PERFORMANCE AND MOLECULAR RESPONSES TO GLUCOSE SUPPLEMENTATION OF MEAT-TYPE CHICKENS RAISED UNDER THERMONEUTRAL OR HEAT STRESS ENVIRONMENT

by

OLUWATOMIDE WILLIAMS ARIYO
(Under the Direction of Samuel E. Aggrey, Ph.D)

ABSTRACT

Heat stress (HS) impairs the performance of broiler chickens by reducing feed intake, suppressing protein synthesis and subsequently reducing carcass yield. The expression of intestinal nutrient transporters and kidney aquaporins (AQPs) are affected by HS. A 2x2 factorial design was used to evaluate the effect of glucose supplementation (Glu-Supplementation) on the performance and carcass parameters of broilers raised under thermoneutral (TN) or HS condition. Factors included glucose supplementation (0 and 6%) and temperature (25^0C and 35^0C). The treatments were TN-0% glucose (TN0); TN-6% glucose (TN6), HS-0% glucose (HS0) and HS-6% glucose (HS6). Heat was applied d28 to d35. Glu-Supplementation improved carcass yield, *Pectoralis major* and drumstick proportions in heat stressed-chickens. Under HS, Glu-Supplementation upwardly expressed monosaccharide (GLUT1 and GLUT5), fatty acid (FATP1, FABP6), and amino acid (rBAT, $b^{0,+}$AT, SNAT2, and SNAT7) transporters; and downwardly expressed kidney AQPs1,2 and 3. Glu-supplementation provided extra-energy and possibly, molecular precursors for enhancing protein synthesis.

INDEX WORDS: Heat stress, glucose supplementation, Aquaporins, nutrient transporters

PERFORMANCE AND MOLECULAR RESPONSES TO GLUCOSE SUPPLEMENTATION OF MEAT-TYPE CHICKENS RAISED UNDER THERMONEUTRAL OR HEAT STRESS ENVIRONMENT

by

OLUWATOMIDE WILLIAMS ARIYO
B.Agric, University of Ilorin, Nigeria, 2014
M.Agric, Federal University of Agriculture Abeokuta, Nigeria, 2018

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2022

PERFORMANCE AND MOLECULAR RESPONSES TO GLUCOSE SUPPLEMENTION OF MEAT-TYPE CHICKENS RAISED UNDER THERMONEUTRAL OR HEAT STRESS ENVIRONMENT

by

OLUWATOMIDE WILLIAMS ARIYO

Major Professor:	Samuel E. Aggrey
Committee:	Laura Ellestad
	Romdhane Rekaya

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
December 2022

ACKNOWLEDGEMENTS

My sincere appreciation goes to Dr. S. E. Aggrey for the support and guidance during my studies. His constant guidance and push helped me during the time of research and writing of this thesis. Dr. Aggrey's continuous effort made the completion of this thesis possible.

My gratitude goes to Dr. Ellestad and Dr. Rekaya, who served on my committee and gave instructions to enhance the best output of this thesis.

A big thank you to Dr. Fuller, Dr. Marie Milfort and Dr. Ahmed Ghareeb for helping with research, sampling and reviewing my writeup. My sincere thanks to my laboratory mates; Josephine Kwakye, Selorm Sovi, Sommer Hipple, Carrienton Stevenson and Bikash Aryal, who were there for me during the experiment. Without their support, this research would not have been successfully conducted.

A gargantuan thank you to my family and loved ones for always supporting me. How will I complete this without you?

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

Exposing broiler chickens to a high ambient temperature causes heat stress (HS). Heat stress remains a global challenge facing the livestock industry because of inducing high mortality and production losses. Poultry production is considered one of the ways to meet the ever-rising demand for animal protein. Heat stress is a major environmental stressor that might be seasonally and geographically restricted to the summer period in temperate regions (Renaudeau *et al*., 2010; Das *et al*., 2016).

Chickens express behavioral and physiological responses to maintain their biological functions under heat condition, including reducing feed intake, panting, increasing relative water intake, and resting (Suganya *et al*., 2015). These strategies also minimize metabolic heat produced by the bird's body. Continuous panting caused by persistent HS (chronic HS) can lead to respiratory alkalosis due to change in blood pH. The alteration in the blood pH disrupts the function of the immune system and hormonal activity (Mack *et al*., 2013). Internal organs' hypoxia may occur, leading to compromised immune response and impaired nutrient digestibility (Gabler and Pearce, 2015; Quinteiro-Filho *et al*., 2010; Oskoueian *et al*., 2014). Heat stress adversely influences the activities of the hypothalamic-pituitary axis and orthosympathic nervous system, resulting in reduced feed consumption that is accompanied by an increased feed conversion ratio and subsequently, economic loss (Piekarski *et al*., 2015).

Heat abatement strategies help to reduce annual economic losses in the US animal industry from $2.4 billion to $1.7 billion. The average poultry production loss due to heat stress is about

$128 million annually (St-Pierre *et al*., 2003). Some of these strategies used to mitigate the impact of HS include nutritional strategies and housing design modifications (Lin *et al*., 2006). Feeding strategies include the use of wet feed, feed restriction and dual feeding (feeding two different diets in sequential order to animals: one highly concentrated in protein and the other in energy). Nutritional strategies employed in poultry production include the use of vitamins, minerals, and amino acids (Min *et al*., 2019). Pen modification involves proper design and orientation of pens (Ambazamkandi *et al*., 2015).

Because birds eat to first satisfy their energy requirement, the reduction in feed consumption and impaired performance induced by HS in birds may result in the inability of the birds to meet nutrient requirements while minimizing their metabolic activities. This is evident when birds maintain basal metabolism by utilizing muscle protein to produce glucose for energy (Ma *et al*., 2021). This affects whole-body metabolism resulting in reduced weight gain. Since feed reduction is inevitable under HS, it is of great importance to ensure that the basal tissue glucose levels are maintained to avoid the breakdown of muscle proteins and a constant supply of energy to meet basal metabolism. Glucose is high in energy content and can undergo several biochemical pathways for energy production, while also supplying the reducing power for nucleotides and amino acid precursors needed for protein biosynthesis (Lunt and Vander Heiden, 2011).

The focus of this study was to determine the molecular and cellular mechanisms by which glucose impacts the performance and nutrient absorption of heat-stressed broiler chickens. The objectives of the study were:

(1) To determine the effect of supplementation of D-glucose on the performance of chickens reared under thermoneutral or HS condition.

(2) To determine the mRNA expression of kidney aquaporins in chickens reared under thermoneutral or HS condition.

(3) To determine the mRNA expression of ilea glucose, amino acid, and fatty acid nutrient transporters in chickens reared under thermoneutral or HS condition.

CHAPTER 2

LITERATURE REVIEW

Heat stress and poultry production

Increases in the human population has increased the global demand for animal protein. This can be met through efficient poultry production because birds have a short generation interval and are affordable. However, this potential in the poultry industry is under threat due to heat stress. The absence of sweat glands makes it inefficient for poultry to dissipate heat effectively, thereby increasing the susceptibility of poultry to HS (Lambert, 2009).

Heat stress has detrimental effects on animal health as it increases morbidity and mortality. This limits the productivity and profitability of livestock, thereby threatening food security. Heat stress is a major global challenge encountered by broiler industry although it is restricted to the summer period in temperate regions (Renaudeau *et al*., 2010; Das *et al*., 2016).

Heat abatement strategies help to reduce annual economic losses in the US animal industry from $2.4 billion to $1.7 billion with losses from poultry production accounting for an average of $128 million (St-Pierre *et al*., 2003) of the latter. More effective strategies should be developed to control HS and/or mitigate its detrimental effect on broiler health and performance.

Heat stress impact on chickens' performance

Chickens exposed to HS express behavioral, physiological, and molecular responses (Gupta *et al*., 2015; Sammad *et al*., 2020). All these responses collectively impair the performance of HS animals. Behaviorally, chickens respond to HS by reducing consumption of feed, restricting

movement, expanding wings to expose feather-free areas, and p anting. The decreased feed intake often results in poor weight gain and feed conversion ratio (ratio of feed intake to weight gain). Heat stress induces physiological and molecular changes, which include alteration in diverse enzymes profile, antioxidants, and metabolites (Sammad *et al*., 2020). Nutrient uptake is hampered when animals are subjected to high ambient temperature (Giri *et al*., 2017). This is because of the modulation in the expression of genes responsible for nutrient transporters. These include genes that encode membrane protein mediating the transportation of monosaccharides, fatty acids, and amino acids. Also, animals respond to HS by changing the expression of genes encoding heat shock proteins (e.g., HSP70 and HSP90), reshuffling the concentration of amino acids, and altering skeletal muscle metabolism (Sammad *et al*., 2020). All these impacts add up to induce performance regression in heat-stressed chickens.

Chickens, raised for meat and egg production, remains a prominent way to satisfy the consistent rise in global demand for animal protein. Recent advances in genetics have made it possible for broilers to reach market size as early as 35 days while improving yearly egg production in layers. HS is a factor that can hamper these characteristics even with adequate nutrition and genetical modification. Awad *et al*. (2020) reported a poorer feed conversion ratio, decreased feed intake and reduced body weight gain in broilers exposed to 34°C for 6 hours daily from d21 to d35. Deeb and Cahaner (2002) reported an increase in water intake in broilers raised under 32°C from d17 to d42. The performance of laying hens is affected by HS, with detrimental effects reported on shell weight, FCR, Haugh unit, weight gain and feed intake when these were subjected to HS of 35°C from week 24 through to 28 (Barrett *et al*., 2019). Nutrient transporter genes are modulated under HS condition and influence weight gain in chicken (Habashy *et al*., 2017). Reduced nutrient intake, digestion, and absorption due to compromised intestinal integrity are also

responsible for poor performance (Liu *et al*., 2016). Anorexia due to the stimulation of appetite-related hormones, like cholecystokinin, may also affect performance (He *et al*., 2018).

Effect of heat stress on blood parameters

Blood enhances the transport of nutrients, gases, and metabolic waste products across the body. Blood is also vital in the diffusion of body heat (Sammad *et al*., 2020). At resting state, the body depends on stored fat to produce energy (Horowitz 2003), but this will be constantly depleted in heat-stressed birds due to continuous reduction in feed intake. Ohara (1981) stated that the thermal conductivity of skin has a linear correlation with the rate at which blood flows in the skin. HS leads to changes in the blood profile, metabolism (Habibian *et al*., 2014), and endocrine system (Quinteiro-Filho*et al*.,2010). The hematological response may include changes in erythrocyte number, hematocrit value, and hemoglobin concentration. Habibian *et al*. (2014) reported increased serum glucose, lower triglyceride, and lower total cholesterol in broilers subjected to a cyclic temperature ranging between 23.9 and 37^0C after 4 weeks. Dervisevik *et al*. (2011) reported increase in blood and heart glucose level of HS mice when subjected to 12 and 24 hours of heat.

The economic impact of heat stress

Heat stress remains a global concern for the livestock industry. Abating the detrimental effect of HS has resulted in modifying the environment in modern housing systems such as installing fans and coolers (Teeter and Belay, 1996). The economic losses accrued to HS in the livestock industry are caused by poor growth rates, impaired fertility, high cost of veterinary treatment, lower carcass quality and imbalance carcass composition, longer time to reach market weights and ever-increasing animal welfare issues (Baracho *et al.,* 2006). Heat stress remains a huge factor contributing to economic loss in the poultry industry. An economic loss ranging

between $1.69 to $2.36 billion is recorded annually in the livestock industry in the United States and poultry industry accounts for $128 to $165 million (St-Pierre, *et al.,* 2003; Wasti et al., 2020). The current trend of rising global temperature will increase the concerns of magnifying poultry industry losses due to HS. For the broiler chicken industry to thrive, HS must be effectively remedied as this will ensure food security, optimum productivity, and job security.

Heat stress and thermoreceptors

The chicken has a high rate of metabolism which results in production of high basal metabolic heat, and makes them susceptible to HS (Kadzere, *et al*., 2002; Tajima *et al*., 2007). "Temperature-sensing neurons" are periphery nerves with thermo-transient receptor potentials. Thermo-transient receptor potentials are a group of temperature sensitive ion channels. The preoptic area of the hypothalamus senses and responds to temperature changes. This area possesses neurons that have been observed at the last phase of embryonic development and reached full maturation 10 days after hatching in Muscovy ducks (Tzschentke and Basta, 2000; Tzschentke *et al*., 2002). There are 3 types of temperature-sensing neurons, including cold, warm, and temperature guardian neurons. Temperature can switch between the cold and warm sensing neurons, while the temperature guardian neurons come into play during extreme temperature conditions (Tzschentke and Basta, 2000). Transient receptor potential -ankyrin 1 (TRPA1) and vanilloid 1 (TRPV1) are expressed in the skin of chickens at body temperature lower and above the average body temperature, respectively (Randall, 1943). The hypothalamus has thermoreceptors that respond in a similar way to cutaneous thermoreceptors. The hypothalamus accepts signals from thermoreceptors and respond by raising or lowering food consumption (Knox *et al*., 1973).

Immune response to heat stress

Birds are easily affected by HS due to the increased production of heat from metabolism, inadequacy in the means of dissipating heat, and a comparative low ratio of body surface to volume. To compensate for this, animals undergo hypoxia, which reduces ATP, induces production of free radicals, and modulates gut integrity (Hall *et al*., 2001). In an healthy state, tight junctions formed between adjacent enterocytes in the gut inhibit the penetration of paracellular bacterial and toxic substrates like endotoxins (Koch *et al*., 2019). Heat stress causes loss of gut integrity, which permits the transport of paracellular bacteria and microbial toxins through the tight junction into the bloodstream. This triggers inflammation resulting from the response of the innate immune system (Hall *et al*., 2001). Inflammation is a biological response by the immune system to combat diseases or infections. Microbial-associated molecular patterns (MAMPs) that penetrate the submucosa will trigger an immune response to initiate an inflammatory response. Pattern recognition receptors like Toll-like receptors on the surface membrane of phagocytes and dendritic cells enable recognition of MAMPs. This transduces a signal that will license T cells, secreting proinflammatory cytokines in the process. This host immune response against pathogens, infections, or toxins (Luster *et al*., 2005) as the inflammatory response. Therefore, HS induces local intestinal and systemic inflammation and oxidative stress.

There is disruption in lymphoid organs growth, and the expression of inflammatory cytokine genes changes due to stress (Zaglool *et al*., 2019). Heat stress suppresses the immune system, thereby causing poor growth performance. This can raise both morbidity and mortality rates (Poorghasemi *et al*. 2013; Gamba *et al*. 2015). Hyperthermia is a resultant effect of having a higher rate of heat production relative to heat dissipation in the body. Heat stress also can result in

encephalopathy and multiple organ failure (Epstein and Roberts, 2011). Heat stress can either be acute or chronic. Cytokines and Toll-like receptors (TLRs) are often used as the main markers of the immune system (Guo and Schluesener, 2007).

Cytokines are extracellular signaling peptides that enable communication between cells to produce an appropriate immune response (Saleh and Al-Zghoul, 2019). Cytokines help in immune regulation and facilitate host defense and homeostasis (Kaiser *et al*., 2009). The advent of HS alters levels of proinflammatory and anti-inflammatory cytokines in both human and animal models (Heled *et al*., 2013). Cytokines are intracellular proteins that are secreted by different cells that trigger inflammation and immune response. Cytokines can be proinflammatory (Interleukin (IL) 1, IL-2, Tumor necrosis factor alpha (TNFα) etc.) or anti-inflammatory (IL6, IL10 etc.). These counter-regulate each other in sequence to maintain homeostasis of the cytokine profile (Pedersen *et al*., 2000; Cannon *et al.,* 2000; Heled *et al*., 2013). How pro- and anti-inflammatory cytokines' function is complex and different experimental models have been used to elucidate this in hyperthermic states. The profiles of plasma cytokines differ based on different hyperthermic states. Some cytokines are unique in their thermoregulatory role and can initiate fever, activate leukocytes and cells of the endothelium, enhance the production of acute phase proteins, stimulate the hypothalamic-pituitary-adrenal axis, and the breakdown of muscle protein (Bouchama and Knochel, 2002; Leon and Helwig, 2010). Several studies have reported modulation in the expression of myriads cytokine genes in the spleen and intestinal tissues of chickens due to HS (Varasteh *et al*., 2015; Ohtsu *et al*., 2015; Song *et al*., 2017; Saleh and Al-Zghoul, 2019). These cytokines play vital roles in avian immune function. For instance, IL-2, IL-15, and IL-21 promote T-cell proliferation, IL-12, and IFN-γ for effective "cell-mediated" immune response, while IL-4 and IL-19 enhance "humoral-mediated" immune response. Some cytokines, like IFN-α, IFN-β,

and IFN-λ, have anti-viral properties (Kaiser and Stäheli, 2014). Cytokines are involved in healing injured tissue, including those caused by heat stroke (Hietbrink *et al*., 2006; Heled *et al*., 2013).

Pro-inflammatory cytokines are actively involved in the inflammatory response to HS (Helwig and Leon, 2011). They may activate immune functions by aiding lymphocytes and macrophage proliferation. There is an increase in the expression of pro-inflammatory cytokines under heat stress, which may induce tissue damage (Varasteh *et al*., 2015; He *et al*., 2019; Goel *et al*., 2021). Suppressing the pro-inflammatory cytokines is known to mitigate the negative effect of HS. For instance, antioxidant vitamins help to suppress the expression of pro-inflammatory cytokines. Niu *et al*. (2009) reported an improved growth performance and immune response in HS broilers supplemented with vitamin C. Yun *et al*. (2012) reported a similar observation when vitamin C was supplemented in Sprague Dawley rat diets.

Toll like receptors (TLRs) are group of DNA molecules that are highly conserved in living systems (Boyd *et al*., 2007). Pathogens have small conserved molecular motifs known as pathogen-associated molecular patterns (PAMPs), which TLRs recognize to protect the innate immune system (Temperley *et al*., 2008). Antiviral compounds and single-stranded RNAs can activate TLRs. Toll-like receptors play unique roles in the immune system. Toll like receptors (TLRs) recognize pathogen-associated molecular patterns (PAMPs), which is processed toward antigen presentation (Temperley *et al*., 2008). HS suppresses immune response via gene regulation. HS alters intestinal barrier integrity, changing the microbiota profile (Song *et al*., 2013). This may allow pathogens to penetrate the membrane of the intestinal barrier, enhancing the presentation of TLRs to naive T-cells. Hypervascular permeability-mediated tissue damage may occur from cytokine-dependent antigen-specific inflammatory responses (Sprague and Khalil, 2009). Of the TLRs, TLR-4 has been reported to have an impact on gene expression in HS broilers.

Toll like receptor-4 is vital in relation to HS in poultry as it aids in the recognition of microbial products like heat shock protein (HSP) and lipopolysaccharides (LPS), which are important in combating HS. TLR-4 gene expression has been observed in the kidney, spleen and intestine of heat-stressed broilers. Heat stress changes the microbiome and integrity of chickens' intestinal epithelium (Song *et al*., 2013). Fifteen (15) day old broilers raised at 38-39 °C for 8 hours each day for 5 days have higher TLR-4 gene expression in their spleen (Varasteh *et al*., 2015) and intestine (jejunum and ileum) tissue (He *et al*., 2019). The entrance of luminal pathogens due to weakened intestinal epithelium, triggers an innate immune response, resulting in TLR-mediated inflammation (Peterson *et al*., 2010). Varasteh *et al*. (2015) observed a lower expression of the TLR-4 gene in the jejunum when compared to the ileum of broilers challenged with HS. This suggests that HS modulates TLR-4 gene expression by altering the permeability of the intestine.

Oxidative stress: a resultant effect of heat stress

Heat stress triggers oxidative stress, which occurs when there is an imbalance between oxidant and antioxidant systems. Oxidative stress induces cellular and tissue damage in chickens (Lin, *et al*., 2006). This imbalance in oxidative status increases the level of expression of apoptosis-related genes while triggering a series of signaling pathways such as activation of endoplasmic reticulum (ER) stress signaling (Ashraf and Sheikh, 2015). The ER is an organelle charged with the responsibility of protein folding, assembly, and maturation in eukaryotic cells. Any physiological or pathological changes to the status of the ER will disrupt its ability to fold proteins. Accumulating misfolded proteins triggers ER stress as a protective response (Walter and Ron, 2011). Persistent severe stress in the ER can orchestrate apoptosis (Oyadomari, and Mori, 2004). Oxidative and ER stress, due to heat stress, accounts for various types of damage to organs, tissues,

and cells of animals. These include apoptosis, immune imbalance in the intestines, testicular damage, and nephropathy (Wang *et al*., 2018; Wu *et al*., 2019). Macromolecule peroxidation occurs due to the generation of free radicals in response to heat exposure. This up-regulates plasma superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Altan *et al*., 2003). Animals under heat stress express the Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 4 (NOX4) gene, an inducer of reactive oxygen species (ROS), that is controlled by antioxidant enzymes such as superoxide dismutase (SOD) and chloramphenicol acetyltransferase (CAT).

Reactive nitrogen species (RNS) and reactive oxygen species (ROS) are produced during inflammation (Zeng *et al*., 2016), which activates antioxidant enzymes to protect the cells. Superoxide dismutase converts superoxide radicals to hydrogen peroxide, which is then converted by catalase and glutathione peroxidase into water molecules (Halliwell, 2006). However, this protection is temporary, as it declines as HS persists (Yang *et al*., 2010; Sahin *et al*., 2010). This has been linked to pathologies like cancer, chronic inflammation, neurodegeneration, and aging (Betteridge, 2000). In gut inflammatory response, the reactive species are released into the intestinal lumen, where nitrogen oxide (NO) is converted into nitrate. Nitrate rich environment may aid the growth of some gram-negative bacteria like *E. coli* due to nitrate reductase genes present in them (Gresse *et al*., 2017). Spees *et al*. (2013) observed an increase in intestinal ROS concentration in conjunction with increased *E. coli* population in piglets 7 days after weaning. Oxygen concentration in the intestine may be influenced by increase in blood flow, aiding the growth of facultative anaerobes (Gresse *et al*., 2017). The dysbiosis that results from this will disturb the microbiota. The nutritional and financial costs of inflammation in chickens are inversely correlated with increased bodyweight and the overall performance (Van Leeuwen *et al*.,

2004). Elevated concentration of proinflammatory cytokines was observed in the plasma of heat-stressed humans and rats (Bouchama *et al*., 1993; Ji *et al*., 2014).

Maintaining the balance (homeostasis) between ROS and antioxidant production is vital for cell survival (Han *et al*., 2020). Oxidative stress may damage tissue due to the imbalance between the production of ROS and the antioxidant defenses (Betteridge 2000). Animal exposure to heat causes formation of excess ROS (Lin *et al*., 2000; Mahmoud and Edens, 2003; Ali *et al*., 2019). This causes oxidative stress, which destroys the antioxidant defense system via lipid peroxidation (LPO) and causes oxidative damage to proteins and DNA (Droge, 2002; Ali *et al*., 2019). In response to this, a quick and temporal protective action is activated through the antioxidant system by releasing enzymes such as SOD, GSH-Px, and CAT. SOD is crucial in the antioxidant system because it is the first defensive enzyme that helps to eradicate superoxide anion free radicals in the body. It converts high-reactive oxygen to low-reactive hydrogen peroxide (Nagami *et al*., 2005). SOD expression is high at the early phase of heat exposure, suggesting its protective activity. CAT gene expression is upregulated in the liver under heat stress (Rimoldi *et al*., 2015). CAT, like SOD, is a vital component of the antioxidant system which helps to stabilize ROS produced by the effect of HS. It may facilitate the production of water and molecular oxygen from the breakdown of hydrogen peroxide produced by SOD. However, prolonged exposure to heat decreases the expression of antioxidant enzymes due to tissue injury formation, cell lesions, and secretion of antioxidant cofactors like Zn, Cu, Se, vitamin C, and vitamin E (Sahin and Kucuk, 2003).

Heat stress induces oxidative stress majorly because of mitochondrial dysfunction (Akbarian *et al*., 2016). About 90% of ATP produced in the cells are accounted for by the mitochondria via oxidative phosphorylation. ROS triggers and modulates the signaling as well as

how cell behaves. The activation of transcription factors including nuclear factor kappa-light-chain enhancer of activated B (NF-κB) and activator protein 1 (AP-1), leads to the culmination of numerous redox-sensitive downstream pathways (Lauridsen, 2019). Reactive oxygen species harm proteins, fatty acids, and nucleic acids (Betteridge, 2000). Under normal physiological conditions, ROS generation is beneficial and controlled by the antioxidant system. The antioxidant system is designed to prevent this damage by converting the free radicals into stable products.

