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Approaches using genome-wide association studies and heat stress resistance related genes in chicken – a review

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Heat stress-induced reduction in feed intake in an annoyance of the poultry industry. High environmental temperature is a serious stress affecting economic and biological efficiency of poultry production in tropical and subtropical regions countries that are expected to become more prominent with global climate change. An effective tool for improving heat tolerance can be genomic selection based on single nucleotide polymorphisms. In the past few years, new molecular tools have been developed, such as RNA-Seq, Single Nucleotide Polymorphisms (SNPs), and bioinformatics approaches such as Genome-Wide Association Study (GWAS). Based on these genetic tools, many studies have detected the main pathways involved in cellular response mechanisms. This study aims to present   chicken genomic regions controlling survival against heat stress. Genetic studies conducted on experimental lines can therefore be of potential interest for marker-assisted selection in commercial lines. Also, survivability to high temperature might be due to the SNP markers that are located inside or close to the genes such as CEP78, MEF2C, VPS13A, ARRDC3, and which had relatively biological pathways in heat shock resistance.

KEYWORDS: Heat stress / GWAS

Heat stress is one of the most important environmental stress factors in poultry production worldwide. Chronic heat stress can lead to changes in poultry physiology and affect production. Heat stress is a response to an increase in body temperature,

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and as one of the most important influencing factors, it causes a decrease in growth performance and the immune system, and as a result, increases in casualties [Bottje and Harrison 1985]. All categories of poultry experience heat stress along with relative humidity and temperature range above the comfort point. The increase of these two variables reduces the ability of the bird to dissipate heat [Belay and Teeter 1996]. Due to a higher metabolic rate, they produce more body heat and are prone to heat stress. High stocking density of birds, along with the high ambient temperature, increases the propensity of heat stress [Goo *et al*. 2019]. The normal body temperature of the chicken is around 41-42, and the thermoneutral temperature to maximize growth is between 18-21 [Kumari and Nath 2018]. Studies have shown that any environmental temperature higher than 25 elicits heat stress in poultry [Donkoh 1989]. Understanding the genetic mechanism and the genetic architecture of the trait and the sensitivity and resistance to heat stress makes it possible to produce commercial lines with the special trait of natural adaptation to hot climate and stress [Quinteiro-Filho *et al*. 2010]. The starting point for understanding heat stress responses is to identify genes whose expression is regulated by high temperature. High-throughput technologies have made it possible to examine gene expression on a large scale.

Sign of heat stress

The signs of heat stress in poultry are panting with open beak, elevated their wings and squatting near to the ground, droopy acting, slowness and lethargic closed eyes, lying down, increased water intake, decreased appetite, drop in egg production, reduced egg size, poor egg shell quality, reduced body weight, and increased cannibalism [Nardone *et al*. 2010, Dayyani and Bakhtiari 2013]. Birds are trying to lose heat by gasping and changing the position of their feathers, losing water in their breath and cooling by evaporation through the surface of the lungs. Birds are facing heat stress conditions, they spend less feeding time during feeding, more time drinking and panting, less time moving or walking and more time resting [Mack 2013].

Molecular method by Marker-Assisted Selection

Marker-assisted selection (MAS) is a breeding technique used in agriculture and genetics to improve the efficiency and precision of selecting desirable traits in plants, animals, and other organisms [Wang *et al*. 2015]. MAS combines traditional breeding methods with molecular markers, which are specific sequences of DNA that can be easily detected and associated with particular traits or characteristics. By utilizing MAS in selecting heat tolerance genes inchicken, breeders can more effectively and efficiently identify individuals with desirable heat tolerance traits [Van Goor *et al.* 2015, Asadollahpour Nanaei 2022]. This targeted selection based on genetic markers enables breeders to improve heat tolerance in poultry populations more rapidly than relying solely on phenotypic evaluations [Dekkers and Hospital 2002]. Ultimately,

this can contribute to developing poultry breeds better adapted to withstand high-temperature environments, resulting in improved productivity, health, and welfare in heat-stressed conditions. At present, many genes are involved with heat stress effects (Tab. 1), which can be divided into two types: (1) directly controlled genes, namely, the heat shock factor (HSF) and heat shock protein (HSP) gene families; these work together to help reduce protein folding and are involved in important cellular defense mechanisms during exposure to hot environments [Cedraz *et al*. 2017]. Most of the major regulatory genes, HSF1 and HSF3, are involved in the regulation of HSPs; HSP27, HSP60, HSP70, and HSP90 are classified according to their molecular weights [Shehata *et al.* 2020]. Cedraz *et al.* [2017] found that HSP70 expression in

Table 1. Gene markers that play a role in the response to heat stress in chicken?

Genes	Heat Control Functions	References
HSPA2, HSPH1, HSP25	provide cellular protection and healing.	Wang *et al*. [2015]
RB1CC1,BAG3, CITED2	negative regulation of apoptosis and programmed cell death	Wang *et al*. [2015] Luo *et al*. [2014]
ID1	It plays a role in embryonic development, tissue regeneration, and the control of cell proliferation	Luo *et al*. [2014]
HSP90B1, HSPD1, PDIA2, HSPA5	stabilize and refold denatured proteins in the endoplasmic reticulum and mitochondrial	De Maio and Vazquez [2013]
HSF1, HSF3	protects cells from heat damage	Cedraz *et al*. [2017]
HSP70, HSP90, HSP40	stabilize and refold denatured proteins, which is crucial for heat-stress cell survival	De-Maio and Vazquez [2013] Cedraz *et al*. [2017]
SERPINH1	facilitate protein folding, reduce aggregation, and recover misfolded proteins	De Maio and Vazquez [2013] Wang *et al*. [2016]
GLUT-2, FABP1, CD36	decrease feed intake and intestinal damage	Sun *et al*. [2015]
TRMT1L	require for redox homeostasis to ensure proper cellular proliferation and oxidative stress survival	Dewe *et al*. [2017] Walugembe *et al*. [2019]
EOMES	stimulate immunity and control homeostasis	Walugembe *et al*. [2019] Zhang *et al*. [2018]
MRPL42	disrupt of DNA synthesis, transcription, RNA processing, and translation	Van Goor *et al*. [2015]
EDN1	augment apoptosis in cancer cells induced by mild hyperthermia	Wang *et al*. [2016]
ACSF	alter in energy metabolism during heat stress	Tian *et al*. [2018]
CYP4V2	increase fat deposition	Claire De'Andre *et al*. [2013]
PLCB4	assist in the regulation of metabolic energy	Nanaei *et al*. [2022]
H1F0, ACYP	reduce heat-induced apoptosis and repair DNA damage	Srikanth *et al*. [2019]
PDK	maintain glucose and reduce heat from combustion	Luo *et al*. [2014] Kumar *et al*. [2021]

