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Original Article

Biomarkers of heat stress in livestock

Dr. Roshna K[1]., Dr. Anandu S[2*] and Gunturu Narasimha Tanuj[3]

[1]MVSc. Scholar, Division of Biochemistry, College of Veterinary and Animal Sciences-KVASU, Mannuthy, Kerala; [2] Ph.D. Scholar, Division of Parasitology; [3]Ph. D Scholar, Division of Animal Biotechnology, ICAR-Indian Veterinary Research Institute, Bareilly, U.P*

***Corresponding author**: drananduivri@gmail.com*

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Abstract
The increase in environmental temperature due to global warming in recent years has created an adverse effect on the health status of all living organisms especially domestic animals. There is a reduced productivity due to the decrease in feed intake, immunity and growth rate. These factors due to stress results in the change in blood constituents and biological pathways, which are known as the biomarkers of heat stress. These biomarkers are effectively utilized as the tool to evaluate heat stress and helps to assess the heat stress management. Here we are discussing about different biomarkers during thermal stress which can be used as tools to evaluate the degree of heat stress in their dairy farm animals.

Keywords: Biomarker, heat stress, livestock

Introduction

Livestock plays an important role in maintaining the global economy. An imbalance between metabolic heat production inside the animal body and its dissipation to the surroundings results in a condition known as Heat Stress/Thermal Stress (HS/TS). It occurs when the ambient temperature lies above thermoneutral zone. TS results in reduced immunity, decreased milk production and composition, reduced growth rate, feed efficiency, reproductive efficiency, carcass composition, meat quality and alters molecular pathway. Temperature Humidity Index (THI) is an important index which combines two of the more important and easily measured climatic elements and its results can be corelated with livestock's productivity. If THI is above 82, it will affect the productivity of cattle or causes even death of the animal. Exposure of ruminant animals to elevated temperatures induces the release of Heat Shock Proteins (HSPs), production of cellular Reactive Oxygen Species (ROS), activation of inflammatory genes and as well as variation in the gene level that affects skin color. In the molecular level there will be variations in plasma hormones like glucocorticoids, thyroid hormones, catecholamines and alterations in concentration of enzymes such as ALP, ACP, AST.

Types of biomarkers

In a broad sense, biomarkers are the substances reflecting an interaction between a biological system and a potential hazard, which can be chemical, physical or biological. Measurements of these substances gives an idea about the body condition of animal. There are different types of biomarkers of heat stress

Physiological biomarkers

Respiration Rate (RR), Pulse Rate (PR), Rectal Temperature (RT), Sweating Rate (SR) and Skin Temperature (ST) are the cardinal physiological variables which helps to maintain the heat balance and homeostasis in the heat stressed animals.

a. *Respiration Rate (RR)* is an Ideal physiological biomarker and an early indicator of a heat stressed cow. > 80 breaths/minute is an indication of high quantum of heat stress. The increased RR of animal during HS condition is an attempt to upsurge the respiratory evaporative cooling mechanisms by dissipating body heat.
b. *Rectal Temperature (RT)* is used as a representative measure of animal's core body temperature. Rising RT even 1ºC or less is enough to reduce the performance in livestock. The increased RT implies the inability of the animal to maintain the normal body temperature during exposure to summer heat stress.

c. *Pulse Rate (PR)* reflects primarily the homeostasis of circulation along with the general metabolic status. The cardio respiratory system is influenced by season, day timings, ambient temperature, humidity and exercise. The exposure of animal to high ambient temperature generally increases the PR. It may act as reliable indicator of livestock in harsh environmental condition.
d. *Skin Temperature (ST)*: The exposure of animals to hot environment increases their skin temperature to maintain the thermal status in their body. The accelerated skin temperature indicates the increased blood flow to the skin from the body core with cutaneous vasodilation to enhance the heat exchange between skin and the surrounding environment. The skin temperature is increased by 0.22 °C per extra degree of ambient temperature exposure.
e. *Sweating Rate (SR):* Dissipation of excess heat from the body through cutaneous evaporative cooling mechanisms is known as sweating. Sweating is influenced by weather parameters such as wind velocity, air temperature, relative humidity, thermal and solar radiation. Whereas other factors that affect the efficacy of evaporative cooling from the skin surface are density and thickness of hair coat, hair length and skin colour etc.

