Review Article

Prospects of HSP70 as a genetic marker for thermo-tolerance and immuno-modulation in animals under climate change scenario

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ABSTRACT

Heat stress induced by long periods of high ambient temperature decreases animal productivity, leading to heavy economic losses. This devastating situation for livestock production is even becoming worse under the present climate change scenario. Strategies focused to breed animals with better thermo-tolerance and climatic resilience are keenly sought these days to mitigate impacts of heat stress especially in high input livestock production systems. The 70-kDa heat shock proteins (HSP70) are a protein family known for its potential role in thermo-tolerance and widely considered as cellular thermometers. HSP70 function as molecular chaperons and have major roles in cellular thermotolerance, apoptosis, immune-modulation and heat stress. Expression of HSP70 is controlled by various factors such as, intracellular pH, cyclic adenosine monophosphate (cyclic AMP), protein kinase C and intracellular free calcium, etc. Over expression of HSP70 has been observed under oxidative stress leading to scavenging of mitochondrial reactive oxygen species and protection of pulmonary endothelial barrier against bacterial toxins. Polymorphisms in flanking and promoter regions in HSP70 gene have shown association with heat tolerance, weaning weight, milk production, fertility and disease susceptibility in livestock. This review provides insight into pivotal roles of HSP70 which make it an ideal candidate genetic marker for selection of animals with better climate resilience, immune response and superior performance.

1. Introduction

Homeotherms in tropical and arid regions have to bear the stress of high temperature. However, homeotherms are gifted with an ability to regulate a steady temperature inside their bodies, regardless of ambient temperature. Their cellular homeostasis is

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likely to be disturbed by heat stress. To combat the drastic outcomes of heat stress, cells are blessed with heat shock proteins (HSP). These proteins are found abundantly in both prokaryotic and eukaryotic cells. Heat shock proteins were reported for the first time in 1962 by an Italian geneticist Ferruccio Ritossa while working on Drosophila at the Genetics Institute in Pavia (Ritossa, 1996). He exposed salivary gland cells of Drosophila to 37 °C for 30 min and then allowed them to return to normal temperature of 25 °C, and found they could recover. During recovery of cells, a "puffing" of genes was observed in the chromosomes, along with an elevation in the expression of proteins of 70 and 26 kDa (Tissieres et al., 1974). Proteins discovered during this experiment were named as "heat shock proteins". These proteins are involved in protecting cells from heat shock by safeguarding the cellular proteins from denaturation (Feder and Hofmann, 1999). The chaperon function of HSP includes prevention of inappropriate protein

aggregation and directing newly synthesized polypeptide for final packaging, degradation, or repair (Kiang and Tsokos, 1998).

Among HSP, the proteins of molecular mass 70 kDa are most abundant and highly conserved proteins and termed as HSP70. All HSP70 have potential to bind with adenosine triphosphate (ATP) molecules (Milarski and Morimoto, 1989). The HSP70 family is encoded by HSP70 gene and includes proteins of molecular masses ranging from 68 to 73 kDa. The bovine HSP70 protein has a molecular weight of 70,190.56 Da, and out of total 641 amino acids, in which 92 are highly basic amino acids, while 82 are highly acidic amino acids. Additionally, 151 amino acids are hydrophilic and 220 amino acids lack affinity for water. Functional parts of HSP70 proteins consist of an amino-terminal ATPase domain (44 kDa) and a carboxylic-terminal region having molecular weight of 25 kDa (Gade et al., 2010). Third functional part of HSP70 is its substrate binding domain, which consists of a 10-kDa helical subdomain and a 25-kDa β sheet subdomain. Studies on cattle, goat and buffalo revealed that HSP70-1 gene has an open reading frame of 1,926 base pairs (Gade et al., 2010). HSP70 are said to be monomeric proteins found in cytosol of prokaryotes, while in eukaryotes they reside in cytosol, endoplasmic reticulum, nuclei, chloroplast and mitochondria. These are also found in extracellular region in a free soluble form or tangled with antigenic peptides. Their half-life is relatively long like 48 h in human epidermoid cells (Kiang and Tsokos, 1998).

2. Genetic variants of HSP70

HSP70 family in bovines further comprises of 4 genes viz., HSP70-1, HSP70-2, HSP70-3 and HSP70-4. Among these, HSP70-1 is a well-studied intron-less gene and is present on chromosome 23 in bovines (Gade et al., 2010). In cattle, HSP70-2 is present in bovine leukocyte antigen region of chromosome 23 band 22, whereas, HSP70-3 is localized at band 34 of chromosome 10 and HSP70-4 is residing at band 13 of chromosome 3. HSP70-1 is found to be firmly attached with HSP70-2 on chromosome 23 (Daniel et al., 1993). Bovine HSP70-1 and HSP70-2 are homologous with HSPA1 and HSPA1L on chromosome 6p21.3 in human while HSP70-3 and HSP70-4 are homologous to unnamed human HSP70 gene on chromosome 14q22-q24 and HSPA-6,-7 genes on chromosome 1, respectively (Grosz et al., 1992).

Almost all variants owe their own unique importance due to their peculiar functions including protection of polypeptides from stress of elevated temperature, packaging and folding of nascent polypeptides, configuration and detachment of protein complexes. They are ATP dependent and work as molecular protector. These are also involved in DNA repair, apoptosis, signal transduction and protein homeostasis. HSP70 also function to serve as tumor-specific target for detection by natural killer cells (NK cells).

3. Functions of HSP70

Having housekeeping functions in the cell, HSP70 are of great importance in living beings as they are mainly involved in cellular protection against heat shock. They also play key roles in modulation of immune system by ensuring proper folding of proteins and regulation of apoptosis. Some of major functions of this protein family are highlighted as follows.

3.1. HSP70 as molecular chaperons

Chaperon means to look after, therefore, HSP70 are considered as molecular chaperons for their function of protecting the cellular compartment from thermal stress. They also protect newly synthesized polypeptides from damage by properly folding and packaging them. The assistance of HSP70 proteins in folding of non-native proteins is further divided into 3 activities including supporting their folding to native state, avoidance of aggregation, solubilization and refolding of aggregated proteins. These activities are used in quality control of misfolded proteins and post-translational folding of nascent polypeptides. HSP70 prevent the aggregation of non-native proteins by association with water repelling patches of substrate molecule, which protects them from intermolecular interactions. This activity is assisted by co-chaperons of J-domain proteins (Mayer and Bukau, 2005). Moreover, HSP70 help in degradation of ineffective or unrecoverable proteins (Yokota and Fujii, 2010).

