Kwaghe International
Journal of Sciences and Technology

ISSN: 1595-7977
Index: Harvard, Boston, Sydney University, Dimensions, Lens, ResearchGet Scilit, Semantic, Google Scholar, Base etc

https://doi.org/10.58578/KIJST.v3i1.9396

Pathway Analysis of the Heat Shock Protein 70 (HSP70) Gene in Some Selected Ruminants

Jacob M., Dauda A., Wafar R. J.
Federal University Wukari, Taraba State, Nigeria
m.jacob@gmail.com

Article Info:

Submitted:	Revised:	Accepted:	Published:
Feb 14, 2025	Mar 14, 2026	Mar 26, 2026	Mar 31, 2026

Abstract

Heat stress poses a major challenge to livestock production, particularly in tropical regions, by threatening animal welfare and productivity; however, pathway-level understanding of the Heat Shock Protein 70 (HSP70) gene across ruminant species remains limited. This study investigated the molecular interactions and regulatory networks of the HSP70 gene under stress conditions in three economically important ruminants—*Bos taurus*, *Ovis aries*, and *Capra hircus*. A bioinformatic pathway analysis was conducted using 34 HSP70 gene sequences retrieved from the NCBI GenBank database, comprising 13 goat, 12 cattle, and 9 sheep sequences. KEGG and STRING databases were employed for pathway enrichment and protein–protein interaction analysis, with confidence scores greater than 0.700 used to define interactions. The results showed that HSP70 functions as a central molecular hub integrating three major biological pathways: the MAPK stress response cascade, the TLR4-mediated innate immune pathway, and the apoptosis regulation pathway. A conserved chaperone cascade involving HSPA1A/HSPA1B/HSPA1L, DNAJB1, HSP90, and HSP70 was identified across all three species, indicating evolutionary conservation of thermotolerance mechanisms. Protein–protein interaction analysis further positioned HSP70 at the center of interactions with co-chaperones such as HSP90, HSP40, DNAJB1, BAG3, and STIP1, forming a coordinated multi-protein repair system. Its anti-apoptotic role was evident through inhibition of caspase activation, suppression of pro-apoptotic BAX activity, and stabilization of anti-apoptotic BCL2. Comparative analysis revealed substantial conservation of pathway architecture among the three species, with only slight variations in the heat shock gene cascade order that may reflect species-specific stress responses. This study provides a comprehensive molecular framework for understanding HSP70-mediated thermotolerance in ruminants and identifies potential genetic targets for marker-assisted selection and genomic breeding strategies to enhance climate resilience and support sustainable livestock production under changing climatic conditions.

Keywords: HSP70; Thermotolerance; Ruminants; Pathway Analysis; Heat Stress

INTRODUCTION

Heat shock proteins (HSPs) play important roles in cellular responses to various environmental stresses. Among them, the Heat Shock Protein 70 (HSP70) gene encodes a family of highly conserved molecular chaperones critical for maintaining protein homeostasis, especially under stressors such as heat, oxidative stress, and inflammation. These proteins assist in proper protein folding, inhibit aggregation of misfolded proteins, and mediate the refolding or proteasomal degradation of denatured proteins (Lindquist, 1986; Kregel, 2002). The HSP70 expression pathway is typically activated by environmental stimuli that initiate a cascade beginning with the activation of Heat Shock Factor 1 (HSF1). Upon stress, HSF1 trimerizes, translocate to the nucleus, and binds to specific DNA sequences known as Heat Shock Elements (HSEs) located in the promoter region of heat shock genes, including HSP70, thereby initiating transcription and subsequent translation of protective HSP70 proteins. These proteins then perform crucial cytoprotective functions to safeguard cellular integrity. At the organismal level, animals respond to stress with a complex interaction of behavioral, metabolic, and physiological adaptations, affecting multiple levels of biological organization, from subcellular compartments to whole-body systems (Collier and Gebremedhin, 2015). This coordinated systemic response is regulated by both the central nervous system and the peripheral nervous system, in conjunction with the endocrine system, which together orchestrate the release of stress mediators such as glucocorticoids and catecholamines (Charmandari *et al.,* 2005).

Functional genomics and bioinformatics tools have facilitated deeper insights into the regulatory networks and signaling pathways involving HSP70. Gene expression profiling, protein-protein interaction (PPI) networks, and transcription factor analyses have helped identify the molecular partners and downstream effects of HSP70 activation under stress. These analyses suggest that HSP70 interacts with key stress-related pathways such as MAPK, PI3K-Akt, and apoptosis regulation, thereby modulating immune function, antioxidant defense, and cellular repair mechanisms in livestock (Sodhi *et al.,* 2013; Singh *et al.,* 2017).

Bioinformatic pathway analysis of the HSP70 gene in ruminants involves computational approaches to understand its molecular roles, interactions, and regulatory networks under heat stress conditions (Mohapatra, *et al.,* 2022). In ruminants such as cattle (*Bos taurus*), sheep (*Ovis aries*), and goats (*Capra hircus*), HSP70 has been identified as a key indicator of thermotolerance and a potential candidate gene for climate-resilient breeding programs (Mohapatra *et al.,* 2022). Advances in bioinformatics have enabled the comprehensive analysis of gene sequences, protein structures, expression profiles, and molecular interactions, thereby offering deeper insights into the functional and regulatory pathways associated with HSP70. Through bioinformatic pathway analysis, researchers can elucidate the roles of HSP70 in various cellular processes, including its involvement in stress signaling pathways, apoptosis, immune response, and protein repair mechanisms. Tools such as KEGG, STRING, Reactome, and Gene Ontology (GO) platforms allow the integration of genomic and proteomic data to map these pathways and understand the gene's regulatory networks (Kumar *et al.,* 2021; Kanehisa *et al.,* 2023). Understanding the pathway architecture and molecular interactions of HSP70 in ruminants is crucial for identifying genetic markers and regulatory elements that can be targeted in marker-assisted selection (MAS) and genomic selection strategies. This bioinformatics-based approach thus holds promise for improving livestock adaptability to heat stress and ensuring sustainable production under changing climatic conditions (Kumaresan *et al.,* 2020).

MATERIALS AND METHODS

Experimental Animal and Sequences

A total of thirty four (34) Heat Shock Protein70 (HSP 70) gene sequences comprising thirteen goat, twelve cattle and nine sheep were obtained from the Gen Bank database National Centre of Biotechnology Information (NCBI) for this study. The sequences were undergo alignment, translation, and comparison using Cluster W, following the protocol described by Larkin *et al.* (2007), with the IUB substitution matrix, a gap opening penalty of 15, and a gap extension penalty of 6.66.