Heat stress and protein biosynthesis

Proteins have highly preserved structures essential for conducting specific cellular activities. In a normal state, protein folding is spontaneous but molecular chaperones such as heat shock proteins are required to aid the folding of a large portion of the newly synthesized proteins to reach their conformational structure and achieve their biological function (Hartl 1996, Vabulas *et al*., 2010). In response to high environmental temperatures, cells produce new or constitutively stimulate molecular chaperones like stress-denatured proteins or HSPs. These molecular chaperones help to prevent misfolding and aggregation of proteins. Stressors affect protein synthesis by disrupting protein homeostasis and proteome quality. In response, affected cells suppress protein synthesis and increase expression of stress-linked genes (Han *et al*., 2020). Molecular systems are triggered by the accumulation of misfolded proteins to maintain the quality of the proteome. This includes the activation of the molecular chaperone network, the ubiquitin-proteasome system (UPS) and autophagy-driven proteolysis. Stressors trigger a molecular chaperone network, which modifies premature misfolding and aggregation of protein by interacting with most nascent polypeptides. Autophagy aids degradation of long-lived cytosolic

proteins and bulk organelles, while UPS degrades short-lived damaged and misfolded proteins (Lilienbaum, 2013).

Proteins, synthesized from free amino acids (AA), are available either from dietary source (digestion) or metabolic origins. Dietary AAs are required for building muscle protein, membrane glycoproteins, and biochemical important enzymes (Wu, 2013). The AAs are catabolized in the liver to integrate into protein, which supplies peripheral tissues (Ma *et al*., 2021). Protein metabolism (breakdown and synthesis) is altered during heat stress because of reduced tissue glucose accruing from reduced feed intake (Ma *et al*., 2021). Heat stress triggers catabolism of proteins in the skeletal muscle and causes changes in the metabolism of amino acids (Tabiri *et al*., 2002; Ma *et al*., 2018, Ma *et al*., 2021). Amino acids are mobilized and undergo catabolism during HS due to decreased food intake and blood glucose. Amino acid in this pathway end up as a precursor of gluconeogenesis (Ma *et al*., 2021) and it contributes to about 40% of the total AA loss in starved animals. Protein synthesis aids the fusion of dietary AA into proteins in the body tissues, while the breakdown of proteins occurs to build amino groups that produce urea or further protein. Amino acids aid RNA/DNA synthesis, hence, the capacity for RNA/DNA synthesis is affected by the protein and amino acid profile of an animal (Fernandez, 2014). In sum, amino acids are used to produce carbon skeleton molecules for glucose and fatty acid production for the release of energy during heat stress condition.

The translation of mRNA occurs when large quantities of unfolded proteins accumulate within the endoplasmic reticulum (ER). This occurs via phosphorylation of eIF2α by protein kinase R (PR)-like ER kinase (PERK). The activation of eIF2 initiates the binding of $\underline{tRNA_i^{Met}}$ to the ribosome utilizing GTP in the process (Wek et al., 2006). Upon completion of the initiation process, eIF2 is released from the ribosome-GDP complex as an inactive binary complex. GDP is

then exchanged for GTP for the subsequent translation process. Regulating mRNA translation of eIF2α protects cells against oxidative stress. Translation increases the difficulty for incomplete polypeptide chains to fold into stable native conformations, hence increasing the risk of misfolding and aggregation of proteins (Vabulas et al., 2010). Aggregation is concentration-dependent (favored in the crowded environment of the cell) and aids binding between folding intermediates via excluded volume effects (Zimmerman and Trach 1991; Ellis and Minton 2006). The guanine nucleotide exchange factor for eIF2 is inhibited by the phosphorylation of eIF2α on serine 51 and slows down the rate of protein translation initiation. This reduces the formation of unfolded proteins entering the ER, hence, conserving ATP and amino acids in ER-stressed cells. The rate of activation of protein translation depends on glucose-induced protein phosphatase -1 (PP1), which inhibits serine 51 phosphorylation of eIF2α. The balance between folding-load-induced phosphorylation and PP1-dependent dephosphorylation reveals the level of eIF2α phosphorylation in β-cells. Dephosphorylation of eIF2α aids glucose tolerance in animal models (Scheuner *et al*., 2005). Zhou *et al*. (1998) revealed that glucose intake may help maintain whole blood viscosity and plasma osmolality under HS condition.

Inhibiting m7 G cap recognition is another mechanism for regulating global protein synthesis. The eukaryotic cap-initiation complex is made up of eIF4E, the cap-binding protein (eIF4G), the scaffold protein (eIF4A), the RNA helicase, and a multiprotein complex (eIF3), which binds to the ribosomal 40S subunit (Gingras *et al*., 1999; Han *et al*., 2020). Cap-dependent initiation is inhibited by eIF4E binding proteins, 4E-BPs, and phosphorylation eliminates affinity between the proteins. The mammalian target of rapamycin (mTOR) is identified as a pivotal signal pathway to regulate muscular hypertrophy and plays an important role in mediating protein synthesis through its downstream targets, p70 ribosomal S6 kinase (p70S6K) and eukaryotic

initiation factor 4E binding protein 1 (4E-BP1) (Ma *et al*., 2018). 4E-BP1 is hyperphosphorylated by mTOR, leading to the inhibition of 4E-BP1 affinity toward eIF4E (Fig 1), hence upregulation of protein synthesis (Hans *et al*., 2020). Under hypoxic conditions, PERK phosphorylates eIF2 and mTOR inhibition (due to decreased insulin signaling) reduces cap-dependent translation (Connolly *et al*., 2006).

One of the molecular chaperones expressed during HS is the family of the HSP70. Glucose-Regulated Protein 78 (GRP78) is a member of HSP70 that is expressed on the membranes of the ER of all eukaryotes. It aids protein folding and assembly while preventing the transport of unfolded proteins (Gething and Sambrook, 1992). ER stress increases the expression of GRP78. It binds to unfolded/misfolded proteins, initiating ER-associated degradation (ERAD) needed to regulate unfolded proteins (Pfaffenbach and Lee, 2011). The binding yields energy that prevents protein aggregation via ATP hydrolysis (Ibrahim *et al*., 2019). GRP78 is naturally found in an inactive form bound to unfolded protein response (UPR) transmembrane stress sensors such as activating transcription factor 6 (ATF6), protein kinase RNA-like endoplasmic reticulum kinase (PERK), and inositol-requiring enzyme 1 (IRE1). Upon exposure to stressors, GRP78 is activated to slow down translation and enhance the proper folding of proteins (Ibrahim *et al*., 2019). Overexpression of GRP78 in the ER causes GRP78 to move to the cell surface where it can act as a multifunctional receptor involved in signaling, proliferation, migration, invasion, apoptosis, inflammation, and immunity of cells.

Insulin aids in the stimulation of skeletal muscle protein synthesis after a meal in growing animals (Davis *et al*., 2002; O'Connor *et al*., 2003). Insulin stimulates whole-body protein synthesis, hind limb protein synthesis, and skeletal muscle protein synthesis in fetal sheep, lamb, and weaned rats (Jeyapalan *et al*., 2007). Increasing insulin during the fed state while maintaining

glucose and amino acids in the fasting state enhanced protein synthesis in the skeletal muscle of neonatal pigs (Davis *et al*., 2002; O'Connor *et al*., 2003). Protein synthesis in tissues was correlated to increasing amino acids in a fed state, and insulin and glucose levels increasing under a fasting condition. This shows that maintaining amino acid composition in tissues during fasted state, (as seen in HS animals), is crucial to achieving optimum performance and productivity in animals. Carbohydrates are largely responsible for the rise in circulatory glucose and insulin after a meal. The rise in insulin level stimulates muscle protein synthesis in the post-weaned rat (Davis *et al.*, 2003). The in vitro reports on the Chinese hamster ovarian cell line (Patel *et al*., 2001) and cardiac myocytes (Yeshao *et al*., 2005) reveal increased glucose levels stimulated protein synthesis independent of insulin. The AMP-activated protein kinase (AMPK) pathway is a viable route for glucose to regulate protein synthesis. AMPK is a sensor of cellular energy, which is activated as the AMP level rises due to energy starvation. This activates the tuberous sclerosis complex ½ (TSC1/2). The activated TSC1/TSC2 inhibits the mammalian target of rapamycin (mTOR) (Fujii *et al*., 2006). mTOR is a protein kinase that regulates the components of translation initiation (Kimball and Jefferson, 2006; Wang and Proud, 2006; Jeyapalan *et al*., 2007). The phosphorylation of mTOR on Ser^{2448} activates the kinase under the stimulation of insulin and amino acids (Mothe-Satney *et al*., 2004). mTOR phosphorylates 70-kDaS6 kinase-1 (S6K1) and eukaryotic initiation factor (eIF)4E- binding protein 1 (4E-BP1), hence regulating mRNA translation (Wang and Proud, 2006; Jeyapalan *et al*., 2007). The activation of S6K1 causes the phosphorylation of the ribosomal subunit (ribosomal protein (rp)S6), which increases the translation of mRNAs encoding proteins in the protein synthetic machinery. The phosphorylation of 4E-BP1 (a repressor protein) permits the dissociation of eIF4E, allowing it to bind to eIF4G. This active complex of eIF4EeIF4G mediates the binding of mRNA to the 40S ribosomal complex

in the initiation of mRNA translation (Kimball and Jefferson, 2006; O'Connor *et al*., 2003, Suryawan *et al*., 2004).

The phosphorylation of eEF2 kinase increases eEF2 phosphorylation on its Thr^{56} residue. This downregulates eEF2, decreasing ribosomal translocation during peptide elongation. Thus, phosphorylation inhibits the elongation step of the translation process. This process is independent of glucose, amino acid, and insulin stimulation, suggesting that initiation rather than elongation step in translation is the primary regulator of protein synthesis in response to feeding (Jeyapalan *et al*., 2007). Raising glucose, insulin, and amino acids increased protein synthesis in most tissues due to the enhanced formation of the eIF4EeIF4G complex and the phosphorylation of PKB, mTOR, S6K1, and 4E-BP1. Jeyapalan *et al*. (2007) reported that increasing only glucose levels in fast-twitch glycolytic muscles increased protein synthesis, due to increased phosphorylation of protein kinase B (PKB) and enhanced formation of the active eIF4EeIF4G complex. The authors observed no change in phosphorylation of AMP-activated protein kinase (AMPK), tuberous sclerosis complex 2 (TSC2), mammalian target of rapamycin (mTOR), 4E-binding protein-1 (4E-BP1), ribosomal protein S6 kinase (S6K1), or eukaryotic elongation factor 2 (eEF2). Protein synthesis in neonatal pigs was stimulated by a postprandial rise in glucose, and not by insulin or amino acids. This response was observed in fast-twitch glycolytic muscle and occurs via AMPK- and mTOR-independent pathways (Jeyapalan *et al*., 2007).

Pentose phosphate pathway (PPP)

The pentose phosphate pathway (PPP) is a fundamental biochemical pathway that maintains carbon homeostasis and supplies the necessary precursors for the formation of nucleotides and amino acids. During this process, NADPH, which serves as the reducing power

for the synthesis of nucleotide and amino acids is produced and helps to combat oxidative stress (Stincone *et al*., 2015). The PPP is divided into two (2) categories, oxidative and non-oxidative branches. The oxidative branch is predominantly active in eukaryotes and yields carbon dioxide, ribulose 5-phosphate, and NADPH from the catabolism of glucose-6-phosphate.The NADPH produced is needed to maintain redox balance under stress situations. The non-oxidative branch utilizes glycolytic intermediates like fructose 6-phosphate, glyceraldehyde 3-phosphate, sedoheptulose sugars, to produce ribose 5-phosphate for the synthesis of the precursors for nucleic acids and sugar phosphates, which in turn are used to produce amino acids. This further support the assertion that glucose supplementation may enhance protein biosynthesis.

Feeding strategies to mitigate heat stress

The focus of feeding strategies to combat HS targets reduction of heat production and facilitating heat dissipation. Reducing heat production can be achieved by methods that improve feed digestibility. It can also be achieved by feed-restriction or manipulating the feed intake of animals such that feed accessibility does not exceed the nutrient requirements. Increasing evaporative cooling through water loss can enhance heat dissipation (Syafwan *et al*., 2011).

Feed restriction or withdrawal is a potent way of reducing heat production in animals. However, it is mostly accompanied by lower body weight due to feed reduction (MacLeod and Hocking, 1993; Abu-Dieyeh, 2006). Feed restriction, during heat stresss, has been reported to have an inconsistent effect on the performance of broiler chickens, with some reporting an improved performance (Yalçin *et al*., 2001; Mohamed *et al*., 2019) and others reporting the opposite (Lozano *et al*., 2006). The timing and extent of feed restriction are suggested to be the reason for the variation in the effect of feed restriction on bird performance (Özkan *et al*., 2003). Feed restriction

reduces mortality (Yalçin *et al*., 2001) and remains a viable means to reduce heat production during HS, and feed removal before the start of the heat stress could help reduce heat production during HS (Fondevila *et al*., 2020). Feed restriction has been used to abate the effect of heat stress in farm animals. It helps to reduce metabolic heat production during the peak hot hours of a day. Heat tolerance is enhanced in poultry birds subjected to 50% feed restriction (Abu-Dieyeh 2006). Broilers are specifically bred for meat purpose and feed restriction will prevent broilers from achieving their optimum performance.

Dual feeding is another feeding strategy used to combat HS. It involves feeding two different diets to animals: one highly concentrated in protein and the other in energy. The feeds are given in a predetermined sequential order or supplied to animals for individual selection. The higher thermogenic property of protein relative to carbohydrates makes it possible to improve the thermotolerance of birds if fed during the peak of the off-HS period (Geraert 1991; Teyssier *et al*., 2022). Dual feeding reduces mortality in broiler chickens under HS condition (Syafwan *et al*., 2012).

Wet feeding (moisturizing feed) was introduced due to the crucial role of water intake under high ambient temperature. There is an increase in water loss through the respiratory tract as a result of panting when birds try to maintain homeostasis under HS (Bruno *et al*., 2011). The improved performance was reported with the use of wet feeding during HS in broilers (Kutlu, 2001; Awojobi *et al*., 2009; Dei and Bumbie, 2011) and laying hens (Tadtiyanant *et al*., 1991). However, the vulnerability of wet feed for mold growth remains a constant challenge to this strategy (Wasti *et al*., 2020).

Feed form and particle size are another feeding strategies used to combat HS in animals. Mash, crumble and pellet are the three forms of feed used for poultry. Pellets are known to improve

feed intake and performance when compared to mash-in HS broilers due to reduction in feed segregation and higher bulk density (Hosseini and Afshar, 2017; Massuquetto *et al*., 2019; Cardoso *et al*., 2022). Coarse feed particle size is reported to increase heat production with a possible mechanism of stimulating the grinding action of the gizzard as compared to finer feed size. More nutrients may be accessible, and energy may be utilized more efficiently if fine particles are used because less heat of digestion will be generated (Hosseini and Afshar, 2017).

Nutritional strategies to mitigate heat stress

Ameliorating the adverse effect of HS through nutritional intervention is a viable means of enhancing the productive performance of poultry (Gelli *et al*. 2017; Shakeri *et al*. 2018). Diets have been recommended for birds during heat stress based on diet composition and time of feeding (Gous and Morris 2005). Heat stress impairs the growth performance of broiler chickens. The hypothalamus is involved in the regulation of feed intake and energy balance while manipulating body temperature and the endocrine system (He *et al*., 2019). The hypothalamus–pituitary–adrenal axis is stimulated under heat stress, thereby increasing blood corticosterone concentration, which reduces feed intake by affecting the center of satiety in the hypothalamus. This slows down the metabolic heat increment associated with digestion and absorption of feed and nutrients. Chronic HS increased the surface temperature of the head and decreased the expression of the orexigenic gene neuropeptide Y, reducing feed intake (FI) in broilers. Reduced FI is a major contributor to the detrimental effect of heat stress as it lowers nutrient uptake and body glucose of the animal. The animal, in turn, utilizes muscle protein as a source of energy for sustenance, thereby disrupting the metabolic activities of the animal.

Several nutritional strategies have been used to combat the effect of heat stress in farm animals. Reducing crude protein (CP) levels in diet and supplementing with specific amino acids is beneficial for heat-stressed animals. Reducing CP levels have been introduced because CP produces heat increment higher than that of fat and carbohydrates. This will ensure that less heat is produced during metabolism. This will encourage better feed intake, production performance and carcass quality (Daghir, 2009; Ratriyanto and Indreswari, 2014). A balanced amino acid profile is preferred over specific crude protein level because birds have been observed to have specific amino acid profile requirement and not CP requirement (Awad *et* al., 2019). Hence, reducing CP and supplementing AAs could alleviate some of the detrimental effects of HS in broiler chickens.

The use of antioxidants (mainly vitamins) to prevent oxidative stress resulting from HS in poultry is well documented. Vitamin A, C, and E are beneficial in poultry as they improves performance and reduce oxidative stress (Sahin *et al*. 2001). Supplementation of 250 mg/kg of vitamin E in a broiler diet reduced the negative effects of heat stress (Sahin *et al*. 2001). Minerals are important in maintaining normal physiological functions in animals. HS increases mineral and body fluid excretion in dairy cows. Hence, supplementation of minerals in diets to maintain body mineral balance might alleviate the adverse effects of heat stress in animals. Chromium (Cr) is effective in ameliorating the detrimental effect of HS. In dairy cows, supplementation of chelated Cr yeast at 4 g/day in the diet increased feed intake from 19.56 to 21.24 kg/day and milk yield from 29.87 to 33.24 kg/day in heat-stressed Holstein dairy cows (Al-Saiady *et al*. 2004). Sodium and potassium are used as metal ion buffers in compensating for reduced feed intake in heat-stressed animals (Sanchez *et al*. 1994; Min *et al*., 2019). Phytochemicals, such as polyphenols, can boost the expression of stress response proteins such as HSP and antioxidant enzymes. This limits

damage caused by reactive oxygen species (ROS) (Hu *et al*. 2019). Vitamins (C and E), carotenoid, and minerals (copper, zinc, and selenium) enhance the antioxidant system and aid the biotransformation process in the body during stressful conditions (Karami *et al*. 2018).

The use of lipids in poultry and swine nutrition is based on the importance of dietary fat as an energy source. They are essential, bioactive nutrients that influence many important physiological processes including lipid metabolism, cell division, and immune function and differentiation. Fat inclusion decreases feed intake since the birds eat to meet the energy requirement (Ferket and Gernat 2006). Relative to protein or carbohydrates inclusion, dietary fat inclusion increases metabolic heat production in birds raised under thermoneutral condition (Musharaf and Latshaw 1999).

Water regulation in the body

The hypothalamic–pituitary–adrenal axis is a major player in controlling the stress response in the body (Collier *et al*., 2008). Upon initiation, it acts on the hypothalamic paraventricular nucleus to stimulate corticotropin-releasing hormone. Adrenocorticotropic hormone is released from the pituitary to stimulate the release of glucocorticoids from the adrenal cortex (Baumgard and Rhoads, 2013). The hypothalamus maintains body homeostasis, which includes regulation of temperature, water and salt balance, blood pressure, reproductive function and other stress responses (Yu *et al*., 2020). Osmoreceptors in the hypothalamus detect changes in plasma osmolarity, altering the diffusion of water in and out of the osmoreceptors. At high plasma osmolarity, osmoregulators send signals to the hypothalamus to initiate a biological sense of thirst. The osmoreceptors simultaneously stimulate the secretion of vasopressin to reduce the high plasma osmolarity to normal levels. Angiotensin II also acts on the hypothalamus to create a sense of

thirst. It releases aldosterone to increase the reabsorption of water in a similar way to vasopressin. Water reabsorption in the descending limb of Henle's loop and the collecting duct is based on active transport in renal tubule segments different from the site of water transport.

The kidney is also important in maintaining homeostasis in the body. Water and salt balance in the body is principally regulated by the kidney. This balance is dependent on the collection of water and solute transporters in the renal tubules and vascular elements in the kidney. Water reabsorption in renal tubules is dictated by the hydration status of the organism as the kidney adjusts osmolality of urine to maintain serum osmolality based on that. The proximal tubules and the thin descending limb have a higher water reabsorption than the distal tubules and collecting ducts. Water reabsorption in the proximal tubules depends on the reabsorption of solutes (mainly NaCl) and the extent to which water can permeate the epithelium. On the other hand, the active transport occurring in the non-water transport segment of the renal tubules aids reabsorption of water in the descending limb of Henle's loop and the collecting duct.

Glucose as a nutritional strategy to mitigate the adverse effect of heat stress

Heat stress raises the concentration of blood corticosterone by stimulating the hypothalamus–pituitary–adrenal axis. This reduces feed intake and slows down metabolic heat increment from digestion and absorption of feed and nutrient (He *et al*., 2019). Chronic HS increased body surface temperature and decreased the expression of the orexigenic gene neuropeptide Y, reducing feed intake in broilers. Reduced feed intake lowers nutrient uptake and body glucose of the animal. It depletes blood and tissue glucose, which affects myriads of metabolic activities and results in poor performance. The animal, in turn, utilizes muscle protein as a source of energy for sustenance, thereby disrupting the metabolic activities of the animal.

However, Protein kinase B (PKB) phosphorylation and enhanced active eIF4E formation in fast-twitch glycolytic muscles resulted in an increase in protein synthesis as observed by Jeyapalan *et al*. (2007) when glucose levels were increased in neonatal pigs. O'Brien *et al*. (2010) reported post-absorptive metabolism of carbohydrate independent of reduced nutrient intake in heat stressed growing Holstein Calves, showing that glucose may have a positive impact on HS animals. This was measured by the basal insulin concentrations and insulin response to a glucose tolerance test in the experimental animals. Chickens under HS increase intestinal Na-dependent glucose transporter 1 activity and thus glucose absorption (Goel *et al*., 2021). Chromium facilitates insulin action on glucose metabolism, and its supplementation has been suggested to alleviate chickens from the deleterious effects of HS. During HS, signaling cascades governed by p38MAPK reprogram glucose metabolism to favor glucose uptake and shift glucose metabolism to an increase in NADPH, which in turn contributes to the buffering of oxidative damage. As a result, we hypothesize that increasing tissue glucose levels and the resultant elevation in tissue NADPH during HS could be an efficient intervention to mitigate the negative consequences of HS resulting from oxidative damage.

LIST OF FIGURES

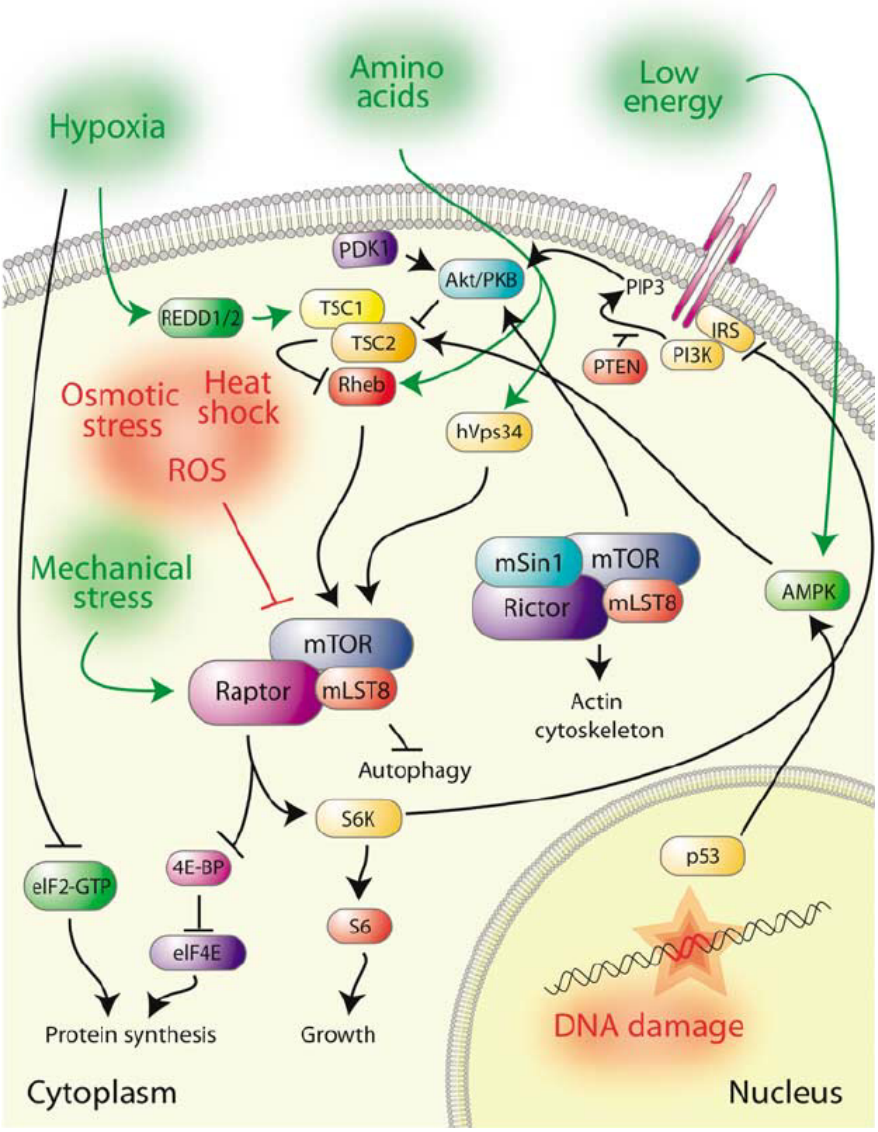

Fig 2.1: role of mTOR in protein synthesis

Source: Reiling and Sabatini, 2006

REFERENCES

Abu-Dieyeh Z. H. M. (2006). Effect of chronic heat stress and long-term feed restriction on broiler performance. *Int. J. Poult. Sci.* 5, 185–190. 10.3923/ijps.2006.185.190.

