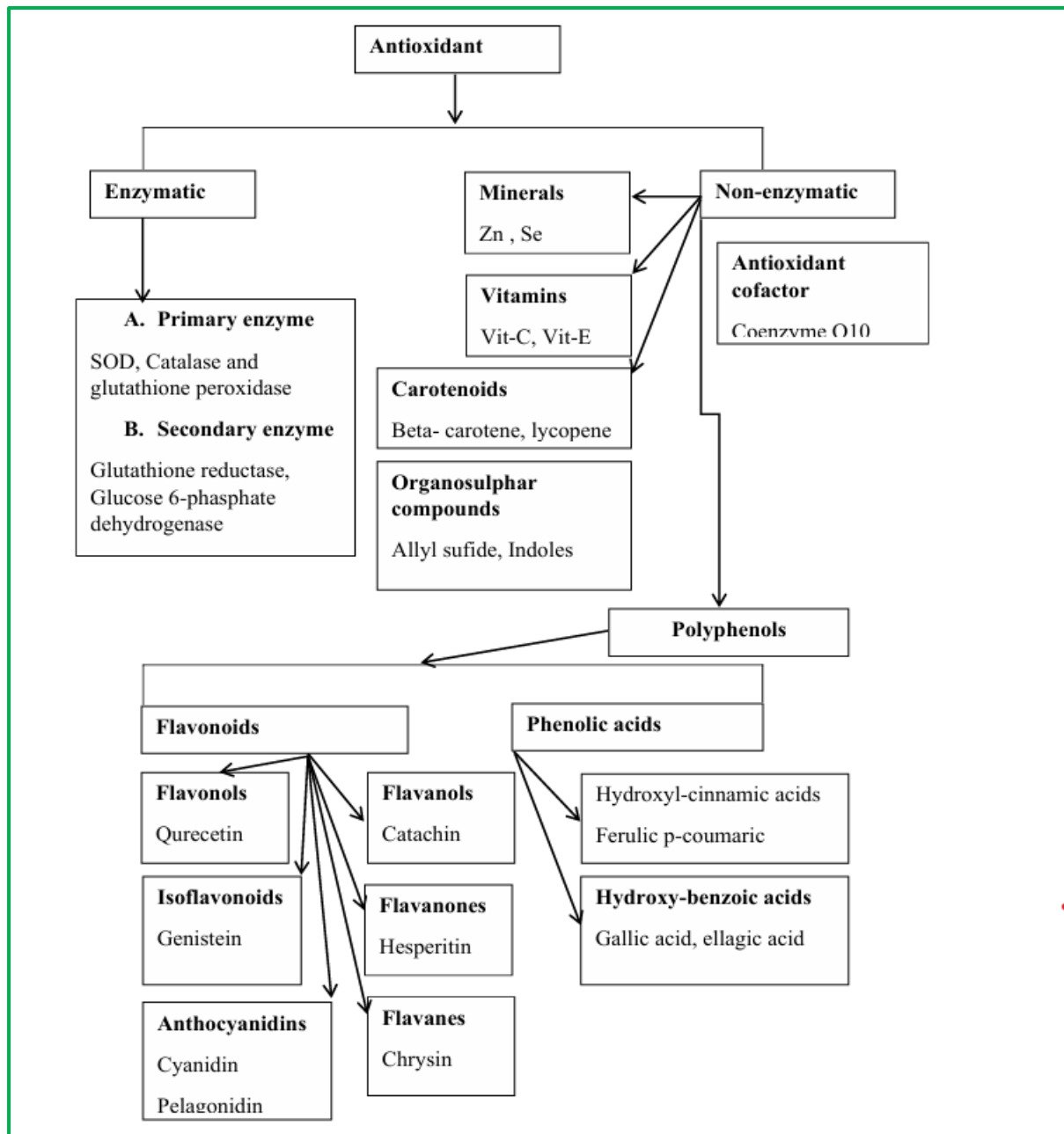


Fig 1: Classification of antioxidants used as additives in semen (Arunpandian et al., 2021)

Conclusion

Livestock are significantly affected by heat stress resulting from climate changes, such as rising temperatures and relative humidity. These environmental factors have a profound impact on both production and reproduction in animals. The article provides a summary of common strategies employed to mitigate heat stress in bulls. These strategies include management practices, nutritional adjustments, and the supplementation of antioxidants to alleviate the effects of heat stress.

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