Pathway Enrichment Analysis

Pathway enrichment analysis using KEGG (Kyoto Encyclopedia of Genes and Genomes), DAVID (Database for Annotation, Visualization, and Integrated Discovery) or Reactome was carried out to understand the broader biological roles of HSP70 in thermotolerance and cellular protection mechanisms.

Pathway Analysis of HSP70 for cattle, sheep and goat

KEGG (Kyoto Encyclopedia of Genes and Genomes) Pathway Database were used to map canonical stress response pathways involving HSP70, including MAPK signaling, Apoptosis regulation, Immune modulation (Kanehisa et al., 2023). STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) Database was used to analyze protein-protein interactions, identifying HSP70 co-regulators and chaperone complexes. Confidence scores >0.700 were set for interaction analysis to ensure high-quality evidence. (Szklarczyk et al., 2021).

RESULTS AND DISCUSSION

Pathway Analysis of HSP70 Protein Gene in Cattle, Sheep and goats

Pathway analysis of HSP70 gene in goat are presented in figure 1 illustrates how HSP70 (Heat Shock Protein 70) gene functions in goats as a key protective protein during stress conditions such as heat exposure, infection, oxidative damage and metabolic imbalance. The pathway are organised into three major biological pathways: stress response (MAPK pathway), immune response, and apoptosis (cell death), with HSP70 located at the centre of all interactions. The pathway shows a chain of heat shock genes which is HSPA1B → HSPA1A → DNAJB1 → HSP90 → HSP70. These genes cooperate to produce HSP70 and activate its protective functions. Upstream kinases such as MAP2K1, MAP2K2, MAPK14 (p38) and MAPK8 (JNK) regulate this pathway. While the immune pathway proceeds as TLR4 → MYD88 → NFKB1 → IL1B → TNF → HSP70 → HSPA1A / HSPA1L. The apoptosis pathway shows how HSP70 protects goat cells fom stress-induced death. Key genes involved include CASP9 and CASP3 (executioner enzymes of apoptosis), BAX (pro-apoptotic) and BCL2 (anti-apoptotic). HSP70 interacts with these molecules to Inhibit caspase activation, Suppress BAX activity and Stabilise BCL2. At the botto onm of the figure, HSP70 is shown as a central hub interacting with other stress proteins such as HSP90, HSP40, DNAJB1, BAG3, MAPK8, BAX and STIP1. The protein STIP1 links HSP70 with HSP90, forming a coordinated chaperone system that enhances protein repair and stress adaptation.

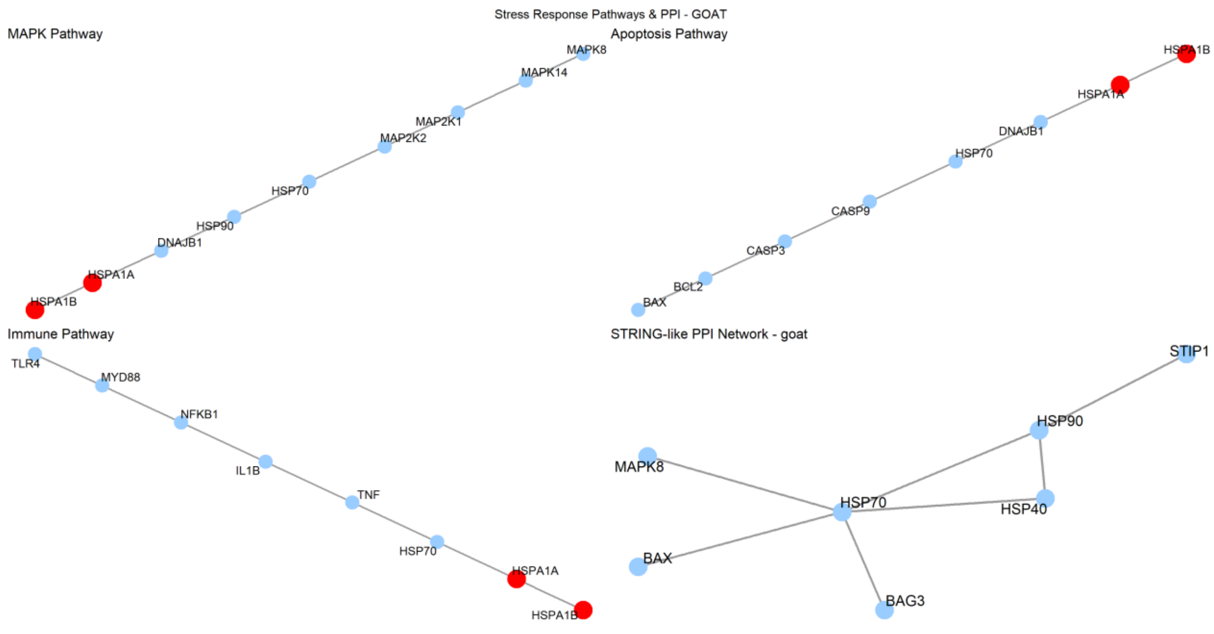

Figure 1: Pathway analysis of HSP70 in goat

Pathway Analysis of HSP70 gene in Sheep are presented in figure 2 shows how HSP70 (Heat Shock Protein 70) gene functions in sheep as a central protective gene that links stress response, immune defence and cell survival mechanisms. The pathway is divided into three main biological processes: the MAPK stress pathway, the immune signalling pathway and the apoptosis (cell death) pathway, with HSP70 acting as the connecting hub. The pathway shows a sequence of heat shock genes which are HSPA1L → HSPA1A → DNAJB1 → HSP90 → HSP70. These genes cooperate to regulate HSP70 production and activity. Upstream signalling molecules such as MAP2K1, MAP2K2, MAPK14 (p38) and MAPK8 (JNK) activate this pathway during heat stress, oxidative damage or metabolic stress. This pathway illustrates the involvement of HSP70 in innate immune defence. It begins with pathogen recognition by TLR4, followed by activation of MYD88 and the transcription factor NFKB1. This results in increased production of inflammatory cytokines IL1B and TNF. The pathway follows the sequence TLR4 → MYD88 → NFKB1 → IL1B → TNF → HSP70 → HSPA1A / HSPA1L. The apoptosis pathway highlights the protective role of HSP70 in preventing cell death. Key regulators in this pathway include CASP9 and CASP3 (which drive apoptosis), BAX (pro-apoptotic) and BCL2 (anti-apoptotic). HSP70 interacts with these molecules to Block caspase activation, Inhibit BAX activity and Stabilise BCL2. The interaction network places HSP70 at the centre of a tightly connected chaperone complex, interacting with HSP90, HSP40, DNAJB1, BAG3, MAPK8, BAX and STIP1. The protein STIP1 acts as a linker between HSP70 and HSP90, forming a coordinated stress-repair system.

