8-2023

Evaluation of Heat Stress and Dietary Amino Acid Density on Nutrient and Energy Partitioning in Broilers

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Evaluation of Heat Stress and Dietary Amino Acid Density on Nutrient and Energy Partitioning in Broilers

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Poultry Science

by

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AgroCampus Ouest
Master of Science in Animal Science, 2016

August 2023
University of Arkansas

This dissertation is approved for recommendation to the Graduate Council.

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ABSTRACT

The simultaneous increase in the global demand for animal-based protein products and the rise in temperature associated with global warming pose important challenges to the poultry industry. Heat stress (**HS**) occurs when an animal's heat production (**HP**) surpasses its capacity to dissipate heat into the surrounding environment, ultimately leading to reduced feed intake (**FI**) and negative impacts on performance. A better understanding of bird responses to elevated temperatures is required to propose effective nutritional strategies that mitigate the adverse effects of HS. Therefore, this dissertation aimed to characterize the nutritional and physiological mechanisms explaining the loss in performance and carcass quality observed in growing broilers under HS conditions, and to determine how these effects can be minimized through nutritional interventions. The first experiment compared the effects on performance, carcass characteristics, and meat quality of chronic constant and cyclic HS models, along with a pair-feeding treatment in which birds were fed the same quantity of feed consumed by HS birds. Constant HS had a more detrimental effect than cyclic HS, with reduced FI accounting for approximately 80% of the reduced body weight gain. Moreover, HS led to reduced breast muscle deposition and increased abdominal fat deposition. Further investigations were conducted to characterize the effect of these chronic HS models, as well as an acute HS model on nutrient digestibility, stress, inflammation, and markers of protein and lipid metabolism. Constant HS induced higher physiological stress compared to the other tested models, and no evidence of adaptation to chronic HS exposure was found when comparing with acute HS birds. Interestingly, reduced digestibility caused by HS only partly explained the reduced performance that occurred independently of the HS-induced reduction in FI. Lipid and protein metabolism were markedly affected by constant HS, although further investigation is needed to explore lipid metabolism

regulation as gene expression markers and carcass observations did not align. Regarding protein metabolism, constant HS downregulated protein synthesis due to the HS-induced reduction in FI, while protein degradation was upregulated due to HS *per se*. In the last experiment, the effects of increased dietary density of select or all essential amino acids (**AA**) on growth and carcass characteristics were evaluated under constant HS conditions, and energy and nitrogen partitioning were quantified using the comparative slaughter technique. Reduced FI and increased energy needs for maintenance functions compromised energy availability in HS birds. Additionally, HS shifted the energy storage from fat to protein due to the lower heat increment associated with fat metabolism. However, increased dietary AA density did not show beneficial effects for HS birds, suggesting that reduced protein deposition and growth are not associated with a lack of digestible AA intake. Instead, additional essential AA may be directed toward non-productive functions, such as the production of energy for maintenance needs or proteins involved in thermoregulation and inflammation processes. Overall, this dissertation provides valuable insights into the detrimental impact of HS beyond the HS-induced reduction in FI. Further research is required to develop feeding strategies that promote protein deposition under HS conditions.

ACNOWLEDGEMENTS

First, I would like to express my sincere gratitude to my advisor, Dr. Samuel Rochell, for his guidance, support, and welcoming nature since the beginning of my program. I am very grateful for the insightful conversations we have had and your constant assistance, professionalism, and open-minded approach. I would like to extend my appreciation to Dr. Mickael Kidd for his mentorship and encouragement in my project, as well as Dr. Sami Dridi and Liz for their support and for giving me the opportunity to work in their lab. Special thanks to the entire team of Adisseo, who collaborated with me on this project and gave me the chance to prove myself. To Aurélie and Pierre, I am grateful for your guidance during my project and for following my adventures in the US. Estelle, I am thankful for motivating me to embark on this journey to the US.

To all the graduate and undergraduate students who contributed to this project, thank you for your cheerful company during long working days. To Kenia, Brooke, Annalise, Trevor, Ben, Jay, Kyle, and everyone involved, I am so grateful to have shared those moments with you. A special thanks to Giorgio for your dynamism and for collaborating with me on the review.

To my friends in France, thank you for your warm welcome whenever I return and for always being there despite the distance. To all the remarkable individuals I met in Arkansas and who made my time here unforgettable, I am grateful to count you as my friends. My heartfelt thanks go to my parents, who gave me the values that have guided me on this journey. And a special thanks to my brother for always being my confidant and source of support even from a distance.

Lastly but certainly not least, I want to give the most thanks to my incredible partner, Alina. I am so grateful to count you in my life. Your love and support over the last 4 years were

extraordinary and those years spent together were unforgettable. Thank you for being my best friend, and for your positivity and vibrant spirit. I look forward to the wonderful adventures that lie ahead for us.

DEDICATION

I would like to dedicate this dissertation to my grandfather, Joseph Teyssier. Your kindness, the countless cherished memories we shared, and the profound values you instilled in me will forever reside in my heart.

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CHAPTER I - INTRODUCTION

This introduction and literature review are adapted from the published manuscript in Frontiers in Physiology 2022:1521.

A Review of Heat Stress in Chickens.

Part II: Insights into Protein and Energy Utilization and Feeding

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The poultry industry continues to play a critical role in meeting the growing demand for animal protein. The global production of chicken and turkey meat has doubled over the last 20 years, reaching 125.5 million tons in 2020 (FAO, 2022). This accounts for approximately 37% of global meat production, while poultry meat only represented 29% in the early 2000s. With the increasing global population projected to rise from 7.8 to 9.9 billion in 2050 (PRB, 2020) and better access to animal products in developing areas, it is predicted that animal-based food demand will grow by nearly 70% in the same timeline (Searchinger et al., 2019). Meanwhile, climate change represents one of the major concerns for livestock production in the coming decades. Some reports indicate that industrialized farming systems may lose 25% of their animal production, and this scenario may be worse for some tropical regions where extensive farming systems are more abundant (Nardone et al., 2010). Emerging estimates by the Intergovernmental Panel on Climate Change emphasized that global warming of more than 2°C will occur during the 21st century unless large reductions in CO_2 and other greenhouse gas emissions are achieved soon (IPCC, 2021). Also, the authors indicated that climate change is already and will continue increasing the frequency and intensity of extreme weather events like hot temperature waves. Therefore, the poultry industry needs to continue adopting technologies and practices that reduce its impact on the environment, but it should also adopt production systems that are resilient in the face of rising global temperatures.

Modern broiler chickens are particularly sensitive to hot temperatures due to their rapid growth rates resulting from genetic selection to enhance production efficiency, as well as from limitations in heat dissipation caused by feathering, an absence of sweat glands, and relatively high stocking densities in intensive commercial rearing facilities (Lara and Rostagno, 2013; Emami et al., 2020). Heat stress (**HS**) occurs when the amount of heat produced by an animal

surpasses its capacity to dissipate the heat to the surrounding environment. When the environmental temperature rises above the thermoneutral zone, birds typically reduce their physical activity and feed intake (**FI**) to limit heat production (**HP**), as well as increase their panting and water consumption to favor heat loss by evaporation (Renaudeau et al., 2012). Indeed, elevated temperatures trigger important physiologic and metabolic changes (Brugaletta et al., 2022), and chronic HS exposure results in significant losses in bird performance, negatively affects welfare, challenges food safety, and reduces the overall economic efficiency of poultry production (Lara and Rostagno, 2013; Pawar et al., 2016). Consequently, HS has been estimated to cause annual economic losses of $128 to $165 million for the US poultry industry (St-Pierre et al., 2003), but these figures probably underestimate current and future losses due to the growth of the poultry industry over the last decade and the worsening of climate change predictions.

The severity of the HS period is typically assessed by the temperature-humidity index, which takes into account the environmental temperature and the relative humidity (Zhang et al., 2020). In chickens, the severity of the stress might differ depending on the breed, as certain breeds are more resistant to hot temperatures than others (Lu et al., 2007). Different durations of stress have led to the classification of various types of HS, with acute HS referring to short periods of heat exposure and chronic HS referring to extended periods of heat exposure (Siddiqui et al., 2020). In addition, chronic HS can either be constant, with a high stress intensity throughout the entire stress period, as observed particularly in tropical regions, or cyclic, characterized by a diurnal pattern of high temperatures during the day and lower temperatures at night, as observed in temperate regions. The birds' response to HS periods depends on the stress conditions (De Souza et al., 2016; Awad et al., 2018), and further investigations comparing

different HS models within the same experiment for market-age broilers reared in floor pens are required.

Moreover, mitigating the adverse effects of hot temperatures in poultry productions requires a holistic and multi-factorial approach. Housing (Oloyo, 2018), management practices (Saeed et al., 2019), genetic selection (Kumar et al., 2021), and feeding and nutrition (Syafwan et al., 2011; Fouad et al., 2016; Sugiharto et al., 2017; Wasti et al., 2020; Chowdhury et al., 2021; Abdel-Moneim et al., 2021) can all provide some benefit to birds under HS conditions and have been the topics of several recent global reviews (Lin et al., 2006; Nawab et al., 2018; Vandana et al., 2021; Goel et al., 2021). From a nutritional perspective, macronutrients serve as the main source of energy in poultry diets. Their oxidation, particularly proteins, results in HP, which needs to be limited under HS (Costa-Pinto and Gantner, 2020). Additional research is needed to investigate adjustments in dietary protein and individual amino acid supplementation to effectively mitigate the detrimental effects of HS.

Therefore, the objective of the first experiment was to compare different chronic HS models to assess their influence on performance, carcass characteristics, and meat quality parameters in broilers, as well as determine the direct effect of constant HS on these parameters independent of decreased FI. Further investigations of these different HS models were conducted in experiment 2, with the objective of characterizing nutrient digestibility and markers of stress, inflammation, and metabolism. Finally, experiment 3 aimed to characterize the effect of constant HS on performance, carcass characteristics, digestibility, and nutrient and energy partitioning in broilers fed diets with different amino acid profiles.

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CHAPTER II - LITERATURE REVIEW

This introduction and literature review are adapted from the published manuscript in Frontiers in Physiology 2022:1521.

A Review of Heat Stress in Chickens.

Part II: Insights into Protein and Energy Utilization and Feeding

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Impact of Heat Stess on the Physiological Stress Response

Heat stress (**HS**) can adversely affect the physiological functions of animals by triggering oxidative stress. In birds, like in other mammals, most of the oxygen consumed is used for energy production and is ultimately converted to water. However, a portion of the consumed oxygen generates reactive oxygen species (**ROS**), which are extremely reactive compounds (Nakai and Tsuruta, 2021). Under thermoneutral conditions, the production of ROS and the antioxidant system are balanced and can adapt to overcome normal challenges. However, both acute and chronic HS disrupt this equilibrium due to an overproduction of ROS by reducing the activity of the mitochondrial respiratory chain (Tan et al., 2010). Ultimately, ROS production can surpass the antioxidant capacity, leading to oxidative damage (Lin et al., 2000, 2006; Azad et al., 2010a; Akbarian et al., 2016). One well-known damage caused by ROS is lipid peroxidation, where ROS attack lipids with carbon-carbon double bonds. This process initiates a chain of reactions that produces multiple breakdown molecules, affects cellular functions, and contributes to the development of various pathological states (Ayala et al., 2014).

A first line of defense against oxidative stress is provided by antioxidant enzymes, including superoxide dismutase (**SOD**) and glutathione peroxidase (**GPX**), which detoxify and decompose free radicals and non-radical toxic products (Surai et al., 2019). Research in broilers has shown that under HS, expression and activity of SOD and GPX enzymes are generally increased in an attempt to compensate for increased production of ROS (Lin et al., 2000, 2006; Tan et al., 2010; Yang et al., 2010; Azad et al., 2010b; Ghazi Harsini et al., 2012; Huang et al., 2015; Cramer et al., 2018; Habashy et al., 2019).

Heat shock proteins (**HSP**) are another defense mechanism against oxidative stress in response to HS. These proteins are known for their chaperone activity, i.e., facilitating the folding and

unfolding of denatured proteins due to stress (Archana et al., 2017).Additionally, they modulate both apoptotic and antiapoptotic signaling pathways, which enhances cellular thermotolerance and regulates cellular redox state (Shehata et al., 2020). In broilers, numerous studies have reported an increase in the expression of different HSP under HS conditions and in different tissues (Gabriel et al., 1996; Liu et al., 2014; Xie et al., 2014; Flees et al., 2017; Cramer et al., 2018; Baxter et al., 2020; Emami et al., 2021; Greene et al., 2022).

In addition to impacting antioxidant enzymes and HSP, HS also affects systemic and local inflammation (Song et al., 2017, 2018). Several factors can explain why HS triggers inflammation, including the overproduction of ROS, the release of stress hormones, impaired function of the gastrointestinal tract and imbalances in gut microbiota, which can potentially lead to leaky gut or dysbiosis (Brugaletta et al., 2022). In an attempt to prevent excessive inflammation, HS was shown to influence the production of cytokines (pro-inflammatory and anti-inflammatory), such as different interleukins, tumor necrosis factor α (**TNFα**), or C-reactive protein (**CRP**) (Ohtsu et al., 2015; Varasteh et al., 2015; Alhenaky et al., 2017; He et al., 2019c; Baxter et al., 2020; Greene et al., 2021b; Alzarah et al., 2021; Emami et al., 2021).

Impact of Heat Stress on Feed Intake Regulation and Nutrient Digestibility

Nearly all studies that have investigated the effects of HS in poultry have observed reductions in feed intake (**FI**) of heat-stressed birds compared with those in thermoneutral conditions, including meta-analyses conducted in broilers (Liu et al., 2020) and laying hens (Mignon-Grasteau et al., 2015). This reduction of FI observed under HS conditions reduces endogenous heat production (**HP**) associated with digestion, absorption, and metabolism of nutrients (Lara and Rostagno, 2013). However, the magnitude of the FI reduction depends on several parameters related to the characteristics of the HS model imposed on the birds, and this

can complicate comparisons among studies. Temperature, length and cyclicality of the heat period, and age of the birds at the beginning and the end of the HS period are all potential factors that can influence the intensity of the FI reduction. Many studies have used a constant HS model with high temperatures applied over a long period of time (Geraert et al., 1996a; Ain Baziz et al., 1996; Bonnet et al., 1997; Faria Filho et al., 2007). However, more recent studies have employed cyclic HS models combining higher temperatures during the day and lower temperatures during the night which may better simulate field conditions in temperate areas of the world (De Souza et al., 2016; Flees et al., 2017; Greene et al., 2021a). When compared within the same experiment, cyclic HS decreased FI by 15% on average, while constant HS resulted in higher reductions ranging from 25 to 45% (De Souza et al., 2016; Awad et al., 2018; Teyssier et al., 2022). Therefore, cyclic HS resulted in a 1.5% reduction in FI per degree Celsius, while the values obtained under constant HS corroborate the expected response proposed by Baziz et al. (1996) of about a 3.5% reduction in FI per degree Celsius increase between 22 and 35°C.

Interestingly, the reduction of growth observed under HS is greater than expected due to the reduced FI alone, leading to a lower feed efficiency (Renaudeau et al., 2012). The use of pair-feeding techniques, where birds under thermoneutral conditions are fed the same amount of feed consumed by heat-stressed birds, have shown that the reduction in growth due to decreased FI ranges between 60 and 99% (Geraert et al., 1996a; Bonnet et al., 1997; Garriga et al., 2006; Lu et al., 2007; Zuo et al., 2015; Zeferino et al., 2016; De Souza et al., 2016; De Antonio et al., 2017; Ma et al., 2021a; Emami et al., 2021; Teyssier et al., 2022). Therefore, the lower FI is the main factor explaining impaired performance of chickens observed under HS, with the remainder of the growth reduction attributable to impaired digestibility or physiological and metabolic

changes that influence feed efficiency (Dale and Fuller, 1980; Geraert et al., 1996a; Renaudeau et al., 2012).

Several studies have reported reduced dry matter (**DM**) digestibility in quails (Orhan et al., 2020) and laying hens (Kim et al., 2020) under HS conditions. In broilers, Bonnet et al. (1997) and De Souza et al. (2016) observed decreases of 1.6 and 3.9% in DM digestibility under constant HS. However, other studies have reported no DM digestibility losses due to HS (Faria Filho et al., 2007; Attia et al., 2016, 2017). At the nutrient level, even though no change in crude protein (**CP**) digestibility were observed by several authors (Faria Filho et al., 2007; Habashy et al., 2017b; Kim et al., 2020), numerous studies have reported decreases in CP or nitrogen digestibility ranging between 1.5 and 10% under hot temperatures (Zuprizal et al., 1993; Bonnet et al., 1997; Soleimani et al., 2010; De Souza et al., 2016; Attia et al., 2016, 2017; Orhan et al., 2020). The detrimental effect of HS has also been measured on amino acid (**AA**) digestibility. Wallis and Balnave (1984) observed a slight decrease in the digestibility for Thr, Ala, Met, Ile, and Leu, with greater impacts in male than in female birds. Standardized and apparent digestibility values of several AA (i.e., Arg, His, Thr, Val, Lys, Ile, Leu, Phe, Cys, Gly, Ser, Ala, Pro, and Tyr) were also reduced by approximately 5.5%, in the study of Soleimani et al. (2010). Regarding other nutrients, none of these studies observed an impact on crude fat digestibility, and only Kim et al. (2020) measured a reduction in neutral detergent fiber digestibility with laying hens.

Several mechanisms have been proposed to explain possible negative effects of HS on nutrient digestibility. Lower expression and activity of digestive enzymes, including trypsin, chymotrypsin, lipase, amylase, and maltase, have been observed in broilers reared under high temperatures (Hai et al., 2000; Song et al., 2018; Al-Zghoul et al., 2019). As described in

Brugaletta et al. (2022) oxidative stress induced by HS aggravates intestinal barrier disorders, and hyperthermia has been associated with a reduction in upper gastrointestinal tract blood flow that can induce degradation of the intestinal mucosa (Song et al., 2014; Chegini et al., 2018). Following hot temperature exposure, the absorptive surface area of the small intestine is decreased due to a reduction in villi height, crypt depth (Song et al., 2018; He et al., 2019b), and relative jejunal weight (Garriga et al., 2006). Heat stress also modulates the gene expression of several macronutrient transporters. Expression of glucose transporters SGLT1 and GLUT2 is downregulated when HS persists for several days (Sun et al., 2015; Habashy et al., 2017b; Al-Zghoul et al., 2019; Abdelli et al., 2021; Goel et al., 2021), whereas the expression of GLUT5 for the transport of fructose is increased (Habashy et al., 2017b). Despite the relatively greater decrease in AA digestibility compared to other macronutrients, several studies observed no influence of HS exposure on expression of AA transporters, including CAT1, y+LAT1, PePT1, and r-Bat (Sun et al., 2015; Habashy et al., 2017b; Al-Zghoul et al., 2019). On the other hand, Habashy et al. (2017a) measured a decrease in expression of several AA transporters (i.e., CAT1, LAT1, SNAT1, SNAT 2, SNAT 7, B0AT) after 12 days of HS. However, this reduction was not consistent with the slight increase in AA digestibility (+3%) observed in the same study. Furthermore, even though HS does not seem to markedly affect fat digestibility, several studies have reported decreased intestinal expression of FABP and CD36 which are both involved in the uptake of fatty acids (Sun et al., 2015; Habashy et al., 2017b; Al-Zghoul et al., 2019), whereas the expression of FATP1 was increased under chronic HS (Habashy et al., 2017b).

While the regulation of nutrient transporter gene expression might be directly related to physiological adaptations to HS, it is important to consider that structural damages and the degradation of the epithelium induced by HS might be a potential factor indirectly causing the

reduction of intestinal transporters (Habashy et al., 2017b). Overall, the slight decrease and inconsistent results regarding nutrient digestibility seem to indicate that reduced digestibility likely explains only a small proportion of reduced feed efficiency under HS conditions.

Impact of Heat Stress on Nutrient Metabolism

***Lipid metabolism*.** Periods of high temperatures have been shown to increase fat retention in birds (Geraert et al., 1996a; Faria Filho et al., 2007; De Souza et al., 2016). The increased fat content in chicken carcasses has been associated with higher abdominal, subcutaneous, inter and intra-muscular fat (Kubena et al., 1972; Ain Baziz et al., 1996; Lu et al., 2007, 2018; He et al., 2015; De Antonio et al., 2017). Interestingly, fat deposition in HS birds depends on the breed, with fast growing breeds showing more abdominal and subcutaneous fat deposition in response to HS than slow growing breeds. In fact, it has been shown that HS tolerant breeds have reduced fat retention at the peripheral body sites which could be an advantage as peripheral fat can be an insulator that limits heat dissipation in the environment (Lu et al., 2007; Renaudeau et al., 2012).

In birds, most of the endogenous body lipids are produced in the liver via the *de-novo* lipogenesis pathway (Goodridge and Ball, 1967; O'Hea and Leveille, 1968), resulting in the production of different types of lipids such as triglycerides, phospholipids, and cholesterol. After synthesis, lipids are combined with specific proteins in the liver to form lipoproteins, such as very low-density lipoproteins (**VLDL**), before being transported to other tissues via the bloodstream (Emami et al., 2020). Interestingly, increased hepatic triglyceride content and plasmatic VLDL levels have been observed in HS broilers, indicating a potential upregulation of *de novo* lipogenesis (Lu et al., 2019b; c; Lan et al., 2022). Furthermore, while HS seems to reduce the mRNA expression of lipogenic enzymes, protein expression and activity of these enzymes appeared to be increased in HS birds (Flees et al., 2017; Lu et al., 2019a; b; c).

Therefore, HS may regulate the transcription and translation processes of lipogenic enzymes, which could partially explain the increased lipid retention in HS birds.

Regarding fat oxidation, increased lipolysis promotes the export of non-esterified fatty acids (**NEFA**) from the adipose tissue. Several studies have demonstrated that HS results in lower serum NEFA levels (Geraert et al., 1996b; Lu et al., 2018; Wang et al., 2021), which is consistent with the increased fat retention observed at the carcass level as it suggests that HS reduces the utilization of fat energy storages. However, inconsistent results have been observed regarding the mRNA expression of lipolytic enzymes in broilers, and further research is needed to understand the regulatory effect of HS on lipolytic pathways (Flees et al., 2017; Huang et al., 2021). Furthermore, contrary to other species where insulin levels are known to inhibit lipolytic pathways (Baumgard and Rhoads, 2013), the insulin signaling pathways are different in chicken abdominal adipose tissue (Dupont et al., 2012). Therefore, the absence of a spike in insulin blood levels in HS birds (Geraert et al., 1996b; Tang et al., 2013; Belhadj Slimen et al., 2016) may coincide with increased fat retention due to the limited antilipolytic effect of insulin in avian species.

Protein metabolism. Furthermore, studies have shown that HS not only increases fat retention, but also affects the composition of broiler carcasses by reducing the proportion of lean tissue, particularly breast meat yield (Geraert et al., 1996a; Ain Baziz et al., 1996; Zuo et al., 2015; Zeferino et al., 2016; Lu et al., 2018; Emami et al., 2021; El-Tarabany et al., 2021). Temim et al. (2000) demonstrated that chronic heat exposure causes a reduction in protein turnover, which is mainly induced by a decrease in protein synthesis rates related to a lower ribosomal capacity, rather than lower protein degradation. However, the effect of HS on muscle growth appears to differ depending on the glycolytic or oxidative nature of muscle fibers. Indeed,

great impacts of HS on yield and protein synthesis have been observed in breast muscles compared to leg muscles (Temim et al., 2000; Zuo et al., 2015; Zeferino et al., 2016; Emami et al., 2021; El-Tarabany et al., 2021), likely because glycolytic fibers rely on glycogen storage, which is depleted following decreased FI in response to HS (Temim et al., 2000; Zeferino et al., 2016).

In chicken, chronic HS exposure downregulates the insulin like growth factor 1 (**IGF1**), thyroid hormones T3 and T4, and mammalian target of rapamycin (**mTOR**) pathways, which play important roles in muscle hypertrophy and hyperplasia (Geraert et al., 1996b; Sohail et al., 2010; Liu et al., 2014; Zuo et al., 2015; Rajaei-Sharifabadi et al., 2017; Roushdy et al., 2018; Ma et al., 2018; Li et al., 2021). Similarly, HS seems to reduce muscle hypertrophy through downregulation of pathways downstream of mTOR, including ribosomal protein S6 kinase (**p70S6K**) and myogenic factor (**MyoG**) (Zuo et al., 2015; Ma et al., 2018, 2021a; Lu et al., 2019a). Furthermore, increased levels of blood metabolites resulting from protein degradation have been measured in HS birds (Zuo et al., 2015; Lu et al., 2018; Hosseini-Vashan and Raei-Moghadam, 2019; He et al., 2019a; Ma et al., 2021a; Kikusato et al., 2021). In addition, the ubiquitin–proteasome pathway plays a major role in the degradation of key muscle proteins in chickens (Lecker et al., 1999; Sacheck et al., 2004). Several studies have reported increased expression of enzymes involved in this pathway in birds exposed to chronic HS, independently from the responses associated with reduced FI induced by HS (Zuo et al., 2015; Lu et al., 2019a; Ma et al., 2021b; Li et al., 2021).