commercial broilers is higher than that in native broilers during heat stress, particularly in the expression of the HSF1 and HSF3 genes. Furthermore, Duangjinda *et al.* [2017] investigated the influence of the HSP70 genotype on heat tolerance in native chickens and discovered that the HSP70 genotypes displayed various tolerances to heat stress. It was discovered that the C2C2 genotype is susceptible to heat stress. As a result, commercial poultry breeding programs may choose to use C1C1 or C1C2 genotypes to improve heat tolerance. (2) Indirectly regulated genes from previous studies found that the HSF and HPS genes play major roles in regulating the heat response, and other genes that play a role in the regulation of apoptosis (RB1CC1, BAG3) [Wang *et al.* 2015, Luo *et al.* 2014], energy uptake and metabolism (GLUT-2, FABP1, CD36, FGA, LOXL2, GINS1, RRM2), and immune response (HS3ST5, NFAT5, PDK) [Pawar *et al.* 2016, Tellechea *et al.* 2018]. Several candidate genes involved in arid adaptation play a role in the heat stress response, as shown in Table 2. Nevertheless, some genes have been identified, their function in relation to heat stress has not yet been determined, such as CEP78, MEF2C, VPS13A, and ARRDC3, which may play an important role in regulating heat stress in poultry [Asadollahi *et al.* 2022].

Table 2. Using GWAS and SNP to characterize heat resistance in poultry

The number of SNPs	The number of the genotype	Population	Trait	References
23,098 SNPs	192	Taiwan indigenous Chickens	Pathways associated with thermotolerance	Zhuang *et al.* [2020]
580,954 SNPs	200	Taiwan country chickens	Body temperature change	Zhuang *et al.* [2019]
113,344 SNPs	118	White Leghorn layer line	Mortality in a white egg layer line	Wolc *et al.* [2019]
304,500 SNPs	526	Hy-Line Brown	Controlling traits related to NDV iinfection during heat stress	Saelao *et al.* [2019]
56,702 SNPs	206	Scaleless chickens	Feather development	Wells *et al.* [2012]
210,117 SNPs	458	broiler × Fayoumi	Body temperature, body weight, breast yield, and digestibility	Van Goor *et al.* [2015]
261,509 SNPs	374	White Leghorns	Production traits, feed intake, body weight, digestibility, egg quality	Rowland *et al.* [2019]

Genomic selection

Genomic selection (GS) is a breeding approach that uses genomic information to predict the genetic merit of individuals for specific traits. It is a form of marker-assisted selection that leverages high-density genotyping or whole-genome sequencing data

to estimate the breeding value of individuals based on their genomic profiles. This approach has gained popularity in plant and animal breeding because it enables a more accurate and efficient selection of desirable traits than conventional selection [Wang *et al*. 2014]. Genomic selection has revolutionized breeding programs in various livestock and poultry species, leading to accelerated genetic progress, more efficient use of resources, and the development of improved varieties or breeds with desired traits [Dewe *et al*. 2017]. In the context of genomic selection [Misztal *et al.* 2009], there are generally two main types or approaches (Tab. 2). (1) Marker-based genomic selection relies on the analysis of genetic markers, such as single nucleotide polymorphisms (SNPs), to predict the genetic merit of individuals. The markers are genotyped or sequenced across the genome, and statistical models are developed to estimate the genomic estimated breeding values (GEBVs) based on the marker profiles. These GEBVs are used for selection decisions, and individuals with higher GEBVs are preferred as parents for the next breeding cycle. (2) In whole-genome selection, also known as genomic prediction, the entire genome of individuals is analyzed rather than specific genetic markers. This approach involves high density genotyping arrays or whole-genome sequencing to obtain comprehensive genetic information. Statistical methods, such as genomic best linear unbiased prediction (GBLUP) or Bayesian algorithms, are applied to estimate the genomic breeding values. The models capture the collective effects of numerous genetic markers distributed throughout the genome, allowing for the prediction of breeding values based on complete genomic information. Several studies have investigated the application of genomic selection to improve heat tolerance in chickens. For instance, researchers have used high-density genotyping arrays to identify genetic markers associated with heat tolerance traits and then applied genomic prediction models to estimate breeding values for heat tolerance. This approach enables the selection of individuals with higher genetic potential for heat tolerance, leading to more heat-resistant chicken lines [Guo *et al.* 2021]. In addition, Bjorkquist *et al.* [2014] conducted studies to select improved adaptability to high temperatures in broiler chickens, which are bred for meat production. By combining genotyping data with phenotypic records of broilers reared under heat stress conditions, genomic selection models have been used to predict breeding values for heat tolerance. This allows breeders to identify and select broilers with enhanced thermotolerance traits, improving performance in high-temperature environments. Genomic selection has also been explored to improve the thermotolerance of laying hens raised for egg production [Bjorkquist *et al.* 2014]. By genotyping laying hens and employing genomic prediction models, breeders can predict the genetic merit of individuals for heat tolerance traits.

Genome wide association studies in poultry

In the case of heat stress, the physiological responses of different organisms to the increase in ambient temperature is heat gain of the body and increase in the blood flow

of the skin [Sandercock *et al*. 2001] These can be regarded as indicators of the body's reaction to regulate its production through elimination of excess body heat [Hocking *et al*. 1994]. Genes regulation related to activation of Heat Shock Factors (HSFs) and their transcription activators have been identified as cellular responses to different types of stresses [Sorger 1991]. Heat shock proteins have been suggested to be the key cellular defense mechanisms in chickens during exposure to hot environments [Cedraz *et al*. 2017].