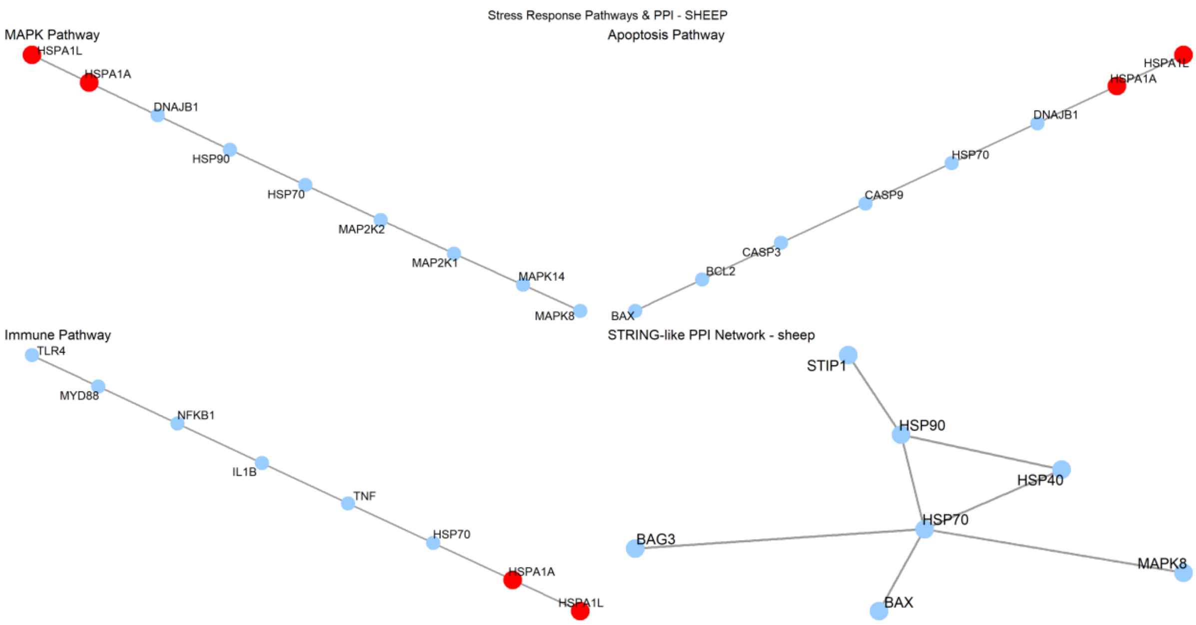

Figure 2: Pathway analysis of HSP70 in sheep

Pathway Analysis of HSP70 gene in Cattle are presented in figure 3 explains how HSP70 (Heat Shock Protein 70) gene functions in cattle as a key protective molecule that helps cells survive heat stress, disease challenge and other environmental pressures. The pathway integrates stress signalling, immune defence and apoptosis control, with HSP70 positioned at the centre of these processes. The MAPK pathway shows how cattle cells detect and respond to heat and oxidative stress. Heat shock proteins such as HSPA1A, HSPA1L, DNAJB1 and HSP90 interact sequentially to regulate the activity of HSP70. These chaperones are controlled by upstream kinases including MAP2K1, MAP2K2, MAPK14 (p38) and MAPK8 (JNK). When cattle experience heat or metabolic stress, these MAPKs are activated and stimulate the transcription of HSP70 genes. This pathway describes the role of HSP70 in cattle innate immunity. It begins with TLR4, which recognises pathogens and activates MYD88, leading to activation of the transcription factor NFKB1. This signalling cascade induces inflammatory cytokines IL1B and TNF. The immune pathway proceeds as TLR4 → MYD88 → NFKB1 → IL1B → TNF → HSP70 → HSPA1A / HSPA1L. Figure 4 also shows how HSP70 regulates apoptosis in cattle. Key genes involved are CASP9, CASP3, BAX and BCL2. HSP70 interacts with these molecules to Inhibit caspase activation (CASP9 and CASP3), Suppress the activity of BAX and Stabilise the anti-apoptotic protein BCL2. The protein–protein interaction network places HSP70 at the centre of interactions with HSP90, HSP40, DNAJB1, BAG3, MAPK8, BAX and STIP1. STIP1 serves as a connecting scaffold between HSP70 and HSP90, ensuring efficient coordination of protein repair and stress response systems.