The HSP70 family consist of both inducible and constitutive forms, which help in stress tolerance by increasing the chaperon activity in the cytoplasm (Lindquist and Craig, 1998). HSP70 help proteins to move across membranes by using ATP binding and hydrolysis. HSP70 improve the overall integrity of cellular proteins. HSP70 are found to be involved in neuroprotection after examining various models of in vivo and in vitro neurodegeneration (Mishra and Palai, 2014).

Bovine HSP70 is found to have an interaction with tubulin protein as well. The sequence of HSP70 obtained from complementary DNA sequence has a region from residues 246 to 264. This region is similar to the tubulin binding motifs of microtubule associated protein (MAP1B). Therefore, a peptide obtained from MAP1B and containing one of the tubulin binding motifs (KKEVVKKEDK) can struggle with HSP70 for binding to polymerized tubulin. Importantly, region of HSP70 having residues from 246 to 264 has a resemblance with tubulin binding motifs of MAP1 proteins. This resemblance is due to the fact that both regions are rich in basic amino acids i.e., arginine and lysine. As such, the binding of HSP70 to tubulin is similar to that of MAP1B peptide. This helps HSP70 in proper folding of tubulin accompanied with chaperonin t-complex polypeptide 1 (TCP-1) (Sanchez et al., 1990).

3.2. Thermal tolerance

Thermal tolerance is the ability of animals to balance thermogenesis and heat dissipation under ambient temperatures above thermoneutral zone (TNZ) of body (e.g. from 32 to 77 °F for healthy cattle). Thermoneutral zone is the range of temperature for animals in which no expenditure of energy is required to maintain normal body temperature. In this regard, heat shock proteins have a major role in heat tolerance and protecting the cellular compartments from adverse effects of heat stress.

3.2.1. Role of HSP70 in cellular thermotolerance

Mammals and birds have a gifted potential to maintain their body temperature. For maintaining homeostasis at cellular level, they have specialized HSP. Among these proteins, HSP with molecular mass of 70 kDa are of great importance. These proteins guard against drastic effects of heat stress in animal sthriving in tropical and arid regions. In case of absence of this protective shield to mediate heat stress, animals become victim of detrimental effects posed by elevated ambient temperature (Mishra and Palai, 2014).

HSP70 are of much importance in cellular thermotolerance and protecting polypeptides from denaturation during heat stress. When a cell is under stress, it cannot perform its normal activities like transport of materials, DNA replication, transcription and polypeptide synthesis. Moreover, stress causes misfolding of proteins at cellular level. In such stressful condition, HSP are activated and save the cell by minimizing accumulation of the denatured or abnormal proteins in the cell. In this way, they enhance the cell

survival and its ability to overcome oxidative and thermal stress (Bhat et al., 2016).

3.2.2. 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). Thermal stress induces expression of HSP70 in cells. In order to protect cells from heat stress, HSP70 bind to affected proteins so that they cannot aggregate within cells. The aggregation of proteins denatured by heat stress can induce several complications within cells with serious consequences. Various experiments have suggested that ability of thermotolerance is directly associated with the rate of HSP70 expression/accumulation in cells. Similarly, thermotolerance 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 thermotolerant (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).

Several post-translational and transcriptional mechanisms are involved in induction and stimulation of cellular stress response. Heat shock factors are present in inactive forms within cells and become active under heat shock by post-translational modification. Their activation increases transcription of HSP (Sorger et al., 1987). Heat shock messages should be translated efficiently in order to guard cell against adverse effects of stress. Therefore, the messenger RNA existing previously should be reserved by translation, so that, competition for translation of new mRNA can be avoided (Lindquist, 1980). During heat shock, those mRNA of HSP70, which are unstable at normal temperatures, become stable (Petersen and Lindquist, 1998). A simple pathway for activation of HSP70 expression under stress is illustrated in Fig. 1.

3.2.3. Polymorphisms in HSP70 associated with thermal stress

Several polymorphisms have been detected in the DNA sequences of different HSP genes to explore their association with ability of heat tolerance in animals. For example Bhat et al. (2016) investigated variants in HSP70 associated with thermotolerance in Tharparkar cattle. They identified a polymorphic fragment of 295 bp by analyzing 3 PCR single-strand conformational polymorphism (PCR-SSCP) patterns. Analysis of nucleotide sequencing alignment of 3 PCR-SSCP patterns revealed a substitution of G to T and G to C at 149th position revealing 2 alleles (allele A with nucleotide T and allele B with nucleotide C) of HSP70 gene. This G to T substitution leads to a change of amino acids (from aspartate to tyrosine) in gene transcript. They concluded that allele A of HSP70 is positively correlated with thermal tolerance, and genotype AA is superior with the highest heat tolerance coefficient. They suggested that such single nucleotide polymorphisms (SNP) could be used as an indicator for selection of thermotolerant cattle. Polymorphisms in the promoter and 3′-untranslated region (UTR) of the HSP70-2 gene have shown association with mRNA stability and stress response in pigs (Schwerin et al., 2001, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3118824/, 2002).

In another study, mutations in selected 5′ flanking region of HSP70 gene were analyzed to check the association of this region with susceptibility of animals to heat stress (Cai et al., 2005). They identified a mutation site in cis-acting element by using PCR-SSCP assay and determined 4 genotypes as, AC, AA, AB and BB. They reported that expression of HSP70 mRNA and Bc1-2 mRNA/Bax-α mRNA in AC genotype was much higher as compared to rest of genotypes. At the 33rd nucleotide of AC genotype, a nucleotide substitution (A→G) was significantly associated withability of dairy cows to resist against heat stress. This study opened up new horizons for exploring other related HSP genes for analyzing molecular genetic marker based susceptibility to heat stress (Cai et al., 2005). Furthermore, presence of SNP (C/- and G/T) in the 5′-UTR region of inducible HSP70-1 showed significant association with HS response and tolerance to heat of bovine peripheral blood mononuclear cells (PBMC) (Basirico et al., 2011). However, they reported monomorphic 3′-UTR of inducible bovine HSP70-1 gene in Italian Holstein population. Contrarily, Adamowicz et al. (2005) reported polymorphic alleles for the same SNP in Holstein Polish population earlier.