Akbarian, A., Michiels, J., Degroote, J., Majdeddin, M., Golian, A., & De Smet, S. (2016). Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. *Journal of animal science and biotechnology*, *7*(1), 1-14.

Al-Saiady, M. Y., Al-Shaikh, M. A., Al-Mufarrej, S. I., Al-Showeimi, T. A., Mogawer, H. H., & Dirrar, A. (2004). Effect of chelated chromium supplementation on lactation performance and blood parameters of Holstein cows under heat stress. *Animal Feed Science and Technology*, *117*(3-4), 223-233.

Ali, Q., Farooq, U., Akhtar, P., Khalid, M.F., Khan, S.U. (2019). Impact of Glucose to Mitigate the Heat Stress in Broiler Chickens. PSM Biol. Res., 4(4): 144-153.

Altan, O., A. Pabuccuoglu, A. Altan, S. Konyalioglu, and H. Bayrak-tar. (2003). Effect of heat stress on oxidative stress, lipid peroxi-dation and some stress parameters in broilers. Br. Poult. Sci.44:545–550.

Ambazamkandi, P., Thyagarajan, G., Sambasivan, S., Davis, J., Shanmugam, S., & Joseph, B. A. (2015). Shelter design for different livestock from a climate change perspective. In *Climate Change Impact on Livestock: Adaptation and Mitigation* (pp. 399-424). Springer, New Delhi.

Ashraf, N. U., & Sheikh, T. A. (2015). Endoplasmic reticulum stress and oxidative stress in the pathogenesis of non-alcoholic fatty liver disease. *Free radical research*, *49*(12), 1405-1418.

Awad, E. A., Najaa, M., Zulaikha, Z. A., Zulkifli, I., & Soleimani, A. F. (2019). Effects of heat stress on growth performance, selected physiological and immunological parameters, caecal microflora, and meat quality in two broiler strains. Asian-Australasian Journal of Animal Sciences, 33(5), 778–787.

Awad, E. A., Najaa, M., Zulaikha, Z. A., Zulkifli, I., & Soleimani, A. F. (2020). Effects of heat stress on growth performance, selected physiological and immunological parameters, caecal microflora, and meat quality in two broiler strains. *Asian-Australasian Journal of Animal Sciences*, *33*(5), 778.

Awojobi H. A., Oluwole B. O., Adekunmisi A. A., Buraimo R. A. (2009). Performance of finisher broilers fed wet mash with or without drinking water during wet season in the tropics. *Int. J. Poult. Sci.* 8, 592–594. 10.3923/ijps.2009.592.594.

Barrett, N. W., Rowland, K., Schmidt, C. J., Lamont, S. J., Rothschild, M. F., Ashwell, C. M., & Persia, M. E. (2019). Effects of acute and chronic heat stress on the performance, egg quality, body temperature, and blood gas parameters of laying hens. *Poultry Science*, *98*(12), 6684-6692.

Baumgard, L. H., & Rhoads Jr, R. P. (2013). Effects of heat stress on postabsorptive metabolism and energetics. *Annu. Rev. Anim. Biosci.*, *1*(1), 311-337.

Betteridge, D. J. (2000). What is oxidative stress?. *Metabolism*, *49*(2), 3-8.

Bouchama, A., & Knochel, J. P. (2002). Heat stroke. *New England journal of medicine*, *346*(25), 1978-1988.

Bouchama, A., Al-Sedairy, S., Siddiqui, S., Shail, E., & Bezeig, M. (1993). Elevated pyrogenic cytokines in heatstroke. *Chest*, *104*(5), 1498-1502.

Boyd, A., Philbin, V. J., & Smith, A. L. (2007). Conserved and distinct aspects of the avian Toll-like receptor (TLR) system: implications for transmission and control of bird-borne zoonoses. *Biochemical Society Transactions*, *35*(6), 1504-1507.\

Bruno L. D. G., Maiorka A., Macari M., Furlan R. L., Givisiez P. E. N. (2011). Water intake behavior of broiler chickens exposed to heat stress and drinking from bell or and nipple drinkers. *Rev. Bras. Cienc. Avic.* 13, 147–152. 10.1590/s1516-635x2011000200009.

Cannons, J. L., Choi, Y., & Watts, T. H. (2000). Role of TNF receptor-associated factor 2 and p38 mitogen-activated protein kinase activation during 4-1BB-dependent immune response. *The Journal of Immunology*, *165*(11), 6193-6204.

Cardoso D. M., Cardeal P. C., Soares K. R., Sousa L. S., Castro F. L. S., Araújo I. C. S., et al. (2022). Feed form and nutritional level for rearing growing broilers in thermoneutral or heat stress environments. *J. Therm. Biol.* 103, 103159. 10.1016/j.jtherbio.2021.103159.

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.

Connolly, E., Braunstein, S., Formenti, S., & Schneider, R. J. (2006). Hypoxia inhibits protein synthesis through a 4E-BP1 and elongation factor 2 kinase pathway controlled by mTOR and uncoupled in breast cancer cells. *Molecular and cellular biology*, *26*(10), 3955-3965.

Daghir, N. J. (2008). Nutrient requirements of poultry at high temperature. *Poultry production in hot climate*, *133*, 60.

Daghir, N. J. (2009). Nutritional strategies to reduce heat stress in broilers and broiler breeders. *Lohmann information*, *44*(1), 6-15.

Das, R., Sailo, L., Verma, N., Bharti, P., & Saikia, J. (2016). Impact of heat stress on health and performance of dairy animals: A review. *Veterinary world*, *9*(3), 260.

Davis, T. A., Fiorotto, M. L., Burrin, D. G., Reeds, P. J., Nguyen, H. V., Beckett, P. R., ... & O'Connor, P. M. (2002). Stimulation of protein synthesis by both insulin and amino acids is unique to skeletal muscle in neonatal pigs. *American Journal of Physiology-Endocrinology and Metabolism*, *282*(4), E880-E890.

Deeb, N., & Cahaner, A. (2002). Genotype-by-environment interaction with broiler genotypes differing in growth rate. 3. Growth rate and water consumption of broiler progeny from weight-selected versus nonselected parents under normal and high ambient temperatures. *Poultry Science*, *81*(3), 293-301.

Dei H. K., Bumbie G. Z. (2011). Effect of wet feeding on growth performance of broiler chickens in a hot climate. *Br. Poult. Sci.* 52, 82–85. 10.1080/00071668.2010.540230.

Dervisevik, M., Dinevska-Kjovkarovska, S., Miova, B., Mitev, S., Velkovski, M., & Susleski, D. (2011). Heat acclimation-induced changes in heart glycogen/glucose metabolism in rats. *The Journal of Physiological Sciences*, *61*(5), 395-402.

Dröge W. Free radicals in the physiological control of cell function. Physiol Rev. 2002 Jan;82(1):47-95. doi: 10.1152/physrev.00018.2001. PMID: 11773609.

Ellis, R. J., & Minton, A. P. (2006). Protein aggregation in crowded environments.

Epstein, Y., & Roberts, W. O. (2011). The pathopysiology of heat stroke: an integrative view of the final common pathway. *Scandinavian journal of medicine & science in sports*, *21*(6), 742-748.

Ferket, P. R., & Gernat, A. G. (2006). Factors that affect feed intake of meat birds: A review. *Int. J. Poult. Sci*, *5*(10), 905-911.

Fernandez, M. S., Pearce, S. C., Gabler, N. K., Patience, J. F., Wilson, M. E., Socha, M. T., ... & Baumgard, L. H. (2014). Effects of supplemental zinc amino acid complex on gut integrity in heat-stressed growing pigs. *Animal*, *8*(1), 43-50.

Fondevila G., Archs J. L., Cámara L., de Juan A. F., Mateos G. G. (2020). The length of the feed restriction period affects eating behavior, growth performance, and the development of the proximal part of the gastrointestinal tract of young broilers. *Poult. Sci.* 99, 1010–1018. 10.1016/j.psj.2019.10.011.

Fujii, N., Jessen, N., & Goodyear, L. J. (2006). AMP-activated protein kinase and the regulation of glucose transport. *American Journal of Physiology-Endocrinology and Metabolism*, *291*(5), E867-E877.

Gabler, N. K., & Pearce, S. C. (2015). The impact of heat stress on intestinal function and productivity in grow-finish pigs. *Animal Production Science*, *55*(12), 1403-1410.

Gamba, P., Testa, G., Gargiulo, S., Staurenghi, E., Poli, G., & Leonarduzzi, G. (2015). Oxidized cholesterol as the driving force behind the development of Alzheimer's disease. *Frontiers in aging neuroscience*, *7*, 119.

Gelli, A., Becquey, E., Ganaba, R., Headey, D., Hidrobo, M., Huybregts, L., ... & Guedenet, H. (2017). Improving diets and nutrition through an integrated poultry value chain and nutrition intervention (SELEVER) in Burkina Faso: study protocol for a randomized trial. *Trials*, *18*(1), 1-16.

Geraert P. A. (1991). Métabolisme énergétique du poulet de chair en climat chaud. *INRA. Prod. Anim.* 4, 257–267. 10.20870/productions-animales.1991.4.3.4340.

Gething, M. J., & Sambrook, J. (1992). Protein folding in the cell. *Nature*, *355*(6355), 33-45.

Gingras, A. C., Raught, B., & Sonenberg, N. (1999). eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. *Annual review of biochemistry*, *68*(1), 913-963.

Giri, A., Heckathorn, S., Mishra, S., & Krause, C. (2017). Heat stress decreases levels of nutrient-uptake and-assimilation proteins in tomato roots. *Plants*, *6*(1), 6.

Goel, A., Ncho, C. M., & Choi, Y. H. (2021). Regulation of gene expression in chickens by heat stress. *Journal of Animal Science and Biotechnology*, *12*(1), 1-13.

Goldstein-Piekarski, A. N., Greer, S. M., Saletin, J. M., & Walker, M. P. (2015). Sleep deprivation impairs the human central and peripheral nervous system discrimination of social threat. *Journal of Neuroscience*, *35*(28), 10135-10145.

Gous, R. M., & Morris, T. R. (2005). Nutritional interventions in alleviating the effects of high temperatures in broiler production. *World's Poultry Science Journal*, *61*(3), 463-475.

Gresse, R., Chaucheyras-Durand, F., Fleury, M. A., Van de Wiele, T., Forano, E., & Blanquet-Diot, S. (2017). Gut microbiota dysbiosis in postweaning piglets: understanding the keys to health. *Trends in microbiology*, *25*(10), 851-873.

Gupta, S. K., Singh, L. V., Chellappa, M. M., & Dey, S. (2015). Toll-like receptor ligands and their combinations as adjuvants-current research and its relevance in chickens. *World's Poultry Science Journal*, *71*(1), 95-110.

Habashy, W. S., Milfort, M. C., Fuller, A. L., Attia, Y. A., Rekaya, R., & Aggrey, S. E. (2017). Effect of heat stress on protein utilization and nutrient transporters in meat-type chickens. *International Journal of Biometeorology*, *61*(12), 2111-2118.

Habibian, M., Ghazi, S., Moeini, M. M., & Abdolmohammadi, A. (2014). Effects of dietary selenium and vitamin E on immune response and biological blood parameters of broilers reared under thermoneutral or heat stress conditions. *International journal of biometeorology*, *58*(5), 741-752.

Hall, D. M., Buettner, G. R., Oberley, L. W., Xu, L., Matthes, R. D., & Gisolfi, C. V. (2001). Mechanisms of circulatory and intestinal barrier dysfunction during whole body hyperthermia. *American Journal of Physiology-Heart and Circulatory Physiology*, *280*(2), H509-H521.

Halliwell, B. (2006). Oxidative stress and neurodegeneration: where are we now?. *Journal of neurochemistry*, *97*(6), 1634-1658.

Han, Q., Zhang, J., Sun, Q., Xu, Y., & Teng, X. (2020). Oxidative stress and mitochondrial dysfunction involved in ammonia-induced nephrocyte necroptosis in chickens. *Ecotoxicology and environmental safety*, *203*, 110974.

Hartl, F. U. (1996). Molecular chaperones in cellular protein folding. *Nature*, *381*(6583), 571-580.

He, X., Lu, Z., Ma, B., Zhang, L., Li, J., Jiang, Y., ... & Gao, F. (2018). Effects of chronic heat exposure on growth performance, intestinal epithelial histology, appetite-related hormones and genes expression in broilers. *Journal of the Science of Food and Agriculture*, *98*(12), 4471-4478.

He, X., Lu, Z., Ma, B., Zhang, L., Li, J., Jiang, Y., ... & Gao, F. (2019). Chronic heat stress alters hypothalamus integrity, the serum indexes and attenuates expressions of hypothalamic appetite genes in broilers. *Journal of thermal biology*, *81*, 110-117.

Heled, Y., Fleischmann, C., & Epstein, Y. (2013). Cytokines and their role in hyperthermia and heat stroke. *Journal of basic and clinical physiology and pharmacology*, *24*(2), 85-96.

Helwig, B. G., & Leon, L. R. (2011). Tissue and circulating expression of IL-1 family members following heat stroke. *Physiological Genomics*, *43*(19), 1096-1104.

Hietbrink, F., Koenderman, L., Rijkers, G. T., & Leenen, L. P. (2006). Trauma: the role of the innate immune system. *World Journal of Emergency Surgery*, *1*(1), 1-11.

Horowitz, J. F. (2003). Fatty acid mobilization from adipose tissue during exercise. *Trends in Endocrinology & Metabolism*, *14*(8), 386-392.

Hosseini, S. M., & Afshar, M. (2017). Effects of feed form and xylanase supplementation on performance and ileal nutrients digestibility of heat-stressed broilers fed wheat–soybean diet. *Journal of Applied Animal Research*, *45*(1), 550-556.

Hu, L., Zhang, Y., Miao, W., & Cheng, T. (2019). Reactive oxygen species and Nrf2: functional and transcriptional regulators of hematopoiesis. *Oxidative medicine and cellular longevity*, *2019*.

Ibrahim, D., El Sayed, R., Abdelfattah-Hassan, A., & Morshedy, A. M. (2019). Creatine or guanidinoacetic acid? Which is more effective at enhancing growth, tissue creatine stores, quality of meat, and genes controlling growth/myogenesis in Mulard ducks. *Journal of Applied Animal Research*, *47*(1), 159-166.

Jeyapalan, A. S., Orellana, R. A., Suryawan, A., O'Connor, P. M., Nguyen, H. V., Escobar, J., ... & Davis, T. A. (2007). Glucose stimulates protein synthesis in skeletal muscle of neonatal pigs through an AMPK-and mTOR-independent process. *American journal of physiology-Endocrinology and metabolism*, *293*(2), E595-E603.

Ji, W. W., Wang, S. Y., Ma, Z. Q., Li, R. P., Li, S. S., Xue, J. S., ... & Ma, S. P. (2014). Effects of perillaldehyde on alternations in serum cytokines and depressive-like behavior in mice after lipopolysaccharide administration. *Pharmacology Biochemistry and Behavior*, *116*, 1-8.

Kadzere, C. T., Murphy, M. R., Silanikove, N., & Maltz, E. (2002). Heat stress in lactating dairy cows: a review. *Livestock production science*, *77*(1), 59-91.

Kaiser, P., & Stäheli, P. (2014). Avian cytokines and chemokines. In *Avian immunology* (pp. 189-204). Academic Press.

Kaiser, P., Wu, Z., Rothwell, L., Fife, M., Gibson, M., Poh, T. Y., ... & Shini, S. (2009). Prospects for understanding immune-endocrine interactions in the chicken. *General and Comparative Endocrinology*, *163*(1-2), 83-91.

Karami, S., Rahimi, M., & Babaei, A. (2018). An overview on the antioxidant, anti-inflammatory, antimicrobial and anti-cancer activity of grape extract. *Biomed Res Clin Pract*, *3*(2), 1-4.

Kimball, S. R. (2006). Jefferson LS. *New functions for amino acids: effects on gene transcription and translation. Am J Clin Nutr*, *83*, 500S-507S.

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*, *116*(21), 10333-10338.

Kutlu H. R. (2001). Influences of wet feeding and supplementation with ascorbic acid on performance and carcass composition of broiler chicks exposed to a high ambient temperature. *Arch. Tierernahr.* 54, 127–139. 10.1080/17450390109381972.

Lambert, G. P. (2009). Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. *Journal of animal science*, *87*(suppl_14), E101-E108.

Lauridsen, C. (2019). From oxidative stress to inflammation: redox balance and immune system. *Poultry science*, *98*(10), 4240-4246.

Leon, L. R., & Helwig, B. G. (2010). Heat stroke: role of the systemic inflammatory response. *Journal of applied physiology*, *109*(6), 1980-1988.

Lilienbaum, A. (2013). Relationship between the proteasomal system and autophagy. *International journal of biochemistry and molecular biology*, *4*(1), 1.

Lin, H., Du, R., & Zhang, Z. Y. (2000). Peroxide status in tissues of heat-stressed broilers. *Asian-Australasian Journal of Animal Sciences*, *13*(10), 1373-1376.

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.

Liu, L., Fu, C., Yan, M., Xie, H., Li, S., Yu, Q., & He, J. (2016). Resveratrol modulates intestinal morphology and HSP70/90, NF-κB and EGF expression in the jejunal mucosa of black-boned chickens on exposure to circular heat stress. Food & Function, 7(3), 1329–1338. https://doi.org/10.1039/c5fo01338k.

Lozano C., De Basilio V., Oliveros I., Alvarez R., Colina I., Bastianelli D., et al. (2006). Is sequential feeding a suitable technique to compensate for the negative effects of a tropical climate in finishing broilers? *Anim. Res.* 55, 71–76. 10.1051/animres:2005047.

Lunt, S. Y., & Vander Heiden, M. G. (2011). Aerobic glycolysis: meeting the metabolic requirements of cell proliferation.

Luster, A. D., Alon, R., & von Andrian, U. H. (2005). Immune cell migration in inflammation: present and future therapeutic targets. *Nature immunology*, *6*(12), 1182-1190.

Ma, B., He, X., Lu, Z., Zhang, L., Li, J., Jiang, Y., ... & Gao, F. (2018). Chronic heat stress affects muscle hypertrophy, muscle protein synthesis and uptake of amino acid in broilers via insulin like growth factor-mammalian target of rapamycin signal pathway. *Poultry science*, *97*(12), 4150-4158.

Ma, B., Zhang, L., Li, J., Xing, T., Jiang, Y., & Gao, F. (2021). Heat stress alters muscle protein and amino acid metabolism and accelerates liver gluconeogenesis for energy supply in broilers. *Poultry science*, *100*(1), 215-223.

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.

MacLeod M. G., Hocking P. M. (1993). Thermoregulation at high ambient temperature in genetically fat and lean broiler hens fed *ad libitum* or on a controlled-feeding regime. *Br. Poult. Sci.* 34, 589–596. 10.1080/00071669308417614.

Mahmoud, K. Z., & Edens, F. W. (2003). Influence of selenium sources on age-related and mild heat stress-related changes of blood and liver glutathione redox cycle in broiler chickens (Gallus domesticus). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *136*(4), 921-934.

Massuquetto A., Panisson J. C., Marx F. O., Surek D., Krabbe E. L., Maiorka A. (2019). Effect of pelleting and different feeding programs on growth performance, carcass yield, and nutrient digestibility in broiler chickens. *Poult. Sci.* 98, 5497–5503. 10.3382/ps/pez176.

Min, L., Li, D., Tong, X., Nan, X., Ding, D., Xu, B., & Wang, G. (2019). Nutritional strategies for alleviating the detrimental effects of heat stress in dairy cows: a review. *International Journal of Biometeorology*, *63*(9), 1283-1302.

Mohamed A. S. A., Lozovskiy A. R., Ali A. M. A. (2019). Strategies to combat the deleterious impacts of heat stress through feed restrictions and dietary supplementation (vitamins, minerals) in broilers. *J. Indones. Trop. Anim. Agric.* 44, 155–166. 10.14710/jitaa.44.2.155-166.

Mothe-Satney, I., Gautier, N., Hinault, C., Lawrence, J. C., & Van Obberghen, E. (2004). In rat hepatocytes glucagon increases mammalian target of rapamycin phosphorylation on serine 2448 but antagonizes the phosphorylation of its downstream targets induced by insulin and amino acids. *Journal of Biological Chemistry*, *279*(41), 42628-42637.

Musharaf, N. A., & Latshaw, J. D. (1999). Heat increment as affected by protein and amino acid nutrition. *World's Poultry Science Journal*, *55*(3), 233-240.

Nagami, H., Yoshimoto, N., Umakoshi, H., Shimanouchi, T., & Kuboi, R. (2005). Liposome-assisted activity of superoxide dismutase under oxidative stress. *Journal of bioscience and bioengineering*, *99*(4), 423-428.

Niu, Z. Y., Liu, F. Z., Yan, Q. L., & Li, W. C. (2009). Effects of different levels of vitamin E on growth performance and immune responses of broilers under heat stress. *Poultry science*, *88*(10), 2101-2107.

O'Connor, P. M., Bush, J. A., Suryawan, A., Nguyen, H. V., & Davis, T. A. (2003). Insulin and amino acids independently stimulate skeletal muscle protein synthesis in neonatal pigs. *American Journal of Physiology-Endocrinology and Metabolism*, *284*(1), E110-E119.

O'hara, T. C. (1981). *Electron-phonon scattering rates in antimony: radio-frequency size effect*. Louisiana State University and Agricultural & Mechanical College.

O'Brien, M. D., Rhoads, R. P., Sanders, S. R., Duff, G. C., & Baumgard, L. H. (2010). Metabolic adaptations to heat stress in growing cattle. *Domestic animal endocrinology*, *38*(2), 86-94.

Ohtsu, H., Yamazaki, M., Abe, H., Murakami, H., & Toyomizu, M. (2015). Heat stress modulates cytokine gene expression in the spleen of broiler chickens. *The Journal of Poultry Science*, 0150062.

Oskoueian, E., Abdullah, N., Idrus, Z., Ebrahimi, M., Goh, Y. M., Shakeri, M., & Oskoueian, A. (2014). Palm kernel cake extract exerts hepatoprotective activity in heat-induced oxidative stress in chicken hepatocytes. *BMC complementary and alternative medicine*, *14*(1), 1-10.

Oyadomari, S., & Mori, M. (2004). Roles of CHOP/GADD153 in endoplasmic reticulum stress. *Cell Death & Differentiation*, *11*(4), 381-389.

Özkan S., Akbaş Y., Altan Ö., Altan A., Ayhan V., ÖZkan K. (2003). The effect of short-term fasting on performance traits and rectal temperature of broilers during the summer season. *Br. Poult. Sci.* 44, 88–95. 10.1080/0007166031000085292.

Patel, J., Wang, X., & Proud, C. G. (2001). Glucose exerts a permissive effect on the regulation of the initiation factor 4E binding protein 4E-BP1. *Biochemical Journal*, *358*(2), 497-503.

Pedersen, B. K., Bruunsgaard, H., Ostrowski, K., Krabbe, K., Hansen, H., Krzywkowski, K., ... & Schjerling, P. (2000). Cytokines in aging and exercise. *International journal of sports medicine*, *21*(Sup. 1), 4-9.

Peterson, C. Y., Costantini, T. W., Loomis, W. H., Putnam, J. G., Wolf, P., Bansal, V., ... & Coimbra, R. (2010). Toll-like receptor-4 mediates intestinal barrier breakdown after thermal injury. *Surgical infections*, *11*(2), 137-144.

Pfaffenbach, K. T., & Lee, A. S. (2011). The critical role of GRP78 in physiologic and pathologic stress. *Current opinion in cell biology*, *23*(2), 150-156.

Poorghasemi, M., Seidavi, A., Qotbi, A. A. A., Laudadio, V., & Tufarelli, V. (2013). Influence of dietary fat source on growth performance responses and carcass traits of broiler chicks. *Asian-Australasian journal of animal sciences*, *26*(5), 705-710.

