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Role of HSP and Leptin in Dairy Animals during Thermal Stress: A Review

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ABSTRACT

Thermal stress is a unique and complex process which alters the normal physiological mechanisms. Heat shock proteins (HSPs) are known to be highly conserved and ubiquitous proteins synthesized in response to several stimuli. The HSP inducers have been categorized as environmental (heat shock, UV radiation, heavy metals, amino acids, oxidative stress, *etc*.) pathological (bacterial or parasitic infections, fever, inflammation, *etc*.) and physiological (growth factors, cell differentiation, hormonal stimulation or tissue development, caloric restriction, *etc*.). The HSPs play an important role for maintaining cellular functioning under environmental challenges and protein denaturation conditions. There is a great potential for using HSP72 expression to detect natural adaptation and exposure to stress in natural populations. Leptin is a 16 kDa polypeptide hormone secreted primarily from the cells of white adipose tissue. Leptin acts through both central and peripheral mechanisms to affect feeding behaviour, lipid and glucose metabolism, thermogenesis, reproductive and endocrine functions. Leptin also plays an important role in the regulation of body weight and composition, *e.g*. reduction in feed intake and increase in energy expenditure. Bovine lymphocytes express Ob-Rb gene (leptin receptor) and also express leptin. There are ample information on the central role of leptin in brain and neural tissue regulation. In dairy cattle, the increase in milk yield has been associated with a negative energy balance and a decrease in fertility during early lactation. The hormone leptin is involved in regulation of nutritional status and reproductive function.

Key words: Dairy cows, HSP, Leptin, Thermal stress.

Heat shock proteins consist of highly conserved stress proteins, expressed in response to stress. Heat shock proteins allows cells to adapt to gradual changes in the environment and are considered to play crucial roles in environmental stress adaptation and thermal balance, particularly protein function (Givskov *et al*., 2003). However, little is known about the tissue-specific responses of these genes *in vivo*, particularly in stress events such as HS, which invokes changes in numerous metabolic and endocrine signalling processes, with consequent physiological and behavioural changes and play crucial roles in environmental stress tolerance and adaptation. The expression level of HSP 70 was reported to increase at the cellular level under HS conditions (Lacetera *et al*., 2006). Heat shock protein 90 gene expression increased after rapid exposure to HS conditions (severe level) and was expressed as a repressor of heat shock transcription factor (HSF) 1 to protect against HS. During the periparturient period in dairy cattle this protein hormone may be a suitable representative for energy metabolism and reproductive functions in periparturient cows.

Heat shock proteins

The heat shock protein was discovered by (Ritossa,1962) who detected a new puffing pattern upon heat shock in the polytene chromosomes of the fruit fly Drosophila. Heat shock proteins are a large protein family consisting of both constitutively expressed and inducible proteins, classified according to their moleculer weight (Kregel, 2002).

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Functions of heat shock proteins

The HSP72 (HSPA1A) one of the members of the HSP70 plays an important role in (i) folding of proteins in various intracellular compartments (ii) maintenance of structural proteins (iii) refolding of misfolded proteins (iv) translocation of proteins across membranes and into various cellular compartments (v) prevention of protein aggregation (vi) degradation of unstable proteins. The role of HSP72 as a chaperone and its potential ability to contribute to cellular

repair processes in response to interventions such as heat, oxidative stress, activation of proteases, release of lysosomal and proteolytic enzymes and alterations of the cytoskeleton have gain so much importance in today's world (Kregel 2002).

Heat shock protein and stress

The cellular response to stress activates a specific set of genes called the heat shock genes which results in the preferential synthesis of a group of proteins known as heat shock proteins. Among the heat shock proteins, HSP70 is the most abundant in both eukaryotes and prokaryotes and it appears as a thermal switch. The HSP70 acts as an indicator of thermo-tolerance retention. Genetic differences in thermotolerence also extend to cellular levels. HSP are broadly classified into five families *viz*, HSP27, HSP 60, HSP 70, HSP 90 and HSP 110/104 depending upon the molecular weight. It was found that HSP 70 is the most stress inducible type. The heat shock response occurs when cells growing at a low temperature are shifted to a higher temperature resulting in the induction of the heat shock proteins (Lindquist and Craig 1988). Patir (2007) reported decreased induction of HSP70 at 45°C in climatic chamber whereas the maximum induction was observed in Murrah buffaloes at $38\frac{238}{92}$C exposure for 48 hr. Mayengbam (2008) also reported an increase in HSP 72 mRNA indairy cattle during peak hot seasons of the year and the level of induction varied depending upon the duration of exposure to stress during natural environment.