Interestingly, recent research suggests that HS may upregulate the gluconeogenesis pathway to compensate for reduced mobilization of fat (Lu et al., 2018; Ma et al., 2018; Kim et al., 2022). Indeed, concomitantly with the reduced energy intake caused by the HS-induced

reduced FI, it is possible that a portion of glucose is utilized for synthesizing fatty acids, resulting in reduced glucose availability and energy production from glucose. Consequently, there may be a stimulation of gluconeogenesis for energy production through AA oxidation due to the shortage of glucose and decline in fatty acid oxidation as an alternative energy source in HS broiler chickens (Kim et al., 2022).

Energy metabolism. From an energetic perspective, the reduction in FI observed in birds exposed to chronic HS results in a substantial reduction in energy intake. Similar to the impact of chronic HS exposure on feed efficiency, where HS induces a higher reduction in performance compared to the decrease in FI, HS results in an increase in apparent metabolizable energy intake when expressed on a metabolic body weight (**mBW**) basis. Ultimately, the energy efficiency of HS birds is reduced compared to birds reared in TN conditions, however, observation in pair-fed birds indicate that this reduced efficiency is mostly explained by the reduced FI (Geraert et al., 1996a; De Souza et al., 2016). In addition, and consistent with the observed effect of HS on lipid and protein metabolism, Geraert et al. (1996a) and Faria Filho et al. (2007) measured an increase energy retention as fat and reduced energy retention in the form of protein.

When expressed on a mBW basis, inconsistent results have been observed regarding the effect of chronic HS on HP (Faria Filho et al., 2007; Syafwan et al., 2011; De Souza et al., 2016). As observed by De Souza et al. (2016), the balance for HP might differ depending on the stress intensity. Indeed, the energy resulting from HP is partitioned between what arises from maintenance (fasting heat production: **FHP**) and what arises from productive functions (Skomiał and Lapierre, 2016). Therefore, under HS, the increase in energy needs for maintenance functions related to thermoregulation (Chowdhury et al., 2021), may be offset to varying degrees by a reduction in heat increment due to lower physical activity and the metabolic use of nutrients

provided by the feed (Skomiał and Lapierre, 2016). Behavioral changes, reduced FI, and changes in metabolism are all strategies implemented to limit heat increment and the concomitant rise in internal body temperature.

Feeding Strategies

Lowering HP and improving heat dissipation are two ways to reduce the adverse effects of HS in poultry. While the reduction of HP is achievable by improving digestibility and by feeding the birds closer to their nutrient and energy requirements, an increased heat dissipation is possible by increasing the amount of water loss by evaporation (Syafwan et al., 2011). Several feeding strategies have been tested to attempt to mitigate the negative impact of hot temperatures through these means.

Feed restriction and withdrawal. Early studies focused on feed restriction before HS exposure, and its effects on HP and performance. In broiler breeders, feed restriction from 44 to 48 weeks before exposure to 4 days of elevated temperatures resulted in 23% decrease in HP compared with ad libitum fed birds. However, fed-restricted birds had a higher HP when adjusted for body weight (**BW**) differences and expressed per unit of mBW ($BW^{0.75}$). The lower BW of fed-restricted birds was therefore responsible for the reduction in HP and not the feed restriction per se (MacLeod and Hocking, 1993). In broilers, no beneficial effect of a preventative feed restriction was measured on performance and carcass quality (Plavnik and Yahav, 1998), but more promising results were obtained when feed restriction was applied during the HS period. Abu-Dieyeh (2006) observed that feed restriction to 75 and 50% of the feed consumption of ad-libitum fed broilers reduced rectal temperature, mortality, and feed conversion ratio (**FCR**). However, feed restriction diminished the rate of BW gain (**BWG**) and delayed marketing age of the birds.

Similarly, feed withdrawal for at least 6 hours during HS decreased the corporal temperature (Yalçin et al., 2001; Özkan et al., 2003; Lozano et al., 2006), mortality (Yalçin et al., 2001) and heterophil-to-lymphocyte ratio (Yalçin et al., 2003) of broilers, indicating a reduction of the adverse effects of HS. Nevertheless, effects on performance were not consistent throughout the studies, with some observing a growth improvement (Yalçin et al., 2001; Mohamed et al., 2019) and others reporting a growth degradation (Lozano et al., 2006) likely due to the timing and magnitude of feed restriction (Özkan et al., 2003). Therefore, a short feed withdrawal during the hottest period of the day appears to be the best strategy to minimize the negative effects of HS on growth and delayed market ages. Removing the feed a few hours before the HS period could also be beneficial to avoid the potential increased in HP induced by anticipatory feeding behavior observed in birds exposed to repeated intermittent fasting (Fondevila et al., 2020).

Dual feeding. Dual feeding is characterized by the distribution of two different diets, one more concentrated in protein and the other more concentrated in energy, that are provided either simultaneously for self-selection or in sequential order. Dietary proteins are known to have a higher thermogenic effect compared with carbohydrates (Geraert, 1991), and feeding high protein diets during the coolest period of the day has been hypothesized to improve the thermotolerance of birds. Sequential feeding of high energy and high protein diets decreased body temperature (De Basilio et al., 2001; Lozano et al., 2006) and mortality (De Basilio et al., 2001), but reduced or did not improve the growth of broilers. Syafwan et al. (2012) tested self-selection under hot temperatures by providing a high-protein diet (CP: 299 g/kg; ME: 2,780 kcal/kg) and a high-energy diet (CP: 150.7 g/kg; ME: 3,241 kcal/kg) and showed that choice-fed and control-fed birds with a standard diet (CP: 215 g/ kg; ME: 2,895 kcal/kg) performed similarly, although the former had 14% lower protein intake and 6.4% higher energy intake.

However, no data on carcass composition were reported, and a lower protein intake could reduce muscle deposition. While a dual-feeding approach might be feasible in tropical areas and less-intensive production systems, Iyasere et al. (2021) estimated that it is not suitable for most commercial production operations due to cost and logistical constraints.

Wet feeding. Water is the most important nutrient in broiler nutrition, and it plays an essential role for thermoregulation under hot temperatures. Heat stress increases water loss through the respiratory tract as birds pant to increase heat loss by evaporative cooling (Richards, 1970; Bruno et al., 2011). In the light of the importance of water for the nutrition and physiology of broilers, wet feeding attempts to maximize water intake and utilization. Several studies have investigated the effect of wet feeding, i.e. the use of high moisture diets, on poultry performance under thermoneutral conditions (Moritz et al., 2001; Shariatmadari and Forbes, 2005; Khoa, 2007) and during HS. In heat-stressed broilers, Kutlu (2001) measured increased BWG, DM intake, carcass weight, protein content, but also increased abdominal fat and lipid content per unit of carcass weight, and reduced DM conversion efficiency (DM intake/BWG), when feed was mixed with the same amount of water. Similarly, Awojobi et al. (2009) and Dei and Bumbie (2011) observed increased BWG with wet-fed birds (addition from 1 to 2 parts of water to 1 part of dry feed) reared in tropical conditions. In laying hens, Tadtiyanant et al. (1991) reported that wet feeding increased DM intake, but no beneficial effects were found on performance. In contrast to these results, egg production and egg weight were increased by wet feeding in Japanese quails (Okan et al., 1996a; b). Despite somewhat positive impacts of wet feeding in poultry, its application in the field remains limited due to an increased risk of fungal growth and resulting mycotoxicosis in birds (Wasti et al., 2020).

Feed form (mash vs. crumble vs. pellets) and feed structure (particle size). Three different forms of feed are generally used in the poultry industry: mash, crumble, and pellets. Under thermoneutral conditions, pelleted feed is known to increase FI and BWG and improve digestibility (Massuquetto et al., 2018, 2019). During summer, increased feed efficiency and egg production of laying hens have been observed for pelleted diets compared with mash diets (Almirall et al., 1997). In broilers exposed to cyclic HS, Cardoso et al. (2022) measured increased FI (+10%), BWG (+8.3%), CP digestibility (+2.3%), and energy utilization (apparent metabolizable energy, **AME** and nitrogen-corrected apparent metabolizable energy, **AMEn**) when feeding a pelleted diet compared with a mash diet. However, pelleting did not improve FCR, livability, or the feed production cost to kg of bird produced ratio. Likewise, Hosseini and Afshar (2017b) observed beneficial effects of pelleting on performance and digestibility when comparing mash, crumbled and pelleted diets under similar cyclic HS conditions. These authors also reported improved carcass weight and yield in heat-stressed broilers fed pelleted diets. Comparable performance improvements were obtained by feeding pelleted diets under thermoneutral and HS conditions (Serrano et al., 2013), so it is likely that mechanisms responsible for the positive effects of pelleting under thermoneutrality can be applied to HS conditions. Pelleting feed has been shown to lower feed wastage (Gadzirayi et al., 2006) and increase feed consumption, while concomitantly reducing physical activity and HP (Skinner-Noble et al., 2005; Latshaw and Moritz, 2009). Furthermore, as observed under thermoneutral (Abdollahi et al., 2011; Serrano et al., 2013) and HS conditions (Hosseini and Afshar, 2017a), pelleted diets reduce the relative weight of the digestive tract compared with birds fed mash diets. The pelleting process can further reduce ingredient particle size, reducing the mechanical stimulation of the gizzard and could therefore lower the energy requirements for maintenance. It

also could release some inaccessible nutrients and enhance energy utilization, which could explain the increase in abdominal fat observed by Hosseini and Afshar (2017b) with pelleted diets fed under cyclic HS. Other potential benefits of feeding pelleted feeds during HS shown by these authors include increased villus length and villus to crypt depth ratio in the jejunum (Hosseini and Afshar, 2017a) as well as decreased breast HSP70 mRNA expression, breast creatine kinase protein level, and heterophil-to-lymphocyte ratio (Hosseini and Afshar, 2017b). Collectively, these reports indicate that pelleting attenuates the harmful effects of high ambient temperature in broiler chickens.

Concerning particle size, the use of coarse particles (2280 μm) of corn increased panting compared to finer particles (605 μm) in broilers fed a mash diet under natural HS conditions (Santos et al., 2019). Similar results were found in laying hens under a semiarid environment, where coarser corn particles increased rectal temperature, respiratory rate, and decreased eggshell quality (De Souza et al., 2015). However, while coarse particles may increase the thermal challenge, they are also known to increase FI and improve performance in broilers under thermoneutral conditions (Amerah et al., 2008; Naderinejad et al., 2016). Thus, more research on broiler performance would be required to fully understand the role of ingredient particle size during HS.

Dietary Energy Density and Lipid Supplementation

The marked decrease in FI and in turn, energy intake, caused by elevated temperatures negatively affects bird performance. The effect of HS on energy utilization of feedstuffs, which is usually represented as AME, is still not well defined. Indeed, responses probably depend on the parameters of the HS imposed and characteristics of the diet, as some studies observed an increase in AME due to hot temperatures (Keshavarz and Fuller, 1980; Geraert et al., 1992),

some observed no difference between thermoneutral and HS conditions (Yamazaki and Zi-Yi, 1982; Faria Filho et al., 2007; De Souza et al., 2016), and some have reported a decrease in AME with HS birds (Bonnet et al., 1997). However, three studies using the comparative slaughter technique with broilers placed under thermoneutral and HS conditions from d 28 to 42 (Geraert et al., 1996a), or d 21 to 42 (Faria Filho et al., 2007; De Souza et al., 2016), indicate a decrease in retained energy and increase in HP per unit of feed when birds are placed under hot temperatures. Similarly, a quadratic effect of the temperature on the energy requirement for maintenance functions was measured by (Sakomura et al., 2005), with the lowest requirements estimated at 25.2°C: $ME_m = BW^{0.75} \times (307.87 + 15.63\ T + 0.31\ T^2)$, with T being the temperature (°C) and $BW^{0.75}$ the mBW. Therefore, the relative contribution of maintenance energy requirements to total energy requirements is partly increased by the lower growth of HS birds, but also directly impacted by the increased temperature, which results in a diminishing effect on feed efficiency.

To compensate for lower energy intake of birds during HS, it has become common for producers in hot climate areas to feed higher energy diets (Wasti et al., 2020). Early studies suggested that high dietary energy concentrations could improve bird performance under constant (Dale and Fuller, 1979) and cyclic HS (Dale and Fuller, 1980), but it should be noted that the CP content of the diets were adjusted to energy levels and thus higher in high energy diets. Nonetheless, more recent studies using isonitrogenous diets have confirmed previous observations and showed that an increase in dietary metabolizable energy (**ME**) between 100 and 200 kcal/kg for broilers improved BWG up to 17% and FCR up to 10% (Raju et al., 2004; Ghazalah et al., 2008; Attia et al., 2011, 2018; Attia and Hassan, 2017) when reared under hot conditions. In addition, decreased skin and rectal temperatures have been observed in HS poultry

fed diets with increased ME content (Al-Harthi et al., 2002; Attia et al., 2011). Increasing dietary ME content also improved ready-to-cook yield (Raju et al., 2004), although no improvement in carcass yield was observed by Ghazalah et al. (2008). However, both research groups reported an increased abdominal fat yield, thus the risk of increasing carcass yield from lipid and not protein deposition is a potential disadvantage of increasing dietary ME in HS broilers.

Increasing ME density in the diet is usually achieved by increasing the concentration of added lipid, and this strategy presents several potential advantages for HS birds. Feeding isocaloric diets with either higher proportions of carbohydrates or fat under HS conditions revealed that broilers had better performance when diets were supplemented with poultry fat, palm oil, or soybean oil compared to no fat supplementation (Zulkifli et al., 2006; Ghazalah et al., 2008). These observations are likely explained by the lower heat increment of fat oxidation compared with carbohydrates and proteins. Indeed, as measured by Fuller and Rendon (1977), high fat diets lead to lower heat increment than low-fat diets. Moreover, lipid inclusion improves nutrient digestion by slowing rate of passage (Mateos et al., 1982) and increasing the energy value of other nutrients (Aardsma et al., 2017). Lipid metabolism also generates more metabolic water than carbohydrate and protein catabolism, which can in turn be used for heat dissipation by evaporation (Barboza et al., 2009). Thus, as suggested by Ghazalah et al. (2008), a potential dietary recommendation for broilers exposed to hot temperatures could be to increase the ME level up to 3,300 kcal/kg, with lipid inclusion up to 5%, especially during the finishing period when birds are the most sensitive to high temperatures.

Although increasing dietary lipid additions has been shown to be a promising way to increase bird performance under HS conditions, less research has been conducted to compare the efficacy of different lipid sources. Zulkifli et al. (2006) did not observe a difference in BWG and

FCR among broilers exposed to 34°C and supplemented either with 8% of palm oil or 8% of soybean oil. Abdominal fat and breast intramuscular fat deposition were also unaffected by the fat source. However, in broilers exposed to HS from 32 to 42 days post-hatch and fed isocaloric diets, improved FCR and BWG were observed when feeding diets with coconut oil or beef tallow than with diets containing olive or soybean oil (Seifi et al., 2020). The fatty acids within coconut oil and tallow are rich in saturated fatty acids and have chain lengths of mainly 12 and 16 carbons, respectively, while olive oil and soybean oil are rich in unsaturated fatty acids and have predominantly 18 carbon fatty acids. Short and medium chain fatty acids (**SCFA/MCFA**), containing up to 12 carbon atoms, are absorbed and metabolized more rapidly than longer chains, as they are transported to the portal vein as free fatty acid and do not require any transporter to get absorbed (Guillot et al., 1993), which could reduce the HP induced by digestion. Recent research also suggests saturated fatty acids, SCFAs, and MCFAs could have a beneficial impact on the mitochondrial metabolism and electron transport chain (Schönfeld and Wojtczak, 2016; Seifi et al., 2018, 2020; Hecker et al., 2021), which are known to be disrupted under HS condition (Akbarian et al., 2016).

Influence of Dietary Crude Protein Content

Proteins have a higher caloric increment than carbohydrates and fat (Musharaf and Latshaw, 1999) and therefore increase the diet-induced HP. When AA are metabolized for energy by birds, much of the HP is caused by deamination reactions and incorporation of nitrogen into uric acid (Smith et al., 1978; Swick et al., 2013). Therefore, optimizing dietary CP composition to better fit bird requirements decreases the heat produced during AA oxidation. So, in an effort to reduce the energy released during digestion, absorption, and metabolism of nutrients, dietary CP reductions have been proposed as a strategy to mitigate the harmful effects

of HS in poultry (Furlan et al., 2004). Numerous studies in broilers have tested the effects of feeding a reduced CP diet versus a standard CP diet under constant HS (Alleman and Leclercq, 1997; Cheng et al., 1999; Gonzalez-Esquerra and Leeson, 2005; Faria Filho et al., 2005; Awad et al., 2018), cyclic HS (Cheng et al., 1999; Liu et al., 2016; Zulkifli et al., 2018; Awad et al., 2018; Lin Law et al., 2019; Amiri et al., 2019; Soares et al., 2020) and hot climates (Zaman et al., 2008; Laudadio et al., 2012; Awad et al., 2014, 2015, 2017; Lin Law et al., 2019; Attia et al., 2020). **Table 1** summarizes 21 HS broiler trials comparing reduced CP diets (ranging from 143 to 190 g/kg CP) and standard CP diets (ranging from 183 to 223 g/kg CP), with both diets in each study formulated to meet or exceed a specific nutritional requirement, such as the Nutrient Requirements of Poultry (NRC, 1994) or breeder recommendations, or to contain similar AA profiles. Approximately half of these studies observed a significant reduction in performance when feeding broilers the reduced CP diet compared to the standard CP diet, while the other half did not observe dietary effects. The response variability can be partly explained by the range of low and standard CP levels, as well as the intensity and duration of the HS period, but it is important to note that feeding a low CP diet without degrading performance is still beneficial for reducing nitrogen excretion. Results for BWG, presented in **Figure 2.1**, indicate that regardless of the HS challenge type, reduced CP diets decreased BWG by 10.8% on average (ranging from a reduction of 40.1% to an improvement of 2.5%). Similar results were obtained with FCR, with an average increase of 6.9% (ranging from a decrease of 0.9% to an increase of 19.7%) when dietary CP was reduced (**Figure 2.2**). Some studies also reported a decreased FI with reduced CP diets (Cheng et al., 1999; Awad et al., 2014, 2015, 2017, 2018). In addition to a reduced CP diet, some researchers tested the effects of a higher CP diet, with CP levels above the standard recommendations. During HS, high CP diets resulted in a decrease (Cheng et al., 1999) or an

increase in BWG (Faria Filho et al., 2005) and a decrease in FCR (Cheng et al., 1999; Gonzalez-Esquerra and Leeson, 2005; Faria Filho et al., 2005). However, other studies reported no effect of high versus standard CP diets (Zaman et al., 2008; Laudadio et al., 2012; Soares et al., 2020) and the increased diet cost associated with high CP diets could result in detrimental economical scenarios (Cardoso et al., 2022).

Feed-grade AA, which are included at higher levels in reduced CP diets to meet digestible AA requirements, allow to provide a balanced AA diet, and minimize the HP caused by AA oxidation, which is not possible to reach when relying on feed sources only. They also do not need enzymes for digestion and, as such, do not contribute to the digestion-related production of body heat (Morales et al., 2020). However, the performance degradations reported with reduced CP diets aligns with the lower HP observed in birds fed a high CP diet (220 g/kg) versus a low CP diet (160 g/kg) under cyclic HS conditions (Soares et al., 2020). Similar results have also been obtained under constant HS when comparing a high (230 g/kg), standard (200 g/kg), and low (170 g/kg) CP diets (Faria Filho et al., 2007). The lack of interaction between the CP level and environmental temperature reported by these authors is supported by studies conducted under thermoneutral conditions, where no difference (Noblet et al., 2003, 2007) or an increase (Swennen et al., 2004) in HP was measured with low CP diets, indicating that HS is not the cause per se of the higher HP with reduced CP diets. These results are surprising due to the higher caloric increment of proteins, but a possible explanation is that standard CP diets are usually formulated with a higher oil inclusion rate to reach the same amount of energy than reduced CP diets which generally have higher inclusion of corn (Soares et al., 2020). The extra-metabolic effect of dietary lipids, where the metabolizable energy value of the lipid exceeds its gross energy value (Aardsma et al., 2017), could compensate for the possible increase in heat

increment derived from protein (Soares et al., 2020). Interestingly, reduced CP diets with AA deficiencies have also been associated with a greater plasma level of triiodothyronine (Carew et al., 1983, 1997; Buyse et al., 1992), which is known for its thermogenic effect (Collin et al., 2003).

Overall, simultaneously increasing dietary energy and CP could be a potentially beneficial strategy to limit the adverse effects of HS on broiler growth and feed efficiency. Indeed, improved performance has been demonstrated under HS conditions when broilers were fed both a high dietary ME and CP contents (Attia et al., 2006; Attia and Hassan, 2017). However, in a similar study in which broilers were exposed to thermoneutral temperatures or cyclic HS from day 19 to 42 and were fed with a dietary ME and CP content of 3,152 kcal/kg and 194.8 g/kg or 3,253 kcal/kg and 210.3 g/kg, respectively, no improvement in performance was observed with the higher nutrient and energy density diet in either environment. Consequently, an economic evaluation actually showed a decrease in overall profitability with the higher density diets (Cardoso et al., 2022).

The conflicting evidence of higher caloric increment of dietary protein and impaired performance of broilers fed reduced CP diets led Gonzalez-Esquerra and Leeson (2006) to conclude that no consensus has been reached on protein requirements of heat-stressed birds. More recent trials on reduced CP diets have shown no performance improvements or amelioration of HP reduction and do not support this dietary strategy under HS conditions. Nonetheless, when following the “ideal protein” concept, where all essential digestible AA are provided in balance (Baker and Chung, 1992), the supplementation of unbound feed-grade AA in reduced CP diets to satisfy the bird’s requirements should result in similar performance as when feeding standard CP diets. Furthermore, most of the studies presented above based their

requirements on NRC or breeder recommendations, albeit broiler's AA requirements under HS conditions still remain undefined. More importantly, although those studies met specific nutritional requirements for essential AA, some potentially limiting AA such as Arg, Thr, Ile, Leu, His, and Phe were not equally balanced between diets. Diets with AA imbalance can lead to adverse effects especially under HS condition as they normally increase HP (Sekiz et al., 1975). Also, the FI reduction triggered by HS reduces the amount of CP and AA ingested by the birds, potentially resulting in deficiency when compared with reduced dietary concentrations. Therefore, even if the inclusion level for all AA was formulated to meet or exceed a target nutritional requirement under thermoneutral conditions, the effective AA consumption may not have reached the bird's requirements for some AA under HS conditions.

Further research on dietary CP and its interaction with energy and AA content would be required to better characterize the biological response induced by those diet changes under HS conditions. This would allow for a better understanding on the utilization of those nutrients in poultry reared under hot temperatures to ultimately facilitate better prediction of economic outcomes associated with nutritional dietary variation.

Supplementation of Amino Acids

Amino acid density. Altering dietary density of essential AA has been shown to have promising results in heat-stressed broilers. In broilers under hot temperatures, Maharjan et al. (2020) fed 5 levels of digestible Lys (**dLys**) from 80 to 120% of the recommended level with all other AA:dLys ratios held constant and observed quadratic responses in average daily gain and FCR up to the 120% dLys level, and no influence on FI. In contrast, the optimal average daily gain and FCR were closer to the 100% recommendation of dLys under thermoneutral conditions. This indicates a potential increase in overall AA requirements under HS, although the authors

concluded that the requirement of AA/Mcal was not different in hot or thermoneutral environments, which was also observed by Hruby et al. (1995). Moreover, Alhotan et al. (2021) fed an AA density ranging from 80 to 110% of breeder recommendations to broilers exposed to cyclic HS. In contrast with the results reported by Maharjan et al. (2020), no interactions between environmental temperature and dietary AA density were observed on performance and processing data. However, linear effects of AA density indicated that BWG, feed efficiency, and breast muscle yield responded to increased AA density in both environments. Even though FCR was numerically improved by 10 points with the 110% AA diet relative to the 100% AA diet, this difference was not statistically significant and may indicate that higher AA levels were above the bird's requirements. In another trial, increasing the density of Met, Lys, and Thr in a reduced CP diet increased production performance of cyclically heat-stressed broilers over the ones obtained with standard CP diet and, in addition, improved intestinal health as indicated by changes in small intestinal morphology and increased mRNA expression of some tight junction proteins (Wang et al., 2022). Therefore, increasing AA density could be beneficial for broilers experiencing HS, especially when achieved with free AA to minimize diet-induced thermogenesis. However, further research is required to better characterize the true AA requirements of birds under HS conditions.