Quinteiro-Filho, W. M., Ribeiro, A., Ferraz-de-Paula, V., Pinheiro, M. L., Sakai, M., Sá, L. R. M. D., ... & 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.

Randall, W. C. (1943). Factors influencing the temperature regulation of birds. *American Journal of Physiology-Legacy Content*, *139*(1), 56-63.

Ratriyanto, A., & Indreswari, R. (2014). Effects of protein levels and supplementation of methyl group donor on nutrient digestibility and performance of broiler chickens in the tropics. *International Journal of Poultry Science*, *13*(10), 575-581.

Reiling, J. H., & Sabatini, D. M. (2006). Stress and mTORture signaling. *Oncogene*, *25*(48), 6373-6383.

Renaudeau, D, C Anais, L Tel, and J L Gourdine. . 2010. Effect of temperature on thermal acclimation in growing pigs estimated using a nonlinear function. *J. Anim. Sci*. 88:3715–3724. doi: 10.2527/jas.2009-2169.

Rimoldi, S., Lasagna, E., Sarti, F. M., Marelli, S. P., Cozzi, M. C., Bernardini, G., & Terova, G. (2015). Expression profile of six stress-related genes and productive performances of fast and slow growing broiler strains reared under heat stress conditions. *Meta gene*, *6*, 17-25.

Sahin, K., & Kucuk, O. (2003). Heat stress and dietary vitamin supplementation of poultry diets. *CABI Reviews*, (2003), 10-pp.

Sahin, K., Orhan, C., Tuzcu, M., Ali, S., Sahin, N., & Hayirli, A. (2010). Epigallocatechin-3-gallate prevents lipid peroxidation and enhances antioxidant defense system via modulating hepatic nuclear transcription factors in heat-stressed quails. *Poultry Science*, *89*(10), 2251-2258.

Sahin, K., Sahin, N., Onderci, M., Yaralioglu, S., & Kucuk, O. (2001). Protective role of supplemental vitamin E on lipid peroxidation, vitamins E, A and some mineral concentrations of broilers reared under heat stress. *VETERINARNI MEDICINA-PRAHA-*, *46*(5), 140-144.

Saleh, K. M., & Al-Zghoul, M. B. (2019). Effect of acute heat stress on the mRNA levels of cytokines in broiler chickens subjected to embryonic thermal manipulation. *Animals*, *9*(8), 499.

Sammad, A., Wang, Y. J., Umer, S., Lirong, H., Khan, I., Khan, A., ... & Wang, Y. (2020). Nutritional physiology and biochemistry of dairy cattle under the influence of heat stress: Consequences and opportunities. *Animals*, *10*(5), 793.

Sanchez, W. K., McGuire, M. A., & Beede, D. K. (1994). Macromineral nutrition by heat stress interactions in dairy cattle: review and original research. *Journal of dairy science*, *77*(7), 2051-2079.

Scheuner, D., Mierde, D. V., Song, B., Flamez, D., Creemers, J. W., Tsukamoto, K., & Kaufman, R. J. (2005). Control of mRNA translation preserves endoplasmic reticulum function in beta cells and maintains glucose homeostasis. *Nature medicine*, *11*(7), 757-764.

Shakeri, M., Cottrell, J. J., Wilkinson, S., Ringuet, M., Furness, J. B., & Dunshea, F. R. (2018). Betaine and antioxidants improve growth performance, breast muscle development and

ameliorate thermoregulatory responses to cyclic heat exposure in broiler chickens. *Animals*, *8*(10), 162.

Song, J., Jiao, L. F., Xiao, K., Luan, Z. S., Hu, C. H., Shi, B., & Zhan, X. A. (2013). Cello-oligosaccharide ameliorates heat stress-induced impairment of intestinal microflora, morphology and barrier integrity in broilers. *Animal feed science and technology*, *185*(3-4), 175-181.

Song, Z., Cheng, K., Zhang, L., & Wang, T. (2017). Dietary supplementation of enzymatically treated Artemisia annua could alleviate the intestinal inflammatory response in heat-stressed broilers. *Journal of Thermal Biology*, *69*, 184-190.

Spees, A. M., Wangdi, T., Lopez, C. A., Kingsbury, D. D., Xavier, M. N., Winter, S. E., ... & Bäumler, A. J. (2013). Streptomycin-induced inflammation enhances Escherichia coli gut colonization through nitrate respiration. *MBio*, *4*(4), e00430-13.

Sprague, A. H., & Khalil, R. A. (2009). Inflammatory cytokines in vascular dysfunction and vascular disease. *Biochemical pharmacology*, *78*(6), 539-552.

St-Pierre N. R., Cobanov B., Schnitkey G. (2003). Economic losses from heat stress by US livestock industries. *J. Dairy Sci.* 86, E52–E77. 10.3168/jds.S0022-0302(03)74040-5

Stincone, A., Prigione, A., Cramer, T., Wamelink, M. M., Campbell, K., Cheung, E., ... & Ralser, M. (2015). The return of metabolism: biochemistry and physiology of the pentose phosphate pathway. *Biological Reviews*, *90*(3), 927-963.

Suganya, T., Senthilkumar, S., Deepa, K., & Amutha, R. (2015). Nutritional management to alleviate heat stress in broilers. *Int J Sci Environ Technol*, *4*(3), 661-6.

Suryawan, A., O'Connor, P. M., Kimball, S. R., Bush, J. A., Nguyen, H. V., Jefferson, L. S., & Davis, T. A. (2004). Amino acids do not alter the insulin-induced activation of the insulin signaling pathway in neonatal pigs. *The Journal of nutrition*, *134*(1), 24-30.

Syafwan S., Kwakkel R. P., Verstegen M. W. A. (2011). Heat stress and feeding strategies in meat-type chickens. *World's. Poult. Sci. J.* 67, 653–674. 10.1017/S0043933911000742.

Syafwan S., Wermink G. J. D., Kwakkel R. P., Verstegen M. W. A. (2012). Dietary self-selection by broilers at normal and high temperature changes feed intake behavior, nutrient intake, and performance. *Poult. Sci.* 91, 537–549. 10.3382/ps.2011-01559.

Tabiri, H. Y., Sato, K., Takahashi, K., Toyomizu, M., & Akiba, Y. (2002). Hepatic tyrosine aminotransferase activity is affected by chronic heat stress and dietary tyrosine in broiler chickens. *British poultry science*, *43*(4), 629-634.

Tadtiyanant C., Lyons J. J., Vandepopuliere J. M. (1991). Influence of wet and dry feed on laying hens under heat stress. *Poult. Sci.* 70, 44–52. 10.3382/ps.0700044.

Tajima, K., Nonaka, I., Higuchi, K., Takusari, N., Kurihara, M., Takenaka, A., ... & Aminov, R. I. (2007). Influence of high temperature and humidity on rumen bacterial diversity in Holstein heifers. *Anaerobe*, *13*(2), 57-64.

Teeter, R. G., & Belay, T. (1996). Broiler management during acute heat stress. *Animal Feed Science and Technology*, *58*(1-2), 127-142.

Temperley, N. D., Berlin, S., Paton, I. R., Griffin, D. K., & Burt, D. W. (2008). Evolution of the chicken Toll-like receptor gene family: a story of gene gain and gene loss. *BMC genomics*, *9*(1), 1-12.

Teyssier, J. R., Brugaletta, G., Sirri, F., Dridi, S., & Rochell, S. J. (2022). A review of heat stress in chickens. Part II: Insights into protein and energy utilization and feeding. *Frontiers in Physiology*, 1521.

Tzschentke, B., & Basta, D. (2000). Development of hypothalamic neuronal thermosensitivity in birds during the perinatal period. *Journal of Thermal Biology*, *25*(1-2), 119-123.

Tzschentke, B., & Basta, D. (2002). Early development of neuronal hypothalamic thermosensitivity in birds: influence of epigenetic temperature adaptation. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *131*(4), 825-832.

Vabulas, R. M., Raychaudhuri, S., Hayer-Hartl, M., & Hartl, F. U. (2010). Protein folding in the cytoplasm and the heat shock response. *Cold Spring Harbor perspectives in biology*, *2*(12), a004390.

Van Leeuwen, P., Mouwen, J. M. V. M., Van Der Klis, J. D., & Verstegen, M. W. A. (2004). Morphology of the small intestinal mucosal surface of broilers in relation to age, diet formulation, small intestinal microflora and performance. *British Poultry Science*, *45*(1), 41-48.

Varasteh, S., Braber, S., Akbari, P., Garssen, J., & Fink-Gremmels, J. (2015). Differences in susceptibility to heat stress along the chicken intestine and the protective effects of galacto-oligosaccharides. *PloS one*, *10*(9), e0138975.

Walter, P., & Ron, D. (2011). The unfolded protein response: from stress pathway to homeostatic regulation. *science*, *334*(6059), 1081-1086.

Wang, X., & Proud, C. G. (2006). The mTOR pathway in the control of protein synthesis. *Physiology*, *21*(5), 362-369.

Wang, Y.; Zhao, H.; Liu, J.; Shao, Y.; Li, J.; Luo, L.; Xing, M. (2018). Copper and arsenic-induced oxidative stress and immune imbalance are associated with activation of heat shock proteins in chicken intestines. *International Immunopharmacology*, 60, 64–75.

Wasti S., Sah N., Mishra B. (2020). Impact of heat stress on poultry health and performances, and potential mitigation strategies. *Animals.* 10, 1266. 10.3390/ani10081266.

Wu, G. (2013). Functional amino acids in nutrition and health. *Amino acids*, *45*(3), 407-411.

Wu, J.; Ibtisham, F.; Niu, Y.F.; Wang, Z.; Li, G.H.; Zhao, Y.; Nawab, A.; Xiao, M.; An, L. 2019. Curcumin inhibits heat-induced oxidative stress by activating the MAPK-Nrf2/ARE signaling pathway in chicken fibroblasts cells. *Journal of Thermal Biolog*y, 79, 112–119.

Yalçin S., Özkan S., Türkmut L., Siegel P. B. (2001). Responses to heat stress in commercial and local broiler stocks. 1. Performance traits. *Br. Poult. Sci.* 42, 149–152. 10.1080/00071660120048375.

Yang, L., Tan, G. Y., Fu, Y. Q., Feng, J. H., & Zhang, M. H. (2010). Effects of acute heat stress and subsequent stress removal on function of hepatic mitochondrial respiration, ROS production and lipid peroxidation in broiler chickens. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *151*(2), 204-208.

Yeshao, W., Gu, J., Peng, X., Nairn, A. C., & Nadler, J. L. (2005). Elevated glucose activates protein synthesis in cultured cardiac myocytes. *Metabolism*, *54*(11), 1453-1460.

Yu, T. Y., Yong, Y. H., Li, J. Y., Fang, B., Hu, C. Y., Wu, L. Y., ... & Ju, X. H. (2020). Proteomic study of hypothalamus in pigs exposed to heat stress. *BMC veterinary research*, *16*(1), 1-11.

Yun, S. H., Moon, Y. S., SoHn, S. H., & Jang, I. S. (2012). Effects of cyclic heat stress or vitamin C supplementation during cyclic heat stress on HSP70, inflammatory cytokines, and the antioxidant defense system in Sprague Dawley rats. *Experimental animals*, *61*(5), 543-553.

Zaglool, A. W., Roushdy, E. M., & El-Tarabany, M. S. (2019). Impact of strain and duration of thermal stress on carcass yield, metabolic hormones, immunological indices and the expression of HSP90 and Myogenin genes in broilers. *Research in veterinary science*, *122*, 193-199.

Zeng, L., Wang, Y. H., Ai, C. X., Zheng, J. L., Wu, C. W., & Cai, R. (2016). Effects of β-glucan on ROS production and energy metabolism in yellow croaker (Pseudosciaena crocea) under acute hypoxic stress. *Fish physiology and biochemistry*, *42*(5), 1395-1405.

Zhou, L., Jang, J. C., Jones, T. L., & Sheen, J. (1998). Glucose and ethylene signal transduction crosstalk revealed by an Arabidopsis glucose-insensitive mutant. *Proceedings of the National Academy of Sciences*, *95*(17), 10294-10299.

Zimmerman, S. B., & Trach, S. O. (1991). Estimation of macromolecule concentrations and excluded volume effects for the cytoplasm of Escherichia coli. *Journal of molecular biology*, *222*(3), 599-620.

CHAPTER 3

THE EFFECT OF GLUCOSE SUPPLEMENTATION ON THE PERFORMANCE OF CHICKENS RAISED UNDER THERMONEUTRAL OR HEAT STRESS CONDITION

Oluwatomide W. Ariyo[1], Ahmed F. A. Ghareeb[1], Marie C. Milfort[1], Bikash Aryal[1], Evan Hartono[1], Josephine Kwakye[1], Selorm Sovi[1], Sommer A. Hipple[1], Carrienton Stevenson[2], Alberta L. Fuller[1], Romdhane Rekaya[3], and Samuel E. Aggrey[1]
To be submitted to *Animal.*

ABSTRACT

A 2x2 factorial design was used to evaluate the effect of glucose supplementation on the performance of broiler chickens raised under thermoneutral (TN) or HS condition. The factors included glucose supplementation (0 and 6%) and temperature (25^0C and 35^0C). The treatments were TN-0% glucose (TN0); TN-6% glucose (TN6), HS-0% glucose (HS0) and HS-6% glucose (HS6); each with 6 replicates of 19 birds each. The heat treatment lasted from d28 to 35. Heat stress reduced ($p<0.05$) FI, BWG and increased FCR and WCR relative to TN groups. HS0 had a lower ($p<0.05$) BW35 compared to TN0 and TN6. Glucose supplementation reduced ($p<0.05$) feed intake under TN and HS conditions. HS0 had a reduced ($P<0.05$) percentage *P. major* and drumstick compared to other treatment groups whereas glucose supplementation improved the *P. major* and drumstick yield of broilers under HS condition. Glucose supplementation improved the carcass yield of broilers under HS condition.

INDEX WORDS: Heat stress, glucose supplementation, performance, carcass yield

INTRODUCTION

The increase in metabolic rate resulting from the genetic selection of broiler chickens for meat production has increased the susceptibility of modern commercial breeds to heat stress (HS). The absence of sweat gland and the inefficiency in heat dissipation has exacerbated the effect of HS in chickens. The physiological response of animals to HS includes a reduction in feed intake, increased rectal temperature, accelerated heart rate and respiration rates, and maximizing water intake (Renaudeau *et al*., 2012). The decreased feed intake often results in lower weight gain and a higher feed conversion ratio (Lara and Rostagno, 2013). This impairment can alter various antioxidants, enzymes, and metabolites like blood glucose levels (Sammad *et al*., 2020). Heat stress has been shown to impair the carcass quality of poultry (Wasti *et al*., 2021).

Heat stress is a constant challenge faced by the poultry sector in the tropics and subtropics but is prominent during the summer period in the temperate regions (Lin et al., 2006; Ghareeb et al., 2022). Heat stress can be acute (short-lived) or chronic (prolonged) and both have an adverse effect on the health and performance of birds (Quinteiro-Filho *et al*., 2012; Habashy *et al*., 2017; Abo Ghanima *et al*., 2020), leading to economic losses in poultry production. Heat stress raises the production rate of reactive oxygen species (ROS) in the mitochondrial causing oxidative damage in muscles (Azad *et al*., 2010; Mujahid *et al*., 2009; Shehata *et al*., 2020) and negatively affects meat quality. The levels of glycogen and glycolysis rate affect the postmortem muscle pH decline rate, leading to affecting carcass muscle pH. Chauhan and England, (2018) reported that these two factors can be affected by the stress prior to slaughtering and muscle temperature. Several nutritional approaches (amino acids, fatty acids, minerals, vitamins, etc.) have been

employed to mitigate HS in broiler chickens (Teyssier *et al*., 2022). Developing an effective HS abating strategy would depend on the understanding of molecular and cellular response of broilers to HS. Birds eat to satisfy their energy and nutritional requirements and resort to using muscle protein to produce glucose to maintain homeostasis under HS, thus declining tissue glucose levels due to a reduction in feed intake. It has been shown that HS disrupts the insulin signaling and pentose phosphate pathways, altering basal metabolism and protein biosynthesis (Ganesan *et al*., 2018). We hypothesize that glucose supplementation could sustain glucose levels in the tissues and prevent the utilization of muscle proteins during HS, and that glucose supplementation would improve the performance of broiler chickens subjected to heat stress.

MATERIALS AND METHODS

This study was carried out under the approved guidelines for the University of Georgia's institutional animal care and use committee. A total of 456 Cobb500 broiler chickens were used for this study. Birds were randomly allocated to 4 treatment groups containing 6 replicates of 19 birds each. Heat application was replicated once i.e., 1 room each for thermoneutral (TN) and heat stress (HS) group. Birds were raised on fresh pine shavings from one day old until 4 weeks before allocating them to the different treatment groups. Birds received crumbled starter, pelleted grower, and pelleted finisher diet from day 0 to 14, d 15 to 28 and d 28 to 42, respectively. Glucose was administered through drinking water. Heat application and glucose supplementation was done at the finisher phase (d28 to d35).

The birds were brooded for 14 days. Lighting and temperature management practices were in accordance with Cobb 500 standards (Cobb, 2018). The birds had unlimited access to feed and water. The temperature was kept at 25^0C for the thermoneutral (TN) room while HS was set at

35^0C from 8 am to 8pm from d28 to d35, after which it was returned to 25^0C. The cycle continued for the duration of the experiment. There were four treatment groups: TN0 and TN6 where birds had glucose supplementation at 0 and 6% respectively and were raised under TN conditions. Subsequently, HS0 and HS6 refer to chickens who had 0 and 6% glucose supplementation, respectively and were raised under HS conditions. The temperature log (^{0}C) and metabolizable energy (ME) intake (kcal/bird/day) of the birds during the duration of the heat stress application is displayed in Table 3.1 and 3.2 respectively.

D-glucose was purchased from Carolina Biological Supply (Burlington, North Carolina, USA) and dissolved in water. D-glucose supplementation was continuously administered via water to the experimental birds at 0% (TN0 and HS0) or 6% (TN6 or HS6). Birds received the glucose water from d28 to d35.

Feed intake was determined as the difference between feed given at the start of the HS treatment (d28) and feed leftover upon the termination of the experiment (d35). Birds were weighed on the first and last day of the experiment, and body weight gain was calculated and averaged per bird for each replicate. We also measured the water intake per pen for the duration of the HS. Feed was weighed in and weighed out at d28 and d35, respectively and feed intake was determined based on the difference between feed given and weighed back. On day 7 post-HS (age=35 days), 3 birds per replicate were randomly selected, wing tagged, and processed at the University of Georgia Processing Plant (Athens, GA United States) to determine the carcass characteristics of the birds. The birds were electrically stunned (11 V, 11 mA for 8 s), slaughtered, and allow to go through the defeathering machine. Birds were eviscerated and manually rinsed before being chilled at 4°C for 24 hours. The cold weight of *Pectoralis* (*P.*) *major*, *P. minor*, thigh, drumstick, and wings were recorded. Based on the variables measured, we calculated feed intake

(FI), feed conversion ratio (FCR), body weight gain (BWG), final body weight (FBW), water intake (WI), and water conversion ratio (WCR).

$$\text{Feed conversion ratio} = \frac{Feed\ intake\ (g)}{Weight\ gain\ (g)}$$

$$\text{Water conversion ratio} = \frac{Water\ intake\ (mL)}{Weight\ gain\ (g)}$$

$$\text{Percent cut part} = \frac{Weight\ of\ cut\ part\ (g)}{Eviscerated\ weight\ (g)}\ X\ 100$$

Statistical analysis

Data were analyzed using a two-way ANOVA design through the SAS® Studio software (SAS Institute Inc., Cary, NC, USA). The analysis was carried out using the generalized linear model (GLM) procedure (SAS, 2018). Multiple comparison between treatment groups was done using the "Tukey-HSD" test with a significant level set at $p < 0.05$.

The model is given below:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where $y_{ijk} = performance\ of\ broiler\ chickens$, μ is the overall mean of the response, α_i is the temperature effect, where i = 1,2; β_j is the effect of glucose, where j = 1,2; $(\alpha\beta)_{ij}$ is the interaction effect between temperature and glucose (class I and j), and the ε_{ijk} is the random error.

RESULTS

Performance

Tables 3.3 and 3.4 show the performance characteristics and carcass quality evaluation of broilers raised with glucose under thermoneutral or heat stress condition. The initial weight of the experimental birds was similar across all treatment groups while HS0 had a significantly ($p<0.05$) lower final weight compared to TN0 and TN6. The HS0 group had a significantly higher FCR, lower BWG and lower FBW compared to the TN0 and TN6. The HS6 groups had a reduced ($p<0.05$) FI and BWG compared to TN0 and TN6. However, the FCR for HS6 birds was similar to TN0 birds. HS0 had a lower BWG and higher FCR when compared to TN0 and TN6. Feed intake was lower in HS0 and HS6 groups relative to the TN0 group (figure 3.3). Glucose supplementation increased ($p<0.05$) final BW of the birds compared to their counterparts whose water was not supplemented and raised under HS. HS0 had a higher ($p<0.05$) water intake relative to TN6. Glucose supplementation reduced ($p<0.05$) feed intake in both TN6 and HS birds. There was a reduction ($p<0.05$) in FI and BWG of birds raised under HS condition as compared to those raised under TN condition. The FCR of HS birds were higher than those under TN condition. Glucose supplementation reduced FI when compared to groups without glucose supplementation. HS0 had a higher ($p<0.05$) WCR compared to TN0 and TN6 and HS raised ($p<0.05$) the WCR relative to TN.

Carcass characteristics

There were no significant differences in the *P. minor*, thigh, wings, and abdominal fat across treatments. HS significantly ($P<0.05$) reduced eviscerated weight with HS0 birds having a reduced eviscerated weight when compared to birds in TN0, TN6 and HS6. Heat stress resulted in

a lower live weight, percentage *P. major* (figure 3.1), and drumstick of the birds (figure 3.2). There was a significant reduction ($p<0.05$) in the live weight, eviscerated weight, percentage *P. major*, and drumstick of HS0 birds compared to TN0 and TN6. Glucose supplementation resulted to an increase ($p<0.05$) in *P. major* proportion of the birds.

DISCUSSION

Under normal conditions, birds eat to meet their energy and nutritional requirements. Glucose is the major determinant of the energy level in the body (Nakrani *et al*., 2022) suggesting that a higher body glucose level will reduce feed consumption and vice versa. In the current study, we supplemented the drinking water of chickens raised under TN or HS with glucose. We observed that, supplementing heat-stressed chickens with a 6% glucose reduced feed intake, which might be due to the increase in body energy composition. As known under HS condition, birds minimize heat production by reducing feed consumption (Wasti *et al*., 2020). This explains why there was a reduced feed intake in birds under HS.

The reduction in the BWG of the HS birds relative to TN birds was expected to be due to the observed reduction in feed intake in this group. Lower feed intake without an external supply of glucose will decrease the energy level in the birds. Under HS, animals convert muscle protein to glucose to meet the basal energy requirement (Ma *et al*., 2021). This diverts protein for meat production towards maintaining the energy status of the animal. Furthermore, the adverse effect of HS on the activities of hypothalamo-pituitary axis and orthosympathic nervous system, impair skeletal development, growth and body temperature regulation (Darras *et al*., 2000).

The higher FCR observed in the HS group is expected to be due to reduced feed intake and lower body weight gain in this group. This is in concordance with results from Ma *et al*. (2021)

and Habashy *et al*. (2017). Glucose supplementation at 6% under TN condition (TN6) reduced feed intake and improved the weight gain of the birds.

Water is a vital nutrient, essential for the thermal homeostasis of birds during heat stress. High loss of water through evaporative cooling via the respiratory tract is a measure to dissipate heat and the resultant higher water intake is a means of maintaining "thermoregulatory balance" (Bruno and Macari, 2002). The higher water consumption observed in the HS relative to the TN group revealed that birds consumed more water due to water loss as a result of HS and to reduce body temperature. The observed higher WCR in the HS group showed that birds utilized as much water as needed for meat production to maintain water balance under HS. This is evident when comparing HS0 to TN0 and TN6 groups. This water increase may also be a result of enhanced vasopressin activities due to increased glucose level (as a result of gluconeogenesis) in the birds. Infusion of vasopressin has been reported to increase hepatic glucose production in rodents (Hems *et al*., 1978) and dogs (Bergen *et al*., 1960). This increase in glucose production will aid the activity of vasopressin in providing a signal to the hypothalamus to create a sense of thirst to reduce the concentration of glucose in the body (Carroll and James, 2019). Also, gluconeogenesis occurs during HS (Ma *et al*., 2021) and increases the glucose composition of the blood. This means that the increased water intake might be a way to regulate the glucose level in the tissues of the HS0 birds.