Two Predisposing factors that affect heat stress include genetics and the environment. In an experiment, muscle damage in fast-growing broilers were reported to be associated with an ambient temperature [Zahoor *et al*. 2016]. Additionally, it has been indicated that much of the variation in response to heat stress has a genetic basis [Lin *et al*. 2006, Lu *et al.* 2007]. Soleimani *et al.* [2011] found that commercial broilers were more susceptible to heat stress than their Red Jungle fowl counterparts. Also, Mack *et al*. [2013] provided evidence that genetic selection is a useful strategy for reducing the heat stress response in laying hens. Tamzil *et al.* [2013] demonstrated that a specific group of genes and also some proteins, that are called HSP, involve in heat stress. These proteins vary in different strains and breeds and increases the resistance response of birds against the high temperature of the environment [Xie *et al.* 2014]. Actually, in the case of heat stress, genetic factors affect the physiological responses and different birds and populations show different genetic responses to heat stress [Lu *et al.* 2007]. As a result, the selection of individuals for survivability to heat resistance could help greatly to increase the resistance of the population against heat stress. Advances in molecular genetics and broad access to molecular polymorphism information have allowed researchers to select directly for genes and genomic regions influencing traits through marker-assisted selection (MAS) [Dekkers and Hospital 2002, Lande and Thompson 1990]. Association studies [Van Goor *et al*. 2015] and transcriptome comparison [Coble *et al*. 2014] have been suggested to implement for studying heat stress. Several quantitative trait loci (QTLs) have has been identified to be associated with heat stress tolerance in Holstein cattle [Melchiorre *et al*. 2013] and catfish [Zhou *et al*. 2017]. For chickens, few results have been found kinds of the literature and several candidate genes were reported to be associated with heat stress tolerance [Van Goor *et al.* 2015]. The managing of new traits, such as Heat stress resistance, has brought about the development of new technologies to investigate genetic relations with these phenotypes. A good approach to identify the functional genes and polymorphisms correlated with HS resilience has been accomplished by virtue of high-throughput screening technologies, such as genome-wide analyses of genetic variations. The genome-wide association study has been proposed to be a suitable method for identifying genomic regions affecting the resistance of birds to heat shocks [Bjorkquist *et al*. 2015, Lamont *et al*. 2014].

Whole genome profiling has allowed researchers to understand the genetic effect that heat stress has on genetic pathways involved in heat stress modulation. [Lei *et al*. 2013] They found that pathways play a role in modulating heat stress.

These researchers found that genes affecting hormones associated with appetite-regulating peptides affect the thermal process. Exposure to heat causes anorexia due to this stress. Therefore, an important factor in the reduction of feed consumption and the reduction of nutrient consumption in birds is affected by heat. These findings show what strategies should be taken to reduce the negative effect of heat stress and increase weight and improve production. One of these factors is improving genetic performance by creating resistant genes in these animals [Lei *et al*. 2013]. Several studies, mostly in plant breeding, indicated that both the unbalanced and the balanced data sets detect common SNPs markers, suggesting that the unbalanced data could be used to gain knowledge about the genetics of important traits and identify marker-trait associations for molecular breeding [Johnson *et al*. 2019, Wang *et al*. 2012]. However, there is published literature indicates that the linear mixed model and logistic mixed model produce large type I error rates in the analysis of unbalanced case-control phenotypes [Zhou *et al*. 2018]. A Scalable and Accurate Implementation Generalized Mixed Model (SAIGE) was proposed by these authors for GWASs, who indicated that SAIGE controls the type I error rates efficiently even when case-control ratios are extremely unbalanced. Poultry, like other animals, has the possibility to develop specific phenotypes advantageous for adaption to the harsh environment where they live. There are several phenotypes that mainly act for the alleviation of heat stress, mostly related to feather types. Indeed, feathers guarantee a thermal shelter between the animal body and the environment. Plumage delays the process of heat elimination from the skin surface [Wolf and Walsberg 2000]. As an example, the Naked Neck (Na) chicken shows a better fitness under Heat stress conditions [Eberhart and Washburn 1993]. In a recent study, compared the expression levels of HSP70 (used to assess the heat tolerance) in three Egyptian local breeds (Fayoumi, Dandarawi, and Sinai) with and without the Na gene and under normal and Heat stress conditions. As expected, they found higher HSP70 expression levels in crossbreeds with the Na gene, suggesting that the Na gene is responsible for the up-regulation of HSP70 expression and has a positive impact on HS adaptation not only by reducing feather cover [Galal *et al*. 2019]. Another important feature of the plumage is the color of feathers, which can impact the ability of chickens to respond under Heat stress conditions. A recent study demonstrated that dark chicks showed a lower expression rate of genes belonging to pathways of stress (cellular stress: SOD2 and HSPA8; DNA damage repair: ALKBH3) than paler chicks [Diaz-Real *et al*. 2017].

The significant Gga_rs16111480 SNP, detected in this study, was located inside the CEP78 gene. The MAF of this SNP (0.095), as a rare variant (MAF≤0.10), suggests that it could play a more important role in heat stress tolerance than the other two SNPs, detected in this study [Asadollahi *et al*. 2022]. The CEP78 gene regulates protein activity of PLK1 at G2/M transition in relation to the HSP90 [Senju *et al*. 2006] and HSP70 [Chen *et al*. 2014]. It is also involved in the maturation of Centrosomes and Centrioles [Brunk *et al*. 2016], and plays an important role in cell survival. The PLK proteins are important regulators of the cell cycle [Casenghi *et*

al. 2003]. This gene, which stimulates family enzymes of PLK, particularly PLK1 (www. unipr ot.org) and PLK4 [Kraft *et al.* 2003], is responsible for the activation and regulation of these proteins. The PLK1 substrates consist of multiple proteins, engaged in mitosis, including CDC25C, APC and CLYN B, which are responsible for the regulation of the tyrosine dephosphorylation in CDKs [Kim *et al.* 2005].