HSPs and mechanism of thermal tolerance

Cellular proteins are adversely affected by heat stress except HSP, which are activated by heat shock. It has been reported that HSP70 showed higher expression levels during heat stress in goats (Dangi *et al*., 2014), buffalo (Kishore *et al*., 2014), sheep (Romero *et al*., 2013) and cattle (Mishra and Palai, 2014). Various experiments have suggested that ability of thermo tolerance is directly associated with the rate of HSP70 expression/accumulation in cells. Similarly, thermo tolerance decays at a rate parallel to HSP70 degradation. In the beginning, HSP70 were not inducible, that is why most of fruit flies, frogs and mammals were hypersensitive to thermal killing. Later on, HSP70 became inducible which made organisms more thermo tolerant (Browder *et al*., 1998). However, induction of HSP70 can be inhibited by some inhibitors like cycloheximide which can interfere with normal cellular stress response (Lindquist, 1986).

Heat Shock Protein 72 mRNA expression

Valenzuela (2000) studied HSP70 expression in five different breeds of cattle and only HSP70 was expressed in lymphocytes. Lisa *et al.* (2003) determined the lower temperature set point for activation of the cellular stress response in T-lymphocytes of mice. Mice were subjected to whole body hyperthermia at various temperatures from 38 to 42°C for 60 minutes. The suppression of lymphocytes cluster formation by inducing hydrogen peroxide in humans was found to be dose dependent (Shinkai *et al*., 2004). Vaidya *et al*. (2019) reported HSP72 mRNA expression was significantly higher in lymphocytes of high producing Karan Fries and Sahiwal cows compared to low producing Sahiwal and KF cows during both the seasons.

Heat shock proteins and energy metabolites

Sordillo *et al*. (2009) suggested that some changes in the intracellular or circulating levels of these multifunctional proteins may occur in dairy cows around calving. Concentrations of HSP72 in peripheral blood mononuclear cell (PBMC) and plasma increased sharply after calving. In the post-calving period, BCS and plasma glucose declined, whereas plasma nonesterified fatty acid (NEFA) increases (Elisabetta *et al*., 2010).

Significance of HSP in livestock adaptation

The HSP70i, an inducible form of HSP70, having a molecular weight of 70kDa has been proposed as a forecaster for thermo tolerance at the cellular level in livestock species. Investigations carried out to find out the association between the heat shock response of mononuclear cells in blood and SNPs at the 5' UTR of HSP 70.1 yielded in understanding the importance of these mutation sites as molecular markers.

HSP72 expression in high and low yielding animals

Vaidya *et al*. (2019) reported during summer season the relative quantity of HSP72 mRNA in high and low yielding Sahiwal cows increased to 268 and 246 per cent respectively on the day of calving from the precalving values (45 day). Whereas in high and low yielding Karan Fries cows the relative quantity of HSP72 mRNA increased to 94.4 and 171.4 per cent respectively on 15th day postpartum compared to per calving values during summer season. During winter season in high and low yielding Sahiwal cows the HSP72 mRNA expression increased to141.4 and 159 per centon 15th day of calving from the pre calving values (45 day). In high and low yielding KF cows the HSP72 mRNA expression increased to 155.9 and 225.4 per cent respectively on 30th day postpartum from the precalving values (45 day) during winter season (Vaidya *et al*. 2019).

Leptin

The *ob* gene was discovered in 1994 by positional cloning techniques by Zhang *et al*., (1994). The 167 amino acid protein product of the *ob* gene was named leptin (derived from the Greek term 'leptos' means 'thin'). Leptin is a 16 kDa protein that is synthesized by adipose tissue and is involved in regulation of feed intake, energy balance, fertility and immune functions (Fruhbeck *et al*., 1998). Leptin binds to a receptor mainly localized on neuropeptide Y neurons, which results in a reduction of feed intake and an increase of energy expenditure. Leptin acts as an energy reserve signal for hypothalamic regions that control feeding behaviour, metabolism and endocrine function so as to maintain energy homeostasis (Chilliard *et al*., 2001). The postpartum leptin reduction is likely to be due to the negative energy balance because plasma leptin remained high in cows not milked after parturition (Block *et al*., 2001).

Leptin level, body weight and feed intake

Halaas *et al*., (1995) observed besides its effect on feed intake *via* hypothalamic NPY neurons, leptin could also play an important role in regulating fat mobilization. The satiety effects of leptin were also observed in ruminants by administration of recombinant human leptin in ewes for 3 days. This treatment caused a decrease in voluntary dry matter intake to approximately a third of the normal intake (Henry *et al*., 1999). Feed deprivation reduced the level of adipose tissue leptin mRNA in cows (Amstalden *et al*., 2000). Injections of leptin caused a rapid decrease in feed intake and body weight in *ob/ob* mice (Campfield *et al*., 1995) and pigs (Barb *et al*., 1998).