Genetic selection for meat production has focused on improving the quality of breast muscle (Zuidhof *et al*., 2014). Heat stress has a grave impact on breast yield in fast-growing broilers (Lu *et al*., 2007). *Pectoralis major* and *P minor* are the two muscles found in the breast muscle of chickens. Heat stress reduced the percentage *P. major* in broiler chickens. However, HS6 birds had a comparable *P. major* yield with birds in the TN groups. A similar result was

observed in the percentage drumstick, suggesting that supplementation of broiler chicken water with glucose improved some carcass yield parameters. The comparable effect of birds in the HS6 group to the control and the significant increase over the HS0 group could be due to the role of glucose in enhancing protein synthesis. A suggested explanation is that glucose might have supported protein biosynthesis as opposed to the breakdown of muscle protein via the insulin signaling pathway and activated pentose phosphate pathway (PPP), to produce NADPH and precursors for nucleotide and amino acid biosynthesis.

CONCLUSIONS

The performance of broiler chicken declines under chronic HS condition. Chronic HS impaired the development of drumstick and *P. major* in broiler chickens. This explains one way in which HS causes economic loss to the poultry industry. Glucose supplementation at 6% showed a noticeable improvement in carcass yield of broilers chicken exposed to chronic HS condition. Glucose supplementation at 6% enhanced the performance of broiler chickens under chronic HS. The continuous supply of heat-stressed broiler chickens with 6% glucose water improved the carcass weight, *P. major*, and drumsticks proportions. The results suggest that D-glucose supplementation might enhance protein synthesis or prevent HS-induced protein breakdown in the muscles of broiler chickens exposed to HS. The observed effect of glucose supplementation proposes a promising approach to reduce the economic losses resulting from the reduction in broiler chicken carcass yield due to HS.

List of tables and figures

Table 3.1: Temperature log (^{0}C) from day 28 to 35

	THERMONEUTRAL (TN)		HEAT STRESS (HS)	
Day	Morning	Evening	Morning	Evening
28	27.00	27.00	27.00	34.06
29	27.67	29.11	27.61	34.06
30	27.22	30.06	27.28	34.06
31	28.11	30.17	34.11	34.11
32	26.94	34.78	26.78	36.06
33	28.61	31.67	27.67	34.83
34	27.39	26.89	27.00	34.61
35	26.89	-	27.28	-

Table 3.2: Metabolizable energy (ME) intake of broiler chickens raised under thermoneutral or heat stress condition

Treatment	FI/b/d(g)	ME from feed	WI (mL/b/d)	(6%*WI)	ME from glucose	Total ME intake (kcal/b/d)
TN0	188.04	578.22	369.34	22.1604	0	578.22
TN6	170.12	523.11	354.73	21.2838	85.14	608.25
HS0	167.91	516.33	491.96	29.5176	0	516.33
HS6	145.94	448.77	356.04	21.3624	85.45	534.22

ME from feed – 3075 kcal/kg

ME from 1 gram of glucose – 4kcal

Table 3.3: Performance characteristics of broilers raised with glucose under thermoneutral or heat stress condition from d28 to 35.

Treatment	FCR	FI (g/bird/day)	BWG (g/bird/day)	Initial weight (g/bird)	FBW	WI (mL/bird/day)	WCR
TN0	2.30^{bc}	188.04^{a}	84.96^{a}	1630.35	2220.96^{a}	369.34^{ab}	4.59^{bc}
TN6	1.94^{c}	170.12^{b}	88.36^{a}	1700.32	2318.85^{a}	354.73^{b}	4.05^{c}
HS0	3.41^{a}	167.91^{bc}	50.06^{b}	1639.76	1990.19^{b}	491.96^{a}	8.3^{a}
HS6	2.84^{ab}	145.94^{c}	54.96^{b}	1681.98	2066.69^{ab}	356.04^{ab}	6.95^{ab}
P value	0.0004	0.0113	0.0004	0.2034	0.0016	0.0441	0.0019
SEM	0.202	7.7451	6.2119	18.6194	53.3621	42.5554	0.7424
Temperature							
TN	2.12^{b}	179.08^{a}	86.37^{a}	1665.33	2269.91^{a}	362.03	4.32^{b}
HS	3.13^{a}	156.93^{b}	52.51^{b}	1660.87	2028.44^{b}	424.00	7.62^{a}
P value	<.0001	0.0104	<.0001	0.8674	0.0003	0.1869	0.0003
SEM	0.143	5.4751	4.3925	18.6001	37.7718	31.5600	0.5249
Glucose levels							
0%	2.86^{a}	178.89^{a}	68.78	1691.98	2105.58	430.65	6.45
6%	2.39^{b}	159.13^{b}	73.18	1634.63	2192.77	355.39	5.50
P value	0.0332	0.0197	0.4841	0.0572	0.1148	0.1165	0.2173
SEM	0.1430	5.4808	4.3970	18.6001	37.7718	31.5600	0.5026

FCR-feed conversion ratio, FI-feed intake, BWG-body weight gain, FBW-final body weight, WI-water intake, WCR-water conversion ratio. Superscripts a,b,c are used to indicate significant differences (p value < 0.05) between parameters across columns.
TN0 – Thermoneutral (25^0C) without glucose supplementation
TN6 - Thermoneutral (25^0C) with 6% glucose supplementation
HS0 – Heat stress (35^0C) without glucose supplementation
HS6 – Heat stress (35^0C) with 6% glucose supplementation

Table 3.4: Carcass quality evaluation of broilers raised with glucose under thermoneutral or heat stress condition from d28 to 35

Treatment	Eviscerated weight (g)	Abdominal fat (g)	*P. major* (%)	*P. minor* (%)	Thigh (%)	Drumstick (%)	Wings (%)
TN0	1675.78[a]	0.015	17.57[a]	3.41	11.82	10.31[a]	7.22
TN6	1682.11[a]	0.014	17.93[a]	3.50	11.62	10.01[a]	7.36
HS0	1514.56[b]	0.019	14.84[b]	3.09	10.96	8.78[b]	6.84
HS6	1654.35[a]	0.020	16.94[a]	3.25	11.86	9.77[ab]	7.18
p value	0.0015	0.2403	0.0005	0.2701	0.1701	0.0129	0.1062
SEM	33.0543	0.0022	0.5295	0.1570	0.3191	0.3352	0.1529
Temperature							
TN	1678.94[a]	0.015	17.75[a]	3.46	11.72	10.16[a]	7.29
HS	1573.83[b]	0.019	15.89[b]	3.17	11.41	9.24[b]	7.01
p value	0.0051	0.0504	0.0007	0.0738	0.3305	0.0100	0.0682
SEM	23.3729	0.0016	0.3744	0.1110	0.2257	0.2370	0.1081
Glucose levels							
0%	1595.17[b]	0.017	16.20[b]	3.25	11.39	9.55	7.03
6%	1668.23[a]	0.017	17.44[a]	3.38	11.74	9.86	7.27
p value	0.0308	0.9800	0.0254	0.4164	0.2744	0.3108	0.1178
SEM	23.3729	0.0016	0.3744	0.1110	0.2257	0.2370	0.1081

Superscripts a,b,c are used to indicate significant differences ($p < 0.05$) between parameters across columns.
TN0 – Thermoneutral (25^0C) without glucose supplementation
TN6 - Thermoneutral (25^0C) with 6% glucose supplementation
HS0 – Heat stress (35^0C) without glucose supplementation
HS6 – Heat stress (35^0C) with 6% glucose supplementation

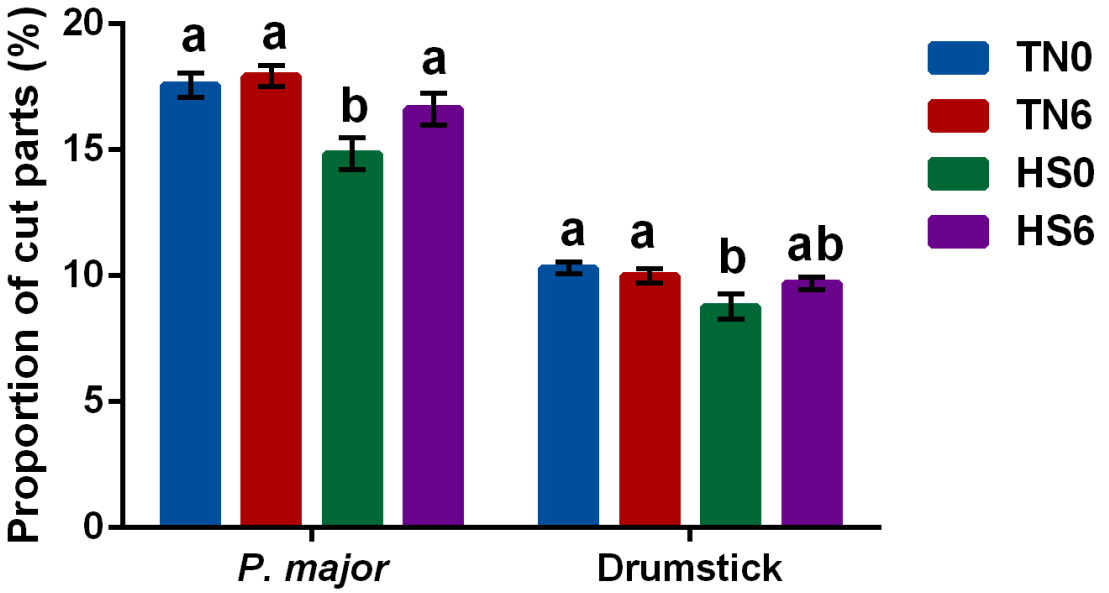

Figure 3.1: Percentage *P. major* and drumstick of broiler chickens raised under HS or TN condition from d28 to 35.
TN0 – Thermoneutral (25^0C) without glucose supplementation
TN6 - Thermoneutral (25^0C) with 6% glucose supplementation
HS0 – Heat stress (35^0C) without glucose supplementation
HS6 – Heat stress (35^0C) with 6% glucose supplementation
Different letters show significant differences ($p<0.05$) between treatment groups. Error bars represent SEM.

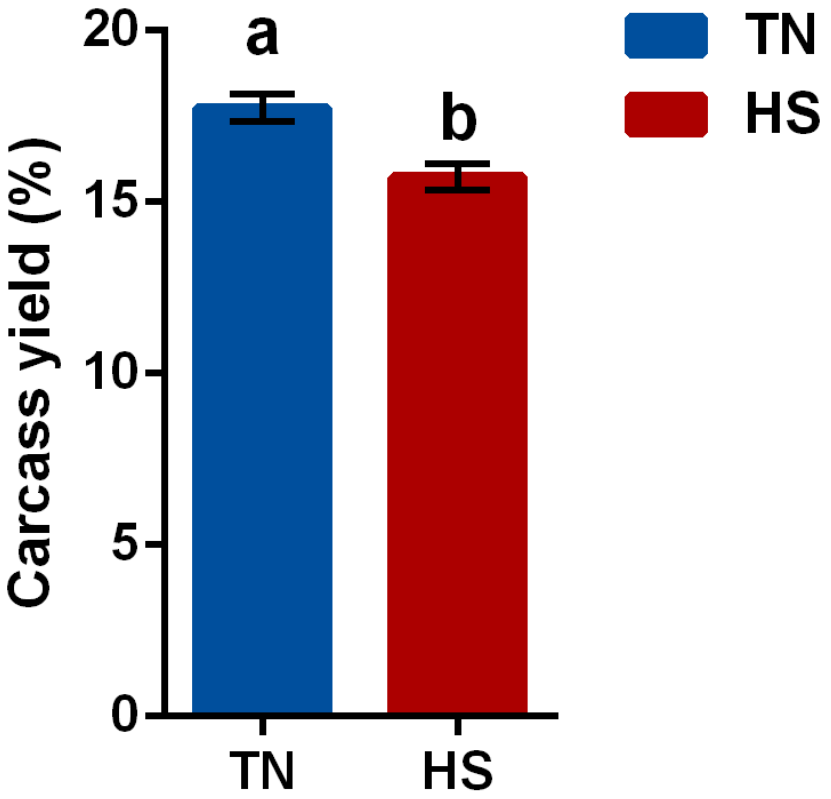

Figure 3.2: Percentage *P. major* component of broiler chickens raised under thermoneutral or heat stress condition from d28 to 35.
TN-Thermoneutral (25^0C)
HS-Heat stress (35^0C)
Different letters show significant differences ($p<0.05$) between treatment groups.
Error bars represent SEM.

REFERENCES

Azad, M. A. K., Kikusato, M., Sudo, S., Amo, T., & Toyomizu, M. (2010). Time course of ROS production in skeletal muscle mitochondria from chronic heat-exposed broiler chicken. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *157*(3), 266-271.

Bergen. S.S., Sullivan. R., Hilton. J.G., Willis, S.W. and Van Itallie, T.B. (1960) Glycogenolytic effect of vasopressin in the canine liver. Amerieaii Jourrial of Phj..fiologj,. 199, 136-1 38.

Bruno LDG and Macari M. Ingestão de água: mecanismos regulatórios. In: Macari M, Furlan RL, Gonzales E, editors. Fisiologia aviária aplicada à frangos de corte. Jaboticabal: FUNEP; 2002. p.201-208.

Carroll, H. A., & James, L. J. (2019). Hydration, arginine vasopressin, and glucoregulatory health in humans: a critical perspective. *Nutrients*, *11*(6), 1201.

Chauhan SS, England EM (2018) Postmortem glycolysis and glycogenolysis: insights from species comparisons. Meat Sci 144:118–126. https://doi.org/10.1016/j.meatsci.2018.06.021

Cobb-Vantress. Cobb 500. Broiler Performance and Nutrition Supplement. 2018. Available online: https://www.cobb-vantress.com/products/cobb500 (accessed on 4 April 2020).

Darras, V. M., Van der Geyten, S., & Kühn, E. R. (2000). Thyroid hormone metabolism in poultry. *BASE*.

Ganesan, S., Summers, C. M., Pearce, S. C., Gabler, N. K., Valentine, R. J., Baumgard, L. H., ... & Selsby, J. T. (2018). Short-term heat stress altered metabolism and insulin signaling in skeletal muscle. *Journal of Animal Science*, *96*(1), 154-167.

Ghanima, M. M. A., Abd El-Hack, M. E., Othman, S. I., Taha, A. E., Allam, A. A., & Abdel-Moneim, A. M. E. (2020). Impact of different rearing systems on growth, carcass traits, oxidative stress biomarkers, and humoral immunity of broilers exposed to heat stress. *Poultry science*, *99*(6), 3070-3078.

Ghareeb AFA, Gustavo H. Schneiders, Jennifer N. Richter, James C. Foutz, Marie C. Milfort, Albert L. Fuller, Jianmin Yuan, Romdhane Rekaya, Samuel E. Aggrey. 2022. Heat stress modulates the disruptive effects of *Eimeria maxima* infection on the ileum nutrient digestibility, molecular transporters, and tissue morphology in meat-type chickens. PLoS ONE 17(6): e0269131. https://doi.org/10.1371/journal.pone.0269131

Habashy W. S., Milfort M. C., Fuller A. L., Attia Y. A., Rekaya R., Aggrey S. E. (2017). Effect of heat stress on protein utilization and nutrient transporters in meat-type chickens. *Int. J. Biometeorol.* 61, 2111–2118. 10.1007/s00484-017-1414-1

Hems. D.A.. Roiirigues, L.M. and Whitton. P.D. (1978) Rapid stimulation by vasopressin oxytocin and angiotensin I1 of glycogen degradation in hepatocyte suspensions. Biochemical Journal, 172, 31 1-312.

Lara, L. J., & Rostagno, M. H. (2013). Impact of heat stress on poultry production. *Animals*, *3*(2), 356-369.

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.

Lu, Q., Wen, J., and Zhang, H. (2007). Effect of chronic heat exposure on fat deposition and meat quality in two genetic types of chicken. Poult. Sci. 86, 1059–1064. doi: 10.1093/ps/86.6.1059

Ma, B., Zhang, L., Li, J., Xing, T., Jiang, Y., & Gao, F. (2021). Heat stress alters muscle protein and amino acid metabolism and accelerates liver gluconeogenesis for energy supply in broilers. *Poultry science*, *100*(1), 215-223.

Mujahid, A., Akiba, Y., & Toyomizu, M. (2009). Olive oil-supplemented diet alleviates acute heat stress-induced mitochondrial ROS production in chicken skeletal muscle. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *297*(3), R690-R698.

Nakrani MN, Wineland RH, Anjum F. Physiology, Glucose Metabolism. [Updated 2022 Jul 25]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK560599/

Quinteiro-Filho, W. M., Rodrigues, M. V., Ribeiro, A., Ferraz-de-Paula, V., Pinheiro, M. L., Sá, L., ... & Palermo-Neto, J. (2012). Acute heat stress impairs performance parameters and induces mild intestinal enteritis in broiler chickens: role of acute hypothalamic-pituitary-adrenal axis activation. *Journal of animal science*, *90*(6), 1986-1994.

Renaudeau D., Collin A., Yahav S., De Basilio V., Gourdine J. L., Collier R. J. (2012). Adaptation to hot climate and strategies to alleviate heat stress in livestock production. *Animal* 6, 707–728. 10.1017/S1751731111002448

Sammad A., Wang Y.J., Umer S., Lirong H., Khan I., Khan A., Ahmad B., Wang Y. 2020. Nutritional physiology and biochemistry of dairy cattle under the influence of heat stress: Consequences and opportunities. *Animals*; 10:793.

SAS Institute Inc. SAS/IML® Studio 15.1 for SAS/STAT® Users; SAS Institute Inc.: Cary, NC, USA, 2018.

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), 2407.

Teyssier, J. R., Preynat, A., Cozannet, P., Briens, M., Mauromoustakos, A., Greene, E. S., ... & Rochell, S. J. (2022). Constant and cyclic chronic heat stress models differentially influence growth performance, carcass traits and meat quality of broilers. *Poultry Science*, 101963.

Wasti S., Sah N., Mishra B. (2020). Impact of heat stress on poultry health and performances, and potential mitigation strategies. *Animals.* 10, 1266. 10.3390/ani10081266

Wasti, S., Sah, N., Lee, C. N., Jha, R., & Mishra, B. (2021). Dietary supplementation of alpha-lipoic acid mitigates the negative effects of heat stress in broilers. *PloS one*, *16*(7), e0254936.

Zuidhof, M. J., B. L. Schneider, V. L. Carney, D. R. Korver, and F. E. Robinson. (2014). Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 2005. Poult. Sci. 94: 1389–1397.

CHAPTER 4
mRNA EXPRESSION OF KIDNEY AQUAPORINS IN MEAT-TYPE CHICKENS REARED UNDER HEAT OR THERMONEUTRSAL CONDITION

Oluwatomide W. Ariyo[1], Ahmed F. A. Ghareeb[1], Marie C. Milfort[1], Bikash Aryal[1], Evan Hartono[1], Josephine Kwakye[1], Selorm Sovi[1], Sommer A. Hipple[1], Carrienton Stevenson[2], Alberta L. Fuller[1], Romdhane Rekaya[3], and Samuel E. Aggrey[1]
To be submitted to *Poultry Science.*

ABSTRACT

Heat stress (HS) causes water homeostasis imbalance. Kidney aquaporins (AQPs) reabsorbs water in the renal tubules to maintain body hydration. The effect of glucose supplementation on blood chemistry and expression of kidney AQPs 1,2,3, and 4 was evaluated in broilers under thermoneutral (TN) or HS condition. The treatments were glucose supplementation at 0 and 6%, both represented at 25^0C (TN0 and TN6) and 35^0C (HS0 and HS6) from d28 to d35. Each treatment had 6 replicates of 19 birds each. AQP-1, -2, and -3 were upwardly regulated ($p<0.05$) in HS0 relative to TN0, TN6, and HS6. Chronic HS lowered blood CO_2, TCO_2, and HCO_3. Blood Na^+ and K^+ were similar across treatments. Chronic HS and glucose supplementation increased ($p<0.05$) blood glucose content. Kidney aquaporins 1,2, and 3 were upwardly expressed in HS0 birds. An increase in water reabsorption and excretion is possible in the birds subjected to HS.

INDEX WORDS: Heat stress, glucose supplementation, Aquaporins, blood chemistry

INTRODUCTION

Heat stress leads to a substantial loss of water due to an increase in respiration and the formation of urine (Bruno *et al*., 2011). Water and salt balance in the body is principally regulated by the kidney. This balance is dependent on the collection of water and solute transporters in the renal tubules and vascular elements in the kidney. Under HS condition, there is an increase in metabolic heat production and the kidney helps to minimize this heat by increasing water reabsorption in the renal tubules and reducing the loss of dissolved sodium (Wideman *et al*., 1994; Sugito *et al*., 2021). Water reabsorption in renal tubules is dictated by the hydration status of the organism as the kidney adjusts the osmolality of urine to maintain serum osmolality. Water reabsorption in the proximal tubules depends on the reabsorption of solutes and the extent to which water can permeate the epithelium. Reabsorption of water in the Henle's loop and the collecting duct is determined by the active transport in the non-water transport site of the renal tubule segments (Jeyaseelan *et al*., 2006). Water can permeate all plasma membranes of mammalian cells, but the extent of permeability differs among tissues.

Aquaporins (AQPs) are a group of pore-forming integral membrane proteins responsible for the regulation of water flow and enabling permeability of biological cell membranes by small molecules (Ishibashi *et al*., 2009; Du *et al*., 2015; Wang *et al*., 2015). AQPs have six membrane-spanning domains, resulting in the formation of five loops (three extracellular: A, C, E; and 2 intracellular: B and D) while leaving the free amine end (N) and the free carboxylic end (C) inside the cell. The B and E loops have an Asp-Pro-Ala (NPA) sequence, which is typical of the membrane's intrinsic protein. The motifs are situated close to the surface of the cell membrane

(one at the luminal side and the other at the cytoplasm side). They extend from opposite sides to form a lipid bilayer and are juxtaposed in the center, to form a pore structure permeable by water (Nielsen and Agre, 1995, Wang *et al*., 2015). The primary and secondary active transport of ions or neutral solutes due to osmotic gradients results in the passive movement of water through the lipid bilayers. Besides facilitation of water movement, AQPs also facilitate the movement of small uncharged particles such as glycerol, urea, and CO_2 across the membranes. Aquaporins expressed in the kidney facilitates osmotic water transport across water-permeable membranes and aid the urinary concentration and dilution process. The amount of AQPs situated at the epithelial tubular cell's membrane determines the extent to which the renal tubules will be permeable by water. The thick ascending limbs are water impermeable because they have no AQPs (Saadoun *et al*., 2005). Aquaporins facilitate the movement of water across cell membranes, and AQPs in the kidney are important for water permeating the renal tubule and in maintaining homeostasis of water in the body.

Environment, as well as metabolism and nutrition, is vital in maintaining the acid-base balance of broiler chickens. Core body temperature and respiratory rates rise in environments having high ambient temperatures and relative humidity. This lowers the CO_2 level in blood, alters the acid-base balance, and ultimately leads to respiratory alkalosis (Ahmad and Sarwar, 2006), which distorts broiler chicken growth performance and survival. All solubilized substances with the ability to conduct an electric current while being broken down by it are considered electrolytes. Birds presumably have an ideal internal electrolyte balance under thermoneutrality, since the body's water and electrolyte balances are kept within fixed bounds (Borges *et al.*, 2003). The production of tissue protein, preservation of intracellular and extracellular homeostasis, electrical

potential of cell membranes, enzymatic processes, osmotic pressure, and acid-base balance all depend on these monovalent minerals (Borges *et al.*, 2003).

Kidney aquaporins are involved water regulation in the body. Heat stress alters salt and water balance as well as acid-base balance in the body. We therefore aim to investigate the mRNA expression of kidney AQP 1, 2, 3, and 4, and acid-base balance in broiler chickens raised under heat stress or thermoneutral conditions and drinking water supplemented with or without glucose.

MATERIALS AND METHODS

We carried out this study under the approved guidelines by the University of Georgia's institutional animal care and use committee. Four hundred and fifty-six (456) Cobb500 broiler chickens were randomly allocated to 4 treatment groups each with 6 replicates of 19 birds each. The experiment was laid out in a 2 x2 factorial design with 2 temperature levels (25^0C - TN and 35^0C - HS) and 2 glucose levels (0 and 6%). Birds were raised on fresh pine shaving from d0 until d28 before randomly allocating them to the different treatment groups. Birds received crumbled starter from d0 to 14, pelletized grower from d 15 to 28 and pelletized finisher diet from d 28 to 35. D-glucose was purchased from Carolina Biological Supply (Burlington, North Carolina, USA) and dissolved in water based on the percentage (%) used. Bird had access to this water from d28 until d35.