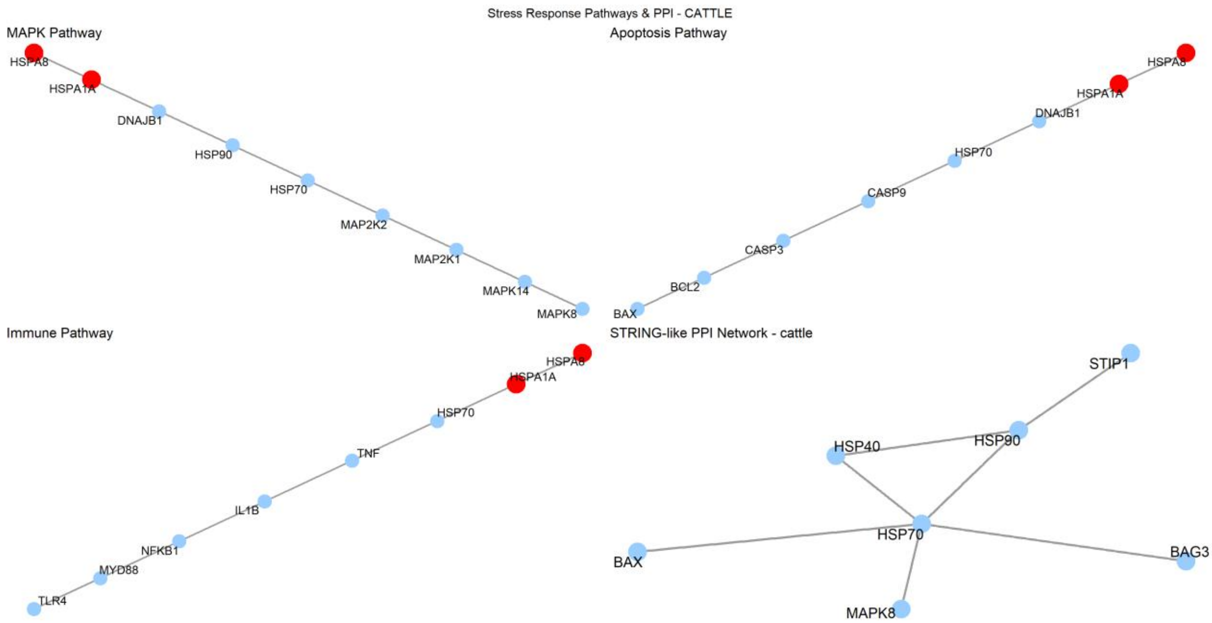

Figure 3: Pathway analysis of HSP70 in cattle.

DISCUSSION

Pathway Analysis of HSP70 Protein Gene in Cattle, Sheep and goats

The pathway analysis diagrams for HSP70 (Heat Shock Protein 70) gene in goats, sheep, and cattle reveal the central and multifunctional role of this chaperone protein in coordinating cellular responses to thermal stress, immune challenges, and apoptotic signals. The integration of stress response, immune defence, and cell survival mechanisms through HSP70 highlights its fundamental importance in livestock adaptation to environmental pressures and provides insights into the molecular basis of heat tolerance in ruminants.

The MAPK (Mitogen-Activated Protein Kinase) stress response pathway demonstrates how HSP70 expression is regulated during thermal and oxidative stress across all three species. The pathway shows a conserved signaling cascade involving upstream kinases MAP2K1, MAP2K2, MAPK14 (p38), and MAPK8 (JNK), which activate transcription of heat shock genes including HSPA1A, HSPA1B, HSPA1L, DNAJB1, and HSP90 (Kamiya *et al.,* 2016; Slimen *et al.,* 2016). The p38 MAPK and JNK pathways are critical stress-activated kinases that respond to cellular stress by phosphorylating heat shock transcription factors (HSFs), leading to enhanced HSP70 transcription (Sakurai and Enoki, 2010; Muralidharan *et al.,* 2016). Research has demonstrated that heat stress activates p38 MAPK in livestock species, resulting in rapid induction of HSP70 family members that protect cells from protein denaturation and aggregation (Banerjee *et al.,* 2021; Kumar *et al.,* 2015).

The sequential activation pattern HSPA1B → HSPA1A → DNAJB1 → HSP90 → HSP70 observed in goats represents a coordinated chaperone network where different HSP family members collaborate to maintain protein homeostasis. HSPA1A and HSPA1B are inducible forms of HSP70 that respond rapidly to stress, while HSPA1L represents a constitutively expressed variant (Radons, 2016). DNAJB1, also known as HSP40, functions as a co-chaperone that stimulates the ATPase activity of HSP70 and delivers client proteins for refolding (Kampinga and Craig, 2010). The cooperation between HSP70 and HSP90 creates a multichaperone complex capable of handling diverse substrates and stress conditions, with HSP90 typically acting on more specialized clients including steroid receptors and signaling kinases (Mayer and Bukau, 2005; Schopf *et al.,* 2017).

The immune response pathway demonstrates HSP70's critical role in innate immunity through the TLR4 → MYD88 → NFKB1 → IL1B → TNF cascade. Toll-like receptor 4 (TLR4) recognizes pathogen-associated molecular patterns and activates the adaptor protein MYD88, which triggers nuclear factor kappa B (NF-κB) signaling (Tao *et al.,* 2015). This results in production of pro-inflammatory cytokines including interleukin-1 beta (IL1B) and tumor necrosis factor alpha (TNF), which are key mediators of acute phase responses and fever (Archana *et al.,* 2017; Collier *et al.,* 2019). HSP70 plays a dual role in this pathway: it is induced by inflammatory signals and simultaneously modulates immune responses by regulating cytokine production and preventing excessive inflammation (Gupta and Knowlton, 2007; Asea *et al.,* 2000).

Studies in heat-stressed livestock have shown that elevated HSP70 expression correlates with reduced inflammatory cytokine levels, suggesting a protective feedback mechanism where HSP70 dampens excessive immune activation that could otherwise cause tissue damage (Dangi *et al.,* 2012; Singh *et al.,* 2020). The connection between HSP70 and inflammatory pathways is particularly important in ruminants, where heat stress can compromise intestinal barrier function and increase systemic inflammation through translocation of bacterial endotoxins that activate TLR4 (Pearce *et al.,* 2013; Koch *et al.,* 2019).

The apoptosis pathway reveals HSP70's anti-apoptotic functions through interactions with key regulators of programmed cell death. HSP70 inhibits apoptosis at multiple points by blocking caspase activation, suppressing pro-apoptotic BAX activity, and stabilizing anti-apoptotic BCL2 (Beere *et al.,* 2000; Ravagnan *et al.,* 2001). Caspase-9 (CASP9) is an initiator caspase activated by cytochrome c release from mitochondria, while caspase-3 (CASP3) is the primary executioner caspase that cleaves cellular substrates during apoptosis (Li and Yuan, 2008). HSP70 can directly bind to and inhibit both caspases, preventing apoptotic cascade progression (Saleh *et al.,* 2000; Parcellier *et al.,* 2003).

The interaction between HSP70 and BAX is particularly significant, as BAX is a pro-apoptotic BCL2 family member that forms pores in mitochondrial membranes, triggering cytochrome c release and apoptosome formation (Antonsson *et al.,* 2000). HSP70 prevents BAX translocation to mitochondria and oligomerization, thereby blocking the intrinsic apoptotic pathway (Stankiewicz *et al.,* 2005). Simultaneously, HSP70 stabilizes BCL2, an anti-apoptotic protein that sequesters pro-apoptotic BCL2 family members and maintains mitochondrial integrity (Pandey *et al.,* 2000; Matsumori *et al.,* 2006). These anti-apoptotic functions are crucial during heat stress, when cellular damage and oxidative stress can trigger widespread apoptosis in sensitive tissues (Slimen *et al.,* 2016; Rhoads *et al.,* 2013).