Individual AA supplementation. Methionine (**Met**) is the first limiting AA in avian species and is considered, along with cysteine (**Cys**), to meet total sulfur AA (**TSAA**) needs for the bird. Because of its importance in maintenance functions and muscle deposition that are greatly impacted during exposure to HS, defining Met requirements is an important step in optimizing poultry nutrition under HS conditions. Indeed, higher requirements of Met have been found in broilers under high temperatures compared to thermoneutral conditions (Silva Junior et al., 2006;

Sahebi-Ala et al., 2021), but this does not appear to be the case in laying hens or pullets (Bunchasak and Silapasorn, 2005; Castro et al., 2019). Several physiological mechanisms have been proposed regarding the importance of Met under HS. First, Met supplementation has been shown to increase the antioxidant capacity of broilers (Del Vesco et al., 2015a; Gasparino et al., 2018; Liu et al., 2019; Santana et al., 2021). Furthermore, Met supplementation affected the inflammation-related gene expression in the liver of broilers placed under high temperature (Liu et al., 2019). Another potential benefit of Met supplementation under HS is its stimulatory effect on protein deposition and inhibition of protein breakdown as indicated by the increased expression of protein synthesis-related genes IGF1, GHR and PI3KR1 in the liver, and decreased expression of protein degradation-related genes atrogin1 and CTSL2 in the breast (Del Vesco et al., 2013, 2015b).

Beneficial effects of increasing the dietary amount of essential AA other than Met are not as well defined. Dietary levels of Lys, the second limiting AA in broiler chicken diets based on corn and soybean meal (Ishii et al., 2019), are closely associated with muscle protein deposition. However, the growth depression under HS does not seem to be ameliorated by supplementing broiler diets with Lys above the thermoneutral requirements (Mendes et al., 1997; Corzo et al., 2003; Attia et al., 2011). Interestingly, when Lys was supplemented in combination with Met in a reduced CP diet, broilers had similar performance and carcass characteristics to those fed a higher CP diet under hot climate conditions (Attia et al., 2020). In this study, additional treatments with supplementation of other essential AA besides Met and Lys did not ameliorate performance reductions caused by HS, emphasizing the potential importance of those two AA under HS conditions.

Threonine is almost invariably the third limiting AA in poultry diets (Kidd et al., 2000). In broilers, the earliest studies on Thr supplementation above the estimated requirements for birds under hot temperatures showed no or minimal benefits on performance (Kidd et al., 2000; Dozier et al., 2000; Ojano-Dirain and Waldroup, 2002; Shan et al., 2003), whereas more recent studies have shown some performance improvements (Debnath et al., 2019; Miah et al., 2022). In laying hens, increasing the supplementation of dietary Thr to 0.66% instead of 0.43% did not improve performance outcomes, but it decreased HSP70 in the ileum (Azzam et al., 2019) and increased SOD concentration in both serum and liver (Azzam et al., 2012), indicating potential antioxidant effects of Thr under HS condition.

Unlike mammals, poultry are highly dependent on dietary Arg supply because of less active *de novo* Arg synthesis pathways in birds (Klose et al., 1938; Tamir and Ratner, 1963; Castro and Kim, 2020). In broilers, the determination of Arg requirements under HS conditions have led to inconsistent results among different age periods. Over-supplementation was detrimental from 1 to 3 weeks of age (Chamruspollert et al., 2004), neutral from 3 to 6 weeks of age (Mendes et al., 1997), and beneficial from 6 to 8 week of age (Brake, 1998). Arg supplementation also improved FCR of Pekin ducks exposed to cyclic HS (Zhu et al., 2014) and enhanced several welfare indicators and decreased corticosterone plasma concentration in laying hens during the hot summer period (Bozakova et al., 2015). Furthermore, increasing dietary Arg improved performance, reproduction, antioxidant status, immunity, and maternal antibody transmission in quails (Kalvandi et al., 2022). The ability of Arg to reduce physiological stress is likely to be attributed to its antioxidative effects (Gupta et al., 2005). Arg is also the only nitrogen donor in the production of nitric oxide, which is involved in vasodilatation to potentially aid thermoregulation of heat-stressed birds (Uyanga et al., 2021). Interestingly, more focus is

being placed on the potential beneficial effects of citrulline (**Cit**), a compound synthetized during Arg catabolism and the formation of nitric oxide. Recent studies have shown that Cit supplementation can effectively increase systemic Arg levels, even more than direct L-Arg supplementation (Morita et al., 2014; Agarwal et al., 2017). Cit concentration in blood has also been shown to be modulated by hot temperatures (Chowdhury et al., 2014; Chowdhury, 2019) and its supplementation may increase nitric oxide synthesis, provide an anti-inflammatory response, and enhance the central regulation of body temperature (Chowdhury et al., 2017; Uyanga et al., 2021, 2022).

Leu, Ile, and Val are three essential AA collectively known as branched-chain AA (**BCAA**). Their roles are diverse and include effects on performance, immunity, and intestinal health. They also serve as signaling molecules in the regulation of glucose, lipid, and protein synthesis (Kim et al., 2022). Kop-Bozbay et al. (2021) investigated the effect of increased BCAA density under HS conditions and did not observe any improvement in growth performance. These authors also tested various dietary Val concentrations and did not observe effects on performance. However, high incorporation of Leu in those diets might have triggered the antagonist effect among BCAA (Ospina-Rojas et al., 2020). Interestingly, in ovo Leu injection improved BWG and thermotolerance of birds during subsequent exposure to HS (Han et al., 2017, 2019, 2020; Chowdhury et al., 2021). With the current increasing availability of feed-grade Val and Ile, further work is needed to define the potential for BCAA to combat HS in poultry.

Trp is an essential AA in poultry diets due to its need for protein synthesis and serotonin, as well as niacin production (Le Floc'h et al., 2011). Few studies have been published on the requirements of Trp under HS conditions, although high dietary concentrations did not improve

performance in broilers (Tabiri et al., 2002; Shan et al., 2003; Badakhshan et al., 2021) or layers (Dong et al., 2012). However, Trp supplementation did decrease rectal temperature and abated corticosterone responses caused by HS in broilers (Badakhshan et al., 2021). Trp supplementation also increased eggshell quality and decreased SOD serum concentration in laying hens during HS (Dong et al., 2012). To our knowledge, no studies on the effect of dietary supplementation of less-limiting essential AA beyond Trp, such as His and Phe, have been conducted in poultry subjected to HS.

The remaining AA are non-essential AA and can be synthesized from other precursors. Besides altering essential AA needs, the reduced FI caused by HS limits the amount of nitrogen consumed by birds, which could potentially lead to a lack of sufficient nitrogen quantity for non-essential AA synthesis (Awad et al., 2014). Feeding low CP diets during hot temperatures could also worsen this nitrogen deficiency. Birds fed a diet with increased essential and non-essential AA concentrations under HS had a better performance than when a diet with only increased essential AA concentrations was fed (Awad et al., 2014, 2015). However, when comparing individual supplementation of several non-essential AA in low CP diets, only Gly improved broiler FCR under both normal and acute HS conditions (Awad et al., 2015, 2018). Recent research also suggests that Gly and Ser, which are normally evaluated together as Gly equivalents, are co-limiting or limiting before some BCAA in low CP diets under thermoneutral conditions (Chrystal et al., 2020; Maynard et al., 2022), which could make Gly equivalents important AA to consider during reduced FI caused by HS.

Therefore, for the essential AA, it seems possible that supplementation of Met and potentially Arg above current requirements could be beneficial under HS condition. However,

further research is required to elucidate the effects of other essential AA, as well as Gly, non-essential AA, and overall nitrogen supply.

Conclusion

Adaptation to rising global temperatures while maintaining production efficiency is an important emerging challenge for the poultry industry. Under hot temperatures, birds reduce their FI to lower HP, and this is the main factor explaining the degradation of bird performance (**Figure 2.3**). Mitigation of those negative effects requires a holistic approach, and adjusting feeding practices and nutritional programs have a critical role to play. Even though some feeding strategies are difficult to implement in the field, especially with intensive rearing systems, several practices discussed in this review have shown beneficial effects to reduce the heat load on poultry. Increasing dietary lipid concentration and maintaining a standard CP level are also recommended to compensate for the FI reduction and better fit the birds' requirements under elevated temperatures. Considering an increase in the density of some AA, like methionine and arginine, to meet the increased AA requirements for maintenance functions could also be advantageous. Therefore, further research is required to characterize nutrient partitioning and requirements of birds under HS conditions to ensure efficient and cost-effective solutions for the poultry industry.

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TABLES AND FIGURES

Table 2.1. Summary of experimental conditions of broiler studies comparing reduced and standard CP diets under HS conditions.

Heat Stress Condition	Heat Stress Length	Average Temperature (°C)	Standard CP (g/kg)	Reduced CP (g/kg)	Age Start (d)	Age End (d)	Duration (d)	Reference
Constant HS	/	34	194	143	22	42	20	Awad et al. (2018)
	/	32	199	160	23	44	21	Alleman and Leclercq (1997)
	/	32.2	198	161	21	49	28	Cheng et al. (1999)
	/	31.4	200	180	21	42	21	Gonzalez-Esquerra and Leeson (2005)
	/	33	200	185	7	21	14	Faria Filho et al. (2005)
Cyclic HS	35 °C for 8 h	29.4	198	161	21	49	28	Cheng et al. (1999)
	33 °C for 6 h	25.5	190	162	22	35	13	Zulkifli et al. (2018)
	32 °C for 8 h	26	200	160	22	42	20	Soares et al. (2020)
	34 °C for 7 h	26.2	183	167	22	42	20	Lin Law et al. (2019)
	/	27.8	213	153	28	42	14	Liu et al. (2016)
	34 °C for 8 h	NA	195	175	0	42	42	Amiri et al., (2019)
	34 °C for 7 h	26.2	194	143	22	42	20	Awad et al. (2018)
Hot climate	/	At least 28.1	223	161	0	21	21	Awad et al. (2015)
	/	At least 28.1	223	162	0	21	21	Awad et al. (2017)
	/	At least 28.3	223 and 194	162 and 135	0	42	42	Awad et al. (2017)
	/	At least 28.5	216 and 187	176 and 156	0	35	35	Lin Law et al. (2019)
	/	At least 28.3	207	177	0	21	21	Awad et al. (2014)
	/	NA	205	185	14	42	28	Laudadio et al. (2012)
	/	NA	210	190	0	28	28	Zaman et al. (2008)
	/	34	190	155	28	49	21	Attia et al. (2020)
	/	35	186	152	30	45	15	Attia et al. (2020)

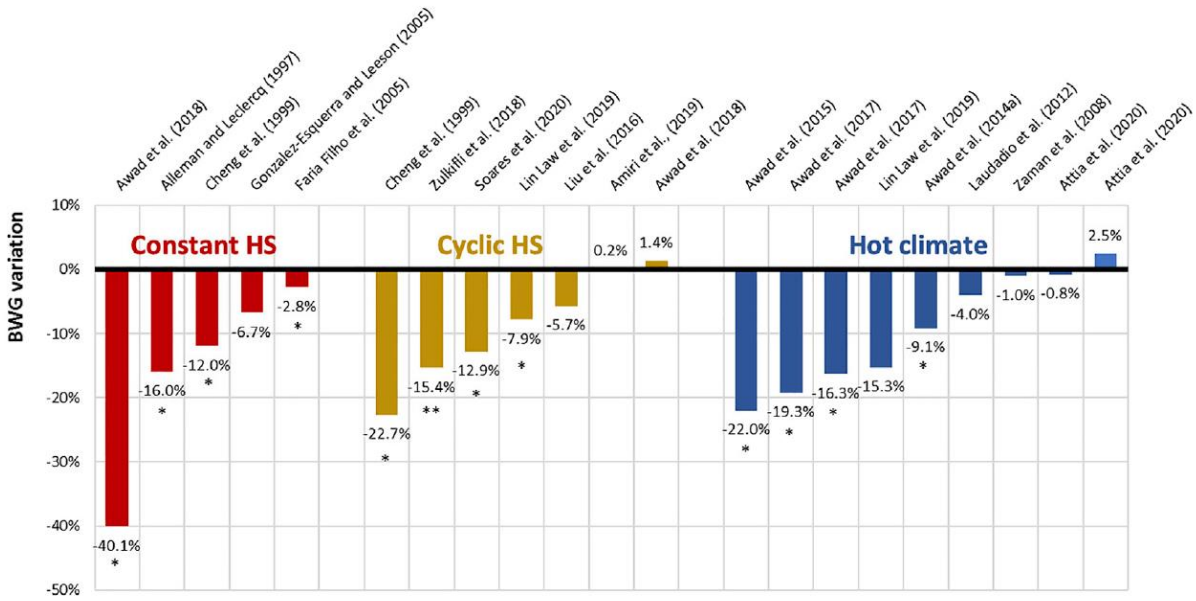

Figure 2.1. Effect of reduced and standard crude protein diets on BW gain of broilers exposed to different heat stress conditions.

Abbreviations: **BWG**: Body weight gain. **CP**: Crude protein

$$BWG\ variation\ = \frac{BWG\ reduced\ CP\ diet - BWG\ standard\ CP\ diet}{BWG\ standard\ CP\ diet}$$

The presence of a * indicates a significant difference in BWG between the reduced and standard CP diet, while ** indicates a significant linear effect across several CP levels

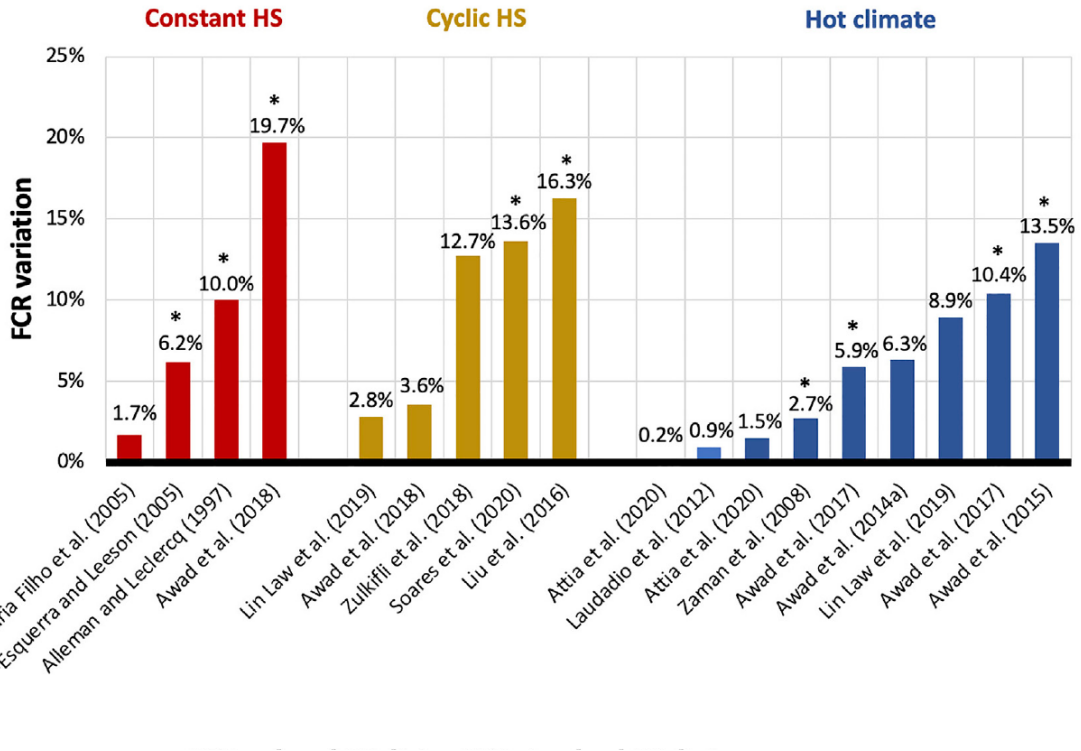

Figure 2.2. Effect of reduced and standard rude protein diets on feed conversion ratio of broilers exposed to different heat stress conditions.

Abbreviations: **FCR:** Feed conversion ratio. **CP**: Crude protein

$$FCR\ variation\ = \frac{FCR\ reduced\ CP\ diet - FCR\ standard\ CP\ diet}{FCR\ standard\ CP\ diet}$$

The presence of a * indicates a significant difference in FCR between the reduced and standard CP diet

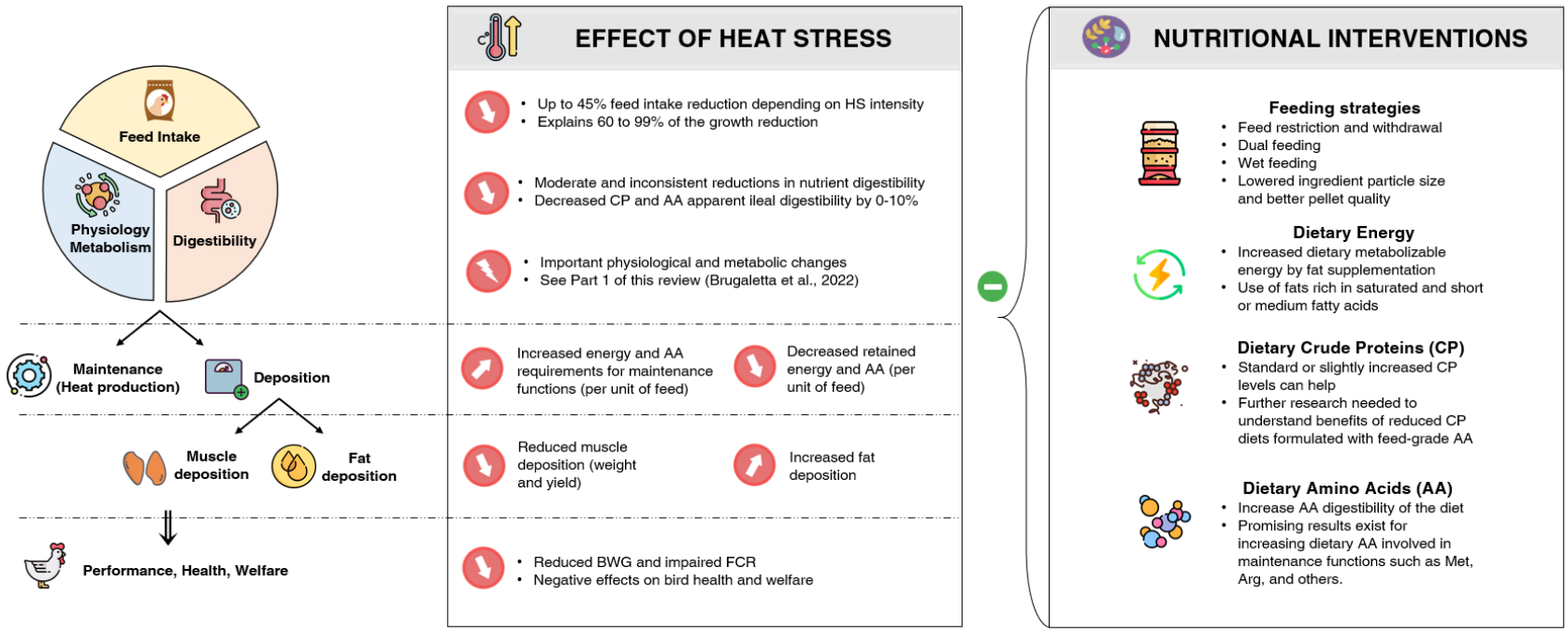

Figure 2.3. Conclusive scheme of the beneficial nutritional interventions on broilers exposed to heat stress conditions.

Abbreviations: **HS:** Heat stress. **CP**: Crude protein. **AA**: Amino acid. **BWG**: Body weight gain. **FCR**: Feed conversion ratio.

CHAPTER III - CONSTANT AND CYCLIC CHRONIC HEAT STRESS MODELS DIFFERENTIALLY INFLUENCE GROWTH PERFORMANCE, CARCASS TRAITS AND MEAT QUALITY OF BROILERS

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Manuscript published in Poultry Science 2022 Aug 1;101(8):101963.

ABSTRACT

This experiment compared the effects of 2 chronic heat stress (**HS**) models, constant (**coHS**) and cyclic (**cyHS**), on broiler performance, carcass characteristics and meat quality. A total of 720 male chicks from a Cobb 500 line were placed in 12 environmentally controlled chambers divided into two pens of 30 birds. Before experimental HS models were applied, chambers temperatures were gradually decreased from 32°C at placement to 24°C on d 20. From 20 to 41 d, 4 chambers were set to 35°C (coHS), and 4 chambers were set to 35°C for 12 h and 24°C for the next 12 h (cyHS). Four thermoneutral chambers were maintained at 24°C with half of the birds pair-fed to equalize feed intake (**FI**) with coHS birds (**TN-coPF**) and half fed ad-libitum (**TN-al**). From 20 to 41 d, FI and BW gain (**BWG**) of cyHS, coHS and TN-coPF birds were decreased ($P < 0.001$), whereas feed conversion ratio (**FCR**) was increased ($P < 0.001$) for coHS and TN-coPF birds compared with TN-al birds. The overall BWG and FCR of coHS birds were lower ($P < 0.001$) than TN-coPF birds. Both HS models reduced ($P < 0.001$) carcass weight, pectoralis major yield, total breast meat yield and increased ($P < 0.001$) wing yield relative to TN-al birds, and each of these measurements was more impacted by coHS than by cyHS. Pair-fed birds had lower ($P < 0.001$) fat pad and a higher total breast meat yield than coHS birds. They also had the lowest ($P < 0.001$) pectoralis major ultimate pH and yellowness, and these parameters were lower ($P < 0.001$) for coHS birds than for TN-al birds. Both HS models reduced ($P < 0.001$) the incidence of woody breast and white striping. Thus, these data indicate that the detrimental effects of HS cannot be entirely explained by reduced FI and that HS *per se* affects metabolic pathways associated with muscle and lipid accretion in broilers.

INTRODUCTION

Poultry production faces several important environmental challenges in meeting the increasing global demand for animal protein. By 2050, predictions estimate the animal based food demand will rise by nearly 70% (Searchinger et al., 2019), and meanwhile, climate change is a major concern for livestock production in the context of global warming. Temperate zones where most of the industrialized farming systems are found may lose 25% of their animal production due to global warming, and this scenario may be worse for some regions of Asia and Africa where extensive farming systems are more abundant (Nardone et al., 2010). By definition, heat stress (**HS**) is a common environmental stressor that occurs when the amount of heat produced by an animal surpasses the animal's capacity to dissipate the heat to its surrounding environment (Lara and Rostagno, 2013). This imbalance between heat production and body heat loss occurs when the environmental temperature rises above the upper critical temperature of the thermoneutral zone (Bernabucci et al., 2010; Lara and Rostagno, 2013).

Birds are particularly sensitive to heat because their capacity for heat loss is limited by feathering and the lack of sweat glands. Furthermore, the genetic selection of high performing birds over several decades has resulted in birds with elevated metabolic rates, making them more sensitive to hot temperatures (Lara and Rostagno, 2013). When environmental temperature rises above the thermoneutral zone, birds decrease their feed intake and physical activity to reduce heat production and increase panting and water consumption to dissipate heat. (McFarlane et al., 1989; Mahmoud et al., 2015). However, at certain temperature thresholds, birds can no longer control their body temperature, leading to welfare problems and detrimental effects on performance, carcass characteristics, and meat quality ensue. From an economical perspective, HS has been estimated to cause total annual economic losses of $128 to $165 million to the US

poultry industry (St-Pierre et al., 2003). However, due to a lack of more recent economic evaluations, these numbers are probably currently underestimated due to growth of the poultry industry during the last decades and worsening of global warming predictions. Though data are lacking for other regions, it is likely that the consequences of HS are even more severe in tropical countries (Pawar et al., 2016).

Numerous studies have investigated the detrimental effect of HS on performance, most often under constant hot temperatures to provide marked responses to model HS (Alleman and Leclercq, 1997; Temim et al., 2000; Lu et al., 2007; Rosa et al., 2007). More recently, several publications have utilized daily cyclical HS to mimic the diurnal pattern observed in temperate countries during summer. These models consistently demonstrate a degradation of body weight gain (**BWG**), feed intake (**FI**), and feed conversion ratio (**FCR**) when compared with birds in thermoneutral conditions (Zhang et al., 2012; De Souza et al., 2016; Awad et al., 2018), with constant HS having a greater effect than cyclic HS. Also, the magnitude of performance reductions observed with different HS models depends on several parameters including the temperature, relative humidity, length, and cyclicality of the heat period, as well as the age of the birds at the beginning of the stress period. Degradations in carcass composition and meat quality have also commonly been observed under cyclic and constant HS (Ain Baziz et al., 1996; Song and King, 2015; Zeferino et al., 2016; De Antonio et al., 2017; Roushdy et al., 2018).