The brooding of the birds was done for 14 days, following the standard management practices for Cobb 500 (Cobb, 2018). The birds were fed *ad libitum*. The temperature was fixed at 25^0C for the TN room and 35^0C for the HS room (starting 8 am to 8 pm), after which it was reverted to 25^0C. The cycle continued for the duration of the experiment. On d 35, blood and kidney samples were collected from 6 birds per treatment. Blood was collected via the jugular vein

using heparin-coated syringe and was analyzed with VetScan ® i-STAT ® handheld blood analyzer (Abott group of companies) and one cartridge was used for each blood sample. The iSTAT measured pH, partial pressure of carbon dioxide (PCO_2), bicarbonate (HCO_3), total carbon dioxide (TCO_2), Na^+, K^+, Cl^-, anion gap (AnGap), glucose, hematocrit (Hct), hemoglobin (Hb) and osmolality of the blood. Osmolality was calculated using the formula below:

$$Osmolality = (2\ XNa^+) + \ ({}^{BUN}/_{2.8}) + ({}^{Glucose}/_{18})$$

BUN = blood urea nitrogen

The kidney was collected after sampled birds were humanely euthanized through cervical dislocation. Tissue samples were snap-frozen in liquid nitrogen and stored at -85˚C until needed.

RNA extraction and RT-PCR

Frozen tissue samples were prepared for total RNA extraction. Samples were coarsely ground, and homogenized in lysing matrix tubes (MP Biomedicals, Santa Ana, CA). Total RNA extraction was done using TRIzol reagents (Invitrogen, Carlsbad, CA), purified with RNeasy Mini Kit (Qiagen, Valencia, CA, USA), and treated with RNase-Free DNase (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. RNA was suspended in DEPC water and concentration was measured and adjusted to 200ng/µl using a NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE). The RNA was stored at -85˚C until cDNA synthesis. Ten (10) microliters (µl) of diluted RNA were reverse transcribed to produce cDNA using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA) based on the manufacturer's protocol. A gradient thermocycler from Eppendorf (Hauppauge, NY) was used for cDNA synthesis. The cDNA synthesis cycle was set to 25˚C for 10 minutes, 37˚C for 120 minutes, and 85˚C for 5 minutes. The concentration of cDNA was taken, and the concentration of all

samples was adjusted to 20 ng/µl using NanoDrop 2000 Spectrophotometer. The cDNA was stored at -82˚C until RT-PCR was carried out. RT-PCR was done using StepOnePlus (Applied Biosystems, Carlsbad, CA). Each RT-PCR reaction consisted of a total volume of 20 µl; made up of 1 µl cDNA, 0.6 µl forward and reverse primer each, 7.4 µl DEPC water, and 10 µl SYBR Green Master Mix (Applied Biosystems, Carlsbad, CA). All samples were replicated 3 times on the same plate. The conditions for RT-PCR were set at 50˚C for 2 minutes and 95˚C for 2 minutes for holding stage; 95˚C for 15 seconds, 58˚C for 15 seconds repeated for 40 cycles, and 72˚C for 1 minute for cycling stage; and 95˚C for 15 seconds, 60˚C for 1 minute and finally 95˚C for 15 seconds for the melt curve stage. At the end of the cycles, a melting temperature curve was determined.

To measure the mRNA expression of AQPs in the kidney, AQP-1, -2, -3 and -4 genes were determined using beta-actin as a housekeeping gene. The . $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) was used to determine the fold difference among groups with data expressed relative to TN0.

Statistical analysis

Data obtained were analyzed using a two-way ANOVA design (2x2 factorial) through the SAS® Studio software (SAS Institute Inc., Cary, NC, USA). The model was carried out using the generalized linear model (GLM) procedure (SAS, 2018). Means were separated using the "Tukey-HSD" test with a significant cut-off set at $p < 0.05$.

The model used is:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where y_{ijk} is the yield (gene expression or blood composition), μ = overall mean of the response, α_i = effect of the ith temperature, where i = 1,2; β_j = effect of jth glucose (class i and j), where j = 1,2; $(\alpha\beta)_{ij}$ = interaction effect between temperature and glucose, and the ε_{ijk} = random error.

RESULTS

The results for AQPs expression in the kidney is shown in Figure 4.1. AQP1, 2, and 3 were upwardly expressed ($p<0.05$) in the HS0 group when compared to TN0, TN6, and HS6 groups. The HS0 and HS6 had a downward ($p<0.05$) expression of AQP4 as compared to TN0 and TN6. Birds under HS had a higher ($p<0.05$) water intake (Figure 4.2) compared to the TN groups. The water conversion ratio (Figure 4.3) of HS0 was lower ($p<0.05$) relative to TN6 and TN0. Table 4.1 shows the blood composition of broiler chickens raised with glucose supplementation under heat stress or thermoneutral conditions. Birds in the HS0 had a lower ($p<0.05$) pCO_2 when compared to TN0 and TN6 groups. Heat stress lowered ($p<0.05$) the pCO_2, TCO_2 and HCO_3 and raised the Cl-, AnGap, and glucose level of the blood. The Na^+, K^+ and Cl^- composition were not significantly ($p>0.05$) affected. Heat stress tended to increase osmolality, but the increment was of no statistical importance at $p=0.05$. Glucose supplementation increased ($p<0.05$) the glucose concentration of the blood.

DISCUSSIONS

The kidney maintains homeostasis of water in the body. Water diffuses through biological membranes at different rates, but urine concentration and dilution primarily occur in the kidney. The reabsorption of water is regulated via different aquaporins in the kidney. The higher water intake in the HS0 groups relative to TN0, TN6 and HS6, coupled with a higher WCR, showed that the HS groups used more water in combating HS. The peripheral blood vessel of birds expands

when subjected to a long period of HS, destroying metabolic and cellular system of the birds (Ali *et al*., 2019). This affects various metabolites in the blood of the birds. Heat stress causes an imbalance between CO_2 and HCO_3 in the blood, leading to pulmonary CO_2 washout that disrupt the acid-base balance and elevates the pH value of the blood. Heat stress lowers blood TCO_2, CO_2 levels and HCO_3, hence raising the pH value into the alkalotic zone. This is in consonance with the report of Pech-Waffenschmidt *et al*. (1995). Painting due to heat stress results to loss of CO_2 (Borges *et al*., 2004; Pech-Waffenschmidt *et al*., 1995). This could be the reason for lower CO_2 levels seen in the HS (HS0 and HS6) birds. The normal value of pCO_2 in chickens ranges from 35 to 45mmHg (Messina and Patrick, 2022) and only HS) appears to fall outside this range. Homeostasis of the respiratory system depends on availability and the removal of oxygen and carbon dioxide, respectively. This help in the regulation of body pH. Birds in the HS0 group had a lower pCO_2 value compared with TN0 and TN6. Decrease in pCO_2 decreases acidity. Decrease in CO_2 production without increase in ventilation results into metabolic alkalosis. The lower pCO_2 in HS0 birds could point to metabolic alkalosis if condition persists for a longer period. Birds in HS0 and HS6 had a lower HCO_3 value. HCO_3 measures the amount of CO_2 in the body and aids the balance between acid and base in the body. The reduction in CO_2 levels under HS agrees with the report of Borges *et al*. (2004). Anion gap blood test (AnGapP checks the acid-base balance of the blood (Lee *et al*., 2020). A higher value can result to acidosis and a lower value can result to alkalosis. However, there is no literature yet to establish the normal range of AnGap in broiler chickens, hence we cannot ascertain the level at which this will occur in broiler chickens (Pandey and Sharma, 2022). The pH of HS group was higher compared with TN group, although not significant and it has a higher AnGap value.

An increase in glucocorticoids is caused by stressors such as heat stress (Borges *et al*., 2004; Kolb, 1984). The elevation in corticosterone (CORT), the major glucocorticoid of chickens, raises the level of body glucose. Once produced, CORT initiates the catabolism of muscle protein and adipose tissue for gluconeogenesis (formation of glucose from non-glucose source) while building resistance to stress (Xu *et al*., 2018). The higher glucose levels observed in the heat-stressed group as opposed to the thermoneutral group could therefore be attributed to an increase in the gluconeogenesis biochemical process in the liver to create an escape route for birds under HS condition. Xie *et al*. (2015) linked higher glucose in HS birds to a measure of survival in which birds that survive higher stress have higher serum glucose.

Heat stress increased water intake in the HS0 birds. The HS6 birds have extra glucose in the drinking water which affected the water intake of those birds even though they were subjected to HS. In addition to drinking more water, the HS0 chickens may have reabsorbed more water from their kidney compared to TN0, TN6 and HS6. This is evidenced by to the higher expression of AQP-1, -2 and -3 in HS0 birds. AQP1 regulates the reabsorption of water in the apical and basolateral membranes of the kidney's proximal tubules, thin descending limb of Henle, and endothelial cells of the descending vasa recta (Wen *et al*., 2019; Pallone *et al*., 1997). AQP1 primarily functions to constitutively absorb over 70% of water in the glomerular filtrate. How effective the kidney is in filtering the waste and excess water is determined by the glomerular filtration rate (Kaufman *et al*., 2022). The glomerular filtrate filters excess water and wastes entering the kidney. The higher expression of AQP1 in HS0 group may show that excess water was filtered in the glomerular filtrate and the lower AQP1 expression in the HS6 group shows a better water utilization in the birds.

The excretion of water is mainly regulated by vasopressin (a peptide hormone), which basically targets AQP-2 for the regulation of water in the body. Aquaporin 2 maintains fluid homeostasis in the body by concentrating and regulating the production of urine (Kwon *et al*., 2013; Brown, 2017). In a healthy state, over 99% of water initially filtered through the glomeruli is reabsorbed by the renal tubules and collecting ducts. AQP2, 3, and 4 are highly expressed in the principal cells of the connecting tubules and collecting ducts that regulate body water homeostasis and urine concentration (Wen *et al*., 2020). The high mRNA expression of AQP2 in the HS0 group may be in response to excess water loss in this group and may be the birds way of maintaining homeostasis. This agrees with the report of Sugito *et al*. (2021) who observed an increase in AQP2 expression in chickens exposed to HS. The higher expression of AQP2 could be a response to rehydrate the cells under heat stress. A lower expression in glucose-supplemented groups (HS6 and TN6) may suggest that glucose supplementation may help in preventing the dehydration of cells under HS.

AQP3 upregulation in HS0 is probably due to a higher amount of water filtered out from the glomerulus filtrate, as evidenced by the upward expression of AQP1. The higher amount of water filtered resulted in higher water absorption by the renal tubules and collecting ducts, hence AQP-3 was highly expressed in the HS0 group. AQP4 was downwardly expressed under HS (HS0 and HS6). However, the lower AQP4 expression in the HS0 group could be a resultant effect of the higher expression of AQP3. Since AQP3 and AQP4 are localized in the same place, a higher expression of one may result in lowering the expression of the other. The result of this study is in accordance with the discovery of Sugimoto *et al*., (2012), who reported upregulation of AQP 1 and 2 and downregulation of AQP-4 in mouse fibroblast cells exposed to 37^0C of heat.

CONCLUSIONS

Glucose supplementation appears to play a vital role in preventing tissue dehydration during HS. The mechanism at which this works should be further evaluated, as this may help to preserve water in broilers exposed to HS during production. Aquaporins -1, -2, and -3 were upwardly expressed under HS compared to chickens raised under TN condition.

The blood chemistry was altered by HS and glucose supplementation. Glucose supplementation increased glucose levels in the blood. This ensured that the energy level is maintained under HS. Heat stress also resulted in an increase in serum glucose level. The increase level of glucose in the serum of HS birds is a response by the birds to combat the negative heat balance between metabolic heat production and heat dissipated. This is because the stress hormone, corticosterone, is released to combat the effect of heat stress, resulting to gluconeogenesis to produce glucose for the cells.

Lists of tables and figures

Table 4.1. Blood composition of broilers chicken supplemented with glucose under thermoneutral or heat stress condition from d28 and 35.

Treatment	pH	pCO_2 (mmHg)	HCO_3	TCO_2	Na^+ (mEq/L)	K^+ (mEq/L)	Cl^- (mEq/L)	AnGap (mEq/L)	Glu (mg/dl)	Hct (%)	Hb (g/dl)	Osmolality (Osm/kg)
TN0	7.36	44.70^{a}	25.22^{a}	26.33^{a}	145.67	5.23	109.33	16.50	240.17^{c}	24.50	8.35	305.68
TN6	7.37	43.42^{a}	24.88^{a}	26.33^{a}	143.50	5.07	107.50	16.17	258.17^{b}	24.33	8.28	302.34
HS0	7.46	31.35^{b}	22.10^{b}	23.17^{b}	145.17	4.95	110.00	18.00	273.00^{ab}	23.17	7.87	306.50
HS6	7.38	36.57^{ab}	21.13^{b}	22.17^{b}	145.33	4.90	111.33	17.50	289.33^{a}	22.50	7.65	307.74
P value	0.0918	0.0058	0.0098	0.0078	0.2762	0.1061	0.1147	0.1201	<.0001	0.2760	0.2507	0.1829
SEM	0.0278	2.6294	0.9097	0.9413	0.8216	0.1330	1.0652	0.5761	5.9393	0.8122	0.2759	1.7299
Temperature												
TN	7.36	44.06^{a}	25.05^{a}	26.33^{a}	144.58	5.15	108.42^{b}	16.33^{b}	249.17^{b}	24.42	8.32	304.01
HS	7.42	33.96^{b}	21.62^{b}	22.67^{b}	145.25	4.93	110.67^{a}	17.75^{a}	281.17^{a}	22.83	7.76	307.12
P value	0.0697	0.0010	0.0012	0.0009	0.4267	0.6656	0.0474	0.0232	<.0001	0.0654	0.0565	0.0872
SEM	0.0196	1.8593	0.6433	0.6656	0.5809	0.0940	0.7532	0.4074	4.1997	0.5743	0.1951	1.2232
Glucose levels												
0%	7.41	38.03	23.66	24.75	145.42	5.09	109.67	17.25	256.58^{b}	23.83	8.11	306.09
6%	7.37	39.99	23.01	24.25	144.42	4.98	109.42	16.83	273.75^{a}	23.42	7.97	305.04
P value	0.2319	0.4632	0.4832	0.6012	0.2377	0.4248	0.8168	0.4779	0.0090	0.6136	0.0565	0.5521
SEM	0.0196	1.8593	0.6433	0.6656	0.5809	0.0940	0.7532	0.4074	4.1997	0.5743	0.1951	1.2232

*pCO_2 means partial pressure of carbon dioxide, HCO_3-bicarbonate, TCO_2 – Total carbon dioxide, Na^+ – sodium, K^+ – potassium, Cl^- – chlorine, AnGap – Anion Gap Blood test, Glu – glucose, Hct – hematocrit, Hb-hemoglobin.

Superscripts a,b,c are used to indicate significant differences ($p<0.05$) between parameters across columns**.**

Table 4.2 Primer pairs used for RT-qPCR analysis of kidney aquaporin gene expression levels

Gene Symbol	Accession Number	Product Size (bp)	Primer Sequence	
			Reverse	5' CACAACCACCACGCTATT 3'
AQP1	NM_001039453	90	Forward	5' AAGTGAGATTGAAGAGCAGTAG 3'
			Reverse	5' GAACAGCCACAGGAACAA 3'
AQP2	NM_001292072	76	Forward	5' GTTACACCGGTTGCTCTATG 3'
			Reverse	5' CCAATGGTCACTGAAGTCTC 3'
AQP3	XM_424500	103	Forward	5' CTGGTATCTTTGCCACCTAC 3'
			Reverse	5' GATAGCCAAGACACAAACAATC 3'
AQP4	NM_001317827	98	Forward	5' CGCTCGCAGCAGCAGTAA 3'
			Reverse	5′AGACATCAGGGTGTGATGGTTGGT3′
B-actin	NM 205518.1 125	125	Forward	5′TCCCAGTTGGTGACAATACCGTGT3′

AQP - Aquaporins

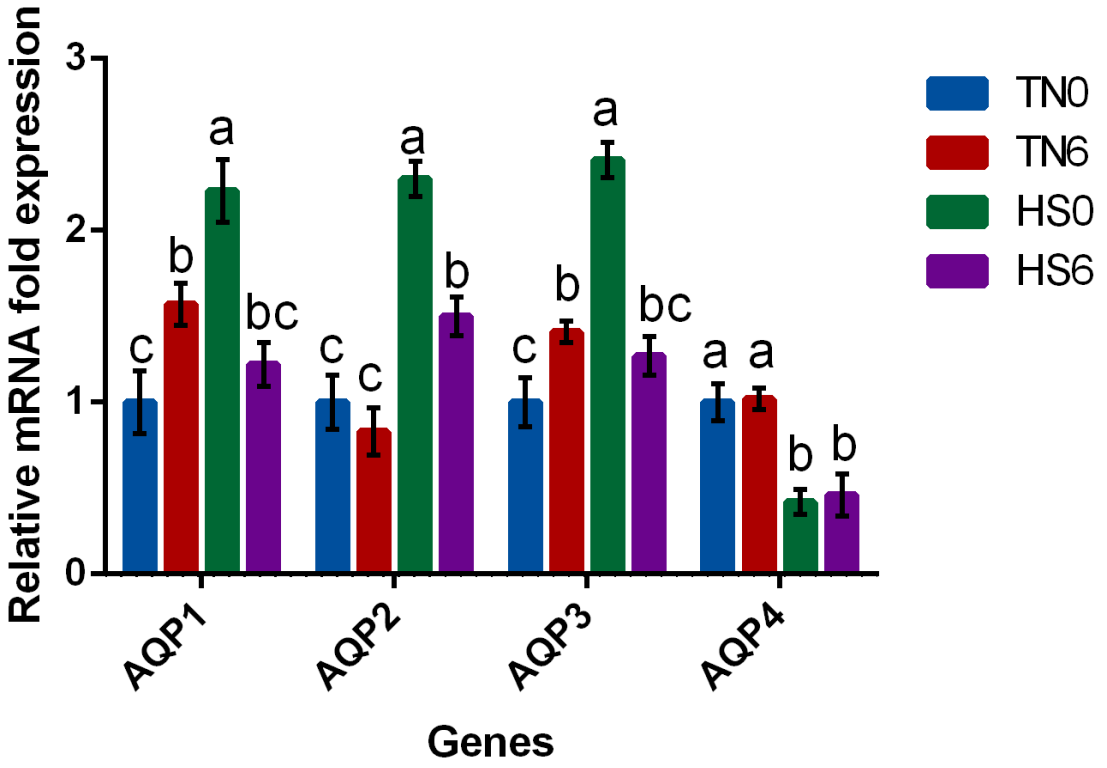

Figure 4.1 mRNA expression of kidney Aquaporins of broiler chickens subjected to heat stress from d28 to d35.
AQP1 – Aquaporin 1, AQP2 – Aquaporin2, AQP3 – Aquaporin3, AQP4 – Aquaporin4
TN0 – Thermoneutral (25^0C) without glucose supplementation
TN6 - Thermoneutral (25^0C) with 6% glucose supplementation
HS0 – Heat stress (35^0C) without glucose supplementation
HS6 – Heat stress (35^0C) with 6% glucose supplementation
Different letters show significant differences ($p<0.05$) between treatment groups.
Error bars represent SEM

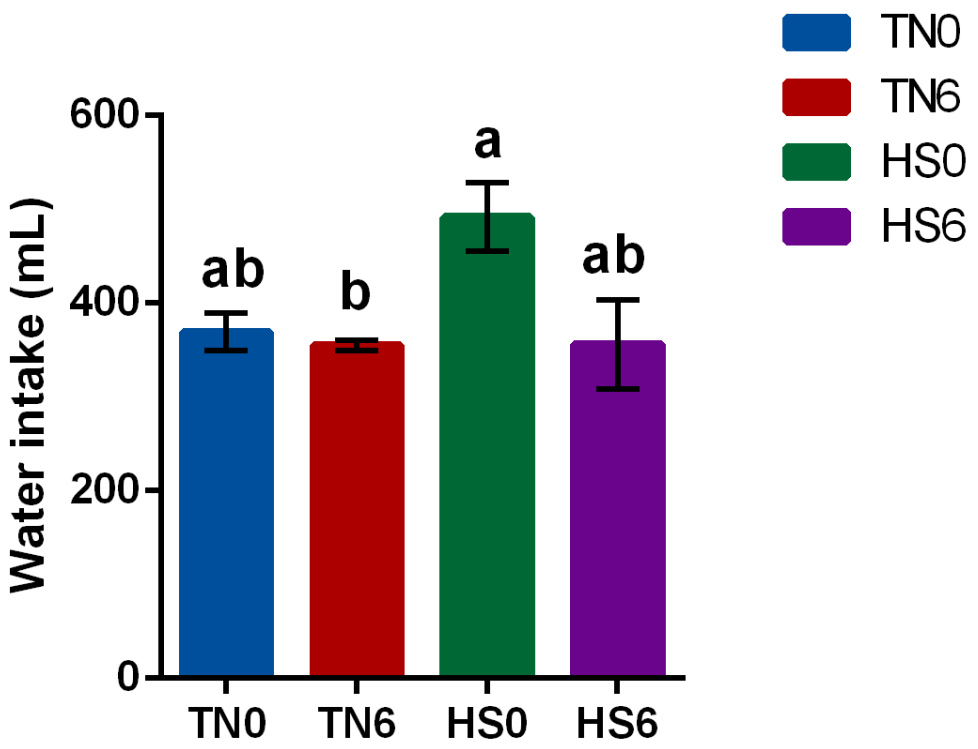

Figure 4.2: Water intake of broiler chickens raised under HS with or without glucose supplementation
TN0 – Thermoneutral (25^0C) without glucose supplementation
TN6 - Thermoneutral (25^0C) with 6% glucose supplementation
HS0 – Heat stress (35^0C) without glucose supplementation
HS6 – Heat stress (35^0C) with 6% glucose supplementation
Different letters show significant differences ($p<0.05$) between treatment groups.
Error bars represent SEM

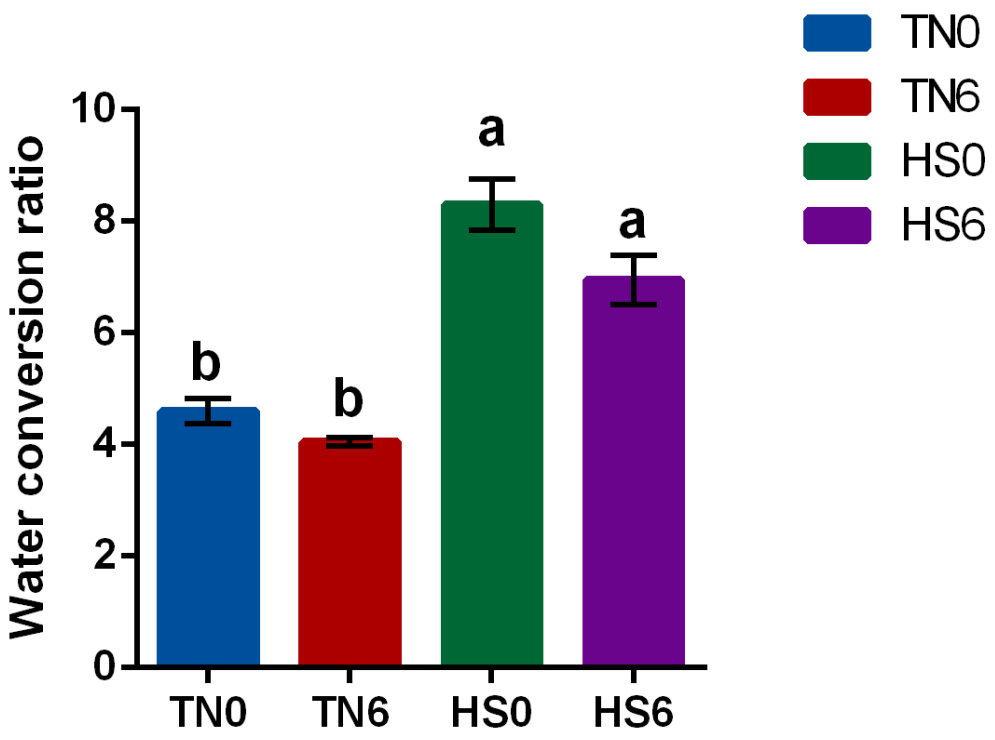

Figure 4.3: Water conversion ratio of broiler chickens raised under HS with or without glucose supplementation
TN0 – Thermoneutral (25^0C) without glucose supplementation
TN6 - Thermoneutral (25^0C) with 6% glucose supplementation
HS0 – Heat stress (35^0C) without glucose supplementation
HS6 – Heat stress (35^0C) with 6% glucose supplementation
Different letters show significant differences ($p<0.05$) between treatment groups.
Error bars represent SEM

REFERENCES

Ahmad, T., & Sarwar, M. (2006). Dietary electrolyte balance: implications in heat stressed broilers. *World's Poultry Science Journal*, *62*(4), 638-653.