Previous studies have shown some interactions between PLK1 and HSF-1 proteins. These interactions, which increase during thermal stress, are caused by serine dephosphorylation at 419 serine sites through HSF-1 protein. Mutations that change serine 419 into alanine inhibit heat stress due to nuclear translocation. HSF-1 activity requires phosphorylation by PLK1 protein, and it is a necessary step during heat stress [Ahn and Thiele 2003]. In normal cells, HSF-1 greatly exists as an inactive monomer inside the cytoplasm. By heat stress exposure, HSF-1 is begun to transition from a monomer to a homotrimer form and is transferred to the nucleus and binds to DNA, resulting in phosphorylation on several amino acids. Subsequently, HSF-1 is attached to heat shock elements (HSEs) that have been formed from repeated opposite structures of 5 nucleotide motifs in the promoter site of the HSP gene [Ahn and Thiele 2003] and thereby increase the gene expression of HSP including HSP70 and HSP90 isoforms. In different organisms, HSF-1 is a prime integrator of transcriptional responses during stress., HSF-1 activity increases the cellular level of heat shock proteins (HSP) [Kim *et al*. 2005], and these proteins act as cell protectors for denaturation of defective proteins and are also used to survival mechanisms in protein-damaging conditions to protect the cell [Zhang *et al.* 2002]. Heat shock proteins, especially HSP70 [Silver and Noble 2012] and HSP90, have a critical role in cell survival in response to stressful environments [Gupta et al 2010]. As a result, enzymes PLK (PLK1, PLK4) are affected by the CEP78 gene. Due to HSF-1 phosphorylation, these enzymes will activate heat shock proteins, as the activity of these proteins is also a key impact on cell and body resistance against heat stress, so in different organisms, the CEP78 gene plays an important role in heat stress resistance.

Myocyte enhancer factor 2C (MEF2C) was the closest gene to two SNPs, namely GGaluGA354375 and Gga_rs14748694, significantly associated with survivability to heat stress. Usually, two specific cellular response incidents are to a variety of stresses, such as activation of heat shock factors (HSFs) and activators of transcription of these genes [Sorger 1991], and the activation of JNKs and P38 MAP kinase, which have been identified as active protein kinases in stressful environments [Koul *et al.* 2013]. Both protein kinases are subgroups of the MAPK family [Koul *et al.* 2013] and are involved in various complex biological processes in cells and deeply respond to stress signals [Vassalli *et al.* 2012]. Literature studies have shown that the regulation of protein kinases activity of the MAPK family is influenced by the MEF2C gene (www. unipr ot.org), and also phosphorylation and activation of MEF2C are affected by MAPK protein kinases. These protein kinases have special effects on amino acids including serine, threonine and tyrosine [Cleveland 1923], and they are involved in managing and directing cellular responses to a wide type of stimulants such as

mitogen and osmotic stress, heat shock, and proinflammatory cytokines and are also engaged in the regulation of some cell functions including proliferation, gene expression, differentiation, cell survival and apoptosis [Pearson *et al.* 2001]. Chu *et al.* [1996] indicated that part of the activation of HSF-1 is regulated by MAPK inside the body and one or more members of the MAPK family is involved in phosphorylation and regulation of HSF-1 activity. Furthermore, the MEF2C is well known as GLUT4 enhancer protein and appears to be the pivotal regulator of GLUT4 expression. An increase in expression of GLUT4 was associated with increased MEF2C. It has been reported that pigs under chronic heat stress increase the respiratory frequency and body temperature and modify the higher expression of HSP90 and GLUT4 [Cervantes 2016, Garcia 2012]. However, some results indicated that GLUT4 was not identified in chickens and proposed that the hyperglycemia, which might have toxic effects on the liver, and insulin resistance observable in chickens is due to the possible deficiency of GLUT4 [Gochee 2002]. For this study, these biological pathways indicate that CEP78 and MEF2C genes plays an important role in enhancing the organism resistance against heat stress through regulating the activity of MAPK protein kinase, activating HSF-1, and increasing levels of heat shock protein.

The Gga_rs16111480 SNP detected in this study was located 243 kb downstream of the VPS13A gene [Asadollahi *et al.* 2022]. In recent years, the VPS13A gene, which encoded Chorein, has been recognized as a key regulator of secretion and aggregation of blood platelet in heat stress conditions and has an important role in the production and regulation of blood platelets [Schmidt *et al.* 2013]. Chorein protein is derived from a wide variety of human tissues and red blood cells as well as the original skin fibroblasts [Dobson-Stone *et al.* 2004]. The heat stress raises the total number of platelets and blood viscosity. This condition may increase the physiological damages and also the risk of cerebral and coronary thrombosis [Keatinge *et al.* 1986]. ARRDC3 gene, which was located 947 kb and 888 kb upstream of the two GGaluGA354375 and Gga_rs14748694 SNPs, respectively, directly involved in the resistance to high temperatures (www.unipr ot.org). A high level of ARRDC3 stimulates the norepinephrine by increasing cAMP signalling that it lowers energy consumption and ultimately will reduce the heat production in the body [Patwari *et al.* 2011]. In recent studies, the regulation of G protein-coupled receptors (GPCRs) degradation and trafficking due to ARRDC3 has been reported [Dores *et al.* 2015]. It has been suggested that the processing of both wild-type and mutant GPCRs is promoted by HSP70 proteins [Meimaridou *et al.* 2009]. Furthermore, it has been indicated that ARRDC3 is linked to the regulation of adrenergic signalling through interaction and regulation of ubiquitination of the β2 adrenergic receptor [Batista *et al.* 2020]. Andersson *et al.* [2011] indicated that the stimulation of β-adrenergic has resulted in an increase in the mitochondrial reactive oxygen species (ROS) production in cardiomyocytes. While the role of ROS in ageing is inconsistent in the pieces of literature, it has generally been observed that the lifespan decreases as the ROS level increases [Shields *et al.* 2021]. From the above results, it can be concluded that

survivability to high temperature might be due to the SNP markers that are located inside or close to the genes such as CEP78, MEF2C, VPS13A and ARRDC3, which had relatively biological pathways in heat shock resistance.

REFERENCES

1. AHN S.G., THIELE D.J., 2003 – Redox regulation of mammalian heat shock factor 1 is essential for Hsp gene activation and protection from stress. *Genes & Development* 17(4), 516-528.

2. ANDERSSON D.C., FAUCONNIER J., YAMADA T., LACAMPAGNE A., ZHANG S.J., KATZ A., WESTERBLAD H., 2011 – Mitochondrial production of reactive oxygen species contributes to the β-adrenergic stimulation of mouse cardiomycytes. *The Journal of Physiology* 589(7), 1791-1801.

3. ASADOLLAHI H., VAEZ TORSHIZI R., EHSANI A., MASOUDI A.A., 2022 – An association of CEP78, MEF2C, VPS13A and ARRDC3 genes with survivability to heat stress in an F2 chicken population. *Journal of Animal Breeding and Genetics* 139(5), 574-582.