A reduction in feed intake (**FI**) in attempt to limit heat production is the main factor explaining degraded performance in response to HS (Syafwan et al., 2011). However, other physiological responses need to be considered, as pair-feeding birds under thermoneutral conditions to equalize FI with those subjected to HS treatments indicates that reduced FI alone does not fully account for the reduction in growth performance associated with HS (De Souza et

al., 2016). Further investigations comparing both chronic constant and cyclical HS models within the same experiment for market-age birds reared in floor pens are required. As such, the objective of this experiment was to compare two chronic HS models (constant and cyclic) to assess their influence on performance, carcass characteristics, and meat quality of broilers reared to 42 days, and to determine the direct effect of constant HS on these parameters independent of decreased FI.

MATERIALS AND METHODS

Animals and Experimental Design

All animal care and procedures were approved by the Institutional Animal Care and Use Committee at the University of Arkansas (protocol #20020).

Seven hundred and twenty male chicks from a Cobb 500 female breeder line were obtained from the Cobb hatchery (Fayetteville, AR). Upon arrival, chicks were selected and weighed to ensure that each group of 30 chicks weights fell within 3% of the expected group weight based on a preliminary weight of approximately one-half of the population. Birds were allocated to 12 environmentally controlled chambers divided into 2 pens. Each chamber measured 2.44 × 3.66 m and was divided by wire paneling into 2 pens of 4.47 m^2 that each housed 30 chicks. Each pen within the chambers were equipped with hanging pan feeders, nipple waters, and concrete floors covered with fresh pine shavings. Before experimental HS models were applied, ambient temperatures of all the chambers were gradually decreased from 32°C at placement to 24°C on d 20.

On d 20, bird numbers were equalized to 25 per pen before application of the experimental treatments and to ensure that each group weight of 25 birds was similar (within 3% of the overall average). From 20 to 41 d, 3 different environmental conditions and 1 pair-feeding

treatment formed a total of 4 treatments: thermoneutral birds fed ad-libitum (**TN-al**), cyclic HS (**cyHS**) birds fed ad libitum in 4 cyclic HS chambers where temperature was maintained at 35°C for 12 h daily (from 7:30 to 19:30) and reduced to 24°C each night., constant HS (**coHS**) birds fed ad libitum in 4 chambers where the temperature was set to and maintained at 35°C, and a group kept in thermoneutral chambers and pair-fed to equalize feed intake to that of coHS bird (**TN-coPF**). These birds were fed one time per day with the same amount of feed consumed by coHS birds over the last 24 hours. Moreover, for the first week of the challenge only and for reasons discussed in the discussion, an additional amount equal to the expected relative daily increase in FI was distributed to the TN-coPF birds according to the following formula: $FI_{d-1} \times \frac{FI_{d-1} - FI_{d-2}}{FI_{d-2}}$, with $FI_{\text{d-2, d-1, d}}$ being the FI of coHS birds measured on day d-2, d-1, and d.

Each chamber was equipped with a Thermochron temperature logger (iButton, DS1922L, Embedded Data Systems, KY) to record environmental temperature hourly (**Figure 3.1**). As described previously (Rajaei-Sharifabadi et al., 2017), two birds per pen were randomly selected and equipped with the same temperature logger for continuous monitoring of core body temperature, but malfunctioning of the loggers prevented data recovery for most birds. Water was provided ad libitum throughout the experiment and a diet based on corn and soybean meal was formulated and fed in 3 phases: starter from d 0 to 13, grower from d 14 to 27, and finisher from d 28 to 42 (**Table 1**). The photoperiod was set at 23L:1D from placement to d 7, 16L:8D from d 8 to 28, and 18L:6D from d 29 until the end of the trial. Light intensity was set at 27 lux from d 1 to 7, 16 lux from d 8 to 14, and 6 lux from d 15 to the end of the experiment.

Performance Measurements and Carcass Characteristics

Feed intake and body weights (**BW**) were measured at 0, 20, 27, 34, and 41 d post-hatch and mortality was recorded daily. On d 42 birds were processed at the University of Arkansas

Pilot Processing Plant (Fayetteville, AR) following an overnight feed withdrawal for 8 h. The weight of each processed bird was individually measured before they were subjected to electrical stunning (11 V, 11 mA for 11 s) and exsanguination via a jugular vein cut. After scalding at 53.8°C, feathers were removed with a commercial inline defeatherer (Foodcraft Model 3; Baker international, MI, USA). Necks, heads, and feet were removed from each bird. Carcasses were then mechanically eviscerated. Carcass and abdominal fat weights were recorded before placing carcasses in ice water for a 4 h chill. Chilled carcasses were weighed, and pectoralis major (**P. major**) and minor (**P. minor**) muscles, wings, and leg quarters were removed and weighed. Total breast meat (**TBM**) was calculated as the sum of the P. major and P. minor weights, and the yield of each part was determined by division of the part weight by the individual back dock live weight. The P. major from each bird was placed on an aluminum tray, covered with plastic wrap and stored at 4°C until 24 h postmortem.

Meat Quality

P. major fillets were immediately scored for woody breast (**WB**) and white striping (**WS**) on a visual scale from 0 to 3 and an increment of 0.5 by a trained individual (Kuttappan et al., 2012b, 2016; Tijare et al., 2016). To simplify data representation, WB scores were categorized as normal (0 to 0.5), mild (1 to 1.5), or severe (2.0 to 3.0). Similarly, WS scores were categorized as normal, faint, or apparent.

At 24h postmortem, P. major color was measured according to the L* a* b* scale using a Minolta colorimeter (CR-400; Konica Minolta Sensing Inc., Sakai Osaka, Japan; size 102 (W) × 217 (H) × 63 (D) mm) with illuminant D65 and a 2.54-cm aperture. Three readings were performed on the ventral side of the right P. major and averaged to obtain the color result. Pectoralis major ultimate pH (**pH_u**) was measured with a temperature-compensating pH meter

(Testo 205; Testo Inc., West Chester, PA) inserted into the cranial region of the right P. major lobe with an average of 3 measurements per each sample (Orlowski et al., 2018).

Statistical Analysis

Each environmental condition was assigned to chambers in completely randomized design. The experimental unit was the pen of 25 birds. Data for the 2 pens within the chambers assigned to the coHS and cyHS treatments were averaged and treated as 1 pen since the treatment was applied to the entire chamber. Thus, while each treatment had a total of 4 replicates, the number of individuals per replicate depended on the environmental condition, with data for birds under HS conditions (cyHS and coHS) consisting of an average of 2 pens in the respective chambers (50 birds total) and data for birds under thermoneutral conditions (TN-al and TN-coPF) representing 1 pen of 25 birds.

Growth performance (FI, BWG, FCR, and morality), carcass characteristics (P. major, P. minor, wings, leg quarters, TBM weights and yields), and meat quality (P. major pH_u and color) were subjected to a one-way ANOVA and means were separated using a Tukey's HSD test. Mean differences were considered statistically significant when $P < 0.05$ and all analyses were performed using R (RStudio 1.3.1093).

RESULTS

Effect of Two Chronic HS Models and Pair-Feeding on Bird Performance

From 0 to 20 d, all birds were reared under the same environmental conditions and received the same diet. Diet analyses indicated that the CP content of the soybean meal may have been higher than estimated, resulting higher analyzed dietary CP than expected, especially during the starter phase before experimental treatments were applied (Table 1). No differences ($P > 0.05$) among each set of 4 chambers were observed on BW, BWG, FI, FCR, and mortality during

this period, and subsequent removal of some birds from each chamber before the beginning of the experimental phase allowed each treatment to have average bird weights within 1.3% of the grand mean weight for all treatments.

The live broiler performance data measured during the three cumulative periods from 20 to 27 d, 20 to 34 d, and 20 to 41 d are presented in **Table 2**. During the first week of the challenge (d 20 to 27), birds in the coHS and TN-coPF treatments had the lowest ($P < 0.001$) FI, while birds in the TN-al had the highest FI, and FI of cyHS birds was intermediate. Compared with the TN-al group, BW and BWG were reduced ($P < 0.001$) by cyHS, further reduced by coHS, and BWG of the TN-coPF group was intermediate to the cyHS and coHS groups. Cyclic HS and TN-al had the lowest ($P < 0.001$) FCR, coHS birds had the highest FCR, and FCR of the TN-coPF treatment was intermediate to these groups.

During the 20 to 34 d cumulative period, the FI of cyHS birds was similar to that of TN-al birds, reduced ($P < 0.001$) in the TN-coPF group, and reduced to a greater extent with coHS. As observed from 20 to 27 d, BW and BWG were reduced ($P < 0.001$) by cyHS relative to the TN-al group, further reduced by coHS, with the TN-coPF group being intermediate to the cyHS and coHS groups. The FCR was lowest ($P < 0.001$) for the TN-al treatment, highest for the coHS treatment, and intermediate for the cyHS and TN-coPF treatments.

During the total cumulative period from 20 to 41 d, compared with TN-al birds, the reduction in FI ($P < 0.001$) was greatest and similar in the coHS and TN-coPF groups, while FI of cyHS bird was intermediate. Compared with the TN-al group, BW and BWG were reduced ($P < 0.001$) by cyHS, further reduced by coHS, with the TN-coPF group being intermediate to the cyHS and coHS groups. The lowest ($P < 0.001$) FCR was observed for TN-al and cyHS groups, the highest observed for coHS birds, with TN-coPF birds having an intermediate FCR. No

differences ($P > 0.05$) in mortality were observed between treatments during any individual period or the total cumulative period.

Effect of Two Chronic HS Models and Pair-Feeding on Carcass Characteristics and Part Weights

The hot and chilled carcass, hot fat pad weight and yields of processed birds are presented in **Table 3**. Hot and chilled carcass weights were the highest ($P < 0.001$) for TN-al birds, intermediate for cyHS birds, and lowest for coHS and TN-coPF birds. The hot and chilled carcass yield of TN-coPF birds was decreased compared to the other treatments, and coHS birds had a higher chilled carcass yield than TN-al birds. Compared to TN-al condition, hot fat pad weights were more drastically reduced ($P < 0.001$) under coHS than with cyHS, and TN-coPF had an even lower abdominal fat weight than coHS birds. Hot fat pad yield was the highest ($P < 0.001$) with coHS birds, intermediate with TN-al and cyHS birds, and the lowest for TN-coPF birds.

Compared with the TN-al group, weights of P. major, P. minor and TBM were reduced ($P < 0.001$) by cyHS, further reduced by coHS, and the TN-coPF group was intermediate to the cyHS and coHS groups. Wing and leg quarter weights followed the same trend ($P < 0.001$) except that there was no difference between coHS and TN-coPF birds. For yields of these parts, coHS birds had a greater ($P < 0.001$) reduction of the P. major yield compared to cyHS birds, while TN-coPF yields were not different than coHS and cyHS birds. Constant HS birds had the lowest ($P < 0.001$) P. minor yield, which was similar among other groups. The TBM yield was the highest ($P < 0.001$) under the TN-al treatment, the lowest under coHS, and intermediate under cyHS and TN-coPF conditions. On the other hand, leg quarter yield was increased ($P < 0.001$) by the two HS models compared to TN-al and TN-coPF conditions. The wing yield was

the lowest ($P < 0.001$) with TN-al birds and the highest with coHS birds. Pair fed and cyHS birds had a lower wing yield than coHS birds, with a reduction more important in cyHS birds.

Effect of Two Chronic HS Models and Pair-Feeding on Meat Quality

No difference ($P > 0.05$) was observed in the incidence of severe WB and apparent WS between the treatments (**Table4**). The incidence of mild WB was highest ($P < 0.001$) for TN-al, intermediate for cyHS, and lowest and not different between coHS and TN-coPF. An inverse relationship was observed for the incidence of normal WB ($P < 0.001$). The incidence of faint WS was highest ($P < 0.001$) and not different for TN-al and cyHS, intermediate for coHS, and lowest for TN-coPF, with an inverse response observed for the incidence of normal WS ($P < 0.001$).

As presented in **Table 5**, compared to TN-al condition, the P. major pH_u was not affected by cyHS or coHS conditions, whereas TN-coPF birds had a lower ($P < 0.001$) pH_u than all other groups. Concerning the P. major color measurements, L* was the highest ($P < 0.001$) for the coHS treatment, but not different among the other groups. Values for a* did not differ ($P > 0.05$) among treatments, whereas P. major b* values were decreased ($P < 0.001$) with coHS birds and to a greater extent in TN-coPF birds.

DISCUSSION

As expected, both coHS and cyHS models impaired BW, BWG, FI, and FCR, with a greater impact resulting from constant versus cyclic HS exposure. Furthermore, markedly reduced performance (67% reduction in BWG and 80% increase in FCR) during the cumulative challenge period indicates that birds were quite stressed when maintained at 35°C continuously. Performance was also negatively affected by cyHS (20% reduction in BWG and 10% increase in FCR), but to a lesser extent than with coHS. With both models, reduced performance was mainly

caused by the decrease in FI induced by hot temperatures; however, the TN-coPF birds presented better performance than the coHS birds, indicating that HS *per se* directly contributes to decreased performance. Similarly, De Souza et al. (2016) noted that the reduced growth under cyHS is more related to decreased FI than directly to HS, while coHS led to greater metabolic impacts.

During the two first weeks of challenge, TN-coPF birds received the same amount of feed consumed by coHS birds plus an amount equal to the expected relative daily increase in feed intake of those same birds. Because the expected daily increases in FI of coHS birds did not occur, this led to a higher FI of TN-coPF birds compared to coHS birds. This was corrected during the third week of the challenge, with no difference in FI observed between the coHS and TN-coPF for the total cumulative challenge period. Comparison of the performance of these pair-fed birds with the coHS birds indicates that, in this experiment, approximately 81% of the degradation of performance under constant high temperature was caused by decreased FI, with the remaining 19% directly associated with the physiologic changes induced by the elevation of temperature. These relative reductions are in the general range of values reported in other studies (Geraert et al., 1996; Bonnet et al., 1997; Faria Filho et al., 2007; De Souza et al., 2016; Habashy et al., 2017). Several physiological changes are responsible for impaired performance following HS *per se* such as oxidative stress, inflammation, and compromised intestinal integrity. From a metabolic standpoint, reduced protein turnover and increased fat deposition have been observed with HS birds compared to PF birds (Geraert et al., 1996; Temim et al., 2000; Faria Filho et al., 2007; De Souza et al., 2016).

Compared with ad libitum fed birds reared under thermoneutral conditions, increased chilled carcass yield under coHS, as observed in the current study, has been previously reported,

as has decreased carcass yield of pair-fed birds (Ain Baziz et al., 1996; Lu et al., 2007; Rosa et al., 2007; Zeferino et al., 2016). Under HS condition, the reduction of relative organ weights (heart, liver) and reduced feathering to improve heat losses could partially explain this increase in carcass yield (Geraert et al., 1993; Zeferino et al., 2016). Concerning TN-coPF birds, reduced carcass yield could partially be explained by increased mobilization of fat as an energy source (Zhan et al., 2007). This hypothesis is supported by the large reduction in abdominal fat deposition of pair-fed birds compared with birds in TN-al condition. Conversely, coHS increased abdominal fat deposition, which agrees with previous studies (Zeferino et al., 2016). Moreover, increased intramuscular fat deposition in birds subjected to coHS observed by others (Zhang et al., 2012) reveals an increase in the amount of energy retained as fat (Geraert et al., 1996; Faria Filho et al., 2007). However, cyHS did not affect abdominal fat yield in the current experiment, which is in agreement with Orlowski et al. (2020) and Greene et al. (2021) and supports the notion that metabolism alterations depend on the HS model.

Whereas an increased proportion of energy appeared to be stored as lipid under coHS, P. major and P. minor yields were decreased. This trend was also observed under cyHS, but to a lower extent. Broilers have been selected to have high P. major yield for decades, and marked decreases in BWG observed under HS should logically result in decreased weights of this highest selective trait (Orlowski et al., 2020). Similarly, less muscle protein deposition (Temim et al., 2000; Zhang et al., 2012) and less energy retention as protein (Geraert et al., 1996; Faria Filho et al., 2007) following HS has been observed in several studies. Furthermore, leg quarter and wing yields are increased under HS, which can be partially explained by the decrease in P. major yield, resulting in increased relative yield of other carcass parts. Additionally, leg quarters are comprised of oxidative fibers whereas P. major muscle is comprised of glycolytic fibers that are

more dependent on glycogen stores, and these are depleted following decreased FI in response to HS (Temim et al., 2000; Zeferino et al., 2016). In the current study, parts yield reductions responded similarly in restricted fed birds as in coHS birds, indicating that the observed effect of HS on carcass part yield is mainly associated with decreased FI. However, leg quarter yield was not increased in TN-coPF birds compared to TN-al birds.

Woody breast and WS are major concerns for the meat industry as they are associated with a decrease in meat quality. White striping is characterized by white striations parallel to muscle, while WB results in a tougher consistency of breast fillets (Kuttappan et al., 2016). Compared to the TN-al treatment, the incidence of WB was reduced to a greater extent by coHS than by cyHS, and WB was completely absent in P. major muscles from broilers in the TN-coPF group. White striping was not decreased by cyHS, but was reduced with coHS, and more importantly with TN-coPF. Orlowski et al. (2020) and Greene et al. (2021) observed similar results under cyHS, but Orlowski also reported a reduction of WS under cyclic HS. The incidence of both WB and WS has been related to rapid growth and high P. major muscle yields (Griffin et al., 2018). Thus, the marked reduction in FI and BWG of coHS and TN-coPF birds could explain the low occurrence of muscle myopathies in those treatments. However, TN-coPF birds presented a lower incidence of these conditions than coHS, despite having higher weight gain and P. major yield. This may have been related to behavioral differences, as WB has been associated with a resting behavior (Norring et al., 2019) that is often displayed by HS birds in an attempt to minimize the heat production through reduced physical activity (Mack et al., 2013).

In our experiment, the pH_u was only decreased under coHS condition and not cyHS. Zhang et al. (2012) observed decreased pH_u in response to both coHS and cyHS, whereas other studies have not shown differences in pH_u in response to cyHS (Orlowski et al., 2020; Greene et

al., 2021) or coHS (Lu et al., 2007, 2017). As observed here, Lu et al. (2007, 2017) and Zeferino et al. (2016) also found that the P. major from coHS birds had higher pH_u than TN-coPF birds. Lowered pH_u during HS could be explained by greater conversion of pyruvate into lactate during chronic HS (Song and King, 2015; Lu et al., 2017). The increased lightness of P. major under constant HS has been observed in previous studies, but responses in redness and yellowness to HS vary in the literature (Lu et al., 2007; Zhang et al., 2012; Zeferino et al., 2016). The lower b* values observed under coHS and TN-coPF conditions could be associated with the less severe WS scores observed in those treatments (Kuttappan et al., 2013a). The higher yellowness observed with severe WS has been associated to higher fat contents (Kuttappan et al., 2012a, 2013c; b; Petracci et al., 2014), but given that restricted fed birds are expected to have low intramuscular fat content, this should not be the case of chronic HS birds (Lu et al., 2017).

In summary, the comparison of performance, carcass characteristics, and meat quality of birds reared under a thermoneutral, cyclic, or constant HS environments within the same experiment have confirmed that the response of birds to HS is largely dependent on the model used. Cooler nights during a diurnal HS seems to improve the ability of the bird to adapt or recover from cyclic HS. Additionally, impaired weight gain resulting from HS is predominantly caused by a decrease in FI, but the use of pair-fed birds confirmed that HS also has direct effects on the measurements reported in this study that are independent of feed intake. Further investigations on oxidative stress and metabolic changes elicited by these conditions are ongoing to better characterize bird responses to the different HS models tested herein.

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TABLES AND FIGURES

Table 3.1. Composition of starter, grower, and finisher diets

Item, % as-fed	Starter (0 to 13 d)	Grower (14 to 27 d)	Finisher (28 to 42 d)
Corn	58.74	62.11	65.56
Soybean meal (47.5%)	37.53	33.98	29.75
Poultry Fat	0.50	1.00	1.59
Limestone	1.10	1.08	1.03
Dicalcium phosphate	0.78	0.62	0.43
Salt	0.40	0.41	0.41
DL-methionine[1]	0.30	0.27	0.23
L-lysine HCl	0.13	0.11	0.09
L-threonine	0.10	0.04	0.02
Choline chloride (60%)	0.11	0.08	0.07
Vitamin and mineral premix[2]	0.25	0.25	0.25
Enzyme blend[3]	0.01	0.01	0.01
Coccidiostat[4]	0.05	0.05	0.05
Titanium Dioxide	0.00	0.00	0.50
Calculated nutrient composition[5]			
AMEn (kcal/kg)	2,991	3,057	3,120
Crude Protein	22.81	21.28	19.45
Digestible Lys	1.22	1.12	1.00
Digestible TSAA	0.92	0.85	0.78
Digestible Thr	0.83	0.73	0.65
Digestible Arg	1.41	1.31	1.18
Digestible Ile	0.89	0.83	0.76
Digestible Val	0.98	0.92	0.85
Total Ca	0.90	0.84	0.76
Total P	0.57	0.53	0.47
Available P	0.45	0.42	0.38
DEB[6] (mEq)	268	250	229
Analyzed nutrient composition[5]			
Crude Protein	25.70	23.33	19.99
Total Ca	0.80	0.80	0.62
Total P	0.56	0.51	0.45

[1] HMTBA-Ca salt (Adisseo France S.A.S., Antony France)

[2] Supplied the following per kg of diet: vitamin A, 6,173 IU; vitamin D3, 4,409 ICU; vitamin E, 44 IU; vitamin B12, 0.01 mg; menadione, 1.20 mg; riboflavin, 5.29 mg; d-pantothenic acid, 7.94 mg; thiamine, 1.23 mg; niacin, 30.86 mg; pyridoxine, 2.20 mg; folic acid, 0.71 mg; biotin, 0.07 mg; manganese, 24 mg; zinc, 14.4 mg; selenium, 0.04 mg; copper, 0.68 mg; iodine, 0.47 mg

[3] Rovabio® AdvancePhy T (Adisseo France S.A.S., Antony France)

[4] BioCox60 (Huvepharma, INC., USA)

[5] Values reported as percentages unless noted otherwise.

[6] DEB: Dietary electrolyte balance

Table 3.2. Cumulative live performance of broilers from 20 to 27 d, 20 to 34 d and 20 to 41 d reared under different environmental conditions and feed regimens.

Period	Parameter[3]	Treatment[1]				SEM[2]	*P*-values
		TN-al	cyHS	coHS	TN-coPF		
20 to 27 d	**20 d BW, kg**	0.975	0.968	0.968	0.965	0.015	0.788
	27 d BW, kg	1.693[a]	1.588[b]	1.361[d]	1.467[c]	0.035	<0.001
	BWG, kg	0.717[a]	0.620[b]	0.390[d]	0.501[c]	0.027	<0.001
	FI, kg	1.039[a]	0.931[b]	0.760[c]	0.880[b]	0.030	<0.001
	FCR	1.448[c]	1.503[c]	1.956[a]	1.761[b]	0.043	<0.001
	Mortality, %	2.00	0.00	0.99	0.00	1.288	0.140
20 to 34 d	**34 d BW, kg**	2.492[a]	2.215[b]	1.633[d]	1.795[c]	0.065	<0.001
	BWG, kg	1.516[a]	1.248[b]	0.663[d]	0.829[c]	0.058	<0.001
	FI, kg	2.314[a]	2.409[a]	1.477[c]	1.619[b]	0.056	<0.001
	FCR	1.492[c]	1.936[b]	2.270[a]	2.016[ab]	0.112	<0.001
	Mortality, %	2.67	1.50	2.51	0.00	2.552	0.557
20 to 41 d	**41 d BW, kg**	3.312[a]	2.841[b]	1.748[d]	2.040[c]	0.099	<0.001
	BWG, kg	2.336[a]	1.873[b]	0.777[d]	1.074[c]	0.094	<0.001
	FI, kg	3.781[a]	3.178[b]	2.069[c]	2.223[c]	0.109	<0.001
	FCR	1.559[c]	1.710[c]	2.806[a]	2.185[b]	0.169	<0.001
	Mortality, %	2.67	3.00	8.14	0.00	3.726	0.082

[1] **TN-al**: Birds reared under continuous 24°C and ad libitum feeding. **cyHS**: Birds reared under cyclic high temperature (8 h at 35˚C and 12 h at 24˚C) and ad libitum feeding. **coHS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-coPF**: Birds reared under continuous 22°C and pair-fed to the coHS treatment.

[2] SEM: pooled standard error of the mean

[2] BW: Body weight; BWG: Body weight gain; FI: Feed intake; FCR: Feed conversion ratio

[a-b] Means within row without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey's multiple comparison test.