3.2.4. Expression dynamics of HSP70 under heat stress

Climatic changes are one of the major challenges faced in livestock production. Tropical zones are under heat stress during summer because temperature rises to almost 44 °C or even higher. Such high temperature is dreadful for livestock, unless they have some heat shock management system in their cells. Heat shock proteins provide this cellular management to overcome the effects of heat stress. Heat stress may be for hours and days. Sometimes, heat stress may become chronic if it prolongs for months (Collier et al., 2006). Former is called short-time heat stress while later is called long-time heat stress. Heat stresses either short-time or long-time triggers the expression of HSP (Dangi et al., 2012). Among all HSP, HSP70 most sensitive and important manager of thermal adaptation in livestock (Dangi et al., 2014).

Experiments conducted to check HSP70 expression in livestock under chronic heat stress revealed that mRNA expression of HSP70 showed 2 peaks on the 2nd and 17th day of the heat stress at 42 °C in cattle. These findings confirmed results of earlier studies on PBMC of goats (Dangi et al., 2015) and lymphocytes of cattle (Kishore et al., 2014). It could be concluded that biphasic expression pattern of HSP70 helps to protect animals against heat stress. Moreover, it might be used as a biomarker of chronic heat stress in livestock (Bharati et al., 2017).

3.2.5. Factors affecting expression of HSP70

The expression of HSP70 is affected by various factors like intracellular pH, cyclic AMP, intracellular free calcium, intracellular inositol 1,4,5-trisphosphate and protein kinase C.

3.2.5.1. Intracellular pH. The latent basal intracellular pH in most cells has a range of 7.3 to 7.5. Many functions of cells are associated with change of intracellular pH. In some systems, alteration in pH range may initiate replication of DNA or cellular proliferation (Grinstein and Smith, 1990). In some cells like Vero and 3T3 cells, a minor rise of pH up to 0.2 can induce growth and tumorigenicity (Perona and Serrano, 1988). Effect of intracellular pH on HSP70 in HeLa cells revealed that heat shock factors (HSF) are activated in cell extracts at a pH of 5.8 to 6.4. Maximum activation of HSF was found at a pH of 6.0 (Mosser and Martin, 1992). However, these studies just focused about activation of HSF instead of HSP70 expression. Later studies conducted on HSP expression revealed that change in resting pH has nothing to do with ability of heat shock to induce HSP70 (Kiang et al., 1994), however, heat shock makes cell acidic (Liu et al., 1996). In cells which possess overexpressed HSP70 due to previous heat shock or HSP70 gene transfection, heat shock still acidifies cells. These findings revealed that there is no clear association between pH and expression of HSP70 (Kiang et al., 1994). However, cells reduce their pH after heat shock because it is necessary to normalize pH to inhibit various deleterious processes. This normalization of pH promotes cell survival
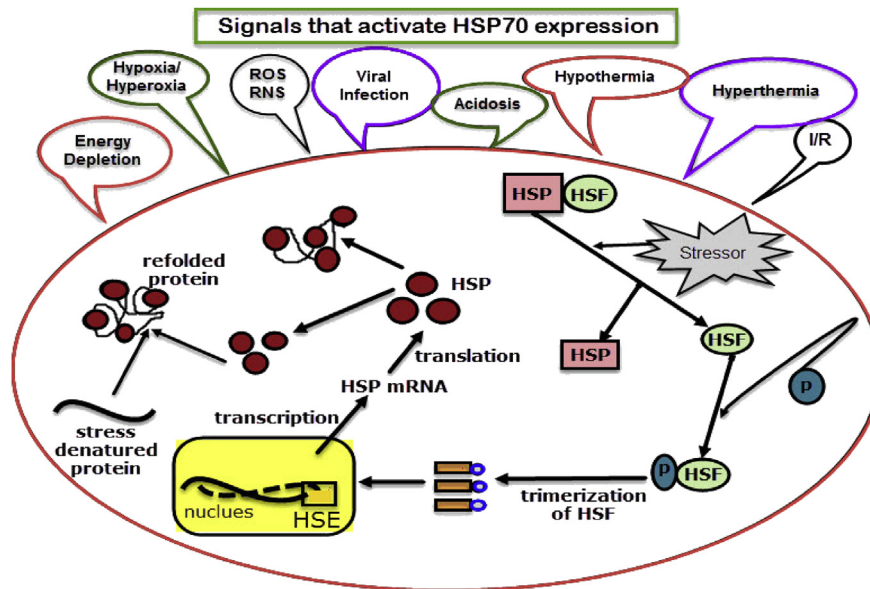
Fig. 1. Different stimuli that activate expression of HSP70 (adapted from Kregel, 2002). HSP = heat shock protein, HSF = heat shock factor, HSE = heat shock element, ROS = reactive oxygen species, RNS = recative nitrogen species, I/R = infra red radiations; P = phosphorylation.

and is thought to be a defensive mechanism of cell survival (Kiang and Tsokos, 1998).

3.2.5.2. Intracellular inositol 1, 4, 5-trisphosphate. Treatment of intracellular inositol 1,4,5-trisphosphate ($InsP_3$) with cholera toxin, forskolin or pertussis toxin increases production of $InsP_3$ (Kiang and McClain, 1993). Enhanced production of $InsP_3$ induces an increase in level of HSP70 mRNA and protein. On the other hand, heat induced increase in *HSP70* expression is diminished by an inhibitor of $InsP_3$ (Kiang et al., 1994). These findings clearly demonstrated that $InsP_3$ synthesis plays a key role in increased *HSP70* expression. However, mechanism by which inhibition of $InsP_3$ causes decrease in HSP70 production is not known. RNA splicing is altered by binding of $InsP_3$ to its receptors that leads to induction of expression of various genes (Nakagawa et al., 1991). Such binding of $InsP_3$ to its receptor may be responsible for induction of HSP70 (Sudhof et al., 1991).

3.2.5.3. Cyclic adenosine monophosphate. Cyclic adenosine monophosphate (cAMP) is considered as a second messenger during cell-signaling process of different cytokines and hormones. It has been reported that cAMP level increases during heat shock in rabbit epididymis (Kampa and Frascella, 1977). Moreover, cAMP and HSP70 level was increased in liver within 3 to 8 h in female mice after injection of 50 mg/kg dibutyryl (Takano et al., 1998).