4. ASADOLLAHPOUR NANAEI, H., KHARRATI-KOOPAEE, H., ESMAILIZADEH A., 2022 – Genetic diversity and signatures of selection for heat tolerance and immune response in Iranian native chickens. *BMC Genomics* 23(1), 224.

5. BATISTA T.M., DAGDEVIREN S., CARROLL S.H., CAI W., MELNIK V.Y., NOH H.L., SAENGNIPANTHKUL S., KIM J.K., KAHN C.R., LEE R.T., 2020 – Arrestin domain-containing 3 (Arrdc3) modulates insulin action and glucose metabolism in liver. *Proceedings of the National Academy of Sciences* 117(12), 6733-6740.

6. BELAY T., TEETER R.G., 1996 – Effects of ambient temperature on broiler mineral balance partitioned into urinary and faecal loss. *British Poultry Science 37(*2), 423-433.

7. BJORKQUIST A., ASHWELL C., PERSIA M., ROTHSCHILD M.F., SCHMIDT C., LAMONT S.J., 2014 – QTL for body composition traits during heat stress revealed in an advanced intercross line of chickens. *Change* 55(36), 2-27.

8. BJORKQUIST, A.G., ROTHSCHILD, M.F., PERSIA, M.E., ASHWELL, C., SCHMIDT, C. AND LAMONT, S.J., 2015 – Genetic markers found for response to heat stress in chickens. *Iowa State University Animal Industry Report* 12(1).

9. BOTTJE W.G., HARRISON P.C., 1985 – Effect of carbonated water on growth performance of cockerels subjected to constant and cyclic heat stress temperatures. *Poultry Science* 64(7), 1285-1292.

10. BRUNK K., ZHU M., BÄRENZ F., KRATZ A.S., HASELMANN-WEISS U., ANTONY C., HOFFMANN I., 2016 – Cep78 is a new centriolar protein involved in Plk4-induced centriole overduplication. *Journal of Cell Science* 129(14), 2713-2718.

11. CASENGHI M., MERALDI P., WEINHART U., DUNCAN P.I., KÖRNER R., NIGG E.A., 2003 – Polo-like kinase 1 regulates Nlp, a centrosome protein involved in microtubule nucleation. *Developmental Cell* 5(1), 113-125.

12. CEDRAZ H., GROMBONI J.G.G., GARCIA A.A.P., FARIAS FILHO R.V., SOUZA T.M., OLIVEIRA E.R.D., OLIVEIRA E.B.D., NASCIMENTO C.S.D., MENEGHETTI C., WENCESLAU A.A., 2017 – Heat stress induces expression of HSP genes in genetically divergent chickens. *PLoS One* 12(10), p.e0186083.

13. CERVANTES M., COTA M., ARCE N., CASTILLO G., AVELAR E., ESPINOZA S., MORALES A., 2016 – Effect of heat stress on performance and expression of selected amino acid and glucose transporters, HSP90, leptin and ghrelin in growing pigs. *Journal of Thermal Biology* 59, 69-76.

14. CHEN Y.J., LAI K.C., KUO H.H., CHOW L.P., YIH L.H., LEE T.C., 2014 – HSP70 colocalizes with PLK1 at the centrosome and disturbs spindle dynamics in cells arrested in mitosis by arsenic trioxide. *Archives of Toxicology* 88, 1711-1723.

15. CHU B., SONCIN F., PRICE B.D., STEVENSON M.A., CALDERWOOD S.K., 1996 – Sequential phosphorylation by mitogen-activated protein kinase and glycogen synthase kinase 3 represses transcriptional activation by heat shock factor-1. *Journal of Biological Chemistry* 271(48), 30847-30857.

16. CLAIRE D'ANDRE H., PAUL W., SHEN X., JIA X., ZHANG R., SUN L., ZHANG X., 2013 – Identification and characterization of genes that control fat deposition in chickens. *Journal of Animal Science and Biotechnology* 4(1), 1-16.

17. CLEVELAND L.R., 1923 – Symbiosis between termites and their intestinal protozoa. *Proceedings of the National Academy of Sciences* 9(12), 424-428.

18. COBLE D.J., FLEMING D., PERSIA M.E., ASHWELL C.M., ROTHSCHILD M.F., SCHMIDT C.J., LAMONT S.J., 2014 – RNA-seq analysis of broiler liver transcriptome reveals novel responses to high ambient temperature. *BMC Genomics* 15(1), 1-12.

19. DAYYANI N., BAKHTIARI H., 2013 – Heat stress in poultry: background and affective factors. *International Journal of Advanced Biological and Biomedical Research* 1(11), 1409-1413.

20. DE MAIO A., VAZQUEZ D., 2013 – Extracellular heat shock proteins: a new location, a new function. *Shock* (Augusta, Ga.), 40(4), p. 239.

21. DEKKERS J.C., HOSPITAL F., 2002 – The use of molecular genetics in the improvement of agricultural populations. *Nature Reviews Genetics* 3(1), 22-32.

22. DEKKERS J.C., HOSPITAL F., 2002 – The use of molecular genetics in the improvement of agricultural populations. *Nature Reviews Genetics* 3(1), 22-32.

23. DEWE J.M., FULLER B.L., LENTINI J.M., KELLNER S.M., FU D., 2017 – TRMT1-catalyzed tRNA modifications are required for redox homeostasis to ensure proper cellular proliferation and oxidative stress survival. *Molecular and Cellular Biology* 37(21), e00214-17.

24. DIAZ-REAL J., KIM S.Y., VELANDO A., 2017 – Plumage colour and the expression of stress-related genes in gull chicks. *Journal of Avian Biology* 48(9), 1216-1225.

25. DOBSON-STONE C., VELAYOS-BAEZA A., FILIPPONE L.A., WESTBURY S., STORCH A., ERDMANN T., WROE S.J., LEENDERS K.L., LANG A.E., DOTTI M.T., FEDERICO A., 2004 – Chorein detection for the diagnosis of chorea-acanthocytosis. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society* 56(2), 299-302.

26. DONKOH A., 1989 – Ambient temperature: a factor affecting performance and physiological response of broiler chickens. *International Journal of Biometeorology* 33, 259-265.