Hormonal biomarkers

a. *Glucocorticoids:* Adrenal corticoids, mainly cortisol, elicit physiological adjustments that enable animals to tolerate stress. The glucocorticoids act as vasodilators to help heat loss and have stimulatory effect on proteolysis and lipolysis, hence providing energy to the animal to help offset the reduced feed intake. The increase of plasma cortisol level during acute heat stress is attributed to the fact that glucocorticoid hormones have hyperglycemic action through the gluconeogenesis process, thus enhancing glucose formation in heat-stressed animals. In chronic heat stress it will reduce because of the fact that cortisol is thermogenic in animals and the reduced adrenocortical activity is a thermoregulatory protective mechanism preventing a rise in metabolic heat production.
b. *Thyroid Hormone:* The plasma thyroxine (T4) and triiodothyronine (T3) levels have been observed to decline under heat stress. Thyroid hormones play an important role in growth, regulation and are essential for maintenance of the basal metabolic rate. The decline in thyroid hormones along with decreased plasma Growth Hormone (GH) level has a synergistic effect to reduce heat production.
c. *Catecholamines:* Epinephrine and norepinephrine are the predominant catecholamine hormones involved in stress response. The concentration of catecholamines is elevated during both acute and chronic thermal stress. Plasma epinephrine levels varies between 285 to 1,575 ng/ml and norepinephrine levels varies from 1,500 to 2,525 ng/ml during summer in crossbred cattle.
d. *Prolactin:* Prolactin levels are observed to increase during thermal stress. Ambient temperature is favorably correlated with prolactin level. Prolactin affect body fluid regulation by maintaining extracellular fluid volume during heat exposure and supporting heat dissipation. Higher circulating prolactin modulate some thermoregulatory process during heat exposure.
e. *Anti-diuretic hormone (ADH):* Concentration of ADH increases to conserve water and increase water intake. Severe dehydration may lead to increased secretion of ADH through activation of renin–angiotensin–aldosterone system. ADH hormone also assists the excretion of concentrated urine in animals suffering from heat stress.
f. *Aldosterone:* Plasma aldosterone concentration decreases significantly during high thermal exposure. Decrease in aldosterone and parathyroid hormone secretion during heat stress in cattle is associated with a rise in body fluids and water turnover rate. The decline in aldosterone level has been observed due to a fall in serum potassium levels mainly because of its increased excretion through sweat. This steroid hormone controls the balance of water and salt in kidney by keeping sodium in and releasing potassium from the body.

Biochemical biomarkers

Blood glucose, amino-acid, urea, protein, creatinine, free fatty acids, haemoglobin, haptoglobin, PCV, cholesterol.

Heat-stressed dairy cattle fail to properly manage glucose utilization mechanisms and there will be an increase in concentration of total amino acids, aspartate, alanine, glutathione, threonine, and glycine. The usage of amino acids in the synthesis of milk protein is decreased because of the involvement of amino-acids in immunological responses and gluconeogenesis. Amino acids (Glu, Gly, Asp, and Val) for gluconeogenesis will be higher in heat-stressed cows. Ala will regulate gluconeogenesis and glycolysis to ensure glucose production during periods of food deprivation and the high concentrations of Thr would be associated with immune response under heat stress. Total blood Hb concentration is another biochemical biomarker which will increase during heat stress so that the animals could meet a higher oxygen requirement. Plasma haptoglobin, which is an acute phase protein to assess the health and inflammatory response of animals will rise during high ambient temperature in dairy cows. Significantly there will be increased levels of PCV, free fatty acids and decreased concentration of plasma protein and cholesterol in livestock exposed to elevated temperatures.