Ali, Q., Farooq, U., Akhtar, P., Khalid, M.F., Khan, S.U. (2019). Impact of Glucose to Mitigate the Heat Stress in Broiler Chickens. PSM Biol. Res., 4(4): 144-153.

Borges, S. A., Da Silva, A. F., Majorka, A., Hooge, D. M., & Cummings, K. R. (2004). Physiological responses of broiler chickens to heat stress and dietary electrolyte balance (sodium plus potassium minus chloride, milliequivalents per kilogram). *Poultry science*, *83*(9), 1551-1558.

Brown, D. (2017). The discovery of water channels (aquaporins). *Annals of Nutrition and Metabolism*, *70*(Suppl. 1), 37-42.

Bruno, L. D. G., Maiorka, A., Macari, M., Furlan, R. L., & Givisiez, P. E. N. (2011). Water intake behavior of broiler chickens exposed to heat stress and drinking from bell or and nipple drinkers. *Brazilian Journal of Poultry Science*, *13*, 147-152.

Du, K. X., Dong, Y., Zhang, Y., Hou, L. W., Fan, D. X., Luo, Y., ... & Lou, J. Y. (2015). Effects of dexamethasone on aquaporin-4 expression in brain tissue of rat with bacterial meningitis. *International journal of clinical and experimental pathology*, *8*(3), 3090.

Ishibashi, K., Koike, S., Kondo, S., Hara, S., & Tanaka, Y. (2009). The role of a group III AQP, AQP11 in intracellular organelle homeostasis. *The Journal of Medical Investigation*, *56*(Supplement), 312-317.

Jeyaseelan, K., Sepramaniam, S., Armugam, A., & Wintour, E. M. (2006). Aquaporins: a promising target for drug development. *Expert opinion on therapeutic targets*, *10*(6), 889-909.

Kaufman DP, Basit H, Knohl SJ. Physiology, Glomerular Filtration Rate. [Updated 2022 Jul 18]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK500032/

Kolb, E. (1984). Fisiologia veterina´ria. 4th ed. Rio de Janeiro: Guanabara Koogan, Rio de Janeiro, Brazil.

Kwon, T. H., Frøkiær, J., & Nielsen, S. (2013). Regulation of aquaporin-2 in the kidney: A molecular mechanism of body-water homeostasis. *Kidney research and clinical practice*, *32*(3), 96-102.

Lee, W., Ha, J. M., & Sugiyama, Y. (2020). Post-translational regulation of the major drug transporters in the families of organic anion transporters and organic anion–transporting polypeptides. *Journal of Biological Chemistry*, *295*(50), 17349-17364.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *methods*, *25*(4), 402-408.

Messina Z, Patrick H. Partial Pressure of Carbon Dioxide. [Updated 2022 Sep 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK551648/

Nielsen S, Agre P (1995) The aquaporin family of water channels in kidney. Kidney Int 48:1057–1068

Pallone, T. L., Kishore, B. K., Nielsen, S., Agre, P. E. T. E. R., & Knepper, M. A. (1997). Evidence that aquaporin-1 mediates NaCl-induced water flux across descending vasa recta. *American Journal of Physiology-Renal Physiology*, *272*(5), F587-F596.

Pandey DG, Sharma S. Biochemistry, Anion Gap. [Updated 2022 Jul 18]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK539757/

Pech-Waffenschmidt, V., Bogin, E., Avidar, Y., & Horst, P. (1995). Metabolic and biochemical changes during heat stress in relation to the feathering degree of the domestic hen. *Avian Pathology*, *24*(1), 33-44.

Saadoun S, Papadopoulos MC, Watanabe H, Yan D, Manley GT, Verkman AS (2005) Involvement of aquaporin-4 in astroglial cell migration and glial scar formation. J Cell Sci 118:5691–5698

SAS Institute Inc. SAS/IML® Studio 15.1 for SAS/STAT® Users; SAS Institute Inc.: Cary, NC, USA, 2018.

Sugimoto, N., Shido, O., Matsuzaki, K., Ohno-Shosaku, T., Hitomi, Y., Tanaka, M., ... & Umehara, H. (2012). Cellular heat acclimation regulates cell growth, cell morphology, mitogen-activated protein kinase activation, and expression of aquaporins in mouse fibroblast cells. *Cellular Physiology and Biochemistry*, *30*(2), 450-457.

Sugito, S., Etriwati, E., Akmal, M., Rahmi, E., Delima, M., Muchlisin, Z. A., & Hasan, D. I. (2021). Immunohistochemical Expression of AQP2 and HSP70 in Broiler Kidney Tissue Treated with Salix tetrasperma Roxb. Extract under Heat Exposure. *The Scientific World Journal*, *2021*.

Wang, Y. H., Liu, T. T., Kung, W. M., Chen, C. C., Wen, Y. T., Lin, I. C., ... & Wei, L. (2015). Expression of aquaporins in intestine after heat stroke. *International journal of clinical and experimental pathology*, *8*(8), 8742.

Wen, L., Zhao, B., Chen, W., Qing, Z., & Liu, M. (2020). Real-time assessment of the behaviour of the bladder neck and proximal urethra during urine leaking in the cough stress test (CST) in supine and standing positions using transperineal ultrasound. *International Urogynecology Journal*, *31*(12), 2515-2519.

Wen, Y., Lu, X., Ren, J., Privratsky, J. R., Yang, B., Rudemiller, N. P., ... & Crowley, S. D. (2019). KLF4 in macrophages attenuates TNFα-mediated kidney injury and fibrosis. *Journal of the American Society of Nephrology*, *30*(10), 1925-1938.

Wideman, R. F., Ford, B. C., May, J. D., & Lott, B. D. (1994). Acute heat acclimation and kidney function in broilers. *Poultry Science*, *73*(1), 75-88.

Xie, J., Tang, L., Lu, L., Zhang, L., Lin, X., Liu, H. C., ... & Luo, X. (2015). Effects of acute and chronic heat stress on plasma metabolites, hormones and oxidant status in restrictedly fed broiler breeders. *Poultry Science*, *94*(7), 1635-1644.

Xu, J., Feng, L., Jiang, W. D., Wu, P., Liu, Y., Jiang, J., ... & Zhou, X. Q. (2018). Different dietary protein levels affect flesh quality, fatty acids and alter gene expression of Nrf2-mediated antioxidant enzymes in the muscle of grass carp (Ctenopharyngodon idella). *Aquaculture*, *493*, 272-282.

CHAPTER 5
MOLECULAR EXPRESSION OF ILEAL NUTRIENT TRANSPORTERS IN CHICKENS RAISED UNDER THERMONEUTRAL OR HEAT STRESS CONDITION.

Oluwatomide W. Ariyo[1], Ahmed F. A. Ghareeb[1], Marie C. Milfort[1], Bikash Aryal[1], Evan Hartono[1], Josephine Kwakye[1], Selorm Sovi[1], Sommer A. Hipple[1], Carrienton Stevenson[2], Alberta L. Fuller[1], Romdhane Rekaya[3], and Samuel E. Aggrey[1]
To be submitted to *Animal.*

ABSTRACT

Heat stress (HS) disrupt nutrients' digestion and absorption causing poor growth performance in chicken. We evaluated the effect of D-glucose supplementation on mRNA expression of ileal monosaccharide, fatty acid, and amino acids (AAs) transporters in broiler chickens. A total of 456 Cobb500 male chickens, randomly assigned to 4 groups (6 replicates/19 birds each): thermoneutral (TN0 and TN6) at 25ºC, HS (HS0 and HS6) at 35ºC (12 hours/day from d28-d35. TN6 and HS6 received 6% glucose-water, TN0 and HS0 received fresh-water. On d35, ileum tissues were collected from 6 random chickens/treatment for gene expression analysis. Birds in HS6 had higher mRNA expression of GLUT1, FATP1, FABP6, rBAT, $b^{0,+}$AT, SNAT2, and SNAT7 compared with TN0, TN6 and HS0. GLUT5 was upwardly expressed in TN6 compared TN0, HS0 and HS6. GLUT 12 and FABP2 were upwardly expressed in HS groups compared to TN groups. D-glucose supplementation enhanced AAs transporters responsible for glutamine absorption.

INDEX WORDS: Heat stress, glucose supplementation, nutrient transporters, gene expression

INTRODUCTION

Several studies have shown that HS causes a decrease in feed intake, as well as impair nutrient digestibility and feed efficiency (Habashy et a., 2017; Ghareeb *et al*., 2022). These factors adversely influence the growth performance of animals under HS (Renaudeau *et al*., 2012). Reduction in feed intake with low nutrient absorption leads to poor growth performance. Nutrient transporters are key markers for measuring nutrient absorption, and their mRNA expression helps to understand the underlying mechanisms behind their functions. Hence, the need to measure the mRNA expression of nutrient transporters in the gastrointestinal tract (GIT) of HS broiler chickens.

The transport of nutrients is facilitated all through the length of the GIT, with nutrient transport occurring mostly in the small intestine, and water and electrolyte transport in the colon. The ileum is the last section of the small intestine, and has a longer retention time permitting better nutrient absorption (Svihus, 2014). Several nutrient transporters and binding proteins are expressed in the ileum, making the ileum a considerable candidate segment to evaluate nutrient absorption.

At the molecular level, animals respond to HS via changes in gene expression of heat shock proteins (e.g., HSP70 and HSP90), reshuffling in amino acid concentration and skeletal muscle metabolism (Sammad *et al*., 2020). Different stressors cause the enterocytes to alter the expression of nutrient transporter to meet body requirements following molecular signals (Sohail *et al*., 2012).

The small intestinal absorption of monosaccharides helps to maintain energy and glucose balance (Dyer *et al*., 2003). Monosaccharides such as glucose must move across tight junctions of epithelial cells to get to the site of absorption (Kellett, 2001). Due to their hydrophilic nature,

monosaccharides cannot passively penetrate cell membranes. For efficient absorption of nutrients, there is a need for the luminal brush border membrane (BBM) and basolateral membrane (BLM) nutrient transporters in the epithelial cells of the small intestine (Koepsell, 2020). Monosaccharides are transported by a group of solute carriers and protein co-transporters that may or may not depend on insulin to be expressed on the enterocytic cell membrane (Byers *et al*., 2017). Facultative glucose transporters (GLUTs) aid the translocation of simple sugars in enterocytes. Active sodium-glucose symporter (SGLT1) is expressed on the enterocyte apical membrane, and it utilizes the Na^+ concentration gradient to move glucose against its concentration (Romereo *et al*., 2009). SGLT1 actively translocates a molecule of D-glucose with two sodium ions into cells using a sodium gradient generated by (Na^+/K^+)-ATPase (Lee *et al*., 1994). Fatty acid-binding proteins (FABPs) and fatty acid transport proteins (FATPs) are expressed on intestinal mucosal cells' apical membrane, where they aid lipid and fatty acid absorption (Yuan *et al*., 2012; Ghareeb *et al*., 2022). Amino acids moves from the intestinal lumen through the plasma membrane of the enterocytes to the bloodstream. This is made possible via amino acid transporters that are unique in function, structure, solute specificity, amino acid specificity and physiological activity (Kiela and Ghishan, 2016).

The detrimental effect of HS on nutrient digestibility in broilers has been reported to be caused by low expression and activity of digestive enzymes such as trypsin, chymotrypsin, lipase, amylase, and maltase (Hai *et al*., 2000; Song *et al*., 2018; Al-Zghoul *et al*., 2019; Teyssier *et al*., 2022). The mRNA expression of nutrient transporters is affected by HS. For instance, Habashy *et al*., 2017 reported a downregulation of FATP1, SGLT1, and GLUT2 in HS broilers. There has been varying reports on the amino acid transporters, ranging from upregulation to downregulation (Sun *et al*., 2015; Habashy *et al*., 2017; Al-Zghoul *et al*., 2019). Several nutritional interventions

have been made to alleviate the effects of heat stress on broiler chickens (Wasti *et al*., 2020). We hypothesize that supplementation of drinking water with glucose can improve the performance of chickens raised under HS. Evaluating nutrient transporter genes can aid in assessing the effect of HS on gut function and lead to better understanding of how nutrients are being absorbed (Habashy *et al*., 2017). We herein study the mRNA expression of nutrient transporters in broiler chickens raised under thermoneutral or HS conditions and the drinking water supplemented with or without glucose.

MATERIALS AND METHODS

This study was performed following the guidelines approved by the University of Georgia's institutional animal care and use committee. A total of 456 Cobb500 broiler chickens were used for this study. This study consisted of 4 treatment groups each with 6 replicates of 19 birds each. Birds were raised on pine shaving and randomly allocated to one of the four treatments on d28. Birds were given crumbled starter, pelletized grower and finisher feed from d0 to 14, d 15 to 28 and d 29 to 56, respectively. D-glucose was purchased from Carolina Biological Supply, Burlington, North Carolina, USA and administered through the water to the experimental birds at 0% (TN0 and HS0) or 6% (TN6 or HS6). Birds had unlimited access to the glucose water from d0 to d35. The brooding of birds lasted for 14 days and the lighting and temperature management practices in accordance with Cobb 500 standards (Cobb, 2018) were followed. The birds were fed *ad libitum*. The temperature was kept constant at 25^0C for the TN room while the HS room was set at 35^0C from 8 am to 8 pm, after which it was returned to 25^0C until 8 am the following day. The cycle continued for the duration of the experiment. On d 7 post-HS (age=35 days), ileum

samples were collected from 6 birds per treatment. Tissue samples were snap frozen in liquid nitrogen and stored at -85˚C until needed.

RNA extraction and RT-PCR

Frozen ileum samples were prepared for total RNA extraction. Samples were coarsely ground, and homogenized in lysing matrix tubes (MP Biomedicals, Santa Ana, CA). Total RNA extraction was done using TRIzol reagents (Invitrogen, Carlsbad, CA), purified with RNeasy Mini Kit (Qiagen, Valencia, CA, USA), and treated with RNase-Free DNase (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. Clean RNA was suspended in DEPC water and concentration was measured and adjusted to 200ng/µl using a NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE). The RNA was stored at -85˚C until cDNA synthesis. Ten microliters (µl) of clean RNA were reverse transcribed to produce cDNA using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA) based on the manufacturer's protocol. A gradient thermocycler from Eppendorf, Hauppauge, NY, was used for cDNA synthesis. The cDNA synthesis cycle was set to 25˚C for 10 minutes, 37˚C for 120 minutes, and 85˚C for 5 minutes and hold at 4^0C. The concentration of cDNA was taken, and the concentration of all samples was adjusted to 20 ng/µl using NanoDrop 2000 Spectrophotometer. The cDNA was stored at -85˚C until RT-qPCR was carried out. RT-qPCR was done using StepOnePlus (Applied Biosystems, Carlsbad, CA). Each RT-PCR reaction consisted of a total volume of 20 µl; made up of 1 µl cDNA, 0.6 µl forward and reverse primer each, 7.4 µl DEPC water, and 10 µl SYBR Green Master Mix (Applied Biosystems, Carlsbad, CA). All samples were replicated 3 times on the same plate. The conditions for RT-PCR were set at 50˚C for 2 minutes and 95˚C for 2 minutes for holding stage; 95˚C for 15 seconds repeated for 40 cycles, and 60˚C

for 1 minute for cycling stage; and 95˚C for 15 seconds, 60˚C for 1 minute and finally 95˚C for 15 seconds for the melt curve stage. At the end of the cycles, a melting temperature curve was determined.

The mRNA expression of the following genes was determined: sodium-glucose linked transporter 1, (SGLT1), glucose transporter protein type 1(GLUT1), glucose transporter protein type 2 (GLUT2), glucose transporter protein type 5 (GLUT5), glucose transporter protein type 8 (GLUT8), glucose transporter protein type 10 (GLUT10), glucose transporter protein type 12 (GLUT12), neutral and basic amino acid transport protein (rBAT), cationic amino acid transporter 1 (CAT1), basic amino acid transporter 1 (BAT1), b(0,+)-type amino acid transporter 1 (b0,+AT1), sodium-coupled neutral amino acid transporter 2 (SNAT2), sodium-coupled neutral amino acid transporter 7 (SNAT7), fatty acid binding protein 1 (FABP1), fatty acid binding protein 2 (FABP2), fatty acid binding protein 6 (FABP6) and fatty acid transport protein (FATP1).The housekeeping gene used was beta-actin and data obtained were analyzed using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) with the TN0 group as the control for the other groups.

Statistical analysis

Data analysis was done using a two-way ANOVA design through the SAS® Studio software (SAS Institute Inc., Cary, NC, USA). The model was done using the generalized linear model (GLM) procedure (SAS, 2018). Multiple comparison between treatment groups was done using the "Tukey-HSD" test with a significant difference set at $p < 0.05$.

The model is given below:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where y_{ijk} $is\ the\ mRNA\ fold\ expression,$ μ is the overall mean of the response, α_i is the effect of the ith temperature, where i = 1,2; β_j is the effect of jth glucose, where j = 1,2; $(\alpha\beta)_{ij}$ is the interaction effect between temperature and glucose (class i and j), and the ε_{ijk} is the random error.

RESULTS

The glucose, amino acids, and fatty acid nutrient transporters used in evaluating the effect of glucose supplementation on HS birds are displayed in Table 5.1. The fold expression of ileal glucose, amino acid, and fatty acid transporters of broiler chickens raised under thermoneutral, or heat stress condition is displayed in Figures 5.1, 5.2, and 5.3 respectively. Birds in the TN6 group elicited a higher ($p<0.05$) expression of GLUT2, GLUT5, GLUT8, and GLUT 10 when compared to the TN0 group. Birds in the HS6 groups had a higher expression of GLUT12 and GLUT1 when compared to birds in the TN0 and TN6 groups. GLUT 12 was highly expressed in the HS0 and HS6 groups relative to the TN0 and TN6 groups. There was a higher expression ($p<0.05$) of rBAT, $bo^{,+}$AT, SNAT2 and SNAT7 in the ileum of the HS6 birds compared to TN0, TN6 and HS0. HS0 had a higher expression of SNAT2 relative to TN0 and TN6. The HS6 birds had higher ileal mRNA expression of SLC7A5 compared to birds in the HS0 group. The HS0 birds had a higher expression of FABP1 and FABP2 when compared to TN0 and TN6. HS6 had a higher expression of FABP6 and FATP1 when compared to TN0 and TN6.

DISCUSSION

Glucose serves as the major source of energy for all living animal cells and various glucose transporters facilitate the diffusion of glucose across the plasma membrane. GLUT1 is the key

molecular transporter that maintains the movement of basal glucose in chickens (Kono *et al*., 2005). The higher expression of GLUT1 in HS6 birds compared to TN0, TN6 and HS0 showed that glucose supplementation at 6% under HS may have provided basal glucose needed for the metabolism of the birds. This might have prevented the utilization of muscle proteins as sources of energy under HS condition. The high expression of GLUT2, GLUT5 and GLUT8, in TN6 compared to TN0, HS0 and HS6 may be due to high glucose level availability in the tissue, which enhanced the uptake of glucose in the ileum. Both GLUT10 and GLUT12 are insulin-sensitive glucose transporters (Byers *et al*., 2017). The higher expression of GLUT10 in the TN6 group compared to TN0 and HS0 may be due to elevated tissue glucose. This triggered insulin and hence, the upregulation of insulin-sensitive glucose transporter 10. Heat stress increases plasma glucose (Lin *et al*., 2000; Garriga *et al*., 2006). The activity of hexokinase increased when broilers were subjected to cyclic heat stress (Zhang *et al*., 2012). This was to ensure energy supply for aerobic metabolism from glucose (Shao *et al*., 2019). This will also increase insulin activity and can explain why insulin sensitive GLUT12 was highly expressed in the HS groups (HS0 and HS6) compared with the TN groups (TN0 and TN6).

SNAT2/SLC38A2 and SNAT7/SLC38A7 are glutamine transporters. Glutamine is a gluconeogenic amino acid that is important for protein synthesis (Schousboe *et al*., 2014). Glutamine can be formed *de novo* from the metabolism of glucose to acetyl CoA (pyruvate dehydrogenase) and oxaloacetate (pyruvate carboxylase). These products react together to produce citrate which will form α-ketoglutarate (α-KG). Aspartate aminotransferase (AAT) then converts α-Ketoglutarate to glutamate, and glutamine is made from the glutamate via glutamine synthetase (GS). The observed higher expression of SNAT2 and SNAT7 in HS6 group compared to HS0 shows that the glucose supplemented may have resulted in the formation of glutamine to ensure

that the muscle proteins are not utilized for energy under HS condition. The rBAT/SLC3A1 (a heavy subunit) forms a complex with SLC7A9/b0,+AT (a light subunit) to yield a heteromeric transporter (Palacin *et al*., 2001). This complex controls the uptake of basic amino acids in exchange for neutral amino acids (Yan *et al*., 2020). There was an upward expression of b0,+AT in HS0 and HS6 group compared with TN0 and TN6 group. This was similar to the report of Ghareeb *et al*. (2022) who observed a higher expression of b0,+AT in HS broilers. CAT1/ SLC7A1 is a nutrient transporter specific for cationic amino acid under physiological condition. When the concentration of cationic amino acids is altered, it impairs the uptake of amino acids involved in protein synthesis (Khwatenge *et al*., 2020). LAT1/SLC7A5 and glycoprotein subunit (4F2hc) forms a dimer to yield a large neutral and aromatic amino acids exchanger that is independent of sodium. This regulates the concentration and balance of large neutral amino acids across the plasma membrane (Meier *et al*., 2002; Fotiadis *et al*., 2013; Ghareeb *et al*., 2022). LAT1's upward expression in HS6 birds compared to HS0 could have resulted from high production of glutamine from glucose, which was specifically retained for protein biosynthesis. The activation of mTORC1 pathway results to protein synthesis. It has been reported that the mTORC1 pathway can be activated by glutamine in a Rag GTPase independent manner (Kim *et al*., 2014; Jewell *et al*., 2015). This putatively improves protein biosynthesis and may help in preserving the storage of muscle protein in heat stressed birds.

Fatty acid transporters and binding proteins expressed in the intestine absorb the bulk of lipids from dietary source (Caspary, 1992; Ghareeb *et al*., 2022). During HS, the energy level of the body is low due to reduced feed consumption and energy need increases due to loss of energy via panting. This may enhance the activity of fatty acid coenzyme A to activate β-oxidation (Hotamisligil and Bernlohr 2015; Habashy *et al*., 2017). The upward expression of FABP1 and

FABP2 in the ileum of HS0 birds compared to TN0 and TN6 could be due to the increased energy need of the birds under HS, resulting to the breakdown of fatty acid to satisfy the energy need. Turcotte *et al*. (1997) reported upregulation of FABPs in skeletal muscles due to fasting (reduced energy level). Habashy *et al*., (2017) reported a higher expression of FABP1 in the *P. major* of heat-stressed broilers on day 1 after initiation of HS. Stahl (2004) observed an improved uptake of long-chain fatty acids due to integral transmembrane protein FATPs. Insulin downregulates FATP1 (Hui *et al*. 1998). Although there was a higher glucose level in the HS6 group, the upward expression of FATP1 could be due to the efficient conversion of glucose to amino acid precursors to ensure that the HS birds can continually synthesize protein to maintain muscle protein mass under HS condition. The observed upward expression of FATP1 in this study is in consonance with the report of Habashy *et al*. (2017).

CONCLUSIONS

Under HS, glucose supplementation at 6% resulted in higher mRNA expression of amino acid transporters $b^{0,+}$AT, rBAT, LAT1, SNAT2 and SNAT7 compared with HS0. However, under thermoneutral conditions, glucose supplementation at 6% resulted in a higher expression of glucose transporters SGLT1, GLUT2, GLUT5, GLUT8 and GLUT10 when compared with TN0. The supplementation of 6% D-glucose water had a positive influence on the expression of nutrient transporters in the ileum of heat stressed broiler chickens compared to their counterparts whose water was not supplemented. This may have resulted to an upward regulation of AAs transporters responsible for the absorption of glutamine and neutral AAs. The enterocytes oxidize glutamine to produce energy and utilizes neutral AAs like cystine to activate glutathione antioxidant to protect cells from HS-induced oxidative damage. This might have enhanced the expression of ileal amino acids transporters of broiler chickens in HS6 compared to HS0. The expression of amino acids in the ileum of HS6 broiler chickens was higher compared to HS0 chickens.