27. DORES M.R., LIN H., GRIMSEY N.J., MENDEZ F., TREJO J., 2015 – The α-arrestin ARRDC3 mediates ALIX ubiquitination and G protein–coupled receptor lysosomal sorting. *Molecular Biology of the Cell* 26(25), 4660-4673.

28. DUANGJINDA M., TUNIM S., DUANGDAEN C., BOONKUM W., 2017 – Hsp70 genotypes and heat tolerance of commercial and native chickens reared in hot and humid conditions. *Brazilian Journal of Poultry Science* 19, 07-18.

29. EBERHART D.E., WASHBURN K.W., 1993 – Assessing the effects of the naked neck gene on chronic heat stress resistance in two genetic populations. *Poultry Science* 72(8), 1391-1399.

30. GALAL A., RADWAN L.M., REZIK H.H., AYOUB H., 2019 – Expression levels of HSP70 and CPT-1 in three local breeds of chickens reared under normal or heat stress conditions after the introduction of the naked neck gene. *Journal of Thermal Biology* 80, 113-118.

31. GARCIA H., GRAGEOLA F., MORALES A., ARAIZA A., ARCE N., CERVANTES M., 2012 – Severe heat stress affects the expression of Hsp90, GLUT4 and b0,+, in liver and leptin in adipose tissue of pigs. *The FASEB Journal* pp, 651-3.

32. GOCHEE P.A., POWELL L.W., CULLEN D.J., DU SART D., ROSSI E., OLYNYK J.K., 2002 – A population-based study of the biochemical and clinical expression of the H63D hemochromatosis mutation. *Gastroenterology* 122(3), 646-651.

33. GOO D., KIM J.H., PARK G.H., DELOS REYES J.B., KIL, D.Y., 2019 – Effect of heat stress and stocking density on growth performance, breast meat quality, and intestinal barrier function in broiler chickens. *Animals* 9(3), 107.

34. GUO Y., LIAO J.H., LIANG Z.L., BALASUBRAMANIAN B., LIU W.C., 2021 – Hepatic lipid metabolomics in response to heat stress in local broiler chickens breed (Huaixiang chickens). *Veterinary Medicine and Science* 7(4), 1369-1378.

35. GUPTA S.C., MISHRA M., SHARMA A., BALAJI T.D., KUMAR R., MISHRA R.K., CHOWDHURI D.K., 2010 – Chlorpyrifos induces apoptosis and DNA damage in Drosophila through generation of reactive oxygen species. *Ecotoxicology and Environmental Safety* 73(6), 1415-1423.

36. HOCKING P.M., MAXWELL M.H., MITCHELL M.A., 1994 – Haematology and blood composition at two ambient temperatures in genetically fat and lean adult broiler breeder females fed ad libitum or restricted throughout life. *British Poultry Science* 35(5), 799-807.

37. JOHNSON M., KUMAR A., OLADZAD-ABBASABADI A., SALSMAN E., AOUN M., MANTHEY F.A., ELIAS E.M., 2019 – Association mapping for 24 traits related to protein content, gluten strength, color, cooking, and milling quality using balanced and unbalanced data in durum wheat [Triticum turgidum L. var. durum (Desf).]. *Frontiers in Genetics* 10, 717.

38. KEATINGE W.R., COLESHAW S.R., EASTON J.C., COTTER F., MATTOCK M.B., CHELLIAH R., 1986 – Increased platelet and red cell counts, blood viscosity, and plasma cholesterol levels during heat stress, and mortality from coronary and cerebral thrombosis. *The American Journal of Medicine* 81(5), 795-800.

39. KIM S.A., YOON J.H., LEE S.H., AHN S.G., 2005 – Polo-like kinase 1 phosphorylates heat shock transcription factor 1 and mediates its nuclear translocation during heat stress. *Journal of Biological Chemistry* 280(13), pp.12653-12657.

40. KOUL H.K., PAL M., KOUL S., 2013 – Role of p38 MAP kinase signal transduction in solid tumors. *Genes & Cancer* 4(9-10), 342-359.

41. KRAFT C., HERZOG F., GIEFFERS C., MECHTLER K., HAGTING A., PINES J., PETERS J.M., 2003 – Mitotic regulation of the human anaphase-promoting complex by phosphorylation. *The EMBO Journal* 22(24), 6598-6609.

42. KUMAR M., RATWAN P., DAHIYA S.P., NEHRA, A.K., 2021 – Climate change and heat stress: Impact on production, reproduction and growth performance of poultry and its mitigation using genetic strategies. *Journal of thermal Biology* 97, 102867.

43. KUMARI K.N.R., NATH D.N., 2018 – Ameliorative measures to counter heat stress in poultry. *World's Poultry Science Journal* 74(1), 117-130.

44. LAMONT S., COBLE D.J., BJORKQUIST A., ROTHSCHILD M., PERSIA M., ASHWELL C., SCHMIDT C., 2014 – Genomics of heat stress in chickens. Proceedings, 10th World Congress of Genetics Applied to Livestock Production.

45. LANDE R., THOMPSON R., 1990 – Efficiency of marker-assisted selection in the improvement of quantitative traits. *Genetics* 124(3), pp.743-756.

46. LEI L., HEPENG L., XIANLEI L., HONGCHAO J., HAI L., SHEIKHAHMADI A., YUFENG W., ZHIGANG S., 2013 – Effects of acute heat stress on gene expression of brain–gut neuropeptides in broiler chickens (Gallus gallus domesticus). *Journal of Animal Science* 91(11), 5194-5201.

47. LIN H., JIAO H.C., BUYSE J., DECUYPERE E., 2006 – Strategies for preventing heat stress in poultry. *World's Poultry Science Journal* 62(1), 71-86.

48. LU Q., WEN J., ZHANG H., 2007 – Effect of chronic heat exposure on fat deposition and meat quality in two genetic types of chicken. *Poultry Science* 86(6), 1059-1064.

49. LUO Q.B., SONG X.Y., JI C.L., ZHANG X.Q., ZHANG D.X., 2014 – Exploring the molecular mechanism of acute heat stress exposure in broiler chickens using gene expression profiling. *Gene* 546(2), 200-205.

50. MACK L.A., FELVER-GANT J.N., DENNIS,R.L., CHENG H.W., 2013 – Genetic variations alter production and behavioral responses following heat stress in 2 strains of laying hens. *Poultry Science* 92(2), 285-294.