The protein-protein interaction network placing HSP70 at the center of interactions with HSP90, HSP40, DNAJB1, BAG3, MAPK8, BAX, and STIP1 illustrates the integrative nature of the cellular stress response. BAG3 (BCL2-associated athanogene 3) is a co-chaperone that modulates HSP70 activity and links the chaperone system to autophagy pathways, providing an alternative mechanism for removing damaged proteins and organelles during stress (Behl, 2016; Knezevic and Knezevic, 2018). The interaction between HSP70 and MAPK8 (JNK) represents a feedback mechanism where HSP70 can modulate stress kinase activity, potentially dampening prolonged JNK activation that could otherwise promote apoptosis (Gabai *et al.,* 1998; Park *et al.,* 2001).

STIP1 (stress-induced phosphoprotein 1), also known as Hop (HSP70-HSP90 organizing protein), serves as a crucial adaptor linking HSP70 and HSP90 in a sequential chaperone system (Odunuga *et al.,* 2004; Prodromou *et al.,* 1999). This coordinated system allows for progressive protein folding and maturation, with HSP70 initially binding unfolded clients and STIP1 facilitating transfer to HSP90 for final maturation steps (Scheufler *et al.,* 2000). The HSP70-STIP1-HSP90 complex is particularly important for folding of complex substrates including signaling kinases and transcription factors that regulate stress adaptation (Li *et al.,* 2012).

Comparative analysis across the three species (goats, sheep, and cattle) reveals remarkable conservation of HSP70 pathway architecture, consistent with the phylogenetic conservation observed in sequence analysis. The similar pathway structures suggest that fundamental mechanisms of thermal adaptation are shared among ruminants, although species-specific differences in expression levels, regulation kinetics, or pathway sensitivity may contribute to observed differences in heat tolerance (Sejian *et al.,* 2018). The slight variation in the heat shock gene cascade order between goats (HSPA1B → HSPA1A) and sheep (HSPA1L → HSPA1A) may reflect differences in which HSP70 family members are most responsive to heat stress in each species (Rout *et al.,* 2017).

The central positioning of HSP70 in these pathways has important implications for understanding heat stress adaptation in livestock. HSP70 serves as a molecular hub integrating information from stress sensors (MAPK pathway), immune signals (TLR4/NF-κB pathway), and survival signals (apoptosis pathway), allowing coordinated cellular responses to multiple simultaneous challenges (Qi *et al.,* 2011). This integrative function explains why HSP70 expression levels correlate strongly with thermotolerance across livestock breeds and species (Deb *et al.,* 2014; Porto-Neto *et al.,* 2014).

From a practical breeding perspective, the pathway analysis identifies multiple potential targets for enhancing heat tolerance through genetic selection. Beyond HSP70 itself, upstream regulators (MAPK components, HSF transcription factors), co-chaperones (DNAJB1, BAG3, STIP1), and downstream effectors (apoptosis regulators, inflammatory mediators) all represent candidates for marker-assisted selection or genomic editing approaches (Collier *et al.,* 2019; Hooper *et al.,* 2015). Understanding these pathway interactions also suggests potential management interventions, such as nutritional strategies or pharmacological agents that could enhance HSP70 activity during heat stress (Min *et al.,* 2016).

The pathway diagrams also highlight the complex crosstalk between stress response, immunity, and cell survival, which has implications for understanding trade-offs between production and stress tolerance in livestock. Enhanced immune activation during heat stress requires metabolic resources that could otherwise support growth and reproduction, while excessive apoptosis can compromise tissue function and productivity (Baumgard and Rhoads, 2013). The regulatory role of HSP70 in modulating all three pathways suggests it may help optimize these trade-offs by preventing excessive inflammation and apoptosis while maintaining cellular repair capacity (Rhoads *et al.,* 2013; Wheelock *et al.,* 2010).

Future research should focus on validating these pathway interactions through functional genomics approaches including gene knockdown/knockout studies, pathway inhibition experiments, and systems biology analyses integrating transcriptomic, proteomic, and metabolomic data from heat-stressed animals (Polsky and von Keyserlingk, 2017; Zachut *et al.,* 2020). Understanding the quantitative dynamics of these pathways including activation kinetics, feedback loops, and threshold responses will be crucial for predicting animal responses to heat stress and designing effective mitigation strategies (Kaufman *et al.,* 2018).

CONCLUSION

This study provides a comprehensive bioinformatic pathway analysis of the Heat Shock Protein 70 (HSP70) gene in selected ruminant species cattle (*Bos taurus*), sheep (*Ovis aries*), and goats (*Capra hircus*) with emphasis on its molecular interactions and regulatory networks under heat stress conditions. The findings clearly demonstrate that HSP70 functions as a central molecular hub integrating stress signaling, immune modulation, and apoptosis regulation in all three species.

The KEGG and STRING pathway analyses revealed that HSP70 operates within a highly conserved MAPK signaling cascade involving upstream kinases such as MAP2K1, MAP2K2, MAPK14 (p38), and MAPK8 (JNK), which regulate its transcription during thermal and oxidative stress. The conserved chaperone cascade (HSPA1A, HSPA1B/HSPA1L, DNAJB1, HSP90) highlights a coordinated multi-protein repair system responsible for maintaining protein homeostasis. This confirms the evolutionary conservation of thermotolerance mechanisms among ruminants.

The immune pathway analysis (TLR4 → MYD88 → NFKB1 → IL1B → TNF → HSP70) further establishes the dual role of HSP70 in both responding to inflammatory stimuli and modulating excessive immune activation. Its regulatory involvement suggests that HSP70 contributes significantly to maintaining immune balance during heat stress, thereby preventing systemic inflammatory damage. The apoptosis pathway findings demonstrate that HSP70 exerts strong anti-apoptotic effects by inhibiting CASP9 and CASP3 activation, suppressing BAX activity, and stabilizing BCL2. These interactions protect cellular integrity during environmental stress, reducing heat-induced tissue damage and supporting survival.