3.2.5.4. Intracellular free calcium. Free calcium can activate the binding of HSF to heat shock elements (HSE) located in *HSP70* gene to mediate transcription (Price and Calderwood, 1991). Increase in level of free calcium can be induced by ionomycin which is a Ca^{2+} ionophore. Consequently, increased Ca^{2+} promotes HSP70 synthesis in rat luteal cells (Khanna et al., 1995).

3.2.5.5. Protein kinase C. Activation of protein kinase C (PKC) indicus *HSP70* expression as reported by many studies (Khanna et al., 1995; Ding et al., 1996). It has been found that treatment of rat luteal cells with phorbol 12-myristate 13-acetate (PMA) induces new HSP70 synthesis. This is because PMA is a potent stimulator of protein kinase C (Khanna et al., 1995).

3.3. Immuno-modulatory effects of HSP70

Many beneficial functions of intracellular HSP70 are known, however, extracellular HSP70 are also important. HSP70 in extracellular fluids are involved in immuno-modulation through signaling of immune cells against invading pathogens by stimulation of increase in neutrophils and macrophages. HSP involved in immuno-modulation exhibit resemblance with pro-inflammatory cytokines and adjuvants. Therefore, they are also termed as "danger signals" and signify some invasions and activate immune responses. It is important to know that HSP have no signal sequences as a leader which can direct their secretions. So, they are considered as intracellular proteins. However, HSP70 in extracellular serum has been found and is mainly due to severity of myocardial infarction, heart failure, atherosclerosis, peripheral and renal vascular disorders (Dybdahl et al., 2005). These may come out of cell after necrosis. Few viable cells can actively secrete HSP70 in extracellular fluid through secretary vesicles or lysosomal endosomes (Mambula and Calderwood, 2006).

Pathogens either bacterial or viral are identified by host pattern recognition molecules like TLR (toll-like receptors), NOD-like receptors (nucleotide-binding oligomerization domain-like receptors) and RIG-I-like receptors (retinoic acid-inducible gene-I-like receptors) through their pathogen-associated molecular patterns (PAMP). HSP in extracellular region serve as PAMP for host immune system. That's why these extracellular HSP can function as "self-adjuvant". In this way, both innate and acquired immunity can be activated. Innate immunity via this pathway includes induced production of inflammatory cytokines, co-stimulatory molecules and cell adhesion molecules while acquired immune system involves enhancement of antigen presentation. Autoantibodies against HSP70 are found in patients with immunological disorders like rheumatic autoimmune disease, atherosclerosis or cardiovascular diseases (Minota et al., 1998). Molecular mimicry between host and bacterial HSP is important for formation of autoantibody. Its importance is due to high sequence homology (50% to 60%) at amino acid level (Yokota and Fujii, 2010).

3.3.1. Role in auto-immune diseases

A cancerous cell or a cell affected by some pathogen usually secretes proteins, which are not a part of normal body proteins. Such proteins are considered as antigen by the body and trigger the immune system. Heat shock proteins alert the immune system against such antigens. This is mainly done by HSP70 and HSP90. They carry these antigens to the antigen-presenting cells (APC) of immune system via surface receptors. These proteins also inhibit inflammatory pathways and make the organism more resistant to diseases. HSP have immuno-regulatory potential because they can promote the formation of anti-inflammatory cytokines. HSP have been found to modulate immune responses in arthritis, graft rejection and colitis. Some members of HSP70 family are linked with autophagy (Borges et al., 2012).

3.3.2. Inhibition of inflammatory pathways

Studies have revealed that HSP70 family interferes with various intracellular inflammatory signaling pathways. A possible pathway of HSP70 activity is through its interaction with dendritic cells, monocytes and myeloid-derived suppressor cells. HSP70 binds to receptors of endocytes and become endocytosed. In this way, they get an access to passages of antigen presentation thus, modulating the cell phenotype towards a tolerogenic one. Finally, it leads to production of anti-inflammatory cytokine interleukin (IL)-10 and consequence is immunosuppression (Borges et al., 2012). IL-10 is the foremost anti-inflammatory and immunosuppressive cytokine (Moore et al., 2001). Modulation of immuno-suppression induced by HSP70 is illustrated in Fig. 2.

3.3.3. Mycobacterial HSP70 as a vaccine for bovine paratuberculosis

Paratuberculosis is an infection caused by *Mycobacterium avium* ssp. paratuberculosis in ruminants. In this disease, victim suffers from chronic granulomatous swelling in small intestine. This disease can occur in cattle worldwide and can cause significant economic losses. Moreover, it is difficult to eradicate due to lack of diagnostic tools. Control measures like vaccination could be used to limit spraed of this fatal disease in animals. Commercially available vaccine for bovine paratuberculosis usually contains various strains of bacteria including some adjuvants to

increase its efficacy (Manning and Collins, 2001). Koets et al. (2006) claimed that they have discovered HSP based vaccine for treatment of bovine paratuberculosis, for the first time (Koets et al., 2006). They reported that vaccine made of recombinant MAP HSP70 leads to significantly reduced shedding of bacteria into bovine fecal matter. Consequently, transmission of infection through feces is reduced. HSP taken from both *M. tuberculosis* and *Mycobacterium bovis* have been used previously in DNA based vaccines for tuberculosis successfully (Skinner et al., 2005). Mycobacterial HSP70 serve as a major T cell antigen in bovine tuberculosis (Koets et al., 2006). reduced bacterial shedding in feces, HSP70 based vaccine has a promising future application in immunization of animals. Moreover, it is inferred that mycobacterial HSP70 has a specific binding behavior towards innate receptors of macrophages and dendritic cells, which ultimately leads to production of pro-inflammatory signals. These signals result in interferon gamma (IFN-γ) production via T and NK cells. This hypothesis was supported by the observation that animals vaccinated with this vaccine exhibited higher levels of monocytes, gradually decreasing with age (Skinner et al., 2005).