Table 3.3. Carcass characteristics and parts weights and yields of broilers reared under different environmental conditions and feed regimens from 20 to 41 d and processed at 42 d.

Parameter		Treatment[1]				SEM[2]	*P*-values
		TN-al	cyHS	coHS	TN-coPF		
Hot Carcass	**Weight, kg**	2.565[a]	2.143[b]	1.365[c]	1.512[c]	0.0909	<0.001
	Yield[3], %	74.9[a]	75.1[a]	75.1[a]	72.5[b]	0.3005	<0.001
Hot Fat Pad	**Weight, kg**	0.0349[a]	0.0283[b]	0.0213[c]	0.0099[d]	0.0015	<0.001
	Yield, %	1.02[b]	1.00[b]	1.24[a]	0.52[c]	0.0678	<0.001
Chilled Carcass	**Weight, kg**	2.617[a]	2.189[b]	1.414[c]	1.555[c]	0.0897	<0.001
	Yield, %	76.4[b]	77.1[ab]	77.9[a]	74.5[c]	0.0049	<0.001
P. major	**Weight, kg**	0.715[a]	0.552[b]	0.312[d]	0.380[c]	0.0313	<0.001
	Yield, %	20.89[a]	19.30[b]	17.03[c]	18.19[bc]	0.5680	<0.001
P. minor	**Weight, kg**	0.134[a]	0.113[b]	0.068[d]	0.084[c]	0.0042	<0.001
	Yield, %	3.91[a]	3.95[a]	3.71[b]	4.02[a]	0.0740	<0.001
TBM[4]	**Weight, kg**	0.849[a]	0.665[b]	0.379[d]	0.464[c]	0.0352	<0.001
	Yield, %	24.80[a]	23.25[b]	20.75[c]	22.21[b]	0.5940	<0.001
Wings	**Weight, kg**	0.259[a]	0.228[b]	0.161[c]	0.174[c]	0.0090	<0.001
	Yield, %	7.56[d]	8.00[c]	8.91[a]	8.36[b]	0.1030	<0.001
Leg quarters	**Weight, kg**	0.800[a]	0.709[b]	0.457[c]	0.487[c]	0.0291	<0.001
	Yield, %	23.36[b]	24.90[a]	24.67[a]	23.34[b]	0.4480	<0.001

[1] **TN-al**: Birds reared under continuous 24°C and ad libitum feeding. **cyHS**: Birds reared under cyclic high temperature (8 h at 35˚C and 12 h at 24˚C) and ad libitum feeding. **coHS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-coPF**: Birds reared under continuous 22°C and pair-fed to the coHS treatment.

[2] SEM: pooled standard error of the mean

[3] Yields calculated relative to live body weight taken immediately prior to processing

[4] TBM: Total breast meat = P. major + P. minor

[a-b] Means within row without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey's multiple comparison test.

Table 3.4. Pectoralis major muscle myopathy distribution of broilers reared under different environmental conditions and feed regimens from 20 to 41 d and processed at 42 d.

Parameter	Score	Treatment[1]				SEM[2]	*P*-values
		TN-al	cyHS	coHS	TN-coPF		
Woody Breast[3] (%)	**Normal**	28.81^{c}	69.67^{b}	87.54^{a}	100.00^{a}	7.1071	<0.001
	Mild	67.74^{a}	30.33^{b}	12.46^{c}	0.00^{c}	7.6967	<0.001
	Severe	3.45	0.00	0.00	0.00	1.9956	0.073
White Striping[4] (%)	**Normal**	1.67^{c}	2.13^{c}	32.67^{b}	83.99^{a}	4.8876	<0.001
	Faint	93.33^{a}	94.70^{a}	67.33^{b}	16.01^{c}	6.0797	<0.001
	Apparent	5.00	3.17	0.00	0.00	3.7671	0.215

[1] **TN-al**: Birds reared under continuous 24°C and ad libitum feeding. **cyHS**: Birds reared under cyclic high temperature (8 h at 35˚C and 12 h at 24˚C) and ad libitum feeding. **coHS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-coPF**: Birds reared under continuous 22°C and pair-fed to the coHS treatment.

[2] SEM: pooled standard error of the mean

[3] P. major fillets were considered normal, mild, or severe for woody breast if the fillet was flexible throughout, stiff in cranial region, or if stiff in the cranial and caudal regions, respectively

[4] P. major fillets were considered normal, faint, or apparent for white striping if they displayed no visible stripes, stripes less than 1 mm, or stripes larger than 1 mm, respectively

[a-b] Means within row without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey's multiple comparison test.

Table 3.5. Pectoralis major pH_u, L*, a* and b* of broilers reared under different environmental conditions and feed regimens from 20 to 41 d and processed at 42 d.

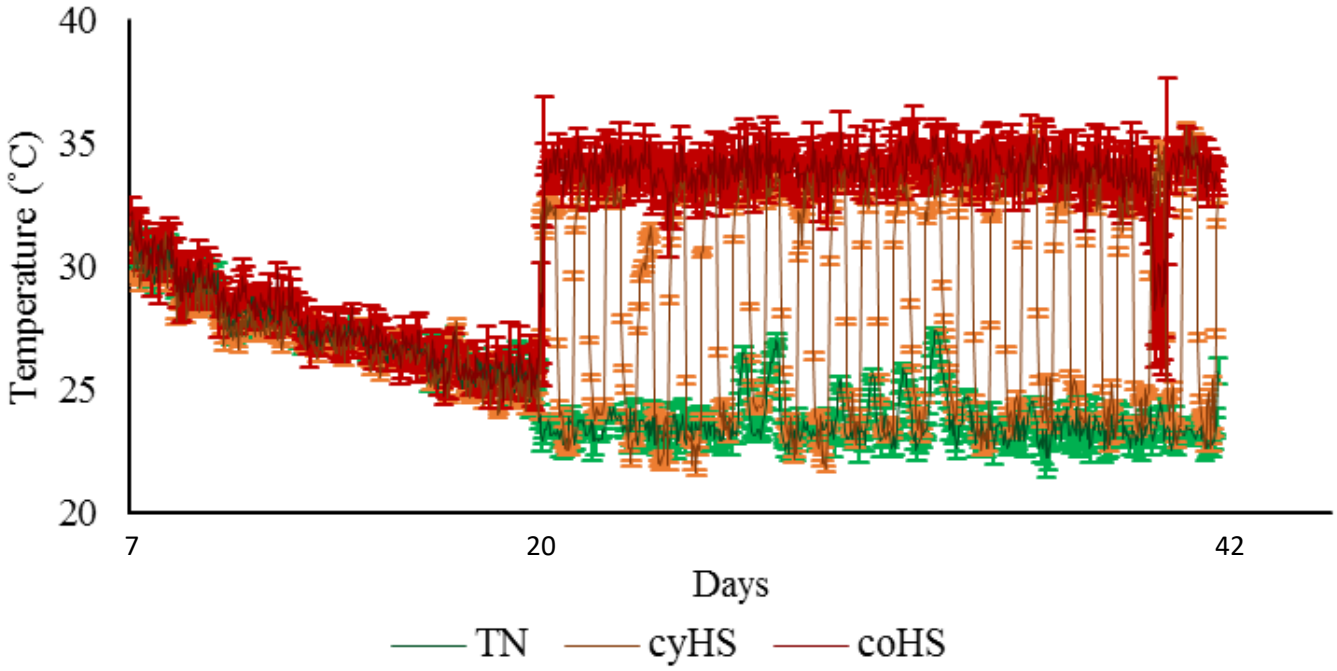
Parameter[3]	Treatment[1]				SEM[2]	*P*-values
	TN-al	cyHS	coHS	TN-coPF		
pH_u	5.89^{ab}	5.94^{a}	5.85^{b}	5.75^{c}	0.039	<0.001
L*	56.5^{b}	56.0^{b}	59.1^{a}	55.0^{b}	0.858	<0.001
a*	2.74	2.43	2.58	2.64	0.233	0.324
b*	9.65^{a}	8.84^{ab}	8.77^{b}	7.07^{c}	0.403	<0.001

[1] **TN-al**: Birds reared under continuous 24°C and ad libitum feeding. **cyHS**: Birds reared under cyclic high temperature (8 h at 35˚C and 12 h at 24˚C) and ad libitum feeding. **coHS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-coPF**: Birds reared under continuous 22°C and pair-fed to the coHS treatment.

[2] SEM: pooled standard error of the mean

[3] pHu: ultimate pH; L*: lightness; a*: redness; b*: yellowness

[a-b] Means within row without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey's multiple comparison test.



TN: Chambers with a continuous 24°C from d20.
cyHS: Chambers with a cyclic high temperature (8 h at 35˚C and 12 h at 24˚C) from d20.
coHS: Chambers with a continuous 35°C from d20

Figure 3.1. Average chamber temperature recorded during the experiment

CHAPTER IV - INFLUENCE OF DIFFERENT HEAT STRESS MODELS ON NUTRIENT DIGESTIBILITY AND MARKERS OF STRESS, INFLAMMATION, LIPID, AND PROTEIN METABOLISM IN BROILERS

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Manuscript under review for publication in Poultry Science

ABSTRACT

This experiment determined the effects of different HS models and pair-feeding (**PF**) on nutrient digestibility and markers of stress, inflammation, and metabolism in broilers. Birds (720 total) were allocated into 12 environmentally controlled chambers and reared under thermoneutral conditions until 20 d. Until 41 d birds were exposed to 4 treatments, including: thermoneutral at 24°C (**TN-al**), daily cyclic HS (12 h at 24 and 12 h at 35°C; **cyHS**), constant HS at 35°C (**coHS**), and PF birds maintained at 24°C and fed to equalize FI with coHS birds (**TN-coPF**). At d 41, ileal digesta were collected to determine nutrient apparent ileal digestibility (**AID**). Blood, liver, and breast tissues were collected from 8 birds per treatment to determine the mRNA expression of stress, inflammation, and metabolism markers. An additional 8 TN-al birds were sampled after acute HS exposure at 35°C for 4 h (**aHS**), and 8 cyHS birds were sampled either right before or 4 h after HS initiation. Data were analyzed by one-way ANOVA and means were separated using Tukey's HSD test. Compared with TN-al birds, AID of nitrogen and ether extract were reduced in coHS birds, and both cyHS and coHS reduced ($P < 0.05$) AID of total essential amino acids. TNFα and SOD2 expression were increased ($P < 0.05$) under aHS, coHS, and TN-coPF conditions. IL6 and HSP70 were increased ($P < 0.05$) under coHS and aHS, respectively. Expression of lipogenic enzymes ACCα and FASN were reduced by coHS and TN-coPF, while coHS increased the lipolytic enzyme ATGL ($P < 0.05$). IGF1 was lowered in coHS birds, and p70S6K and MyoG were reduced under coHS and TN-coPF ($P < 0.05$). Interestingly, MuRF1 and MAFbx were increased ($P < 0.05$) under coHS only. Overall, these results indicate that coHS has a greater impact on nutrient digestibility and metabolism than aHS and cyHS. Interestingly, increased protein degradation during HS appears to be mostly driven by HS *per se* and not the reduced FI.

INTRODUCTION

Average global temperatures are projected to warm by 1.5°C of over the next 20 years, leading to an increased frequency of heat waves, longer warm seasons, and shorter cold seasons (Masson-Delmotte et al., 2021). Climate change has wide-ranging impacts on agriculture and is an important challenge for poultry production (Bhattacharyya et al., 2020), especially given that much of the global increase in poultry production is expected to occur in hot climate areas (Moekti, 2020). Poultry are particularly sensitive to high temperatures due to their feathering and the absence of sweat glands, making panting and wing movement (evaporative cooling) their primary means for heat dissipation. Furthermore, genetic selection for birds with the highest performance, and in turn, the highest metabolic rates and heat production, also contributes to the sensitivity of birds to hot environmental temperatures (Deeb and Cahaner, 1999; Rosa et al., 2007; Lara and Rostagno, 2013). Physiological heat stress (**HS**) occurs when the balance between heat production and dissipation is disturbed and body heat production surpasses heat loss capacities in the surrounding environment (Bernabucci et al., 2010; Akbarian et al., 2016). In an attempt to reduce heat production and its effects, birds generally decrease their feed intake (**FI**) and undergo several metabolism changes that reduce performance and can ultimately increase mortality. These HS-induced physiological changes cause extensive economic losses, making HS one of the most significant challenges for the poultry industry worldwide (St-Pierre et al., 2003; Zhang et al., 2017).

Several models of HS have been used to mimic the different types of high environmental temperature conditions that birds may encounter in the field. We previously presented the effects of chronic cyclic and constant HS on performance, carcass characteristics, and meat quality in broilers (Teyssier et al., 2022b). Cyclic and constant HS reduced FI by 16 and 45%, respectively,

corresponding with 20 and 67% reductions in body weight gain (**BWG**). Observations of birds that were pair-fed the same amount of feed as birds subjected to constant HS showed that approximately 81% of the BWG reduction was caused by decreased FI, with the remaining 19% caused by physiological changes induced by HS *per se*. Similarly, 36% of the reduction in breast meat yield was attributed directly to HS, further reflecting the dramatic potential that HS can have on lean protein accretion in broilers (Teyssier et al., 2022b).

From a nutritional perspective, reduced nutrient digestibility and altered post-absorptive metabolism, including oxidative stress, can impair the performance of HS birds. Under thermoneutral (**TN**) conditions, the production of reactive oxygen species (**ROS**) and the antioxidant systems in broiler chickens are balanced and can adapt to overcome moderate challenges. Major enzymes, including glutathione peroxidase (**GPX**) and superoxide dismutase 1 (**SOD1**) and 2 (**SOD2**), detoxify ROS soon after their formation (Surai et al., 2019). However, acute and chronic HS have been shown to disturb this equilibrium due to an overproduction of ROS that surpasses the bird's antioxidant capacity, leading to oxidative stress (Lin et al., 2000, 2006; Azad et al., 2010a; Akbarian et al., 2016). Another cellular line of defense against HS is the increased synthesis of heat shock proteins (**HSP**), which function as chaperones proteins to inhibit the aggregation of non-native and misfolded proteins. They enhance cellular thermotolerance by modulating apoptotic and antiapoptotic signaling pathways and regulating cellular redox conditions (Shehata et al., 2020). In addition, systemic and local inflammation can occur with high temperatures (Song et al., 2017, 2018). Therefore, HS is an important stressor for poultry, particularly on the liver which is highly susceptible to external stressor due to its important role in avian metabolic activity and has high rates of substrate metabolism and energy expenditure in broilers (Sanchez-Valle et al., 2012; Emami et al., 2020). Indeed, the liver is the

main site of de-novo lipogenesis in avian species (Leveille, 1969), and increased fat deposition of broilers during HS (Geraert et al., 1996; Faria Filho et al., 2007; Zeferino et al., 2016; Lu et al., 2019b; Teyssier et al., 2022b) could be attributed to hepatic stress (Emami et al., 2020). In contrast to the increased energy deposition as lipid, HS results in a lower protein deposition (Temim et al., 2000; Faria Filho et al., 2007; Zhang et al., 2012; De Souza et al., 2016). Therefore, alterations in bird metabolism play an important role in the degradation of bird performance.

This study aimed to characterize the effects of 3 HS models: constant, cyclic, and acute, and one pair-feeding treatment on nutrient digestibility and markers of stress, inflammatory, and metabolism in broilers. We hypothesized that the expression of some heat shock proteins, cytokines, and HS markers will be differently impacted depending on the HS model. Our previous study revealed that a large proportion of the decrease in breast meat yield and increase in fat pad deposition was not explained by the decrease in FI, and thus, this paper addresses markers of protein and lipid metabolism. We also hypothesized that constant HS will have a greater impact on digestibility and cause more changes in protein and lipid synthesis/degradation pathways than reduced FI alone.

MATERIALS AND METHODS

All procedures involving live animals were approved by the Institutional Animal Care and Use Committee at the University of Arkansas. Descriptions of the facilities, diets, and animals, as well as the results for growth performance, carcass characteristics, and meat quality of broilers used in this experiment were previously reported by Teyssier et al. (2022). A brief summary and additional information on our experimental design as well as the methodology

applied for the measurement of nutrient digestibility, stress, lipid, and protein metabolism markers are provided below.

Animals and Experimental Design

Seven hundred twenty male chicks from a Cobb 500 female breeder line were distributed into 12 environmentally controlled chambers with 60 chicks per chamber at 0 d post-hatch. Each chamber was divided by wire paneling into 2 pens of 4.47m2, with each equipped with 2 hanging pan feeders and nipple waters. Birds were kept under standard conditions from d 0 to d 20, with the ambient temperatures of all the chambers gradually decreasing from 32 to 24°C on d 20. The experimental period was set from d 20 to d 41.

On d 20, an appropriate number of birds were removed from each pen to obtain groups of 25 birds/pen with similar weights across all treatment groups. From d 20 to 41, 3 different environmental conditions and 1 pair-fed (**PF**) treatment were applied. These included TN birds fed ad-libitum and kept at a constant 24°C temperature (**TN-al**); cyclic HS (**cyHS**) birds fed ad libitum in 4 cyclic HS chambers for which the temperature was maintained at 35°C for 12 h daily (from 7:30 to 19:30) and reduced to 24°C each night; constant HS (**coHS**) birds fed ad libitum in 4 chambers for which the temperature was set to and maintained at 35°C; and a group kept in TN chambers and PF to equalize feed intake to that of coHS bird (**TN-coPF**). Moreover, at sampling at 41 d, cyHS birds were sampled during both the early morning at the end of the cool phase (**cyHS$_{cool}$**) and 4 hours after the beginning of the hot phase (**cyHS$_{hot}$**). Additionally, 2 birds from each TN-al pen were randomly selected and exposed at 35°C for 4 h to assess acute HS (**aHS**) in birds not previously exposed to HS. Birds were euthanized by CO_2 inhalation and immediately dissected for whole blood and pectoralis major (**P. major**) collection.

Apparent Ileal Digestibility

On d 41, four birds per replicate from the TN-al, cyHS, coHS, and TN-coPF groups were randomly selected for sampling. Birds were euthanized by CO_2 inhalation and immediately dissected for digesta collection. One-half of the ileum proximal to the ileocecal junction was flushed for collection of digesta samples, which were pooled per replicate, placed on ice, frozen, lyophilized, and ground using an electric coffee grinder. Dry matter (**DM**), ether extract (**EE**), nitrogen (**N**) concentration, and gross energy (**GE**) content of the diet and digesta were analyzed by a commercial laboratory (ATC scientific, Little Rock, AR). Amino acid (**AA**) concentrations were analyzed at the University of Arkansas with a high-performance liquid chromatography instrument (Waters Corporation, Milford, MA; methods 994.12; AOAC International, 2005).

Titanium dioxide concentration, used as an indigestible marker, was determined according to the procedures of Short et al. (1996). Apparent ileal digestibility (**AID**) of DM, EE, N, GE, and AA was calculated using the following formula:

$$AID, \% = \frac{\left({}^{X}/_{TiO_2}\right)_{diet} - \left({}^{X}/_{TiO_2}\right)_{digesta}}{\left({}^{X}/_{TiO_2}\right)_{diet}} \times 100\,,$$

where ${}^{X}/_{TiO_2}$ = ratio of nutrient or energy concentration (%) to TiO2 (%) in the diet or ileal digesta. Energy digestibility (%) values obtained from the equation above were multiplied by the gross energy content of the diet to calculate apparent ileal digestible energy (**IDE**) in units of kcal/kg.

Blood and Pectoralis Major Sampling

Blood was collected from the heart immediately post-mortem, from 8 birds per group. One drop of whole blood was placed in TRIzol reagent (catalog #15596018, Life Technologies, Carlsbad, CA) for RNA extraction, snap-frozen in liquid N, and stored at -80°C for further analysis on the mRNA expression of HSP, cytokines, and oxidative stress markers. Moreover, approximately 3mL of blood was stored on ice in heparin tubes before being centrifuged at 1300 x g for 15 min at 4°C and plasma was harvested. Plasma samples were stored at -80°C for subsequent analysis of total protein (**TP**) and uric

acid (**UA**) concentration. Additionally, a small sample (approximately 2 grams) of the left P. major muscle was collected, and frozen in liquid N and stored at -80°C for future mRNA expression analysis.

Gene Expression in the Whole Blood and Pectoralis Major

Total RNA was extracted from the whole blood and P. major muscle using Trizol reagent (catalog #15596018, Life Technologies, Carlsbad, CA) according to the manufacturer's recommendations. For each sample, RNA concentrations and purity were determined by Take3 Micro-Volume Plate using a Synergy HT multimode microplate reader (BioTek, Winooski, VT). Ribonucleic acid samples were RQ1 DNase treated and reverse transcribed using qScript cDNA Synthesis SuperMix (catalog #95048-100, Quanta Biosciences, Gaithersburg, MD). The RT products (cDNA) were amplified by real-time quantitative PCR (Applied Biosystems 7500 Real-Time PCR System) with PowerUp SYBR green master mix (catalog #4312074, Life Technologies, Carlsbad, CA) as previously described (Rajaei-Sharifabadi et al., 2017; Greene et al., 2021a). Relative expressions of target genes were determined by the $2^{-\Delta\Delta Ct}$ method and normalization was performed with the 18S rRNA as a housekeeping gene (Schmittgen and Livak, 2008). Oligonucleotide primer sequences specific for HSP27, HSP60, HSP70, HSP90, SOD1, SOD2, GPX1, IL6, IL10, IL18, tumor necrosis factor alpha (**TNFα**), C-reactive protein (**CRP)**, adipose triglyceride lipase (**ATGL**), lipoprotein lipase (**LPL**), acetyl-CoA carboxylase (**ACC**) alpha, ATP-citrate lyase (**ACLY**), fatty acid synthase (**FASN**), insulin like growth factor 1 (**IGF1**), phosphoinositide 3-kinase (**PI3K**), extracellular signal-reduced protein kinase 1 (**ERK1**), AKT Serine/Threonine Kinase (**AKT**) 1, AMP-activated protein kinase (**AMPK**) alpha 1, mammalian target of rapamycin (**mTOR**), ribosomal protein S6 kinase (**p70S6K**), myogenic factor (**MyoG**), muscle RING-finger protein (**MuRF**) 1, and muscle atrophy F-box (**MAFbx**), also known as atrogenin-1 are presented in **Table 1**.

Total Protein and Uric Acid Concentrations in the Plasma

Uric acid and TP concentrations were measured in the plasma by a commercial laboratory (Veterinary Medical Diagnostic Laboratory, University of Missouri, Columbia, MO).

Statistical Analysis

All data were subjected to a one-way ANOVA and means were separated using a Tukey's HSD test. Digestibility data were available for 4 treatments with 4 replicates per treatment, with each replicate corresponding to a pool of the 4 sampled birds from the same pen. Gene expression data analysis was performed on 6 treatments of 4 replicates, corresponding to the average value of the 2 sampled birds per replicate. Gene expression data were log-transformed to achieve a normal distribution for statistical analysis. Those data are presented as antilog of the geometric means and upper and lower 95% confidence interval of the log-transformed data (Olivier et al., 2008). Model residuals were inspected for outliers using histograms and QQ-plots. Mean differences were considered significant when the p-value < 0.05, and analysis was performed using R (RStudio 2022.07.2).

RESULTS

The complete performance, carcass characteristics, and meat quality results from this experiment can be found in Teyssier et al. (2022b). A summary of these data is presented in **Table 2**.

Apparent Ileal Digestibility

Apparent ileal digestibility values (%) of DM, EE, N, and energy, as well as ileal digestible energy values (kcal/kg) are presented in **Table 3**. The different environmental conditions did not affect the DM AID ($P = 0.428$), but the EE AID was decreased ($P = 0.039$) under coHS compared to TN-coPF conditions, and the N AID was decreased ($P = 0.032$) under coHS compared to TN-al conditions. No effects were observed on energy AID values expressed as percentage digestibility ($P = 0.417$) or IDE expressed in kcal/kg of diet ($P = 0.415$).

In our study, AID values of total AA (essential plus non-essential AA) for broilers under coHS and cyHS were 4.4% units lower ($P = 0.001$; **Table 4**) than those of TN-al birds and intermediate for TN-coPF birds (-2.3% units). The same trend was observed for total essential

AA digestibility ($P < 0.001$) and AID of Met ($P = 0.005$) and Lys ($P = 0.017$), but different responses were found among other individual essential AA. Specifically, leucine ($P = 0.026$) and histidine ($P = 0.027$) digestibility values were reduced under coHS compared to TN-al conditions and intermediate for cyHS and TN-coPF birds, and phenylalanine AID was reduced ($P < 0.001$) under cyHS and coHS compared to TN-al and TN-coPF conditions. Additionally, the AID of isoleucine ($P = 0.049$) was different among the treatments, but the Tukey HSD test did not result in separation of those means. Threonine ($P = 0.189$), valine ($P = 0.074$), and arginine ($P = 0.243$) AID were not affected by the different environmental treatments.