REFERENCES

Antonsson, B., Montessuit, S., Sanchez, B., & Martinou, J. C. (2001). Bax is present as a high molecular weight oligomer/complex in the mitochondrial membrane of apoptotic cells. *Journal of Biological Chemistry, 276*(15), 11615–11623. https://doi.org/10.1074/jbc.M010810200

Archana, P. R., Aleena, J., Pragna, P., Vidya, M. K., Niyas, A. P. A., Bagath, M., Krishnan, G., Manimaran, A., Beena, V., Kurien, E. K., Varma, G., & Bhatta, R. (2017). Role of heat shock proteins in livestock adaptation to heat stress. *Journal of Dairy, Veterinary & Animal Research, 5*(1), 00127. https://doi.org/10.15406/jdvar.2017.05.00127

Asea, A., Rehli, M., Kabingu, E., Boch, J. A., Bare, O., Auron, P. E., Stevenson, M. A., & Calderwood, S. K. (2002). Novel signal transduction pathway utilized by extracellular HSP70: Role of Toll-like receptor (TLR) 2 and TLR4. *Journal of Biological Chemistry, 277*(17), 15028–15034. https://doi.org/10.1074/jbc.M200497200

Banerjee, D., Upadhyay, R. C., Chaudhary, U. B., Kumar, R., Singh, S., Ashutosh, Polley, S., Mukherjee, A., Das, T. K., & De, S. (2021). Seasonal variation in expression pattern of genes under HSP70 family in heat- and cold-adapted goats (*Capra hircus*). *Cell Stress and Chaperones, 26*(3), 621–634. https://doi.org/10.1007/s12192-021-01211-9

Baumgard, L. H., & Rhoads, R. P. (2013). Effects of heat stress on postabsorptive metabolism and energetics. *Annual Review of Animal Biosciences, 1*, 311–337. https://doi.org/10.1146/annurev-animal-031412-103644

Beere, H. M., Wolf, B. B., Cain, K., Mosser, D. D., Mahboubi, A., Kuwana, T., Tailor, P., Morimoto, R. I., Cohen, G. M., & Green, D. R. (2000). Heat-shock protein 70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome. *Nature Cell Biology, 2*(8), 469–475. https://doi.org/10.1038/35019501

Behl, C. (2016). Breaking BAG: The co-chaperone BAG3 in health and disease. *Trends in Pharmacological Sciences, 37*(8), 672–688. https://doi.org/10.1016/j.tips.2016.04.007

Charmandari, E., Tsigos, C., & Chrousos, G. (2005). Endocrinology of the stress response. *Annual Review of Physiology, 67*, 259–284. https://doi.org/10.1146/annurev.physiol.67.040403.120816

Collier, R. J., Baumgard, L. H., Zimbelman, R. B., & Xiao, Y. (2019). Heat stress: Physiology of acclimation and adaptation. *Animal Frontiers, 9*(1), 12–19. https://doi.org/10.1093/af/vfy031

Collier, R. J., Collier, J. L., Rhoads, R. P., & Baumgard, L. H. (2008). Invited review: Genes involved in the bovine heat stress response. *Journal of Dairy Science, 91*(2), 445–454. https://doi.org/10.3168/jds.2007-0540

Collier, R. J., & Gebremedhin, K. G. (2015). Thermal biology of domestic animals. *Annual Review of Animal Biosciences, 3*, 513–532. https://doi.org/10.1146/annurev-animal-022114-110659

Dangi, S. S., Gupta, M., Maurya, D., Yadav, V. P., Panda, R. P., Singh, G., Mohan, N. H., Bhure, S. K., Das, B. C., Bag, S., Mahapatra, R. K., Sharma, G. T., & Sarkar, M. (2012). Expression profile of HSP genes during different seasons in goats (*Capra hircus*). *Tropical Animal Health and Production, 44*(8), 1905–1912. https://doi.org/10.1007/s11250-012-0155-8

Deb, R., Sajjanar, B., Singh, U., Kumar, S., Singh, R., Sengar, G., & Sharma, A. (2014). Effect of heat stress on the expression profile of Hsp90 among Sahiwal (*Bos indicus*) and Frieswal (*Bos indicus* × *Bos taurus*) breed of cattle: A comparative study. *Gene, 536*(2), 435–440. https://doi.org/10.1016/j.gene.2013.11.086

Gabai, V. L., Meriin, A. B., Mosser, D. D., Caron, A. W., Rits, S., Shifrin, V. I., & Sherman, M. Y. (1997). Hsp70 prevents activation of stress kinases: A novel pathway of cellular thermotolerance. *Journal of Biological Chemistry, 272*(29), 18033–18037. https://doi.org/10.1074/jbc.272.29.18033

Gupta, S., & Knowlton, A. A. (2007). HSP60 trafficking in adult cardiac myocytes: Role of the exosomal pathway. *American Journal of Physiology-Heart and Circulatory Physiology, 292*(6), H3052–H3056. https://doi.org/10.1152/ajpheart.01355.2006

Hooper, H. B., Silva, P. P., de Oliveira, S. A., Merighe, G. K., & Mello Souza Alves, J. A. (2015). Trial of heat shock protein (Hsp) 70 gene expression in Nelore and Caracu cattle. *African Journal of Agricultural Research, 10*(18), 2004–2011. https://doi.org/10.5897/AJAR2015.9578

Kamiya, Y., Mizuno, T., Komenoi, S., Ogawa, H., & Ando, H. (2016). Activation of conventional and novel protein kinase C isozymes by different pathways. *Biochemical Journal, 473*(18), 2749–2758. https://doi.org/10.1042/BCJ20160603

Kampinga, H. H., & Craig, E. A. (2010). The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nature Reviews Molecular Cell Biology, 11*(8), 579–592. https://doi.org/10.1038/nrm2941

Kanehisa, M., Furumichi, M., Sato, Y., Kawashima, M., & Ishiguro-Watanabe, M. (2023). KEGG for taxonomy-based analysis of pathways and genomes. *Nucleic Acids Research, 51*(D1), D587–D592. https://doi.org/10.1093/nar/gkac963

Kaufman, J. D., Saxton, A. M., & Ríus, A. G. (2018). Short communication: Relationships among temperature-humidity index with rectal, udder surface, and vaginal temperatures in lactating dairy cows experiencing heat stress. *Journal of Dairy Science, 101*(7), 6424–6429. https://doi.org/10.3168/jds.2017-13799

Knezevic, T., & Knezevic, N. (2018). BAG3: A new player in the pathogenesis of heart failure? *Heart Failure Reviews, 23*(6), 909–920. https://doi.org/10.1007/s10741-018-9704-6

Koch, F., Thom, U., Albrecht, E., Weikard, R., Nolte, W., Kuhla, B., & Kuehn, C. (2019). Heat stress directly impairs gut integrity and recruits distinct immune cell populations into the bovine intestine. *Proceedings of the National Academy of Sciences of the United States of America, 116*(21), 10333–10338. https://doi.org/10.1073/pnas.1820130116