Leptin and lactation

The suckling stimulus itself did not appear to influence the decrease in leptin concentration. Pickavance *et al*. (1998) observed that the food intake-induced leptin increase was eliminated during lactation and they speculated that the hypoleptinemia may be an important factor promoting the hyperphagia of lactation. Eliminating the energetic costs of lactation by preventing milk delivery in rats and cows caused an increase in plasma leptin levels together with an increase in energy balance (Block *et al.,* 2001).

Levels of leptin during dry period and early lactation

In ruminants and other animals, leptin is synthesized in proportion to the overall degree of adiposity and acts on the central nervous system to reduce voluntary feed intake (Ehrhardt *et al*., 2000). Leptin, mainly produced in adipose tissue, inhibits feed intake and down-regulates adipose tissue deposition. Block *et al*. (2001) observed that leptin is negatively correlated with the amount of non-esterified fatty acids, which reflects the amount of fat mobilization and also stated that plasma leptin level was reduced by approximately 50 per cent after calving, and remained depressed during lactation. During pregnancy the plasma leptin level increases significantly in several species, due to either the enhanced expression of placental leptin mRNA (Primates and rat), or a dramatic elevation in plasma content of soluble leptin receptors which serve as binding proteins in circulation and decrease the renal leptin clearance (Islami and Bischof, 2003 and Waddell and Smith, 2003).

Leptin and negative energy balance (NEBAL)

During fasting, the plasma concentration of leptin is reduced rapidly, concomitant with changes in neuroendocrine signals that promote fasting metabolism and the attenuation of dispensable, energy-dependent functions such as reproduction and immune response (Lord *et al*.,1998). During the peri-parturient period, high yielding dairy cows experience major changes in energy metabolism (Bauman, 2000). With the onset of lactation, the added metabolic activities of the mammary gland increase the total energy requirements by approximately fourfold. A pronounced energy deficit develops because voluntary feed intake is insufficient to meet this increased energy expenditure and is met by mobilizing lipids from white adipose tissues (Bauman, 2000). Ahima and Flier (2003) observed that changes in the plasma concentration of leptin could also be an important adaptation, particularly given the role of WAT in support of early lactation in dairy cattle. In ruminants and other animals,leptin is synthesized in proportion to the overall degree of adiposity (Delavaud *et al*., 2000), and acts on the central nervous system (CNS) to reduce voluntary feed intake (Schwartz *et al*., 2000). Because leptin therapy partly reverses these adaptations in fasted animals (Nagatani *et al*., 2000), an equally important role of leptin is to signal the energy status of the periphery to the CNS (Ahima and Flier, 2003).

Relationship between leptin with HSP72

Zhang *et al*. (1994) observed Leptin, the adipocyte-derived hormone and the key regulator of feed intake and energy homeostasis and alsofound that alteration of HSP70 gene expression is accompanied by high plasma leptin levels and a reduction in feed intake. Oates *et al*. (2000) observed in developing pups that effect of leptin and of leptin-reduced feed intake maintaining low stress response on HSP70 gene expression. Continuous infusion of recombinant chicken leptin (8 µg/kg per hour) at a constant rate of 3 ml/h for 6 h in 3-week-old broiler chickens significantly ($P<0.05$) decreased feed intake and HSP-70 mRNA levels in liver and hypothalamus, but not in muscle (Denise *et al*., 2007).

Leptin expression in high and low yielding animals

During summer season the mean relative quantity of leptin mRNA expression in high and low yielding Sahiwal cows decreased to 89.3 and 80.7 per cent on 15th day of calving, whereas in Karan Fries the relative quantity of leptin mRNA expression decreased to 84.6 and 79.5 per cent on the day of calving and 15th day of postpartum from the precalving values (45 day) respectively. During winter season the quantity of leptin mRNA expression in high and low yielding Sahiwal cows decreased to 74 and 75.7 per cent on 30th day postpartum and on the day of calving from the pre calving values (45 day). In high and low yielding Karan Fries cows the quantity of leptin mRNA expression lowered to 70.7 and 86.2 per cent respectively on 45th day postpartum from the precalving values (45 day) during winter season (Vaidya *et al* 2019).

CONCLUSION

It is concluded that, the levels of leptin andHSP72 during and after parturition implies the stress on the lactating cattle due to insufficient energy level (glucose) in the animals and leptin helps in mobilization of the fat for fulfilling the energy requirement around parturition. However, the molecular chaperones are up regulated on the day of parturition at cellular level of the HSP72 gene expression indicating more cellular damage on the day of parturition and metabolic, thermal and production stresses. Therefore, these

parameters could be used to monitor the metabolic status of the animals around parturition for improvement in health and productivity of bovine species. It is further indicated that in coming era of climate change and elevated temperatures across the globe, further study on leptin and HSP72 will be beneficial for the thermoregulation and overall management of dairy animals.

Conflict of interest: None.

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