Lists of tables and figures

Table 5.1 Primer pairs used for RT-qPCR analysis of ileum glucose transporters gene expression levels

Description	Gene	GeneBank Accession Number	Product size (bp)	Align.	Primer sequence
Glucose transporters	SGLT1 (SLC5A1)	NM_001293240	97	Forward	5′GAGGAGAAACCCGATGAAAGAG3′
				Reverse	5′CTAAGCCACAGAACCAGTTGTA3′
	GLUT1 (SLC2A1)	NM_205209.1	105	Forward	5′CTTCTGCATACACTCCTTCTCC3′
				Reverse	5′TGGACGTGAAACCAGCTAAA3′
	GLUT8 (SLC2A8)	AB083371	309	Forward	5′GCAGCAGAGGTTATTCGCGCC3′
				Reverse	5′GCCTCCCAGTATTCCTCCAGC3′
	GLUT10 (SLC2A10)	XM_417383.5	133	Forward	5′CCGCTGCAGATGAGGTATTT3′
				Reverse	5′GTTTCTTCTCAGAGCCGTAGTG3′
	GLUT12 (SLC2A12)	XM_419733.5	110	Forward	5′AGAGAGTGGGGAGGTTCCC3′
				Reverse	5′TCAGGACGAGCCAAGACA3′
Fructose transporters	GLUT2 (SLC2A2)	NM_207178.1	577	Forward	5′ATGCTGGTGGTCAATGTCCTCTC3′
				Reverse	5′TGATGCCTGAGAACTGCTGCGAT3′
	GLUT5 (SLC2A5)	XM_417596.6	108	Forward	5′AGGCTGATCTCTGCCTTTG3′
				Reverse	5′GTCGATGTAGGTTCGGTTGTAG3′
B-actin		NM 205518.1 125	125	Forward	5′AGACATCAGGGTGTGATGGTTGGT3′

	Reverse	5′TCCCAGTTGGTGACAATACCGTGT3′

Table 5.2 Primer pairs used for RT-qPCR analysis of ileum fatty acids transporters gene expression levels

Description	Gene	GeneBank Accession Number	Product size (bp)	Align.	Primer sequence
Fatty Acids transporters	FABP1	NM_204192.3	94	Forward	5′CCAGAAGGGTAAGGACATCAAG3′
				Reverse	5′GGTCATTACTTTGGAGCCAGTA3′
	FABP2	NM_001007923.1	77	Forward	5′AAAGATAATGGAAAAGTACTCACAGCAT3′
				Reverse	5′CCTTCGTACACGTAGGTCTGTATGA3′
	FABP6	XM_015293653.2	59	Forward	5′CGGTCTCCCTGCTGACAAGA3′
				Reverse	5′CCACCTCGGTGACTATTTTGC3′
	FATP1 (SLC27A1)	NM_001039602.2	119	Forward	5′TGCCTTTACGCCAGGATTT3′
				Reverse	5′GAGGGTCTCACGTTGCTTATC3′
B-actin		NM 205518.1 125	125	Forward	5′AGACATCAGGGTGTGATGGTTGGT3′
				Reverse	5′TCCCAGTTGGTGACAATACCGTGT3′

Table 5.3 Primer pairs used for RT-qPCR analysis of the ileum amino acids transporters gene expression levels

Nutrient Transporter	Transporter Gene	Gene bank Accession Number	Size	Align.	Primers Sequences
Apical amino acids	$b^{0,+}$AT (SLC7A9)	NM_001199133.1	113	Forward	5′GATCCCTGGAGCCTGAATTAC3′
				Reverse	5′CTCCTTTCTGTTGTCCTGTTCT3′
	rBAT (SLC3A1)	XM_004935370.3	119	Forward	5′CTGAGAGCATCACAGCCTATTC3′
				Reverse	5′GCCAGGTTCACTGCTGTATT3′
Basolateral amino acids	CAT1 (SLC7A1)	NM_001145490.1	111	Forward	5′CGAACAACAGAGGAGACAGATAA3′
				Reverse	5′GGGACACAGTATGGCTTTGA3′
	LAT1 (SLC7A5)	NM_001030579.2	98	Forward	5′GCCTTCTCCAATGACATCTTCT3′
				Reverse	5′TAACGCAGCCACATCATACC3′
	SNAT2 (SLC38A2)	NM_001305439.1	127	Forward	5′GAACAAGTAGGGCCCTGTAATC3′
				Reverse	5′GGGCAGAGCTTGATGTTATCT3′
	SNAT7 (SLC38A7)	XM_025154307.1	93	Forward	5′CAAGTTCACCATCAGCATCAC3′
				Reverse	5′CTCAGAGAGCTGGCGTATTT3′
B-actin		NM 205518.1 125	125	Forward	5′AGACATCAGGGTGTGATGGTTGGT3′
				Reverse	5′TCCCAGTTGGTGACAATACCGTGT3′

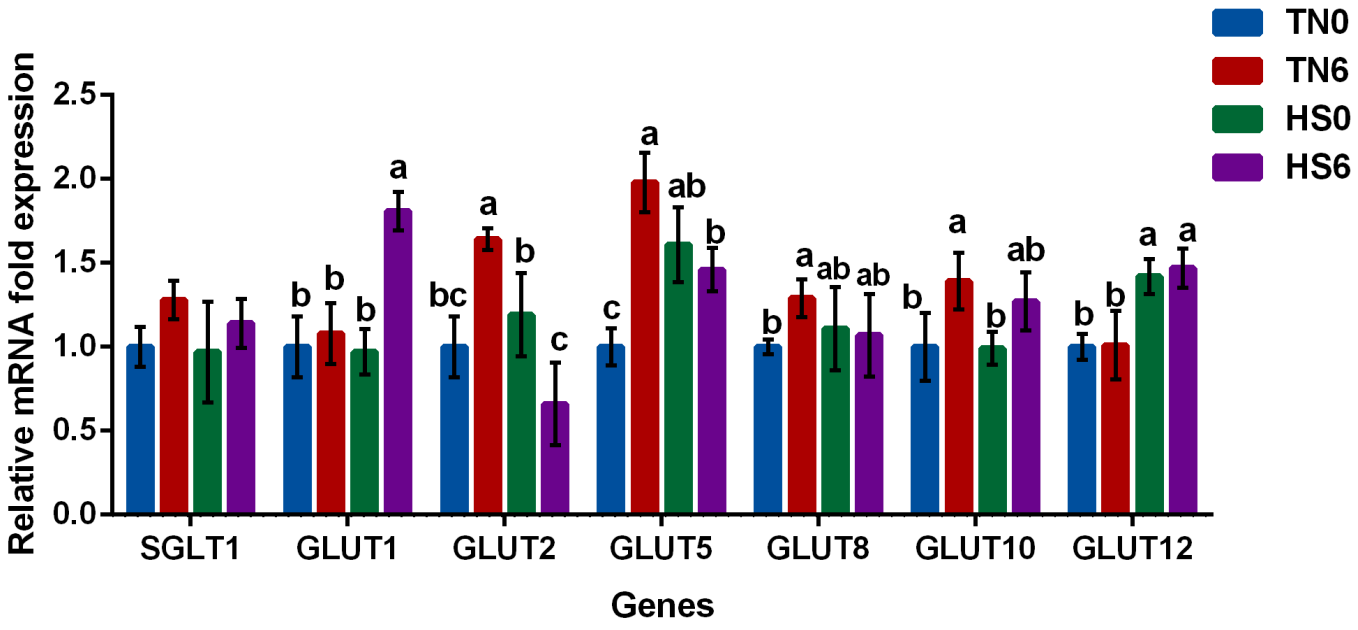

Fig. 5.1 The mRNA expression of ileal glucose transporters. TN0 = thermoneutral control (0% glucose), TN6 = Thermoneutral + 6% glucose, HS0 = heat stress with no glucose supplementation, HS6 = Heat stress + 6% glucose. Different letters show significant differences ($p<0.05$) between treatment groups. Error bars represent SEM.

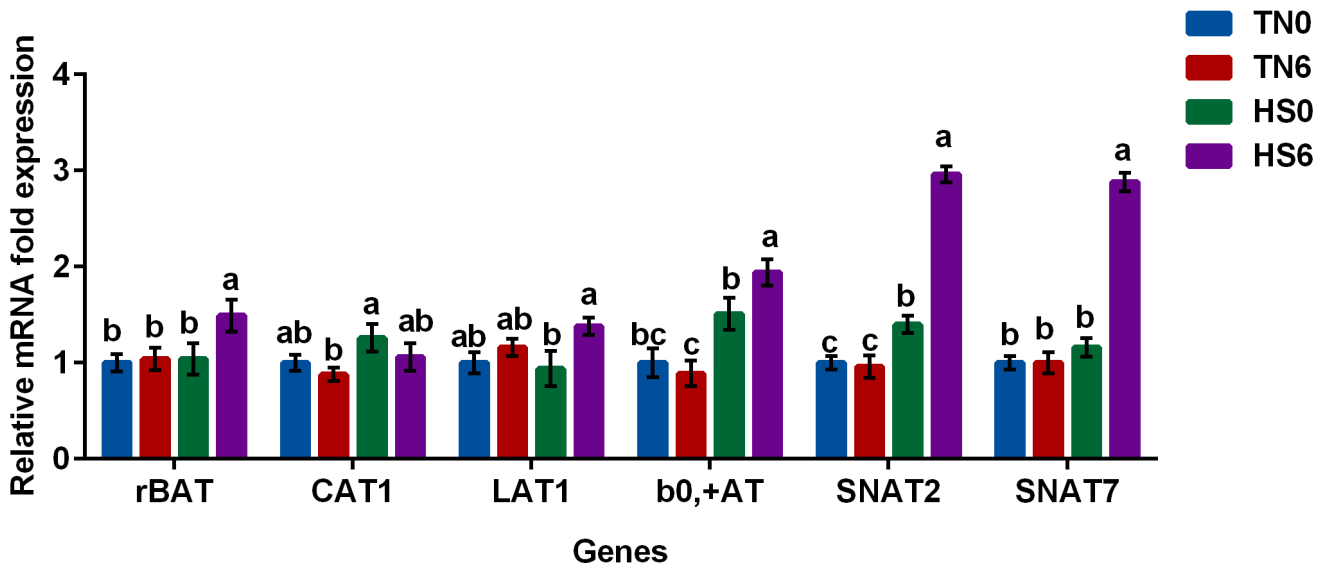

Fig. 5.2. The mRNA expression of ileal amino acid transporters. TN0 = thermoneutral control (0% glucose), TN6 = Thermoneutral + 6% glucose, HS0 = heat stress with no glucose supplementation, HS6 = Heat stress + 6% glucose. Different letters show significant differences ($p<0.05$) between treatment groups. Error bars represent SEM.

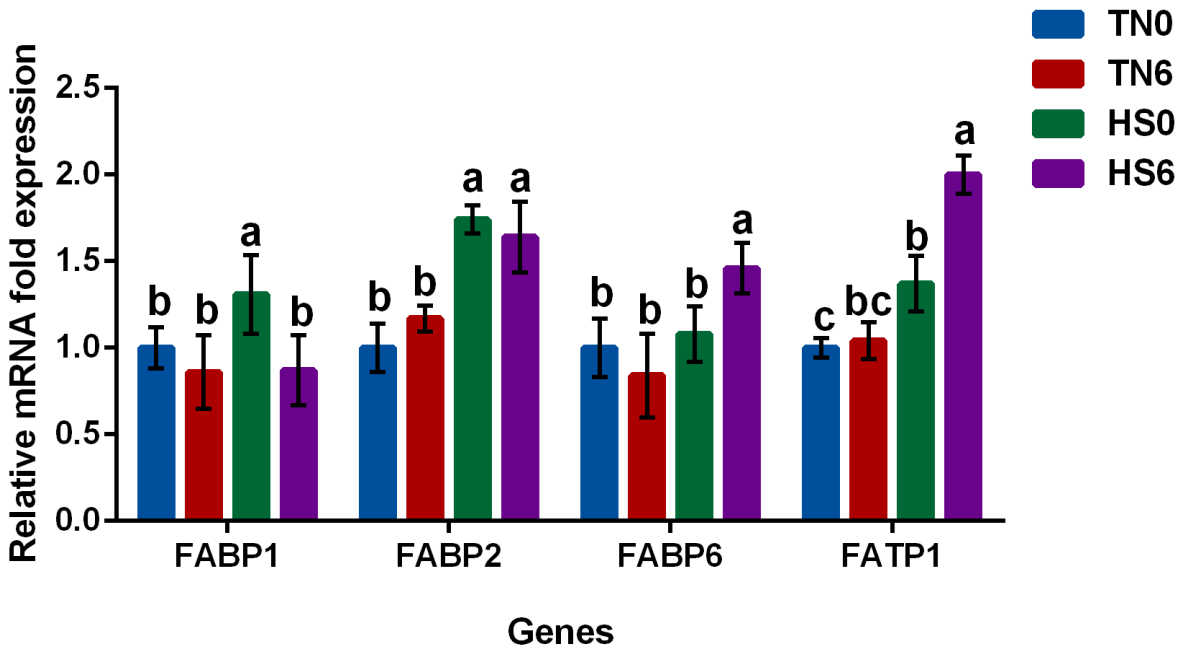

Fig. 5.3 The mRNA expression of ileal fatty acid transporters. TN0 = thermoneutral control (0% glucose), TN6 = Thermoneutral + 6% glucose, HS0 = heat stress with no glucose supplementation, HS6 = Heat stress + 6% glucose. Different letters show significant differences ($p<0.05$) between treatment groups. Error bars represent SEM.

REFERENCE

Al-Zghoul M. B., Alliftawi A. R. S., Saleh K. M. M., Jaradat Z. W. (2019). Expression of digestive enzyme and intestinal transporter genes during chronic heat stress in the thermally manipulated broiler chicken. *Poult. Sci.* 98, 4113–4122. 10.3382/ps/pez249

Byers, M. S., Howard, C., & Wang, X. (2017). Avian and mammalian facilitative glucose transporters. *Microarrays*, *6*(2), 7.

Caspary, W. F. (1992). Physiology and pathophysiology of intestinal absorption. *The American journal of clinical nutrition*, *55*(1), 299S-308S.

Cobb-Vantress. Cobb 500. Broiler Performance and Nutrition Supplement. 2018. Available online: https://www.cobb-vantress.com/products/cobb500 (accessed on 4 April 2020).

Dyer, J. (2003). Vayro (Joint First) S, King TP, Shirazi-Beechey SP. *Glucose sensing in the intestinal epithelium. Eur J Biochem*, *270*, 3377-3388.

Fotiadis, D., Kanai, Y., & Palacín, M. (2013). The SLC3 and SLC7 families of amino acid transporters. *Molecular aspects of medicine*, *34*(2-3), 139-158.

Garriga, C., Hunter, R. R., Amat, C., Planas, J. M., Mitchell, M. A., & Moretó, M. (2006). Heat stress increases apical glucose transport in the chicken jejunum. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *290*(1), R195-R201.

Ghareeb AFA, Gustavo H. Schneiders, Jennifer N. Richter, James C. Foutz, Marie C. Milfort, Albert L. Fuller, Jianmin Yuan, Romdhane Rekaya, Samuel E. Aggrey. (2022). Heat stress modulates the disruptive effects of Eimeria maxima infection on the ileum nutrient digestibility, molecular transporters, and tissue morphology in meat-type chickens. PLoS ONE 17(6): e0269131. https://doi.org/10.1371/journal.pone.0269131

Habashy W. S., Milfort M. C., Fuller A. L., Attia Y. A., Rekaya R., Aggrey S. E. (2017). Effect of heat stress on protein utilization and nutrient transporters in meat-type chickens. *Int. J. Biometeorol.* 61, 2111–2118. 10.1007/s00484-017-1414-1

Hai L., Rong D., Zhang Z. Y. (2000). The effect of thermal environment on the digestion of broilers. *J. Anim. Physiol. Anim. Nutr.* 83, 57–64. 10.1046/j.1439-0396.2000.00223.x

Hotamisligil GS, Bernlohr DA (2015) Metabolic functions of FABPs— mechanisms and therapeutic implications. Nat Rev Endocrinol 11: 592–605

Hui, T. Y., Frohnert, B. I., Smith, A. J., Schaffer, J. E., & Bernlohr, D. A. (1998). Characterization of the murine fatty acid transport protein gene and its insulin response sequence. *Journal of Biological Chemistry*, *273*(42), 27420-27429.

Jewell, J. L., Kim, Y. C., Russell, R. C., Yu, F. X., Park, H. W., Plouffe, S. W., ... & Guan, K. L. (2015). Differential regulation of mTORC1 by leucine and glutamine. *Science*, *347*(6218), 194-198.

Kellett GL. (2001) The facilitated component of intestinal glucose absorption. J Physiol 531:585–595. 10.1111/j.1469-7793.2001.0585h.x

Khwatenge CN, Kimathi BM, Nahashon SN. Transcriptome Analysis and Expression of Selected Cationic Amino Acid Transporters in the Liver of Broiler Chicken Fed Diets with Varying Concentrations of Lysine. Int J Mol Sci. (2020) Aug 5;21(16):5594. doi: 10.3390/ijms21165594. PMID: 32764289; PMCID: PMC7460557.

Kiela, P. R., & Ghishan, F. K. (2016). Physiology of intestinal absorption and secretion. *Best practice & research Clinical gastroenterology*, *30*(2), 145-159.

Kim, B. R., Yoon, K., Byun, H. J., Seo, S. H., Lee, S. H., & Rho, S. B. (2014). The anti-tumor activator sMEK1 and paclitaxel additively decrease expression of HIF-1α and VEGF via mTORC1-S6K/4E-BP-dependent signaling pathways. *Oncotarget*, *5*(15), 6540.

Koepsell, H. (2020). Glucose transporters in the small intestine in health and disease. *Pflügers Archiv-European Journal of Physiology*, *472*(9), 1207-1248.

Kono, T., Nishida, M., Nishiki, Y., Seki, Y., Sato, K., & Akiba, Y. (2005). Characterisation of glucose transporter (GLUT) gene expression in broiler chickens. *British poultry science*, *46*(4), 510-515.

Lee, W. S., Kanai, Y., Wells, R. G., & Hediger, M. A. (1994). The high affinity Na+/glucose cotransporter. Re-evaluation of function and distribution of expression. *Journal of Biological Chemistry*, *269*(16), 12032-12039.

Lin, H., Du, R., Gu, X. H., Li, F. C., & Zhang, Z. Y. (2000). A study on the plasma biochemical indices of heat-stressed broilers. *Asian-Australasian Journal of Animal Sciences*, *13*(9), 1210-1218.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *methods*, *25*(4), 402-408.

Meier, C., Ristic, Z., Klauser, S., & Verrey, F. (2002). Activation of system L heterodimeric amino acid exchangers by intracellular substrates. *The EMBO journal*, *21*(4), 580-589.

Palacín, M., Fernaández, E., Chillarón, J., & Zorzano, A. (2001). The amino acid transport system bo,+ and cystinuria. *Molecular membrane biology*, *18*(1), 21-26.

Renaudeau D., Collin A., Yahav S., De Basilio V., Gourdine J. L., Collier R. J. (2012). Adaptation to hot climate and strategies to alleviate heat stress in livestock production. *Animal* 6, 707–728. 10.1017/S1751731111002448.

Romero, A., Gomez, O., Terrado, J., & Mesonero, J. E. (2009). Expression of GLUT8 in mouse intestine: identification of alternative spliced variants. *Journal of cellular biochemistry*, *106*(6), 1068-1078.

Sammad, A., Wang, Y. J., Umer, S., Lirong, H., Khan, I., Khan, A., ... & Wang, Y. (2020). Nutritional physiology and biochemistry of dairy cattle under the influence of heat stress: Consequences and opportunities. *Animals*, *10*(5), 793.

SAS Institute Inc. SAS/IML® Studio 15.1 for SAS/STAT® Users; SAS Institute Inc.: Cary, NC, USA, 2018.

Schousboe, A., Scafidi, S., Bak, L. K., Waagepetersen, H. S., & McKenna, M. C. (2014). Glutamate metabolism in the brain focusing on astrocytes. In *Glutamate and ATP at the Interface of Metabolism and Signaling in the Brain* (pp. 13-30). Springer, Cham.

Shao, D., Wang, Q., Hu, Y., Shi, S., & Tong, H. (2018). Effects of cyclic heat stress on the phenotypic response, meat quality and muscle glycolysis of breasts and thighs of yellow-feather broilers. *Italian Journal of Animal Science*.

Sohail, M. U., Hume, M. E., Byrd, J. A., Nisbet, D. J., Ijaz, A., Sohail, A., ... & Rehman, H. (2012). Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. *Poultry science*, *91*(9), 2235-2240.

Song, Z. H., Cheng, K., Zheng, X. C., Ahmad, H., Zhang, L. L., & Wang, T. (2018). Effects of dietary supplementation with enzymatically treated Artemisia annua on growth performance, intestinal morphology, digestive enzyme activities, immunity, and antioxidant capacity of heat-stressed broilers. *Poultry Science*, *97*(2), 430-437.

Stahl, A. (2004) A current review of fatty acid transport proteins (SLC27). Pflügers Arch-Eur J Physiol 447:722–727.

Sun X., Zhang H., Sheikhahmadi A., Wang Y., Jiao H., Lin H., *et al*. (2015). Effects of heat stress on the gene expression of nutrient transporters in the jejunum of broiler chickens (Gallus gallus domesticus). *Int. J. Biometeorol.* 59, 127–135. 10.1007/s00484-014-0829-1

Svihus, B. (2014). Starch digestion capacity of poultry. *Poultry science*, *93*(9), 2394-2399.

Teyssier J. R., Preynat A., Cozannet P., Briens M., Mauromoustakos A., Greene E. S., *et al*. (2022). Constant and cyclic chronic heat stress models differentially influence growth performance, carcass traits and meat quality of broilers. *Poult. Sci.* 10196. 10.1016/j.psj.2022.101963.

Turcotte, L. P., Srivastava, A. K., & Chiasson, J. L. (1997). Fasting increases plasma membrane fatty acid-binding protein (FABPPM) in red skeletal muscle. *Molecular and cellular biochemistry*, *166*(1), 153-158.

Wasti S., Sah N., Mishra B. (2020). Impact of heat stress on poultry health and performances, and potential mitigation strategies. *Animals.* 10, 1266. 10.3390/ani10081266.

Yan, R., Li, Y., Shi, Y., Zhou, J., Lei, J., Huang, J., & Zhou, Q. (2020). Cryo-EM structure of the human heteromeric amino acid transporter b0,+ AT-rBAT. *Science advances*, *6*(16), eaay6379.

Yuan, J., Zhang, B., & Guo, Y. (2012). Poultry fat decreased fatty acid transporter protein mRNA expression and affected fatty acid composition in chickens. *Journal of animal science and biotechnology*, *3*(1), 1-6.

Zhang, Z. Y., Jia, G. Q., Zuo, J. J., Zhang, Y., Lei, J., Ren, L., & Feng, D. Y. (2012). Effects of constant and cyclic heat stress on muscle metabolism and meat quality of broiler breast fillet and thigh meat. *Poultry science*, *91*(11), 2931-2937.

CHAPTER 6

SUMMARY

Heat stress in chicken houses continues to be a major threat to poultry industry and food security and the advancement of the broiler industry. Several methods have been deployed to combat the adverse effect of heat stress, but none have been able to address how to maintain the glucose and protein composition of broiler chickens under HS conditions. Heat-stressed birds express changes in behavior and performance, which is a resultant effect of the communication between various organs and tissues. Heat stress reduced feed consumption and body weight gain while increasing the water conversion ratio in birds. Heat stress leads to a reduction in body weight gain, *P. major* and drumstick that are cut parts of economic importance. The proportion of both *P. major* and drumstick was improved with 6% glucose supplementation. Incorporating glucose at 6% in poultry production may be a promising way to enhance profitability under HS condition since *P. major* can be preserved.

Aquaporins help to regulate water balance in the body. The observed result in the HS0 group showed that there is an increase in the water demand of birds under HS condition. Glucose supplementation can prevent tissue dehydration in a mechanism yet to be known during HS. This may be important in developing strategies to minimize water wastage by poultry production in areas under high water demand.

Heat stress and glucose supplementation altered the blood chemistry of broilers under HS condition. The higher serum glucose level observed in HS relative to TN groups is a survival strategy for the birds. Increasing the serum glucose level in heat-stressed birds is a response by

which the birds combat the negative heat balance between metabolic heat production and heat dissipated.

Glucose supplementation enhanced the expression of ileal sugars and amino acids transporters, hinting at the possibility of these nutrients being more available to birds. Glucose supplementation improved carcass yield under HS condition and might be a subtle pointer to the efficacy of glucose in either preventing protein catabolism or enhancing protein synthesis. This may elucidate the mechanism by which 6% glucose supplementation improved the proportion of both *P. major* and drumstick of heat-stressed broiler chickens. The findings of this thesis project establish a novel nutritional approach to controlling the deleterious effect of HS on the broilers' carcass quality and meat yield and propose further molecular studies to delineate the influence of extra glucose supplementation on protein synthesis, hormonal dynamic, and immune response in heat-stressed broiler chickens.