Kregel, K. C. (2002). Heat shock proteins: Modifying factors in physiological stress responses and acquired thermotolerance. *Journal of Applied Physiology, 92*(5), 2177–2186. https://doi.org/10.1152/japplphysiol.01267.2001

Kumar, A., Ashraf, S., Goud, T. S., Grewal, A., Singh, S. V., Yadav, B. R., & Upadhyay, R. C. (2015). Expression profiling of major heat shock protein genes during different seasons in cattle (*Bos indicus*) and buffalo (*Bubalus bubalis*) under tropical climatic condition. *Journal of Thermal Biology, 51*, 55–64. https://doi.org/10.1016/j.jtherbio.2015.03.006

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution, 35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Kumaresan, A., Ramesha, K. P., & Sinha, S. (2020). Heat stress and livestock adaptation: Role of heat shock proteins. *Indian Journal of Animal Sciences, 90*(5), 615–622.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J., & Higgins, D. G. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics, 23*(21), 2947–2948. https://doi.org/10.1093/bioinformatics/btm404

Li, J., & Yuan, J. (2008). Caspases in apoptosis and beyond. *Oncogene, 27*(48), 6194–6206. https://doi.org/10.1038/onc.2008.297

Li, J., Soroka, J., & Buchner, J. (2012). The Hsp90 chaperone machinery: Conformational dynamics and regulation by co-chaperones. *Biochimica et Biophysica Acta, 1823*(3), 624–635. https://doi.org/10.1016/j.bbamcr.2011.09.003

Lindquist, S. (1986). The heat-shock response. *Annual Review of Biochemistry, 55*, 1151–1191. https://doi.org/10.1146/annurev.bi.55.070186.005443

Matsumori, Y., Hong, S. M., Aoyama, K., Fan, Y., Kayama, T., Sheldon, R. A., Vexler, Z. S., Ferriero, D. M., Weinstein, P. R., & Liu, J. (2005). Hsp70 overexpression sequesters AIF and reduces neonatal hypoxic/ischemic brain injury. *Journal of Cerebral Blood Flow & Metabolism, 25*(7), 899–910. https://doi.org/10.1038/sj.jcbfm.9600080

Mayer, M. P., & Bukau, B. (2005). Hsp70 chaperones: Cellular functions and molecular mechanism. *Cellular and Molecular Life Sciences, 62*(6), 670–684. https://doi.org/10.1007/s00018-004-4464-6

Miller, L., & Qureshi, M. A. (1992). Heat-shock protein synthesis in chicken macrophages: Influence of in vivo and in vitro heat shock, lead acetate, and lipopolysaccharide. *Poultry Science, 71*(6), 988–998. https://doi.org/10.3382/ps.0710988

Min, L., Cheng, J., Zhao, S., Tian, H., Zhang, Y., Li, S., Yang, H., Zheng, N., & Wang, J. (2016). Plasma-based proteomics reveals immune response, complement and coagulation cascades pathway shifts in heat-stressed lactating dairy cows. *Journal of Proteomics, 146*, 99–108. https://doi.org/10.1016/j.jprot.2016.06.017

Mohapatra, A., Yadav, B., & Singh, G. (2022). Functional annotation and gene ontology analysis of thermotolerant genes in ruminants: A bioinformatics perspective. *Theriogenology Insight, 12*(1), 34–46.

Muralidharan, S., Hanley, P. J., Liu, E., Chakraborty, R., Bollard, C., Shpall, E., Rooney, C., Savoldo, B., Rodgers, J., & Dotti, G. (2011). Activation of Wnt signaling arrests effector differentiation in human peripheral and cord blood-derived T lymphocytes. *Journal of Immunology, 187*(10), 5221–5232. https://doi.org/10.4049/jimmunol.1101585

Odunuga, O. O., Longshaw, V. M., & Blatch, G. L. (2004). Hop: More than an Hsp70/Hsp90 adaptor protein. *BioEssays, 26*(10), 1058–1068. https://doi.org/10.1002/bies.20107

Pandey, P., Saleh, A., Nakazawa, A., Kumar, S., Srinivasula, S. M., Kumar, V., Weichselbaum, R., Nalin, C., Alnemri, E. S., Kufe, D., & Kharbanda, S. (2000). Negative regulation of cytochrome c-mediated oligomerization of Apaf-1 and activation of procaspase-9 by heat shock protein 90. *EMBO Journal, 19*(16), 4310–4322. https://doi.org/10.1093/emboj/19.16.4310

Parcellier, A., Gurbuxani, S., Schmitt, E., Solary, E., & Garrido, C. (2003). Heat shock proteins, cellular chaperones that modulate mitochondrial cell death pathways. *Biochemical and Biophysical Research Communications, 304*(3), 505–512. https://doi.org/10.1016/S0006-291X(03)00623-5

Park, H. S., Lee, J. S., Huh, S. H., Seo, J. S., & Choi, E. J. (2001). Hsp72 functions as a natural inhibitory protein of c-Jun N-terminal kinase. *EMBO Journal, 20*(3), 446–456. https://doi.org/10.1093/emboj/20.3.446

Pearce, S. C., Mani, V., Boddicker, R. L., Johnson, J. S., Weber, T. E., Ross, J. W., Rhoads, R. P., Baumgard, L. H., & Gabler, N. K. (2013). Heat stress reduces intestinal barrier integrity and favors intestinal glucose transport in growing pigs. *PLoS ONE, 8*(8), e70215. https://doi.org/10.1371/journal.pone.0070215

Polsky, L., & von Keyserlingk, M. A. G. (2017). Invited review: Effects of heat stress on dairy cattle welfare. *Journal of Dairy Science, 100*(11), 8645–8657. https://doi.org/10.3168/jds.2017-12651

Porto-Neto, L. R., Reverter, A., Prayaga, K. C., Chan, E. K. F., Johnston, D. J., Hawken, R. J., Fordyce, G., Garcia, J. F., Sonstegard, T. S., Bolormaa, S., Goddard, M. E., Burrow, H. M., Henschel, J. M., James, A. S., & Barendse, W. (2014). The genetic architecture of climatic adaptation of tropical cattle. *PLoS ONE, 9*(11), e113284. https://doi.org/10.1371/journal.pone.0113284