Regarding the total non-essential AA, and as observed with the essential AA, a decrease ($P = 0.005$) in AID was observed under cyHS and coHS compared to TN-al conditions, with intermediate values for AID of non-essential AA for TN-coPF birds. Alanine ($P = 0.043$) and tyrosine ($P = 0.030$) AID was reduced under coHS compared to TN-al conditions, and values for cyHS and TN-coPF birds were intermediate. Additionally, the AID of glutamic acid ($P = 0.001$) and serine ($P = 0.028$) was reduced under both coHS and cyHS compared to TN-al conditions, with the TN-coPF treatment presenting intermediate values. Also, the AID of aspartic acid ($P = 0.006$) and proline ($P = 0.031$) was only reduced under cyHS compared to TN-al conditions, and the AID of those two AA was intermediate under cyHS and TN-coPF. Finally, no difference was observed among treatments for cysteine ($P = 0.089$) or glycine ($P = 0.068$) AID, despite large numerical differences in AID values between coHS and TN-al groups (-9.2% and -5.5% units, respectively).

Gene Expression of Stress and Inflammatory Markers in the Whole Blood

Gene expression data for HSP and oxidative stress (SOD1, SOD2, and GPX1) markers in whole blood, including the aHS group for which digestibility data were not determined, are

presented in **Figure 1**. Compared to TN-al conditions, aHS increased blood gene expression of HSP70 ($P = 0.018$), and HSP90 tended to increase ($P = 0.058$) under coHS conditions. Both HSP27 ($P = 0.152$) and HSP60 ($P = 0.110$) were unaffected by the environmental conditions. For the oxidative stress markers, SOD2 gene expression was increased ($P = 0.010$) by aHS, coHS, and TN-coPF conditions, but no difference was observed for the gene expression of SOD1 ($P = 0.074$) and GPX1 ($P = 0.180$) among groups.

Concerning the inflammation markers presented in **Figure 2**, aHS, coHS, and TN-coPF conditions increased ($P < 0.001$) the gene expression of TNFα, and coHS birds had a higher ($P = 0.005$) CRP gene expression compared to $cyHS_{cool}$ and $cyHS_{hot}$ birds. Environmental condition did not affect IL10 ($P = 0.099$) or IL18 ($P = 0.245$) gene expression, but IL6 was upregulated ($P = 0.009$) under coHS compared to TN-al conditions.

Gene Expression of Lipid Metabolism Markers in the Liver

Gene expressions of lipid metabolism markers are presented in **Figure 3**. Constant HS increased ($P < 0.001$) ATGL expression and decreased ACCα ($P < 0.001$), ACLY ($P = 0.010$), and FASN ($P = 0.010$) expression compared to TN-al conditions, with ACCα and FASN also decreased for TN-coPF birds. LPL gene expression was increased ($P = 0.002$) under TN-coPF compared to aHS and cyHS conditions. Compared to TN-al birds, except for the lower ($P = 0.010$) gene expression of ACLY under aHS conditions, none of the lipid markers were affected by the aHS, $cyHS_{cool}$, and $cyHS_{hot}$ treatments.

Gene Expression of Protein Metabolism Markers in the Pectoralis Major Muscle

Gene expressions of protein metabolism markers are presented in **Figure 4**. Upstream of the mTOR pathway, IGF1 gene expression was decreased ($P = 0.001$) by coHS, and PI3Kα gene expression was increased ($P < 0.001$) by aHS, cyHShot, coHS, and TN-coPF compared to TN-al

conditions. However, treatments did not affect the gene expression of ERK1 (P = 0.857), AKT1 (P = 0.707), AMPKα1 (P = 0.106), or mTOR (P = 0.894). Both coHS and TN-coPF conditions reduced the gene expression of p70S6K (P < 0.001) and MyoG (P <0.001), as well as $cyHS_{cool}$ for p70S6K. In addition, TN-coPF birds had a higher MyoG gene expression than coHS birds. Gene expression of the two protein catabolism markers MuRF1 and MAFbx was increased (P < 0.001) by coHS compared to TN-al conditions, but not affected by the other treatments.

Total Protein and Uric Acid Concentrations in the Plasma

No differences in TP (P = 0.146) and UA (P = 0.101) concentrations in the plasma were observed among the different environmental conditions (**Figure 5**).

DISCUSSION

Apparent Ileal Digestibility

Heat stress impairs nutrient digestibility by compromising intestinal barrier function, reducing upper gastrointestinal tract blood flow, and lowering expression and activity of digestive enzymes and several macronutrient transporters (Brugaletta et al., 2022; Teyssier et al., 2022a). In broilers, Bonnet et al. (1997) and De Souza et al. (2016) observed reductions of 1.6% and 3.9% in DM digestibility under constant HS, and Laganá et al. (2007) reported 1.6% lower DM digestibility under cyclic HS compared with TN bird. Although a similar magnitude of reduction in DM digestibility was observed in our experiment, this reduction was not statistically different due to the observed variability. Other studies have also reported no DM digestibility losses due to HS (Faria Filho et al., 2007; Seven and Seven, 2008; Attia et al., 2017). In addition, no difference was obtained in AID energy in our experiment, which is similar to what was observed in other studies reporting apparent metabolizable energy (Bonnet et al., 1997; Faria Filho et al., 2007; De Souza et al., 2016).

We observed a 5.3% reduction in ileal N digestibility under coHS in the current experiment. Other studies have reported decreases in ileal or fecal N digestibility ranging between 1.5% and 10% in broilers reared under hot temperatures (Zuprizal et al., 1993; Bonnet et al., 1997; Seven and Seven, 2008; Soleimani et al., 2010; Attia et al., 2017), whereas other authors observed no effects on N digestibility (Laganá et al., 2007; Faria Filho et al., 2007; Habashy et al., 2017b; Ghareeb et al., 2022). Similar to our study, De Souza et al. (2016) observed a significant decrease in AID of N under constant HS that was not observed under cyclic HS, reflecting that the magnitude or duration of HS can influence its effects on N digestibility. These authors also observed no difference in N digestibility between PF and HS conditions. However, similarly to De Souza et al. (2016), fat digestibility was reduced under constant HS compared to PF conditions indicating that in contrast with proteins, fat digestibility may be more directly impacted by the heat *per se* than the reduced FI.

Our study also revealed the detrimental effect of HS on AA digestibility, with an average reduction of 5% in AID of total AA. Standardized and apparent digestibility values of several AA (i.e., Arg, His, Thr, Val, Lys, Ile, Leu, Phe, Cys, Gly, Ser, Ala, Pro, and Tyr) were also reduced by approximately 5.5% in a study by Soleimani et al. (2010). Similarly, Wallis and Balnave (1984) observed a slight decrease in the digestibility for Thr, Ala, Met, Ile, and Leu under coHS at 31°C, but Habashy et al. (2017a) did not observe any effects on AA digestibility under constant HS at 35°C. Despite the relatively greater decrease in AA digestibility compared to other macronutrients, several studies observed no influence of HS exposure on expression of AA transporters (Sun et al., 2015; Habashy et al., 2017b; Al-Zghoul et al., 2019). However, Habashy et al. (2017a) reported a decrease in expression of several AA transporters (Habashy et

al., 2017a), but this reduction was not consistent with the slight increase in AA digestibility (+3%) observed in the same study.

The reduction in FI appeared to be the main factor explaining the performance degradation induced by HS and accounted for 81% of the BWG reduction for coHS birds, whereas reduced nutrient digestibility appears to have only minimally contributed to impaired feed efficiency under HS. Therefore, a more important role may be played by alterations in post absorptive nutrient utilization pathways, especially regulatory mechanisms of protein metabolism, as TBM yield were highly affected by both cyHS (-6.3%) and coHS (-16.3%) in the current experiment.

Gene Expression of Stress and Inflammatory Markers in the Whole Blood

Heat stress is known to be a major stressor leading to excess ROS production. Both SOD and GPX are major antioxidant enzymes that detoxify and decompose free radicals and non-radical toxic products (Surai et al., 2019). Superoxide dismutase enzymes are involved in the conversion of superoxide free radicals into hydrogen peroxide ($\mathbf{H_2O_2}$) and molecular oxygen (Fridovich, 1995; Surai et al., 2019). Research in broiler chickens has shown that SOD enzyme activity was increased in plasma, heart, liver, and skeletal tissues under acute and chronic HS (Lin et al., 2000, 2006; Tan et al., 2010; Yang et al., 2010; Azad et al., 2010b; Ghazi Harsini et al., 2012; Huang et al., 2015; Habashy et al., 2019). However, the influence of HS on SOD activity is probably organ and time-dependent, as other studies did not observe increased SOD activity or mRNA expression under high temperatures (Lin et al., 2000; Willemsen et al., 2011; Xie et al., 2014; Rimoldi et al., 2015; Cramer et al., 2018; Emami et al., 2021; Chen et al., 2023). In our study, we found that blood mRNA expression of SOD2, the manganese-dependent SOD, was increased under aHS, coHS, and TN-coPF conditions, which could imply that SOD2

expression is upregulated by acute exposure to high temperature and prolonged feed restriction. Similar to our findings, Greene et al. (2021b) did not observe increased blood SOD2 mRNA expression in broilers reared under cyclic HS, although SOD1 mRNA expression, the copper and zinc-dependent SOD, was increased by cyclic HS. After SOD enzymes convert superoxide into H_2O_2, GPX enzymes catalyze the reduction of H_2O_2 into water and convert lipid peroxides to their corresponding alcohols (Ighodaro and Akinloye, 2018). In our study, HS did not influence GPX1 mRNA expression, which is similar to the observations of Greene et al. (2021b) and Emami et al. (2021) of broilers under cyclic HS, and Azad et al. (2010a; b) on GPX activity for broilers under constant HS. However, GPX activity was increased in the blood and liver of broilers by acute HS (Yang et al., 2010), and decreased in the blood and skeletal muscle by chronic HS (Huang et al., 2015; Cramer et al., 2018). Collectively, these responses in SOD and GPX indicate that metabolism of birds exposed to HS may be altered in attempt to compensate for the increased production of ROS. However, the response might be organ-dependent, with whole blood being potentially less responsive than other tissues and differences also due to the cyclicity, temperature, and duration of the stress.

Heat shock proteins are another defense mechanism to maintain the function and structural integrity of cells in response to HS. Among the HSP family, the four measured in this experiment are the most extensively researched (Shehata et al., 2020) and are believed to have a role in HS response due to their chaperone activity that allows for the folding and unfolding of stress-denatured proteins (Archana et al., 2017). In our study, only aHS increased HSP70 mRNA expression and HSP90 tended to be upregulated under coHS. In broilers, increased expression of HSP70 and HSP90 have also been observed in different tissues under acute and chronic HS (Gabriel et al., 1996; Xie et al., 2014; Flees et al., 2017; Baxter et al., 2020; Emami et al., 2021).

Interestingly, both these HSP are widely expressed in chicken breeds that are naturally exposed to high temperatures and proven to be resistant to HS (Cedraz et al., 2017). Several studies also indicate that HSP70 plays an important role in preventing deleterious effects caused by oxidative stress (Guo et al., 2007; Hao et al., 2012). Even though we did not observe treatment effects on HSP27 and HSP60 in our study, other studies reported upregulation of these HSP under chronic HS in different tissues of broilers (Liu et al., 2014; Cramer et al., 2018; Emami et al., 2021; Greene et al., 2022), with differences among studies like due to variations in intensity and duration of HS and individual bird variability.

Heat stress has also been shown to impact production of several cytokines that regulate inflammatory and anti-inflammatory processes. In birds, TNFα and IL6 are pro-inflammatory cytokines, IL10 is an anti-inflammatory cytokine, and IL18 can have both inflammatory or anti-inflammatory effects based on the duration of the stress (Shini and Kaiser, 2009). In our study, IL6 was the only interleukin upregulated by HS, which occurred during constant exposure only, while IL10 and IL18 were not affected by the environmental conditions. Increased mRNA expression of some interleukins in the blood, spleen, and digestive tract tissues of broilers following HS has also been reported in other studies (Ohtsu et al., 2015; Varasteh et al., 2015; He et al., 2019b; Baxter et al., 2020), while some other authors found no response or a decrease in the mRNA expression of some circulating interleukins (Greene et al., 2021b; Emami et al., 2021). Compared to other cytokines, TNFα seems to more consistently respond to HS across studies (Alhenaky et al., 2017; He et al., 2019b; Baxter et al., 2020; Greene et al., 2021b; Alzarah et al., 2021; Emami et al., 2021), and our results suggest that among the inflammation markers measured in our study, TNFα appeared to be the most sensitive cytokine marker to both acute and constant HS conditions. Interestingly, beyond its pro-inflammatory effect, TNFα has

also been shown to decrease muscle response to IGF1 (Frost and Lang, 2007), and promising results indicate that TNFα interacts with the antioxidant system by repressing SOD1 gene (Afonso et al., 2006). Moreover, the different HS conditions tested in our study did not influence mRNA expression of CRP, an acute-phase protein activated by pro-inflammatory cytokines. As explained for interleukins, CRP responses to HS have been shown to vary across studies (Baxter et al., 2020; Greene et al., 2021b).

Overall, compared to TN-al conditions, aHS and coHS caused a greater stress response than cyHS, as evidenced by increased levels in HSP70, SOD2, and TNFα under aHS conditions, and higher expression of HSP90, SOD2, TNFα, and IL6 under coHS. The absence of effects of cyHS in our study seems to indicate that cyclic exposure to hot temperature may only have moderate effects on the expression of the measured HSP, inflammatory, and oxidative stress markers. Furthermore, similar mRNA expression profiles in aHS birds and $cyHS_{hot}$ birds, which were both sampled 4h after HS exposure on the sampling day, indicate that all the stress and inflammatory markers measured in our study were not subject to physiological adaptation due to chronic HS exposure. Also, similar responses between coHS and TN-coPF birds suggest that those markers were not specific to HS *per se* but may have been associated with reduced FI. Interestingly, for all the stress and inflammation markers measured in our study, the lowest variability was generally obtained with the TN-al group, while the highest variability was observed in the coHS or aHS groups. This may reflect varying capacities of individual birds to adapt and cope with HS via the pathways evaluated in this experiment.

Gene Expression of Lipid Metabolism Markers in the Liver

In birds, *de novo* lipogenesis within the liver plays an important role in lipid metabolism since this pathway is responsible for most of the endogenous body lipids (Goodridge and Ball,

1967; O'Hea and Leveille, 1968). The ACLY enzyme converts citrate into acetyl-CoA, which is in turn converted into malonyl-CoA by ACC and used by the FASN enzyme for fatty acid biosynthesis by condensation with acetyl-CoA or fatty acyl-CoA (Smink, 2012). In the current study, mRNA expression of these three enzymes was decreased under coHS, and ACLY was reduced under aHS. This results appears contradictory to the observed increased abdominal fat at the carcass level (Teyssier et al., 2022b), as well as the up-regulation of FASN and ACC mRNA levels observed under less intensive cyclic HS conditions (Kim et al., 2022) and after 7 or 14 days of constant HS (Lu et al., 2017, 2019a; b; c). Interestingly, as observed in our study, Flees et al. (2017) measured a reduction in the mRNA expression of these enzymes under constant and acute HS, but did report an increase in protein levels for ACLY under constant HS, and ACLY and FASN under acute HS. Comparable changes were observed by Lu et al. (2019a; b; c) at the protein level with an increase in ACC and FASN expression under constant HS, and a non-targeted transcriptomic and metabolomic analysis indicated an increase in ACC levels under cyclic HS compared to TN conditions (Jastrebski et al., 2017). Therefore, the reduction in gene expression observed for these markers may not directly reflect protein expression and is an important limitation to the interpretation of genomic data. Heat stress might affect the regulation of transcription and translation processes, and the pool of existing lipogenic mRNA could be quickly translated or degraded while the proteins accumulate due to their high half-life (Schwanhäusser et al., 2011; Flees et al., 2017). Additionally, we observed similar levels of mRNA expression under coHS and TN-coPF treatments, which is in agreement with findings of Lu et al. (2019b) in birds after 14 d of HS exposure, indicating that mRNA expression regulation of these lipogenic enzymes is not induced by the heat *per se*, but rather by the decreased FI. However, regulation at the protein level could be directly related to the increase in temperatures

as suggested by research from Lu et al. (2019b), who obtained a lower protein expression of FASN and ACC under PF compared to HS conditions.

Lipoprotein lipase is also associated with fat deposition (Fried et al., 1993; Cai et al., 2009), as it breaks down triglycerides found in chylomicrons and very low-density lipoproteins into free fatty acids, monoglycerides, and other by-products, which can then either be stored or used by the body (Fraser et al., 1986; He et al., 2018; Papah and Abasht, 2019). Although Flees et al. (2017) observed a decrease in LPL expression in birds exposed to constant HS, there were no differences in LPL mRNA expression between any HS treatments and TN-al conditions in our study. Nevertheless, as observed with lipogenic enzymes, contrasting results have been measured at the protein level, with an increased LPL activity in the adipose tissue of birds exposed to constant HS (Lu et al., 2019a), which again highlights the potential differences between mRNA expression and protein activity or expression for markers of lipid metabolism.

Concerning lipid degradation, the ATGL enzyme catalyzes the initial step in triglyceride hydrolysis, which releases a diglyceride and a non-esterified fatty acid (Zimmermann et al., 2004). Under coHS, we observed an upregulation of the mRNA expression of ATGL, which agrees with the study of Flees et al. (2017) for HS birds maintained at 35°C for 3 weeks. However, this is contradictory to the increase in fat accumulation observed for HS birds in these studies, and with the reduction in mRNA expression of ATGL observed by Huang et al. (2021) after exposure of preadipocyte cells at 41.5°C compared to 37°C for 2 and 8 days. Therefore, further research investigating lipolytic enzyme expression in liver and adipocyte tissues is still required to understand their role under HS conditions.

Ultimately, the mRNA expression results of the different markers associated with lipogenesis and lipolysis do not explain the results at the carcass level which indicate that

chronic HS birds are energy-deficient due to the reduced FI but are prone to effectively store fat (Teyssier et al., 2022b). Similarly, gene expression results do not corroborate with the results at the protein level from other studies and regulation mechanisms during the translation and/or traduction processes need to be further investigated.

Gene Expression of Protein Metabolism Markers in the Pectoralis Major Muscle

Several markers of protein metabolism were measured to explain the decrease in total breast meat yield observed under chronic HS and PF conditions in the first part of our study (Teyssier et al., 2022b), as well as the reduced protein deposition and protein synthesis observed under HS in other studies (Temim et al., 2000).

In broiler chickens, IGF1 is known to play an important role in muscle hypertrophy and hyperplasia during development and is consequently considered a marker of muscle growth and protein synthesis (McMurtry et al., 1997; Guernec et al., 2003; Duclos, 2005). Interestingly, IGF1 can also affect protein degradation by limiting the expression of the two ubiquitin ligase enzymes, MuRF1 and MAFbx (Stitt et al., 2004; Sacheck et al., 2004). In agreement with the reduced protein deposition caused by hot temperatures, we observed that coHS reduced IGF1 mRNA expression in chicken breast. Similarly, other studies found that constant HS, but also cyclic HS, results in a decrease in IGF1 mRNA expression in the breast and the liver, as well as in IGF1 blood concentration (Liu et al., 2014; Zuo et al., 2015; Roushdy et al., 2018; Ma et al., 2018). However, some studies have reported no differences in IGF1 expression or blood concentration under different chronic HS conditions (Chang et al., 2020; Ma et al., 2021a). Additionally, contrary to what was observed in our study, other studies have reported that PF conditions increased mRNA expression of IGF1 over the corresponding HS condition,

suggesting that the heat *per se* could directly affect IGF1 expression (Zuo et al., 2015; Ma et al., 2018).

The binding of IGF1 to its receptor activates several intracellular signaling cascades including the PI3K/AKT and MAPK/ERK pathways (Weeks et al., 2017; Józefiak et al., 2021). The activation of PI3K, which is involved in the control of cell proliferation and apoptosis, promotes phosphorylation of AKT, which subsequently activates mTOR (Hemmings and Restuccia, 2012). It can also inhibit the activation the MURF1 and MAFbx enzymes (Del Vesco et al., 2015) and promote fat deposition in broilers by activation of FASN expression (Chen et al., 2022). On the other hand, MAPK/ERK pathway activation also promotes cell differentiation and proliferation (Józefiak et al., 2021), and recent evidence shows that ERK is involved in hepatic lipogenesis in broilers (Greene et al., 2020; Ramser and Dridi, 2022). Complex interactions exist between these pathways and both have been shown to regulate each other via cross-inhibition and cross-activation (Mendoza et al., 2011). Interestingly, the increase in IGF1 expression observed under coHS in the current study did not result in increased PI3K expression. Conversely, PI3K mRNA expression was increased under aHS, $cyHS_{hot}$, coHS, and TN-coPF birds compared with TN-al birds. Zuo et al. (2015) also observed an increase in PI3K mRNA expression in the thigh muscle under constant HS, while IGF1 was not increased in the same tissue. However, the PI3K expression in the breast was reduced, corresponding with a reduction in IGF1 expression in the same tissue. The upregulation of PI3K observed in our study could be a negative feedback mechanism caused by reduced IGF1 expression or increased protein degradation markers to balance the loss of protein caused by HS and enhance survival (Zuo et al., 2015). Additionally, no difference on AKT, ERK, and mTOR mRNA expression was observed in the current study, while Li et al. (2021) reported lower AKT and mTOR mRNA

expression under constant HS. However, some research have shown that the quantity of phosphorylated, (i.e., activated) ERK and AKT was increased in the spleen of birds exposed to cyclic HS, while mRNA and protein expression of mTOR, PI3K, and ERK were not (He et al., 2019b), highlighting that protein phosphorylation mechanisms also play an important role in the regulation of the expression of those protein markers.

With mTOR, AMPK is considered a master regulator of cell metabolism that controls autophagy, protein synthesis, metabolism, and mitochondrial function (Garza-Lombó et al., 2018). The interaction between AMPK and mTOR is complex, but it is considered that the activation of AMPK leads to the inhibition of mTOR activity (Agarwal et al., 2015). AMPK also affects lipid metabolism by negatively regulating ACC activity and hepatic lipid content, with the inhibition of AMPK leading to increased hepatic lipid accumulation (Zang et al., 2004). Inconsistent results have been obtained under HS, with some researchers observing increased AMPK mRNA expression and phosphorylation in the breast under chronic HS (Lu et al., 2017; Chen et al., 2023), or similar to our study, no effect or downregulation under acute and chronic HS in the hypothalamus or liver (Rajaei-Sharifabadi et al., 2017; Lu et al., 2019b).

In chickens, muscle fiber numbers are determined during embryonic development and cannot be increased. Subsequent muscle growth occurs via hypertrophy, with fiber size increasing as a result of protein deposition (Nawaz et al., 2021). Downstream of the mTOR pathway, p70S6K plays an important role in protein synthesis and muscle hypertrophy (Zanchi and Lancha, 2008). In our study, mRNA expression of p70S6K was reduced in the breast muscle under cyHS$_{cool}$, coHS, and TN-coPF conditions, which is in agreement with results from other studies under constant HS (Zuo et al., 2015; Ma et al., 2018, 2021a; Lu et al., 2019a). In addition, in agreement with Zuo et al. (2015), we did not observe differences between the coHS

and TN-coPF treatments, whereas Ma et al. (2018, 2021a) observed an intermediate expression under PF conditions compared to TN and constant HS conditions. Nevertheless, reduced p70S6K expression observed under constant HS appears to be mainly caused by reduced FI. Myogenin is a protein from the family of myogenic regulatory factors (MRFs) that controls the determination and differentiation of myocytes into myoblasts and myotubes cells during embryogenesis and postnatal myogenesis (Hernández-Hernández et al., 2017; Nawaz et al., 2021). Its direct involvement in muscle cell differentiation makes MyoG the most direct marker of protein synthesis among the pool of markers measured in our study. The reduction in MyoG mRNA expression observed under coHS is in agreement with the reduction observed in IGF1 mRNA expression, which has been reported to be a positive regulator of myogenesis (Clemmons, 2009). Other researchers have observed a decrease in MyoG mRNA expression in embryos exposed to high temperatures (Gabriel et al., 2003), in the breast muscle of growing birds under constant HS (Ma et al., 2018), and in the liver of birds subjected to cyclic HS (Zaglool et al., 2019). Therefore, it seems that HS reduces muscle hypertrophy through downregulation of pathways downstream from mTOR, including p70S6K, which could regulate MRFs expressions (Ma et al., 2018). Also, although we observed an increase in MyoG mRNA expression under TN-coPF conditions compared to coHS conditions, the magnitude of the decrease in p70S6K and MyoG seems to indicate that most of the reduction in protein synthesis is explained by reduced FI.