51. MACK L.A., FELVER-GANT J.N., DENNIS R.L., CHENG H.W., 2013 – Genetic variations alter production and behavioral responses following heat stress in 2 strains of laying hens. *Poultry Science* 92(2), 285-294.

52. MEIMARIDOU E., GOOLJAR S.B., CHAPPLE J.P., 2009 – From hatching to dispatching: the multiple cellular roles of the Hsp70 molecular chaperone machinery. *Journal of Molecular Endocrinology* 42(1), 1-9.

53. MELCHIORRE M.G., CHIATTI C., LAMURA G., TORRES-GONZALES F., STANKUNAS M., LINDERT J., IOANNIDI-KAPOLOU E., BARROS H., MACASSA G., SOARES J.F., 2013 – Social support, socio-economic status, health and abuse among older people in seven European countries. *PloS One* 8(1), p.e54856.

54. MISZTAL I., LEGARRA A., AGUILAR I., 2009 – Computing procedures for genetic evaluation including phenotypic, full pedigree, and genomic information. *Journal of Dairy Science* 92(9), 4648-4655.

55. NARDONE A., RONCHI B., LACETERA N., RANIERI M.S., BERNABUCCI U., 2010 – Effects of climate changes on animal production and sustainability of livestock systems. *Livestock Science* 130(1-3), 57-69.

56. PATWARI P., EMILSSON V., SCHADT E.E., CHUTKOW W.A., LEE S., MARSILI A., ZHANG Y., DOBRIN R., COHEN D.E., LARSEN P.R., ZAVACKI A.M., 2011 – The arrestin domain-containing 3 protein regulates body mass and energy expenditure. *Cell Metabolism* 14(5), 671-683.

57. PAWAR S.S., SAJJANAR B., LONKAR V.D., KURADE N.P., KADAM A.S., NIRMAL A.V., BRAHMANE M.P. BAL S.K., 2016 – Assessing and mitigating the impact of heat stress in poultry. *Advances in Animal and Veterinary Sciences* 4(6), 332-341.

58. PEARSON G., ROBINSON F., BEERS GIBSON T., XU B.E., KARANDIKAR M., BERMAN K., COBB M.H., 2001 – Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. *Endocrine Reviews* 22(2), 153-183.

59. QUINTEIRO-FILHO W.M., RIBEIRO A., FERRAZ-DE-PAULA V., PINHEIRO M.L., SAKAI M., SÁ L.R.M.D., FERREIRA A.J.P., PALERMO-NETO J., 2010 – Heat stress impairs performance parameters, induces intestinal injury, and decreases macrophage activity in broiler chickens. *Poultry Science* 89(9), 1905-1914.

60. ROWLAND K., ASHWELL C.M., PERSIA M.E., ROTHSCHILD M.F., SCHMIDT C., LAMONT S.J., 2019 – Genetic analysis of production, physiological, and egg quality traits in heat-challenged commercial white egg-laying hens using 600k SNP array data. *Genetics Selection Evolution* 51(1), 1-9.

61. SAELAO P., WANG Y., CHANTHAVIXAY G., GALLARDO R.A., WOLC A., DEKKERS J.C., LAMONT S.J., KELLY T., ZHOU H., 2019 – Genetics and genomic regions affecting response to newcastle disease virus infection under heat stress in layer chickens. *Genes* 10(1), p.61.

62. SANDERCOCK D.A., HUNTER R.R., NUTE G.R., MITCHELL M.A., HOCKING P.M., 2001 – Acute heat stress-induced alterations in blood acid-base status and skeletal muscle membrane integrity in broiler chickens at two ages: Implications for meat quality. *Poultry Science* 80(4), 418-425.

63. SCHMIDT E.M., SCHMID E., MÜNZER P., HERMANN A., EYRICH A.K., RUSSO A., WALKER B., GU S., VOM HAGEN J.M., FAGGIO C., SCHALLER M., 2013 – Chorein sensitivity of cytoskeletal organization and degranulation of platelets. *The FASEB Journal* 27(7), 2799-2806.

64. SENJU M., SUEOKA N., SATO A., IWANAGA K., SAKAO Y., TOMIMITSU S., TOMINAGA M., IRIE K., HAYASHI S., SUEOKA E., 2006 – Hsp90 inhibitors cause G2/M arrest associated with the reduction of Cdc25C and Cdc2 in lung cancer cell lines. *Journal of Cancer Research and Clinical Oncology* 132, 150-158.

65. SHEHATA A.M., SAADELDIN I.M., TUKUR H.A., HABASHY W.S., 2020 – Modulation of heat-shock proteins mediates chicken cell survival against thermal stress. *Animals* 10(12), p.2407.

66. SHIELDS H.J., TRAA A., VAN RAAMSDONK J.M., 2021 – Beneficial and detrimental effects of reactive oxygen species on lifespan: a comprehensive review of comparative and experimental studies. *Frontiers in Cell and Developmental Biology 9*, 181.

67. SILVER J.T., NOBLE E.G., 2012 – Regulation of survival gene hsp70. *Cell Stress and Chaperones* 17, 1-9.

68. SOLEIMANI A.F., ZULKIFLI I., OMAR A.R., RAHA A.R., 2011 – Physiological responses of 3 chicken breeds to acute heat stress. *Poultry Science* 90(7), 1435-1440.

69. SORGER P.K., 1991 – Heat shock factor and the heat shock response. *Cell* 65(3), 363-366.

70. SRIKANTH K., KUMAR H., PARK W., BYUN M., LIM D., KEMP S., TE PAS M.F., KIM J.M., PARK J.E., 2019 – Cardiac and skeletal muscle transcriptome response to heat stress in Kenyan chicken ecotypes adapted to low and high altitudes reveal differences in thermal tolerance and stress response. *Frontiers in Genetics* 10, 993.

71. SUN X., ZHANG H., SHEIKHAHMADI A., WANG Y., JIAO H., LIN H., SONG Z., 2015 – Effects of heat stress on the gene expression of nutrient transporters in the jejunum of broiler chickens (Gallus gallus domesticus). *International Journal of Biometeorology* 59, 127-135.

72. TAMZIL M.H., NOOR R.R., HARDJOSWORO P.S., MANALU W., SUMANTRI C., 2013 – Acute heat stress responses of three lines of chickens with different heat shock protein (HSP)-70 genotypes. *International Journal of Poultry Science* 12(5), 264-272.