Prodromou, C., Panaretou, B., Chohan, S., Siligardi, G., O'Brien, R., Ladbury, J. E., Roe, S. M., Piper, P. W., & Pearl, L. H. (1999). The ATPase cycle of Hsp90 drives a molecular 'clamp' via transient dimerization of the N-terminal domains. *EMBO Journal, 19*(16), 4383–4392. https://doi.org/10.1093/emboj/19.16.4383

Radons, J. (2016). The human HSP70 family of chaperones: Where do we stand? *Cell Stress and Chaperones, 21*(3), 379–404. https://doi.org/10.1007/s12192-016-0676-6

Ravagnan, L., Gurbuxani, S., Susin, S. A., Maisse, C., Daugas, E., Zamzami, N., Mak, T., Jäättelä, M., Penninger, J. M., Garrido, C., & Kroemer, G. (2001). Heat-shock protein 70 antagonizes apoptosis-inducing factor. *Nature Cell Biology, 3*(9), 839–843. https://doi.org/10.1038/ncb0901-839

Rhoads, R. P., Baumgard, L. H., Suagee, J. K., & Sanders, S. R. (2013). Nutritional interventions to alleviate the negative consequences of heat stress. *Advances in Nutrition, 4*(3), 267–276. https://doi.org/10.3945/an.112.003376

Rout, P. K., Kaushik, R., & Ramachandran, N. (2017). Differential expression pattern of heat shock protein 70 gene in tissues and developmental stages in Osmanabadi goat. *Cell Stress and Chaperones, 22*(3), 389–395. https://doi.org/10.1007/s12192-017-0778-4

Sakurai, H., & Enoki, Y. (2010). Novel aspects of heat shock factors: DNA recognition, chromatin modulation and gene expression. *FEBS Journal, 277*(20), 4140–4149. https://doi.org/10.1111/j.1742-4658.2010.07829.x

Saleh, A., Srinivasula, S. M., Balkir, L., Robbins, P. D., & Alnemri, E. S. (2000). Negative regulation of the Apaf-1 apoptosome by Hsp70. *Nature Cell Biology, 2*(8), 476–483. https://doi.org/10.1038/35019510

Scheufler, C., Brinker, A., Bourenkov, G., Pegoraro, S., Moroder, L., Bartunik, H., Hartl, F. U., & Moarefi, I. (2000). Structure of TPR domain-peptide complexes: Critical elements in the assembly of the Hsp70-Hsp90 multichaperone machine. *Cell, 101*(2), 199–210. https://doi.org/10.1016/S0092-8674(00)80830-2

Schopf, F. H., Biebl, M. M., & Buchner, J. (2017). The HSP90 chaperone machinery. *Nature Reviews Molecular Cell Biology, 18*(6), 345–360. https://doi.org/10.1038/nrm.2017.20

Sejian, V., Bhatta, R., Gaughan, J. B., Dunshea, F. R., & Lacetera, N. (2018). Review: Adaptation of animals to heat stress. *Animal, 12*(S2), s431–s444. https://doi.org/10.1017/S1751731118001945

Singh, K. M., Singh, S., Ganguly, I., Nachiappan, R. K., Ganguly, A., Venkataramanan, R., Chopra, A., & Narula, H. K. (2017). Heat shock protein genes and their role in livestock adaptation to heat stress. *Journal of Thermal Biology, 67*, 1–8. https://doi.org/10.1016/j.jtherbio.2017.04.006

Singh, S., Hooda, O. K., Kundu, S. S., Kirar, V., & Nanda, V. (2020). Heat stress and thermoregulation: Molecular mechanisms in ruminants. In V. Sejian, J. Gaughan, L. Baumgard, & C. Prasad (Eds.), *Climate change and livestock production: Recent advances and future perspectives* (pp. 55–66). Springer. https://doi.org/10.1007/978-981-15-1964-5_5

Slimen, I. B., Najar, T., Ghram, A., & Abdrrabba, M. (2016). Heat stress effects on livestock: Molecular, cellular and metabolic aspects, a review. *Journal of Animal Physiology and Animal Nutrition, 100*(3), 401–412. https://doi.org/10.1111/jpn.12379

Sodhi, M., Mukesh, M., Kishore, A., Mishra, B. P., Kataria, R. S., & Joshi, B. K. (2013). Comparative expression profile of HSP70 gene in native cattle breeds exposed to heat stress. *Molecular Biology Reports, 40*, 6699–6706.

Sodhi, M., Mukesh, M., Kishore, A., Mishra, B. P., Kataria, R. S., & Joshi, B. K. (2013). Structural and functional characterization of molecular variants of bovine HSP70.1 gene. *Molecular Biology Reports, 40*(3), 2019–2029. https://doi.org/10.1007/s11033-012-2264-7

Stankiewicz, A. R., Lachapelle, G., Foo, C. P., Radicioni, S. M., & Mosser, D. D. (2005). Hsp70 inhibits heat-induced apoptosis upstream of mitochondria by preventing Bax translocation. *Journal of Biological Chemistry, 280*(46), 38729–38739. https://doi.org/10.1074/jbc.M509497200

Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., Simonovic, M., Doncheva, N. T., Morris, J. H., Bork, P., Jensen, L. J., & von Mering, C. (2019). STRING v11: Protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Research, 47*(D1), D607–D613. https://doi.org/10.1093/nar/gky1131

Tao, X., Xu, Z., Men, X., Xu, L., & Zhang, Z. (2015). Histopathological changes and expression of heat shock protein 70 and glucose-regulated protein 94 in goat lungs infected with *Pasteurella multocida*. *Journal of Comparative Pathology, 153*(4), 196–205. https://doi.org/10.1016/j.jcpa.2015.08.003

Wheelock, J. B., Rhoads, R. P., VanBaale, M. J., Sanders, S. R., & Baumgard, L. H. (2010). Effects of heat stress on energetic metabolism in lactating Holstein cows. *Journal of Dairy Science, 93*(2), 644–655. https://doi.org/10.3168/jds.2009-2295

Zachut, M., Kra, G., Livshitz, L., Portnick, Y., Yakoby, S., Friedlander, G., & Levin, Y. (2017). Seasonal heat stress affects adipose tissue proteome toward enrichment of the Nrf2-mediated oxidative stress response in late-pregnant dairy cows. *Journal of Proteomics, 158*, 52–61. https://doi.org/10.1016/j.jprot.2017.02.011