Enzyme biomarkers

Malondialdehyde, Superoxide Dismutase (SOD), glutathione peroxidase (GPx), Acid Phosphatase (AP), Alkaline Phosphatase (ALP), Aspartate Amino Transferase (AST) and *Alanine Amino Transferase (ALT)* are the major enzyme biomarkers involved in heat stressed livestock. Antioxidant enzymes such as SOD and GPx are synthesized in the body to provide protection from reactive oxygen species generated during heat stress. AP and ALP are two major enzymes associated with the metabolic activities in animals. The levels of these enzymes are generally low in heat stressed animals, which could be attributed to a metabolic shift in the animals. AST and ALT are two important metabolic enzymes that increase during heat stress.

Molecular Biomarkers

Heat stress was found to alter several molecular functions such as DNA synthesis, replication and repair, cellular division, nuclear enzymes and DNA polymerases functions. There are changes in the expression patterns of certain genes that are fundamental for thermo-tolerance at the cellular level in animals. The classical *Heat Shock Protein (HSP) gene, Apoptotic gene, Cytokines*, *Toll-Like Receptors (TLRs), MC1R (melanocortin 1 receptor), PMEL (Premelanosome protein),* Inflammatory gene *(NF-κ/nuclear factor kappa B and tissue TNF-α/tumor necrotic factor) , MicroRNAs (miRNAs),* Genes - associated with thermo-tolerance in ruminant livestock such as *superoxide dismutase, nitric oxide synthase, thyroid hormone receptor* and *prolactin receptor genes* were found to be associated with thermo-tolerance in ruminant livestock.

a. *HSPs* are considered to be protective agents against stress factors. Elevated HSP70 and HSP90 during heat stress exposure in ruminant are identified to be ideal molecular marker for quantifying heat stress response.

b*. Inflammatory gene (NF-κB and tissue TNF-α):* High ambient temperature resulted in increased expression of proinflammatory mediators (such as TNF-α), possibly leading to permeation of lipopolysaccharides into the blood circulatory system. Moreover, it has been reported that elevated expression of TNF-α is involved in promoting systemic inflammatory responses, that are associated with heat stress.

c. *Skin colour genes (PMEL / premelanosome protein and Melano-Cortin 1 Receptor MC1R):* The gene expression of PMEL and MC1R is upregulated during cold and heat stress, however high environmental temperatures apparently have a greater effect than cold stress. Animals live in a hot climatic condition (such as Zebu cattle) have increased expression of color genes in skin cells so it usually shows dark pigmentation as compared to animals in cold areas, and also, they have a subsequent increase in skin protective capability and decreasing in oxidative injury under environmental heat stress.

d. *MicroRNA:* Alterations in the miRNAs profile could potentially be used as diagnostic biomarkers for resilience to increased high temperatures and climate change. miRNA-19a/b and miRNA26a have regulatory roles in innate immunity and thermotolerance in cattle via the targeting of TLR 2 and HSP genes, respectively. The miRNA-27b has a direct effect in the synthesis of HSPs and cell survival under adverse environmental conditions such heat stress. miRNA-103, which is over-expressed in peak summer and it can show a protective function in terms of maintenance of cellular homeostasis and inhibiting cell proliferation. Heat stress increased lipid accumulation in the blood or different tissues via overexpression of miRNA-103.

e. Toll Like Receptors (TLRs): During heat stress, Toll-like receptor 4 (TLR4) and Toll-like receptor 2 (TLR2) identify the damage-associated molecular patterns to produce many pro-inflammatory cytokines triggering the host immune response. Powerful link may exist between TLR and HSP expression by heat stress. Heat shock stress might influence TLRs expression in immune cells to stimulate all adaptive and innate immune systems.

Conclusion

The potential environmental heat stress has negative impacts on biochemical pathways, immune and inflammatory responses, proteomics, physiologic and performance traits in livestock and poultry. Heat stress becomes evident when the amount of heat generated by an animal's body exceeds the ability of the body to distribute heat to its surroundings. Accurate and measurable biomarkers of heat stress in dairy cows can be taken as tools to evaluate the degree of heat stress in their dairy farm thereby assessing heat stress management. Biomarkers those emerge in this pathway may serve as useful indicators of animal welfare in the changing climatic condition.

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