Protein degradation in chickens is mainly induced by the ubiquitin–proteasome pathway and associated with the increased expression of MAFbx and MuRF1, which catalyze the ubiquitination and degradation of key muscle proteins (Lecker et al., 1999; Sacheck et al., 2004). In the current study both MAFbx and MuRF1 were highly expressed under constant HS, with no differences in expression of these markers among other treatments. These findings are in

agreement with other studies reporting increased MuRF1 and/or MAFbx mRNA expression in muscles of broilers reared under constant HS (Zuo et al., 2015; Lu et al., 2019a; Ma et al., 2021a; b; Li et al., 2021). Nonetheless, Ma et al. (2021a) did not observe an effect on MuRF1 expression, and Zuo et al. (2015) did not observe an effect on these markers in the breast muscle but did observe differences in the thigh muscle. In agreement with our finding, Temim et al. (2000) directly measured an increase in protein degradation under constant HS, which could be at least partly explained by increased expression of MuRF1 and MAFbx. During HS, protein degradation may serve to mobilize AA as substrates for gluconeogenesis in the liver (Ma et al., 2021a). Additionally, in agreement with our findings, all the previously mentioned studies (Zuo et al., 2015; Lu et al., 2019a; Ma et al., 2021b; Li et al., 2021) observed increased MuRF1 and MAFbx mRNA expression for HS birds not observed in TN or PF conditions, except for Ma et al. (2021a) who observed a downregulation of those markers under PF conditions. This indicates that, contrary to protein synthesis, increased protein breakdown during HS could be directly associated with HS *per se* and not the reduced FI. Further analyses and research are required at the gene and protein level to elucidate the different interactions occurring in protein synthesis and degradation pathways.

Total Protein and Uric Acid Concentrations in the Plasma

Uric acid is a major end product of N metabolism in birds, and increased plasma UA concentrations could be caused by protein breakdown and the mobilization of AA for hepatic gluconeogenesis (Sun et al., 2015; Hosseini-Vashan and Raei-Moghadam, 2019). Plasma UA could also be increased as a mechanism for attenuating oxidative damage since it can act as an endogenous antioxidant (Kikusato et al., 2021; Akinyemi and Adewole, 2022). In this study, we did not observe an effect of any treatment on plasma UA or TP concentration. In several other

studies, chronic HS increased serum or plasma UA concentrations and decreased TP (Zuo et al., 2015; Hosseini-Vashan and Raei-Moghadam, 2019; He et al., 2019a; Kikusato et al., 2021), whereas some studies only observed increases in UA concentration (Azad et al., 2010a; Willemsen et al., 2011; Sun et al., 2015; Ma et al., 2018, 2021b; Akinyemi and Adewole, 2022). Inconsistent results among the current and previous studies (Xie et al., 2015; Bueno et al., 2017; Ma et al., 2021a) could indicate that those markers are highly variable and time-dependent (Willemsen et al., 2011; Zuo et al., 2015).

Conclusion

In summary, HS markedly impairs broiler performance, mainly due to a reduction in FI in an attempt to minimize the endogenous heat production associated with nutrient digestion, absorption, and metabolism. Overall, constant HS resulted caused greater aberrations in digestibility and markers of metabolism (i.e., mRNA expression levels) than cyclic HS. Part of this observation can be explained by the lower physiological stress response induced by cyclic exposure to hot temperatures, as suggested by the absence of difference in stress and inflammation markers between TN-al and cyHS birds. In addition, our study indicates that the reductions in digestibility observed on N, EE, and some AA induced by constant HS are independent of the reduced FI and driven by the heat *per se*. However, considering the magnitude of performance losses caused by constant HS, this reduced nutrient digestibility appears to have limited contribution in the impaired feed efficiency. Regulatory mechanisms of protein and lipid metabolism were also highly impacted by constant HS exposure. However, further research is required to explain the absence of correlation between the reduced lipogenic activity measured in our study under constant HS at the mRNA level and the expected increase in fat deposition observed at the carcass level. Interestingly, downregulation of protein synthesis

mechanisms under constant HS was associated also occurred with PF, whereas upregulation of protein degradation markers was directly associated with HS *per se* and not reduced FI. Furthermore, some of the stress, inflammation, and metabolism markers measured in this study were impacted by acute HS exposure when compared to TN-al conditions, but similar mRNA expression profiles between aHS and $cyHS_{hot}$ birds, that were both sampled 4 h after the increase in temperature, do not support a possible physiologic adaptation to chronic high-temperature exposition. Similarly, except for the ATGL mRNA expression, comparable mRNA expression profiles between $cyHS_{cool}$ and $cyHS_{hot}$ birds indicate an absence of acute regulatory mechanisms when birds are cyclically exposed to chronic high temperatures. In summary, our study emphasizes the importance of managing HS in broiler production by implementing strategies that consider the effects of HS on nutrient utilization and metabolic regulation, and aim to minimize the physiological stress response induced by acute and chronic exposure to high temperatures.

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TABLES AND FIGURES

Table 4.1. Oligonucleotide qPCR primers.

Gene	Accession number[1]	Primer sequence (5'→3')	Orientation	Product size (bp)
HSP27	XM_001231557	TTGAAGGCTGGCTCCTGATC	For	58
		AAGCCATGCTCATCCATCCT	Rev	
HSP60	NM_001012916	CGCAGACATGCTCCGTTTG	For	55
		TCTGGACACCGGCCTGAT	Rev	
HSP70	J02579	GGGAGAGGGTTGGGCTAGAG	For	55
		TTGCCTCCTGCCCAATCA	Rev	
HSP90	X07265	TGACCTTGTCAACAATCTTGGTACTAT	For	68
		CCTGCAGTGCTTCCATGAAA	Rev	
SOD1	NM_205064	TGGCTTCCATGTGCATGAAT	For	58
		AGCACCTGCGCTGGTACAC	Rev	
SOD2	NM_204211	GCTGGAGCCCCACATCAGT	For	61
		GGTGGCGTGGTGTTTGCT	Rev	
GPX1	NM_001277853	TCCCCTGCAACCAATTCG	For	57
		AGCGCAGGATCTCCTCGTT	Rev	
TNFα	NM_204267	CGTTTGGGAGTGGGCTTTAA	For	61
		GCTGATGGCAGAGGCAGAA	Rev	
CRP	NM_001039564	AAGCTCAGGACAACGAGATCCT	For	71
		TTTCCCCCCCACGTAGAAG	Rev	
IL6	NM_204628	GCTTCGACGAGGAGAAATGC	For	63
		GGTAGGTCTGAAAGGCGAACAG	Rev	
IL10	NM_001004414	CGCTGTCACCGCTTCTTCA	For	63
		CGTCTCCTTGATCTGCTTGATG	Rev	
IL18	GU119895	TGCAGCTCCAAGGCTTTTAAG	For	63
		CTCAAAGGCCAAGAACATTCCT	Rev	
ATGL	EU240627	GCCTCTGCGTAGGCCATGT	For	60
		GCAGCCGGCGAAGGA	Rev	
LPL	NM_205282	GACAGCTTGGCACAGTGCAA	For	62
		CACCCATGGATCACCACAAA	Rev	
ACCα	NM_205505	CAGGTATCGCATCACTATAGGTAACAA	For	74
		GTGAGCGCAGAATAGAAGGATCA	Rev	
ACLY	NM_001030540	CTTTTAAGGGCATTGTTAGAGCAAT	For	65
		CCTCACCTCGTGCTCTTTCAG	Rev	
FASN	J03860	ACTGTGGGCTCCAAATCTTCA	For	70
		CAAGGAGCCATCGTGTAAAGC	Rev	
IGF1	NM_001004384	GCTGCCGGCCCAGAA	For	56
		ACGAACTGAAGAGCATCAACCA	Rev	
PI3K	NM_001004410	GCCATCTTACTCCAGGCGTATC	For	70
		GAGGGACTTGGCTGTAGCTTCTC	Rev	
ERK1	NM_204150	CGGACCATGATCACACAGGAT	For	63
		CAGGAGCCCTGTACCAACGT	Rev	
AKT1	AF039943	TTCAACGGTGATCTTTTGACTGA	For	64
		CGGGAATGTCTCTTGGTGGAT	Rev	
AMPKα1	NM_001039603	CCACCCCTGTACCGGAAATA	For	68
		GGAAGCGAGTGCCAGAGTTC	Rev	
mTOR	XM_417614.5	CATGTCAGGCACTGTGTCTATTCTC	For	77
		CTTTCGCCCTTGTTTCTTCACT	Rev	
p70S6K	NM_001109771	GTCAGACATCACTTGGGTAGAGAAAG	For	60
		ACGCCCTCGCCCTTGT	Rev	
MyoG	NM_204184	GGAGAAGCGGAGGCTGAAG	For	62
		GCAGAGTGCTGCGTTTCAGA	Rev	
MuRF1	XM_015297755	TGGAGAAGATTGAGCAAGGCTAT	For	64
		GCGAGGTGCTCAAGACTGACT	Rev	
MAFbx	NM_001030956	CCTTCCACCTGCTCACATCTC	For	59
		CACAGGCAGGTCCACAAA	Rev	
18S	AF173612	TCCCCTCCCGTTACTTGGAT	For	60
		GCGCTCGTCGGCATGTA	Rev	

[1] Accession number refer to GenBank (NCBI).

Table 4.2. Cumulative live performance and carcass characteristics of broilers reared under different environmental conditions and feed regimens from 20 to 41 d (summarized from Teyssier et al., 2022b).

Parameter[3]	Treatment[1]				SEM[2]	*P*-values
	TN-al	cyHS	coHS	TN-coPF		
41 d BW, kg	3.312[a]	2.841[b]	1.748[d]	2.040[c]	0.099	<0.001
BWG, kg	2.336[a]	1.873[b]	0.777[d]	1.074[c]	0.094	<0.001
FI, kg	3.781[a]	3.178[b]	2.069[c]	2.223[c]	0.109	<0.001
FCR	1.559[c]	1.710[c]	2.806[a]	2.185[b]	0.169	<0.001
Mortality, %	2.67	0.00	3.00	8.14	3.726	0.082
Hot Fat Pad Yield, %	1.02[b]	1.00[b]	1.24[a]	0.52[c]	0.068	<0.001
TBM[4] Yield, %	24.80[a]	23.25[b]	20.75[c]	22.21[b]	0.594	<0.001

[1] **TN-al**: Birds reared under continuous 24°C and ad libitum feeding. **cyHS**: Birds reared under cyclic high temperature (8 h at 35˚C and 12 h at 24˚C) and ad libitum feeding. **coHS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-coPF**: Birds reared under continuous 22°C and pair-fed to the coHS treatment.

[2] SEM: pooled standard error of the mean

[3] BW: Body weight; BWG: Body weight gain; FI: Feed intake; FCR: Feed conversion ratio

[4] TBM: Total breast meat = Pectoralis major + Pectoralis minor

[a-b] Means within row without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey's multiple comparison test.

Table 4.3. Ileal digestible energy and apparent ileal digestibility values of dry matter, ether extract, and nitrogen of broilers reared under different environmental conditions and feed regimens from 20 to 41 d with ileal content sampled at 41 d.

	Treatment[1]				SEM[2]	*P*-values
Parameter[3]	TN-al	cyHS	coHS	TN-coPF		
Dry matter (%)	74.1	70.8	69.9	70.9	3.70	0.428
Ether extract (%)	94.2^{ab}	93.8^{ab}	91.8^{b}	94.8^{a}	1.37	0.039
Nitrogen (%)	84.6^{a}	80.3^{ab}	79.3^{b}	82.3^{ab}	2.27	0.032
Energy (%)	78.3	75.1	73.6	75.9	3.89	0.417
IDE[4] (kcal/kg)	3,167	3,036	2,975	3,069	157	0.415

[1] **TN-al**: Birds reared under continuous 24°C and ad libitum feeding. **cyHS**: Birds reared under cyclic high temperature (8 h at 35˚C and 12 h at 24˚C) and ad libitum feeding. **coHS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-coPF**: Birds reared under continuous 24°C and pair-fed to the coHS treatment.

[2] SEM: pooled standard error of the mean.

[3] Values are means of 4 replicates per treatment (pool of 4 birds per replicate).

[4] IDE: Ileal digestible energy

[a-b] Means within row without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey's multiple comparison test.

Table 4.4. Apparent ileal digestibility coefficients of amino acids of broilers reared under different environmental conditions and feed regimens from 20 to 41 d and ileal content sampled at 41 d.

Parameter[3]	Treatment[1]				SEM[2]	*P*-values
	TN-al	cyHS	coHS	TN-coPF		
Essential amino acids						
Methionine	93.6[a]	89.8[b]	90.2[b]	92.2[ab]	0.94	0.005
Lysine	91.7[a]	88.2[b]	88.5[b]	89.7[ab]	1.29	0.017
Threonine	81.5	76.7	77.5	77.7	3.13	0.189
Valine	84.8	80.4	79.8	81.2	2.60	0.074
Isoleucine	86.0	81.4	81.4	82.9	2.30	0.049
Leucine	87.0[a]	83.0[ab]	81.9[b]	84.7[ab]	2.12	0.026
Arginine	92.6	90.4	90.4	92.1	1.78	0.243
Phenylalanine	88.3[a]	83.4[b]	84.3[b]	87.7[a]	1.26	<0.001
Histidine	88.5[a]	84.9[ab]	83.9[b]	87.1[ab]	2.04	0.027
Total essential amino acids	88.2[a]	83.9[b]	84.2[b]	86.1[ab]	1.15	<0.001
Non-essential amino acids						
Alanine	86.8[a]	83.0[ab]	81.8[b]	83.9[ab]	2.22	0.043
Aspartic acid	85.2[a]	80.4[b]	83.2[ab]	83.0[ab]	1.50	0.006
Cysteine	80.5	73.6	71.3	79.0	4.36	0.089
Glutamic acid	90.0[a]	86.3[b]	87.3[b]	88.2[ab]	1.00	0.001
Glycine	82.7	77.6	77.2	79.0	2.86	0.068
Proline	85.0[a]	80.0[b]	80.7[ab]	82.4[ab]	2.22	0.031
Serine	86.5[a]	82.5[b]	82.6[b]	83.3[ab]	1.83	0.028
Tyrosine	90.4[a]	87.0[ab]	83.8[b]	86.8[ab]	2.61	0.030
Total non-essential amino acids	85.9[a]	81.3[b]	81.0[b]	83.2[ab]	1.68	0.005
Total[4]	87.1[a]	82.7[b]	82.7[b]	84.8[ab]	1.34	0.001

[1] **TN-al**: Birds reared under continuous 24°C and ad libitum feeding. **cyHS**: Birds reared under cyclic high temperature (8 h at 35˚C and 12 h at 24˚C) and ad libitum feeding. **coHS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-coPF**: Birds reared under continuous 24°C and pair-fed to the coHS treatment.

[2] SEM: pooled standard error of the mean.

[3] Values are means of 4 replicates per treatment (pool of 4 birds per replicate).

[4] Total = Sum of all reported essential and non-essential amino acids.

[a-b] Means within row without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey's multiple comparison test.

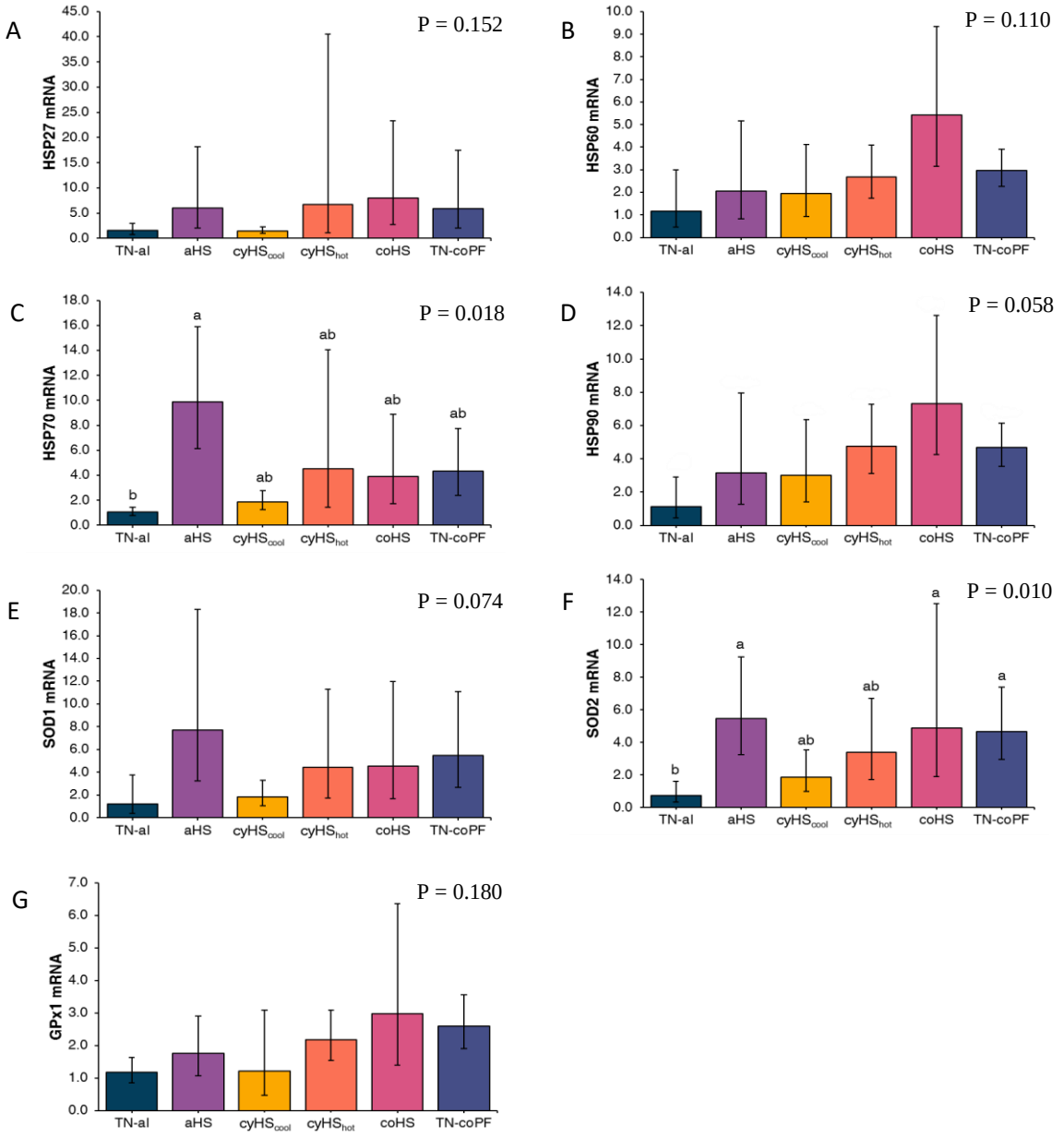

Figure 4.1. Effect of different environmental conditions and feed regimen on blood mRNA expression of stress related transcription factors in broilers.

Relative abundances of HSP27 (**A**), HSP60 (**B**), HSP70 (**C**), HSP90 (**D**), SOD1 (**E**), SOD2 (**F**), and GPX1 (**G**) mRNA was determined by real-time quantitative PCR and analyzed by the $2^{-\Delta\Delta Ct}$ method using the TN-al group as calibrator. Data were log-transformed and are presented as antilog of the geometric means with error bars representing the 95% confidence interval: $\mu \pm 1.96 \times \mathrm{sd}/\sqrt{n}$.

[a-b] Different letters indicate significant difference at $P < 0.05$ by a Tukey's multiple comparison test.

TN-al, birds reared under continuous 24°C and ad libitum feeding; **aHS**, birds reared under acute heat stress for 4 h on d 41; **cyHS$_{cool}$**, birds reared under cyclic high temperature (12 h at 35˚C and 12 h at 24˚C), ad libitum feeding, and sampled at the end of the cool phase of the night; **cyHS$_{hot}$**, same as cyHS$_{cool}$, but sampled after 4 h at 35°C; **coHS**, birds reared under continuous 35°C and ad libitum feeding; **TN-coPF**, birds reared under continuous 24°C and pair-fed to the coHS treatment.

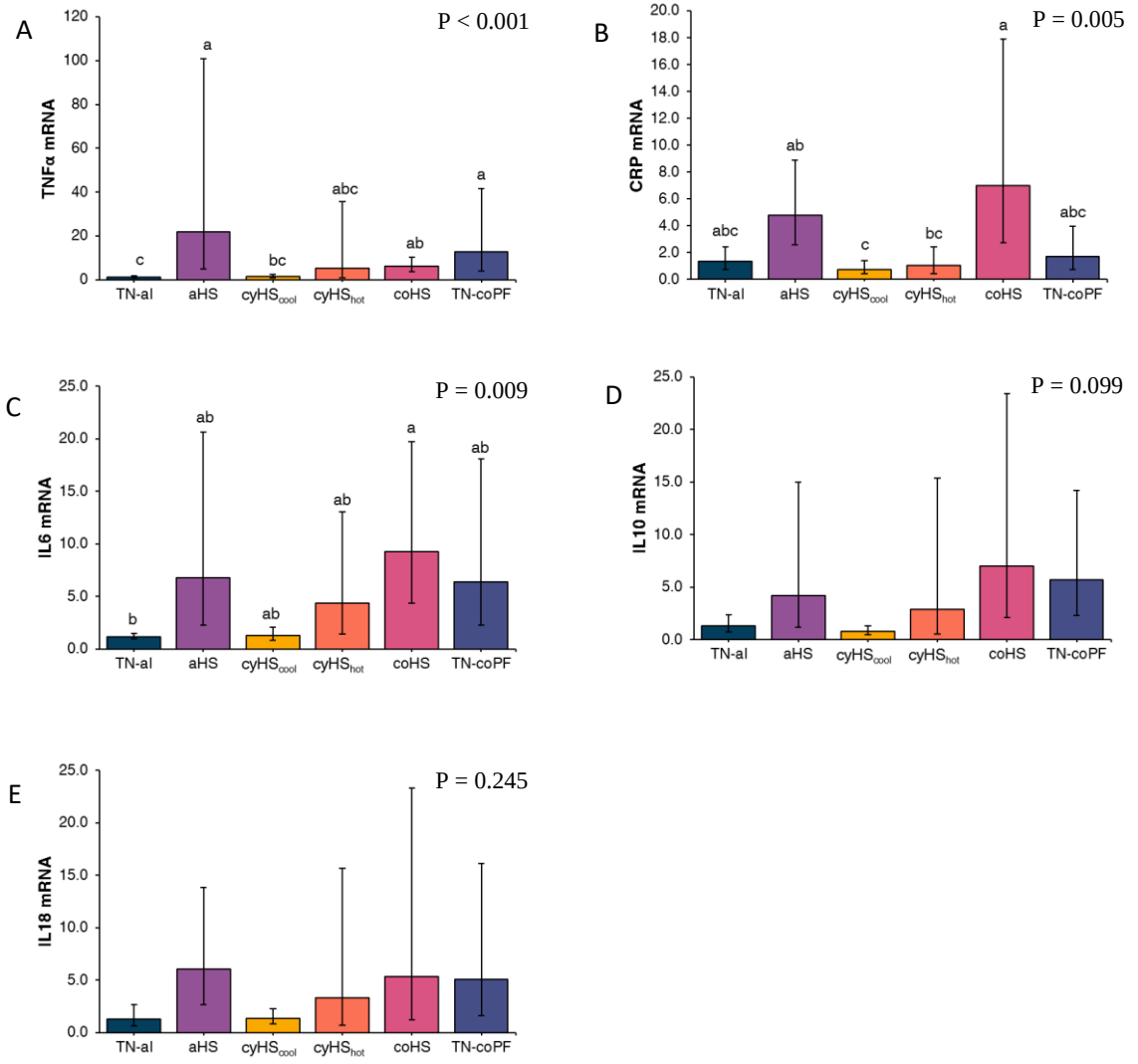

Figure 4.2. Effect of different environmental conditions and feed regimen on blood expression of inflammatory related transcription factors in broilers.

Relative abundances of TNFα (**A**), CRP (**B**), IL6 (**C**), IL10 (**D**), and IL18 (**E**) mRNA was determined by real-time quantitative PCR and analyzed by the $2^{-\Delta\Delta Ct}$ method using the TN-al group as calibrator. Data were log-transformed and are presented as antilog of the geometric means with error bars representing the 95% confidence interval: $\mu \pm 1.96 \times \mathrm{sd}/\sqrt{n}$.

[a-b] Different letters indicate significant difference at P < 0.05 by a Tukey's multiple comparison test.