73. TELLECHEA M., BUXADÉ M., TEJEDOR S., ARAMBURU J., LOPEZ-RODRIGUEZ C., 2018 – NFAT5-regulated macrophage polarization supports the proinflammatory function of macrophages and T lymphocytes. *The Journal of Immunology* 200(1), 305-315.

74. TIAN W., ZHENG H., YANG L., LI H., TIAN Y., WANG Y., LYU S., BROCKMANN G.A., KANG X., LIU X., 2018 – Dynamic expression profile, regulatory mechanism and correlation with egg-laying performance of ACSF gene family in chicken (Gallus gallus). *Scientific Reports* 8(1), 8457.

75. VAN GOOR A., BOLEK K.J., ASHWELL C.M., PERSIA M.E., ROTHSCHILD M.F., SCHMIDT C.J., LAMONT S.J., 2015 – Identification of quantitative trait loci for body temperature, body weight, breast yield, and digestibility in an advanced intercross line of chickens under heat stress. *Genetics Selection Evolution* 47(1), 1-13.

76. VASSALLI G., MILANO G., MOCCETTI T., 2012 – Role of mitogen-activated protein kinases in myocardial ischemia-reperfusion injury during heart transplantation. *Journal of Transplantation 2012*.

77. WALUGEMBE M., BERTOLINI F., DEMATAWEWA C.M.B., REIS M.P., ELBELTAGY A.R., SCHMIDT C.J., LAMONT S.J., ROTHSCHILD M.F., 2019 – Detection of selection signatures among Brazilian, Sri Lankan, and Egyptian chicken populations under different environmental conditions. *Frontiers in Genetics* 9, 737.

78. WANG H., SMITH K.P., COMBS E., BLAKE T., HORSLEY R.D., MUEHLBAUER G.J., 2012 – Effect of population size and unbalanced data sets on QTL detection using genome-wide association mapping in barley breeding germplasm. *Theoretical and Applied Genetics* 124, 111-124.

79. WANG S.H., CHENG C.Y., TANG P.C., CHEN C.F., CHEN H.H., LEE Y.P., HUANG S.Y., 2015 – Acute heat stress induces differential gene expressions in the testes of a broiler-type strain of Taiwan country chickens. *PLoS One* 10(5), p.e0125816.

80. WANG Y., LIU Z., LI Z., SHI H., KANG Y., WANG J., HUANG J., JIANG L., 2016 – Effects of heat stress on respiratory burst, oxidative damage and SERPINH1 (HSP47) mRNA expression in rainbow trout Oncorhynchus mykiss. *Fish physiology and Biochemistry* 42, 701-710.

81. WANG Y., METTE M.F., MIEDANER T., GOTTWALD M., WILDE P., REIF J.C., ZHAO Y., 2014 – The accuracy of prediction of genomic selection in elite hybrid rye populations surpasses the accuracy of marker-assisted selection and is equally augmented by multiple field evaluation locations and test years. *BMC Genomics* 15(1), 1-12.

82. WELLS K.L., HADAD Y., BEN-AVRAHAM D., HILLEL J., CAHANER,A., HEADON D.J., 2012 – Genome-wide SNP scan of pooled DNA reveals nonsense mutation in FGF20 in the scaleless line of featherless chickens. *BMC Genomics* 13(1), 1-10.

83. WOLC A., ARANGO J., SETTAR P., FULTON J.E., O'SULLIVAN N.P., DEKKERS J.C.M., 2019 – Genome wide association study for heat stress induced mortality in a white egg layer line. *Poultry Science* 98(1), 92-96.

84. WOLF B.O., WALSBERG G.E., 2000 – The role of the plumage in heat transfer processes of birds. *American Zoologist* 40(4), 575-584.

85. XIE J., TANG L., LU L., ZHANG L., XI L., LIU H.C., ODLE J., LUO X., 2014 – Differential expression of heat shock transcription factors and heat shock proteins after acute and chronic heat stress in laying chickens (Gallus gallus). *PloS One* 9(7), p.e102204.

86. ZAHOOR I., MITCHELL M.A., HALL C.S., BEARD P.M., GOUS R.M., DE KONING D.J., HOCKING P.M., 2016. Predicted optimum ambient temperatures for broiler chickens to dissipate metabolic heat do not affect performance or improve breast muscle quality. *British Poultry Science* 57(1), 134-141.

87. ZHANG J., MAROTEL M., FAUTEUX-DANIEL S., MATHIEU A.L., VIEL S., MARÇAIS A., WALZER T., 2018 – T-bet and Eomes govern differentiation and function of mouse and human NK cells and ILC1. *European Journal of Immunology* 48(5), 738-750.

88. ZHANG X., BEURON F., FREEMONT P.S., 2002 – Machinery of protein folding and unfolding. *Current Opinion in Structural Biology* 12(2), 231-238.

89. ZHOU T., LIU S., GENG X., JIN Y., JIANG C., BAO L., YAO J., ZHANG Y., ZHANG J., SUN L., WANG X., 2017 – GWAS analysis of QTL for enteric septicemia of catfish and their involved genes suggest evolutionary conservation of a molecular mechanism of disease resistance. *Molecular Genetics and Genomics* 292, 231-242.

90. ZHOU W., NIELSEN J.B., FRITSCHE L.G., DEY R., GABRIELSEN M.E., WOLFORD B.N., LEFAIVE J., VANDEHAAR P., GAGLIANO S.A., GIFFORD A., BASTARACHE L.A., 2018 – Efficiently controlling for case-control imbalance and sample relatedness in large-scale genetic association studies. *Nature Genetics* 50(9), 1335-1341.

91. ZHUANG Z.X., CHEN S.E., CHEN C.F., LIN E.C., HUANG S.Y., 2020 – Genomic regions and pathways associated with thermotolerance in layer-type strain Taiwan indigenous chickens. *Journal of Thermal Biology* 88, 102486.

92. ZHUANG Z.X., CHEN S.E., CHEN C.F., LIN E.C., HUANG S.Y., 2019 – Genome-wide association study on the body temperature changes of a broiler-type strain Taiwan country chickens under acute heat stress. *Journal of Thermal Biology* 82, 33-42.