Recent studies recommended that upregulation of HSP70 could be used as a potential supportive therapy in respiratory infections to protect lung endothelial barrier from bacterial toxins. The promising feature of this modality involves upregulation of HSP70 with chemicals (pharmacological agents) leading to protection against wider range of bacterial toxins and hyperoxia. Integrity of endothelial barriers is much important for health as damages to endothelial barrier can lead to mitochondrial dysfunction, production of reactive oxygen species and cell death (Li et al., 2018). This highlights the promising potential application of HSP70 in animal health sector as an effective preventive and curative strategy.

4. Association of HSP70 with performance traits in animals

Studies have revealed association of polymorphism in HSP with performance traits in animals. Genetic polymorphism in HSP70 has been found to be associated with calving traits of crossbred Brahman cows as revealed by SNP in the promoter region at 895, 1,125 and 1,128 nucleotide positions (Rosenkrans et al., 2010). Similarly, association between SNP in highly polymorphic 5′-UTR of HSP70 and post-partum anestrus (PPA) in Murrah buffaloes was determined using custom sequencing and restriction digestion analysis (Kumar et al., 2017). Among these 7 SNP including 4 transversions and 3 transitions, detected in 5′-UTR region of HSP70, the T+38G transversion revealed significant association with PPA condition in Murrah buffaloes. A restriction site is created for restriction enzyme *BglI* (5′-GCCNNNNNGGC-3′) due to transversion of T to G. Furthermore, restriction enzyme analysis revealed that animals with nucleotide sequence 5′-GCCGTTTAGGC-3′ between position +30 and + 40 showed PPA. Such genetic linkage would be helpful in replacing problematic animals from herd at an early stage because such animals suffer from PPA after first calving and are more prone to heat stress in summer season. Maintaining health of such animals is quite costly, if considered at a larger scale. The SNP identified in 5′-UTR region of HSP70 are mainly associated with milk production, thermal stress and disease vulnerability (Deb et al., 2013; Sodhi et al., 2013). Highly polymorphic variants in *HSP70* gene have been identified in Angus, Brahman and their crossbreds cows with little effect on milk protein and milk fat (Brown et al., 2010). Association of HSP variants with performance of animals mainly stems from relief achieved in terms of adverse effects of heat stress and restoration of normal physiology.
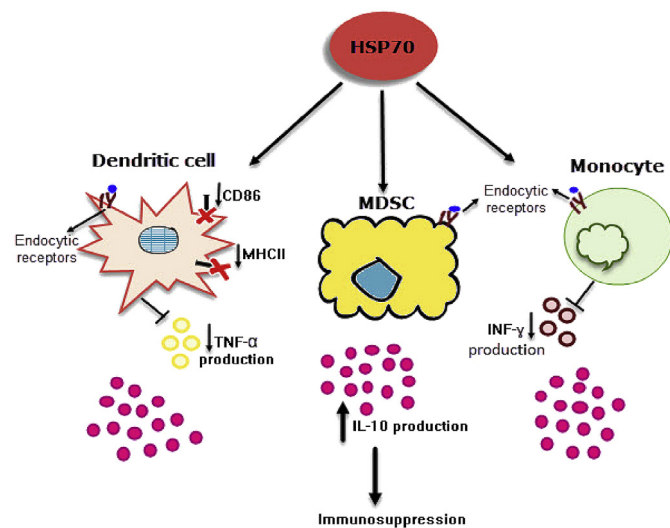

Fig. 2. Pathway for immunosuppression induced by HSP70 (Adapted from Borges et al., 2012). CD86 = dendritic cells 86, MDSC = myeloid-derived suppressor cells, MHCII = major histocompatability complex-II, TNF-α = tumour necrosis factor alpha, INF-γ = interferon gamma, IL-10 = interleukin 10.

5. Role of HSP70 in livestock under heat stress

Environmental heat stress is one of most challenging conditions in an animal's life that adversely affects not only its productive and reproductive performance but also poses threats to its survival. Despite the availability of latest technologies to control environment of livestock farms like cooling pads and mist spraying, still management of heat stress is a costly intervention for global livestock industry (St-Pierre et al., 2003). This is particularly becoming more serious concern under climate change scenario in tropics and subtropics. Animals need defensive mechanism to adapt/mitigate this stress to survive and maintain homeostasis to achieve physiological harmony. Dairy cattle are particularly more prone to heat stress as it disrupts its endocrine status (Rhoads et al., 2009; O'Brien et al., 2010; Bernabucci et al., 2014). Environment induced heat stress not only decreases milk production (35% to 40%) in dairy cows (West, 2003) but also poses health and metabolic challenges (Wheelock et al., 2010; Bernabucci et al., 2014). It is reported that ambient temperature above 35 °C initiates stress response in dairy cattle (Berman, 2005) through activation of HSF leading to increased expression of *HSP* (Collier et al., 2008). This is due to the fact that HSP function as a first line of defense to mitigate adverse effects of heat stress (Trinklein et al., 2004; Page et al., 2006). Due to this crucial ability of HSP, they are associated with thermotolerance and climatic resilience abilities of animals that make them better adapted to harsh environmental heat stress (Feder and Hofman, 1999).

The role of HSP70 to mitigate different type of stress conditions in animals is well established as it enables animals to tolerate variety of stresses like environmental stress (heat stress), heavy metal toxicity, osmotic stress (Iwama et al., 1998), as well as physical strain and physiologic stresses like oxidative stress and ischemia (Nowak et al., 1990; Iwaki et al., 1993). Many studies have revealed increased extracellular levels of HSP70 in animals under heat stress conditions (Kavanagh et al., 2011; Gaughan et al., 2013; Min et al., 2015). Furthermore, HSP70-1 and HSP70-2 have shown higher relative abundance and temperature sensitivity than other HSP70 proteins. The role of HSP70 in cellular thermotolerance and survival of animals under stress is also well established. (Beckham et al., 2004; King et al., 2002).

5.1. HSP70 as a biomarker for heat stress in animals

Onset of stress factors especially heat stress induces expression of *HSP70* in many cell types enabling animals to withstand adverse effects of heat stress. HSP70 has been categorized into constitutive and inducible form (HSP70i) which alleviates stress by their molecular chaperon activity in the cytoplasm (Nollen et al., 1999). The inducible form of HSP70 has been recognized as biomarker for cellular thermotolerance by monitoring its serum levels in animals which are significantly associated with stress tolerance ability (Li and Mak, 1989; Flanagan et al., 1995; Min et al., 2015). The expression of *HSP70i* has been observed in bovine lymphocytes (Lacetera et al., 2006; Liu et al., 2010; Mishra et al., 2011) and goat kidneys (Zulkifli et al., 2010). Variable expression of *HSP70* has been observed in caprine PBMC as elevated levels were observed in peak summer season as compared to winter in both tropical and temperate regions (Dangi et al., 2012). HSP70 not only improves overall protein integrity but also inhibits cellular apoptosis which basically protects cells from stress (Beere et al., 2000).

Role of HSP70 in neuroprotection has also been observed in many *in vitro* and *in vivo* neurodegenerative animal models (Gifondorwa et al., 2007). It has been observed that in addition to heat stress, other physical stress factors can also induce *HSP* expression, for example elevated levels of HSP70 has been observed

in renal cells of Boer goats after transportation under hot and humid conditions (Zulkifli et al., 2010). Under stress conditions, even a 200-fold increase in serum level of HSP70 has been observed in calves of Murrah buffalo (Mishra et al., 2011). Similarly, buffalo heifers have shown induction of HSP70 in response to thermal exposure simultaneously exhibiting decrease in lymphocyte proliferative response and IL-2. This revealed that HSP70 is a potential biomarker for heat stress and compromised immune status in buffalo heifers (Patir and Upadhyay, 2010).

Variations in levels of HSP70 in different animals may be potentially due to variable ability of thermotolerance among species (Agnew and Colditz, 2008). Higher expression of *HSP70* in goat PBMC under heat stress exhibited potential role of HSP70 in alleviating heat stress while maintaining cellular homeostasis (Dangi et al., 2012). Synthesis of HSP70 in rainbow trout has been observed under temperature stress (Currie and Tufts, 1997). Plasma concentration of HSP70 is strongly associated with ambient temperature but not with body temperature of animals owing to intermediate messengers that respond to changes in ambient temperature leading to increased transcription of HSP (Horowitz, 2001; Gaughan et al., 2013). However, expression of *HSP70* could be used as a useful indicator for change in body temperature when it is more than 38.6 °C. Moreover, HSP70 is suggested as a reliable biomarker of chronic stress but in case of multiple stresses, it is not a reliable indicator of single stressor (Gaughan et al., 2013). Similarly serum concentrations of HSP70 and HSF could also be used as a potential indicator of heat stress in animals with good sensitivity and accuracy (Min et al., 2015).

5.2. Cytoprotection by HSP70 in animals under heat stress

Cytoprotection mediated by HSP70 has been observed in many organs of animals such as intestine, kidney and embryo (Bhat et al., 2016). During heat stress HSP70 mediates inhibition of cytotoxic protein complexes by interacting with unfolded/misfolded (denatured) proteins leading to restore cellular protein homeostasis (Mayer and Bukau, 2005). The chaperonic activity of HSP70 is responsible for restoring normal function of stress denatured protein. It has also exhibited crucial role in cytotoxic protection as elevated expression of *HSP70* has been observed in animals under higher body temperatures, circulatory shock and cerebral ischemia during heat shock (Gaughan et al., 2010). The HSP possess chaperonic activity that mediates folding, unfolding and refolding of stress-denatured proteins. Binding of HSP70 with hydrophobic sequences of denatured proteins, prevents loss of function of these proteins by avoiding interaction of these protein with other neighbor proteins (Deb et al., 2015; Bhat et al., 2016). HSP70 stabilizes unfolded proteins and facilitates protein transportation across membranes within the cell (Borges and Ramos, 2005).

5.3. HSP70 facilitates thermal adaptation in animals

HSP70 like other HSP, is a highly conserved protein being activated by different stresses including thermal stress (Lindquist and Craig, 1998). Under heat stress, a major proportion of dietary energy is directed to sustain thermal equilibrium by maintaining normal body temperature through heat acclimatization in order to ameliorate adverse effects of stress. This heat acclimatization diverts most of the energy to heat dissipation by vasodilation of blood vessels. The major role of HSP70 under heat stress is to inhibit cellular apoptosis to prevent fatal effects of stress.

Generally HSP70 is inducible by different stress stimuli and present in all animals, but still individual animal show variable capacity to cope heat stress. This is partly due to naturally occurring nucleotide variations in the flanking regions 5′- and 3′-UTR of

HSP70. These genetic variations are responsible for differential inducibility, degree of expression and/or stability of *HSP7*0 mRNA, ultimately associated with variable tolerance of stress in individual animals (Archana et al., 2017). Moreover, a rapid increase in synthesis of lymphocyte HSP has been observed as a thermo adaptive response in different farm animals (Guerriero and Raynes, 1990). Initiation of cellular response is a primary pathway for thriving under challenging conditions of heat stress in farm animals. The end product of this crucial cellular response is synthesis and release of HSP, like HSP70, which helps animals to survive under heat stress.

6. Regulation of steroid hormone secretion

The steroid hormones play a key role in controlling metabolism, inflammation, salt and water balance etc. Major HSP involved in regulation of steroid hormone is HSP90 but, the binding of HSP90 with various steroid receptors is aided by HSP70. Moreover, HSP70 has been found to bind with steroid hormone receptors like progesterone receptor (PR) in chicken and human (Onate et al., 1991). In Chinese hamster ovary cells, HSP70 has been found to bind with over-expressed glucocorticoid receptor (GR). However, binding of HSP70 with GR has not been found in mouse L cells (Sanchez et al., 1990). HSP70 has the ability to bind with relatively hydrophobic regions of unfolded proteins in reversible pattern, during ATP dependent process (Flynn et al., 1991).

Two types of associations have been observed between chick PR and HSP70 viz., stable interaction and transient interaction. In stable interaction, purified and inactive chick PR contains HSP70 which is able to bind with elements in the receptor steroid binding domain (Kost et al., 1989). Such binding of HSP70 requires ATP and divalent cations for effective dissociation, *in vitro*. However, *in vivo* observation showed that dissociation of HSP70 from PR is rather gradual, as compared to rest of receptor-associated proteins (RAP).

During transient interaction, additional levels of HSP70 have been found in native PR assemblies using rabbit reticulocyte lysate as a medium of reconstitution. These additional HSP70 associate transiently with newly formed HSP90-PR complexes (Smith et al., 1992). The presence of relatively elevated level of HSP70 binding in nascent PR complexes was found to have a link with 60-kDa protein (p60). These observations support the hypothesis that both HSP70 and p60 fasciliate transient binding of HSP90 with receptors. Recovery of large pool of HSP90 in reticulocyte lysate and some other tissues with HSP70 and p60, also supports this evidence. Moreover, HSP90 binding was inhibited by the addition of anti-HSP70 monoclonal antibody to reticulocyte lysate. Therefore, it is concluded that both HSP70 and p60 help in formation of HSP90 complexes *in vitro*.

7. Role of HSP70 in cellular apoptosis

A variety of environmental, physical or chemical stresses can induce a molecularly regulated cell death called apoptosis. It involves a series of events which result in self-destruction of a cell. Apoptosis may have been evolved as a result of stress response or activity of HSP, to ensure effective cellular recovery. Both of these involve different mechanisms but, they functionally interact to decide whether a cell should die or live. Latest findings revealed that HSP promote survival via suppressing apoptosis. The exposure of cells to a mild hyperthermia made them less susceptible against more severe heat shock (Gerner and Schneider, 1975). Such cells were considered thermotolerant due to induced expression of HSP, mainly the HSP70 (Li and Werb, 1982). The thermotolerant cells appeared to oppose apoptosis which is mainly due to induced expression of HSP70 (Mosser and Martin, 1992). Various studies

involving mammalian cells indicated that protective effect of HSP70 is partly due to inhibition of apoptosis. HSP70 also protect cells from apoptotic stimuli such as UV radiations, DNA injury, certain chemotherapeutic agents and extraction of serum (Samali and Orrenius, 1998). However, actual mechanism of such anti-apoptotic effects remained unclear.

Apoptosis is generally characterized by certain morphological indicators such as membrane protrusion, chromatin concentration and nuclear fragmentation. These morphological changes are governed by biochemical activities including endonuclease mediated DNA fragmentation and externalization of phosphatidylserine residues. These events are usually observed in cells under apoptosis collectively termed as "apoptotic execution" which is being organized by caspases. Caspases include a family of cysteine proteases and have specificity for aspartate residues (Wolf and Green, 1999). For inhibition of apoptosis, HSP70 requires to inhibit caspase activity. Latest studies have suggested that, HSP70 possess strong apoptotic inhibitory activity and mainly acts downstream of cytochrome c release while upstream of caspase-3 activation (Beere et al., 2000; Li et al., 2000). HSP70 inhibited caspase processing and substrate fractionation, when external cytochrome c and deoxyadenosine triphosphate (dATP) were added to extracts of cells, in order to induce caspase activity (Liu et al., 1996). These findings revealed potential of HSP70 for lowering caspase activation downstream of the mitochondrial liberation of cytochrome c.

The inhibition of caspase activity by HSP70 requires an active C-terminal peptide binding domain (Beere et al., 2000). *In vitro* studies revealed inhibition of cytochrome c-mediated procaspase 9 processing by HSP70 (Mosser et al., 2000). Moreover, HSP70 can suppress activity of caspase through direct interaction with Apaf-1, intercepting the enrollment of procaspase-9 to the apoptosome complex (Beere et al., 2000; Saleh et al., 2000). However, Beere et al. (2000) observed cytochrome c/dATP-dependent self-association of Apaf-1 in presence of HSP70. They further observed that procaspase-9 and procaspase-3 were not able to associate with partially assembled aggregation of cytochrome c/dATP of Apaf-1. These findings revealed that HSP70 can mediate inhibition of heat induced apoptosis through suppression of cytochrome c release and inhibition of caspase activity (Beere et al., 2000). Moreover, HSP70 interrupt apoptotic pathway at different points through inhibition of JNK (c-Jun N-terminal protein kinase) activation, blocking cytochrome c release and interrupting apoptosome formation by binding to cytochrome c, suppression of Apaf-1 oligomerization and inhibition of procaspase enrollment (Beere and Green, 2001). Recent studies revealed greater contribution of HSP70 as compared to other HSP proteins in cell survival under apoptotic conditions (Vasaikar et al., 2015).

8. Factors affecting induction of HSP70

Heat shock is not the sole reason for induction of HSP70. Other stimuli which can cause an increase in level of HSP include; exposure to amino acid analogs (Kelley and Schlesinger, 1978), glucose analogs (Pouyssegur et al., 1977), heavy metals (Levinson et al., 1980), protein kinase C stimulators (Ding et al., 1996), calcium increasing mediators, ischemia, sodium arsenite (Levinson et al., 1980), microbial illness, nitric oxide, hormones and antibiotics (Fig. 3).

9. Potential of HSP70 as a tool for selection of thermo-tolerant animals

Extensive literature have reported strong evidence of higher expression of HSP70 during heat stress in different animals to protect against adverse effects of stress (Deb et al., 2013; Romero
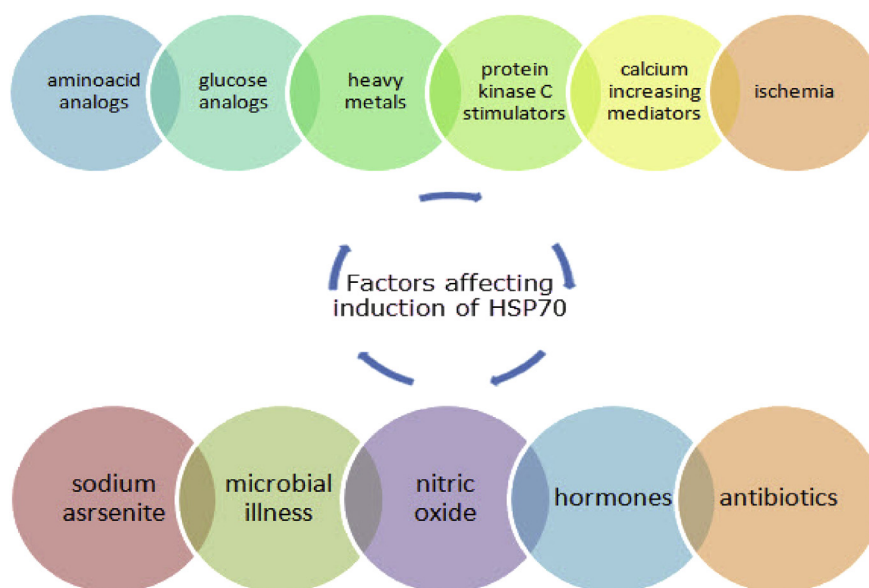
Fig. 3. Factors influencing induction of HSP70.

et al., 2013; Dangi et al., 2014; Kishore et al., 2014). This peculiar role of HSP70 family makes it a potential candidate genetic marker for selective breeding of thermo-tolerant animals. Under climate change scenario, it seems imperative to enhance thermo-tolerance of our animals to mitigate adverse effects of ever increasing global ambient temperature. One possible way to reduceimpact of heat stress on animal's productivity is to select animals with better climatic resilience. The genetic alterations for cellular thermotolerance are orchestrated by HSP. Expression of *HSP* and their transcripts give an indication of cellular response and intensity of stress in animls (Neuer et al., 2000). At transcription level, persuaded expression of *HSP70* genes is coordinated as upstream elements at promoter region directly control the expression profiles of *HSP70* (Tsukiyama et al., 1994).

It has been reported that gene expression of bovine *HSP70-1* gene is affected by SNP at promoter region which has been associated with thermotolerance and higher milk production in Frieswal cattle (Flynn et al., 1991). In swine, the 3′-UTR region of *HSP70* is associated with stability of mRNA along with stress response (Schwerin et al., 2001). Various studies have reported association of HSP with respiratory rate and body temperature under stress (Liu et al., 2011). The association of genetic variants in HSP70 promoter region with reduced productive life of bovine has also been reported (Schwerin et al., 2003). Moreover, different studies reported effect of polymorphism in promoter region of HSP70 on various reproductive variables such as, calf weaning weights, pregnancy rate and fertility in dairy animals, particularly cattle (Starkey et al., 2007; Rosenkrans et al., 2010). Conclusively, importance of SNP in promoter region of HSP70 as a reference to select dairy cattle with respect to thermos-tolerance can not be denied (Deb et al., 2014). A more recent study reported association of SNP in 5′-UTR of *HSP70* gene in cattle with number of service per conception in Pasundan cattle (Said and Putra, 2018). Studies conducted on reproductive traits revealed that expression of HSP has been associated with folliculogenesis, embryonic development and gestation (Britt, 1992; Sagirkaya et al., 2006; Wilkerson and Sarge, 2009). Various mutations detected in HSP genes has shown association with different reproductive traits in bovines (Schwerin et al., 2003; Rosenkrans et al., 2010; Basirico et al., 2011; Deb et al., 2013; Sodhi et al., 2013; Xiong et al., 2013).

Studies have been carried out to explore mutational variants at 5′-UTR of *HSP70-1* gene and their association with heat shock response of blood cells. Such variants are potential candidates for selection of better thermotolerance in cattle (Schwerin et al., 2003; Basirico et al., 2011). No doubt it is quite challenging to deduce variations in ability of animals to withstand heat stress due to diverse phenotypic and genotypic differences among breeds. But availability of latest biotechnological developments in molecular genetics, marker assisted selection and transgenesis have made possible to dissect genomic variations in animals and utilizing them for selective breeding to produce better adapted next generations. Genetic improvement of future generations in terms of thermo-tolerance is more sustainable and intelligent strategy than improving managemental aspect of animal production. Thus it is recommended that different variants of HSP70 in flanking and UTR regions may be used as genetic marker for selection of more adaptive animals which will be definitely help to improve overall performance and welfare of animals.

10. Adverse effects of HSP70 in animals

Besides numerous beneficial aspects, there are some negative aspects attributed to HSP70. It is found that uninterrupted expression of HSP70 can affect cell growth and capability. Continuous expression of *HSP70* reduces growth rate in many cell lines (Jaattela et al., 1992). Similar effect was observed in cell line of Drosophila melanogaster, in which *HSP70* expression is controlled by metallothionein promoter (Feder et al., 1992). To investigate the specific domain of HSP70 responsible for such reduction in growth, Mosser et al. (1993) compared rate of growth for each of the clone in both induced and non-induced state and concluded that cell growth was inhibited due to overexpression of HSP70 leading to increased cell death. Moreover, *HSP* have been found to act as oncogene, especially *HSP70-1* and *HSP70-2*. Overexpression of *HSP70-1* was found to be involved in causing tumorigenicity in mouse fibrosarcoma cells. Moreover, this overexpression makes these cells prone to be killed by cytotoxic T-cells and macrophages *in vitro* (Jaattela, 1995). Overexpression of *HSP70-1* within T-cells of transgenic mice causes an increase in T-cell lymphoma. Overexpression of *HSP70-2* has also been observed in breast cancer cells in human (Rohde et al., 2005). Similarly, overexpression of *HSP70* is

considered as a marker for early hepatocellular and prostate cancer. These studies show that *HSP70* can function as oncogene, if overexpressed.

11. Conclusions

It is evident from extensive literature that HSP70 are a pivotal protein family due to its dominant role in maintaining a steady cellular environment under high thermal stress in animals. Over expression of *HSP70* enables animals to mitigate adverse effects of heat stress to survive under prolonged periods of high ambient temperatures. All of functional variants of HSP70 family are involved in safeguarding functional proteins from thermal stress, packaging and folding of newly formed polypeptides, configuration and detachment of protein complexes. Their molecular chaperon nature enables them to act as molecular protector in DNA repair, apoptosis, signal transduction and protein homeostasis. HSP70 in extracellular fluids are involved in immuno-modulation through signaling of immune cells against invading pathogens by stimulating synthesis of neutrophils, macrophages and anti-inflammatory cytokines like IL-10. Nucleotide variations in 5′-UTR and promoter region of HSP70 have shown their association with various productive and reproductive traits in animals like calf weaning weights, pregnancy rate and fertility especially in dairy cattle. These highly polymorphic regions in *HSP70* genes associated with thermotolerance and performance traits make them potential candidate for marker assisted selection of animals. Under climate change scenario, selective breeding to improve future generations in terms of thermotolerance is envisioned as a more sustainable and intelligent strategy than improving managemental aspect of animal production. Moreover, serum and plasma levels of HSP70 have been recognized as a biomarker for heat stress and thermo-tolerance in animals. Therefore, HSP70 family could be used as a potential marker for selection of climate resilient animals with superior thermo-tolerance and better immune response to enhance livestock productivity globally.

Conflicts of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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