TN-al, birds reared under continuous 24°C and ad libitum feeding; **aHS**, birds reared under acute heat stress for 4 h on d 41; **cyHS$_{cool}$**, birds reared under cyclic high temperature (12 h at 35˚C and 12 h at 24˚C), ad libitum feeding, and sampled at the end of the cool phase of the night; **cyHS$_{hot}$**, same as cyHS$_{cool}$, but sampled after 4 h at 35°C; **coHS**, birds reared under continuous 35°C and ad libitum feeding; **TN-coPF**, birds reared under continuous 24°C and pair-fed to the coHS treatment.

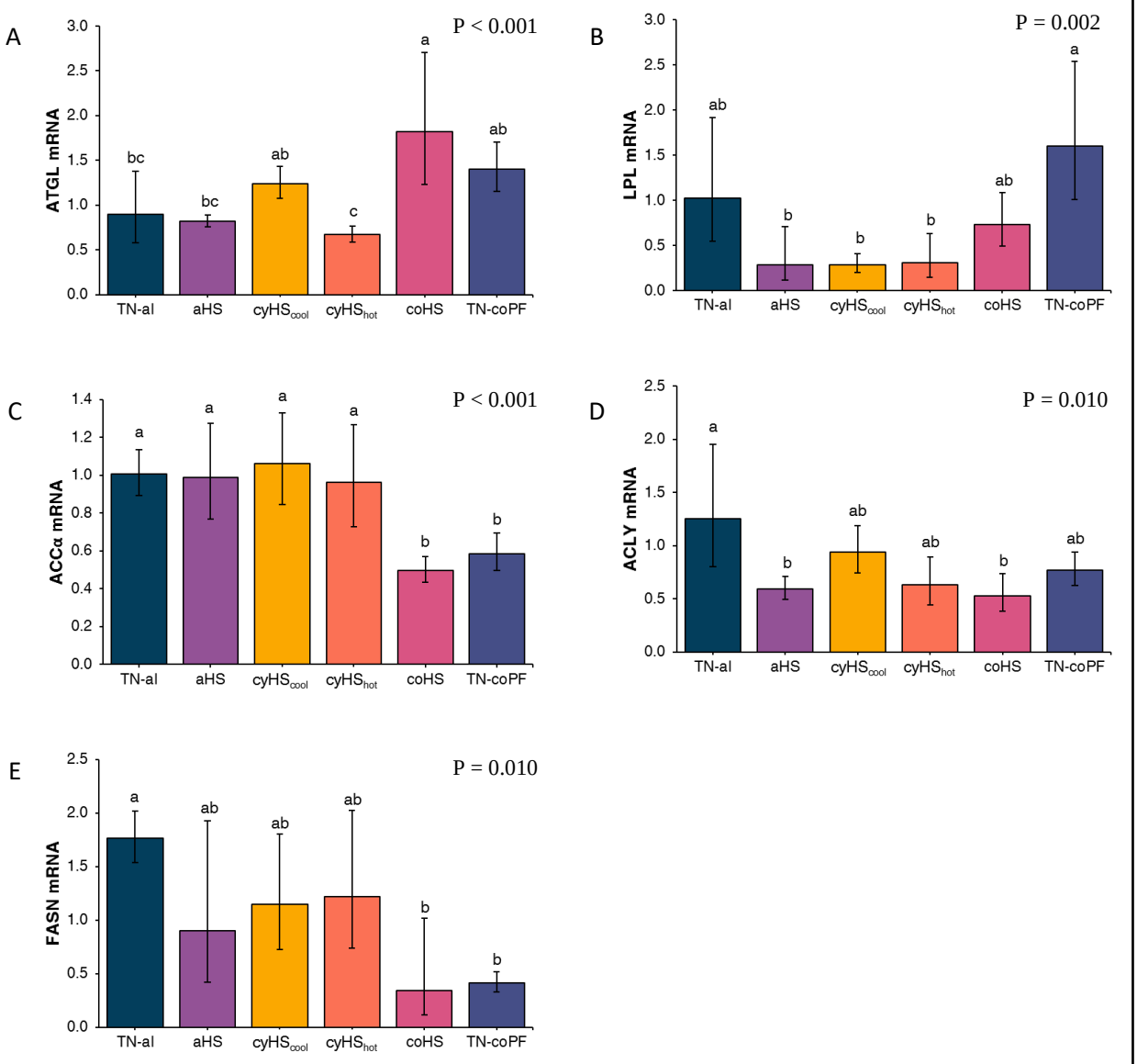

Figure 4.3. Effect of different environmental conditions and feed regimen on liver expression of lipid metabolism related transcription factors in broilers.

Relative abundances of ATGL (**A**), LPL (**B**), ACCα (**C**), ACLY (**D**), and FASN (**E**) mRNA was determined by real-time quantitative PCR and analyzed by the $2^{-\Delta\Delta Ct}$ method using the TN-al group as calibrator. Data were log-transformed and are presented as antilog of the geometric means with error bars representing the 95% confidence interval: $\mu \pm 1.96 \times \mathrm{sd}/\sqrt{n}$.

[a-b] Different letters indicate significant difference at $P < 0.05$ by a Tukey's multiple comparison test.

TN-al, birds reared under continuous 24°C and ad libitum feeding; **aHS**, birds reared under acute heat stress for 4 h on d 41; **cyHS$_{cool}$**, birds reared under cyclic high temperature (12 h at 35˚C and 12 h at 24˚C), ad libitum feeding, and sampled at the end of the cool phase of the night; **cyHS$_{hot}$**, same as cyHS$_{cool}$, but sampled after 4 h at 35°C; **coHS**, birds reared under continuous 35°C and ad libitum feeding; **TN-coPF**, birds reared under continuous 24°C and pair-fed to the coHS treatment.

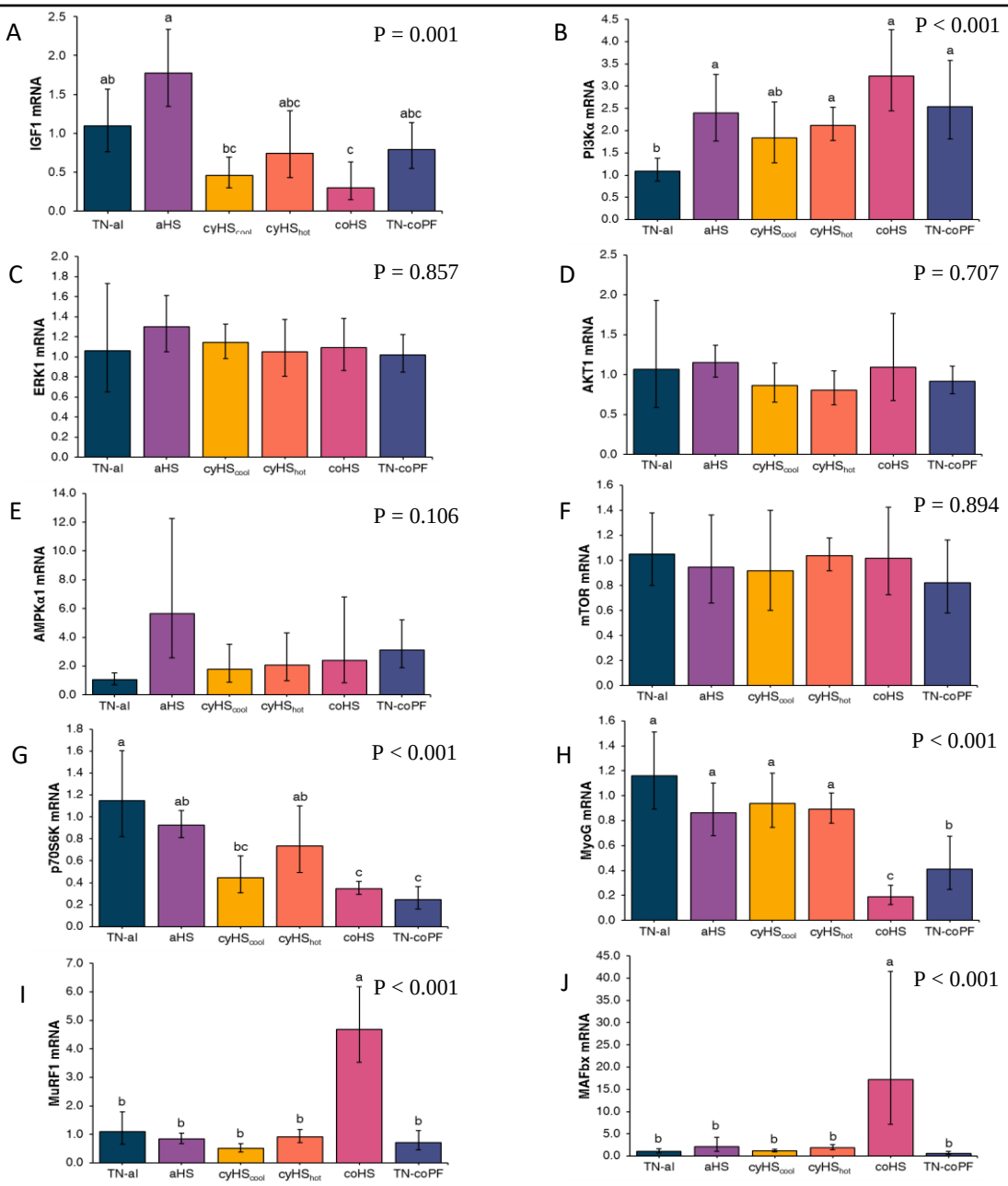

Figure 4.4. Effect of different environmental conditions and feed regimen on pectoralis major expression of protein metabolism related transcription factors in broilers.

Relative abundances of IGF1(**A**), PI3Kα (**B**), ERK1 (**C**), AKT1 (**D**), AMPKα1 (**E**), mTOR (**F**), p70S6K (**G**), MyoG (**H**), MuRF1 (**I**), and MAFbx (**J**) mRNA was determined by real-time quantitative PCR and analyzed by the $2^{-\Delta\Delta Ct}$ method using the TN-al group as calibrator. Data were log-transformed and are presented as antilog of the geometric means with error bars representing the 95% confidence interval: $\mu \pm 1.96 \times \mathrm{sd}/\sqrt{n}$.
[a-b] Different letters indicate significant difference at $P < 0.05$ by a Tukey's multiple comparison test.
TN-al, birds reared under continuous 24°C and ad libitum feeding; **aHS**, birds reared under acute heat stress for 4 h on d 41; **cyHS$_{cool}$**, birds reared under cyclic high temperature (12 h at 35˚C and 12 h at 24˚C), ad libitum feeding, and sampled at the end of the cool phase of the night; **cyHS$_{hot}$**, same as cyHS$_{cool}$, but sampled after 4 h at 35°C; **coHS**, birds reared under continuous 35°C and ad libitum feeding; **TN-coPF**, birds reared under continuous 24°C and pair-fed to the coHS treatment.

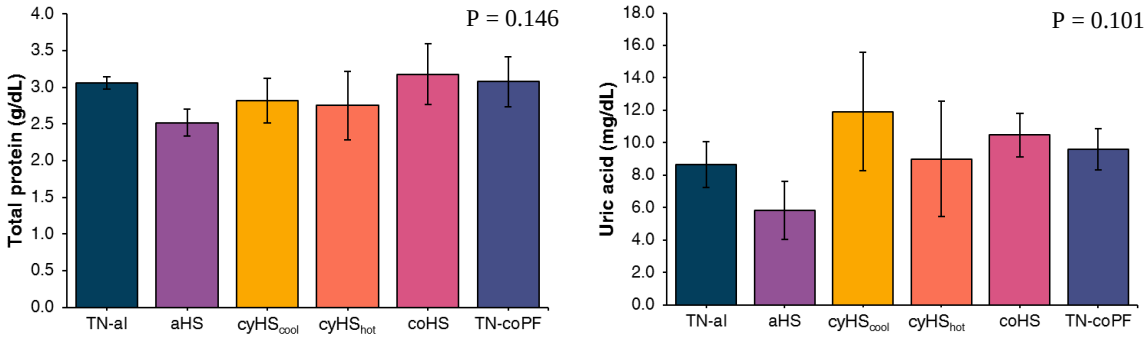

Figure 4.5. Effect of different environmental conditions and feed regimen on plasma total protein and uric acid concentration in broilers.

Data are presented as mean and error bars represent the 95% confidence interval: $\mu \pm 1.96 \times \mathrm{sd}/\sqrt{n}$. **TN-al**, birds reared under continuous 24°C and ad libitum feeding; **aHS**, birds reared under acute heat stress for 4 h on d 41; **cyHS$_{cool}$**, birds reared under cyclic high temperature (12 h at 35˚C and 12 h at 24˚C), ad libitum feeding, and sampled at the end of the cool phase of the night; **cyHS$_{hot}$**, same as cyHS$_{cool}$, but sampled after 4 h at 35°C; **coHS**, birds reared under continuous 35°C and ad libitum feeding; **TN-coPF**, birds reared under continuous 24°C and pair-fed to the coHS treatment.

CHAPTER V - EFFECTS OF DIETARY AMINO ACID DENSITY ON PERFORMANCE, DIGESTIBILITY, CARCASS CHARACTERISTICS, AND NITROGEN AND ENERGY PARTITIONING IN BROILERS REARED UNDER THERMONEUTRAL, HEAT STRESS, AND PAIR-FEEDING CONDITIONS

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To be submitted to Poultry Science

ABSTRACT

Poultry have a limited capacity to dissipate heat, and heat stressed (**HS**) broilers decrease feed intake (**FI**) and alter their metabolism to reduce heat production (**HP**). This study evaluated the effect of dietary amino acids (**AA**) on performance, digestibility, carcass characteristics, and energy and nitrogen partitioning under different environmental conditions. At 20 d post-hatch, Cobb male birds (n=1,536) were allocated into 8 houses, each divided into 16 pens with 12 birds per pen. Experimental environments were applied from 20 to 42 d post-hatch, with 4 houses maintained at 24°C (thermoneutral, **TN**), and the remaining 4 houses at 35°C to induce chronic HS. Four isocaloric and isonitrogenous diets were formulated with 100% recommended AA levels (**CTL**), or 135% of recommended levels of either all 10 essential AA (**AA+**), total sulfur AA, Arg, Thr (**MRT+**), or total sulfur AA, Arg, Thr, Ile, Val (**MRTIV+**) and were distributed to 4 pens per house. In each TN house, 2 pens per diet were pair-fed (**TN-PF**) to equalize the FI of HS birds, while 2 pens per diet were fed ad-libitum (**TN-AL**). This resulted in a split-plot design of 3 environments (whole-plot factor) × 4 diets (sub-plot), totaling 12 treatments. Data were analyzed by ANOVA followed by Tukey's means separation. Heat stress impaired ($P < 0.001$) BW gain and feed efficiency. Digestibility of essential AA was reduced ($P = 0.031$) by HS conditions, but supplementation of unbound AA mitigated this reduction ($P < 0.001$). Heat stress increased ($P < 0.001$) fasting HP and reduced ($P = 0.014$) the heat increment relative to total HP, partly because of the reduced FI and the shift in energy metabolism from protein to fat. The elevated dietary density of some or all essential AA did not alleviate the adverse effects of HS, indicating that adequate amounts of dietary AA were provided under HS conditions. Further research is required to understand the metabolic shift towards fat deposition and the fate of AA in HS broilers.

INTRODUCTION

Heat stress (**HS**) occurs when animals are not able to dissipate their heat production (**HP**) (Leithead and Lind, 1964; Bernabucci et al., 2010; Akbarian et al., 2016). To maintain homeostasis, increased panting rate, wing flapping and reduced feed consumption are commonly observed behaviors in birds exposed to high temperatures. Additionally, HS induces physiological and metabolic adaptations, including alteration of the gastrointestinal epithelium, which can ultimately lead to leaky gut syndrome and reduced digestibility, as well as alterations in protein and lipid metabolism (Brugaletta et al., 2022). Though the reduce feed intake (**FI**) explains most off the performance reduction observed under HS conditions (Teyssier et al., 2022a), the use of pair-feeding (**PF**) techniques have demonstrated that HS *per se* reduces growth performance and protein deposition, and increases carcass fat content (Zeferino et al., 2016; Teyssier et al., 2022b). These shifts may reflect higher relative nutrient requirements for maintenance functions associated with body temperature regulation (Geraert et al., 1996; Faria Filho et al., 2007; De Souza et al., 2016). Ameliorating these adverse effects of HS requires a holistic approach, from genetic selection to bird management and dietary interventions.

Numerous dietary strategies have been investigated to mitigate the detrimental effects of HS and its associated metabolic alterations in birds (Teyssier et al., 2022a). Increasing dietary energy and lipid inclusion improves broiler performance under HS (Ghazalah et al., 2008). Higher fat content reduces HP because of its lower heat increment compared to carbohydrates and protein (Fuller and Rendon, 1977) and enhances heat dissipation through factors like metabolic water generation and improved nutrient digestion (Mateos et al., 1982; Barboza et al., 2009; Aardsma et al., 2017). However, high dietary energy can lead to elevated abdominal fat deposition (Raju et al., 2004; Ghazalah et al., 2008), which may exacerbate the shift of energy

deposition toward the lipid form. Adjusting the dietary protein fraction may be more beneficial for maximizing protein deposition. Interestingly, despite the high caloric increment of protein, low crude protein (**CP**) diets supplemented with unbound amino acid (**AA**) have not successfully mitigated HS impact on performance (Alleman and Leclercq, 1997; Cheng et al., 1999; Gonzalez-Esquerra and Leeson, 2005; Faria Filho et al., 2005; Liu et al., 2016; Zulkifli et al., 2018; Awad et al., 2018; Lin Law et al., 2019; Amiri et al., 2019; Soares et al., 2020; Attia et al., 2020). According to the "ideal protein" concept (Baker and Chung, 1992) meeting the adequate amount of essential AA should satisfy the requirements of birds regardless of the dietary CP content. Therefore, HS likely alters AA requirements. Interestingly, increased dietary AA density under HS conditions has shown beneficial effect on feed efficiency (Maharjan et al., 2020), and breast yields (Alhotan et al., 2021). However, further research is required to determine which AA can be beneficial under HS conditions and to evaluate the interactive effects of AA density, temperature, and feed consumption.

Additionally, an increasing number of studies are investigating the potential benefits of individual AA supplementation, which is crucial for defining AA requirements under HS conditions. For example, broilers under HS have been found to have higher Met requirements compared to those under TN conditions (Silva Junior et al., 2006; Sahebi-Ala et al., 2021), potentially attribute to its antioxidant properties (Del Vesco et al., 2015a; Gasparino et al., 2018; Liu et al., 2019; Santana et al., 2021), its modulation of inflammation (Liu et al., 2019), and its stimulatory effects on protein deposition and inhibition of protein breakdown (Del Vesco et al., 2013, 2015b). In contrast, supplementation beyond the standard recommendations of Lys, the second limiting AA in poultry diets and closely related to protein deposition, has not yet shown beneficial effects under HS conditions (Mendes et al., 1997; Corzo et al., 2003; Attia et al., 2011,

2020). Inconsistent results have been obtained with Thr and Arg, but recent studies have shown an improvement of performance under HS conditions when Thr was supplemented above standard requirements (Debnath et al., 2019; Miah et al., 2022). Arginine and its catabolic product citrulline, are believed to be potentially beneficial under HS due to their antioxidant effect (Gupta et al., 2005) and role in the nitrogen oxide synthesis, which is involved in vasodilatation to support thermoregulation (Uyanga et al., 2021). While Brake (1998) observed a positive effect of Arg on performance under HS, more recent results are sparse. Furthermore, with the increased availability of feed-grade Val and Ile, recent studies have investigated the potential benefits of their supplementation above standard levels, but no improvements in performance have been observed under HS conditions (Kop-Bozbay et al., 2021; Kim et al., 2022b).

Therefore, additional research is needed to investigate dietary protein and individual amino acid supplementation adjustments to effectively mitigate the detrimental effects of HS. This experiment aims to further investigate the effect of constant HS, PF, and thermoneutral (**TN**) conditions from 20 to 42 d on performance, carcass characteristics, digestibility, and nutrient partitioning in broilers fed diets with different AA profiles. We hypothesized that diets supplemented with specific or all essential AA will help fulfill the increased AA maintenance requirements caused by HS, ultimately leading to the mitigation of adverse effects on performance, carcass characteristics, digestibility, and nutrient partitioning.

MATERIALS AND METHODS

Animals and Experimental Design

All procedures involving live animals were approved by the Institutional Animal Care and Use Committee at the University of Arkansas.

A total of 1,920 male chicks from a Cobb 500 female breeder line were obtained from the Cobb hatchery in Siloam Springs, Arkansas. Chicks were placed in 24 pens with 80 chicks per pen, each pen measuring 1.50 × 3.20 m (4.80 m^2). Pens were equipped with two hanging pan feeders, nipple waters, and concrete floors covered with fresh pine shavings. Before the start of the experimental period on d 20, the ambient temperature was gradually decreased from 32°C at placement to 24°C on d 20. A common starter (d 0 to d 11) and grower (d 12 to d 20) feed were distributed ad-libitum (**Table 5.1**).

On d 19, all birds were tagged and individually weighed. Additionally, a total of 1,536 birds were re-allocated into 8 individual environmentally controlled houses based on their individual BW, with 192 chickens per house. Birds were reared until they reached 42 d old. Each house consisted of 16 pens measuring 1.22 × 0.91 m (1.11 m^2) with 12 birds per pen. Pens were equipped with hanging pan feeders, nipple waters, and placed on concrete floors covered with fresh pine shavings. On d 21, 4 houses were set to a TN temperature of 24°C, while the remaining 4 houses were set to 35°C to induce chronic constant HS. The FI of HS birds was measured daily, and in each TN house, birds from 8 pens were pair-fed (**TN-PF**) to equalize their FI to that of HS birds on the previous d. Feed distribution adjustments were performed between birds receiving the same diet. Pair-fed birds were fed 3 times per day to minimize feed outage periods. Birds from the remaining 8 pens were fed ad-libitum (**TN-AL**). Four diets based on corn and soybean meal were formulated and distributed to 4 pens per house. The first diet (**CTL**) contained 100% of the recommended AA levels according to breeder recommendations. The other three diets were formulated with 135% of the recommended levels of either TSAA, Arg, and Thr (**MRT+**), or TSAA, Arg, Thr, Ile, and Val (**MRTIV+**), or all 10 essential AA (**AA+**). DL-Methione only, without addition of Cys, was used to adjust dietary TSAA concentrations.

Diets were formulated to have the same inclusion of SBM, and to be isocaloric and isonitrogenous, with the nitrogen levels balanced using glutamic acid. The increase in AA density of 135% was calculated based on data from a previous experiment (Teyssier et al., 2022b) in an attempt to equalize the AA intake of birds under HS conditions with that of birds under TN-AL conditions and fed the CTL diet. Water was provided ad libitum throughout the experiment, and lighting schedules were implemented according to primary breeder management guidelines.

Each house was equipped with a sensor (Model HT.w, SensorPush, New York, NY) to instantly monitor and record environmental temperature and humidity every minute. Additionally, following the method described in previous study (Rajaei-Sharifabadi et al., 2017), one bird per pen receiving the CTL diet was randomly selected and equipped with a Thermochron temperature logger (iButton, DS1922L, Embedded Data Systems, Lawrenceburd, KY) to monitor core body temperature hourly from d 33 to 41.

Performance Measurements and Sampling Procedures

Feed intake and BW were measured, and feed conversion ratio (**FCR**) was calculated per pen at 20 and 41 d post-hatch. Additionally, daily mortality was recorded throughout the experiment.

On d 20, before the HS protocol or experimental diets were implemented, 24 birds with BW representative of the population average were euthanized for initial carcass composition. At the end of the experimental period, all birds from the TN and PF pens were used for sampling on d 41 or processing on d 42, while only half of the HS pens were collected to reach an equal number of pens sampled per combination of environmental condition and dietary treatment (n=8). From each sampling pen, 5 birds were randomly chosen for ileal digesta collection

(n=480), 1 bird was selected for carcass composition (n=96), and the remaining birds (6 birds when there was no mortality in the pen) were processed (n=540).

Apparent Ileal Digestibility

On d 41, after measuring the performance, birds selected for ileal digesta collection were euthanized by CO2 inhalation and immediately dissected for digesta collection. One-half of the ileum, proximal to the ileocecal junction, was flushed for collection of digesta samples. Sample from each replicate were pooled, placed on ice, frozen, lyophilized, and ground using an electric coffee grinder. The feed and digesta samples were analyzed for dry matter (**DM**) (AOAC Method 930.15 "Moisture in Animal Feed"), ether extract (**EE**) (AOCS Approved Procedure Am 5-04 "Rapid Determination of Oil/Fat Utilizing High Temperature Solvent Extraction", 2004), nitrogen (**N**) (AOAC 968.06-1969 "Protein (Crude) in Animal Feed") concentration, and gross energy (**GE**) (ISO 1928 "Solid Mineral Fuels – Determination of Gross Calorific Value by the Bomb Calorimetric Method, and Calculation of Net Calorific Value") using a bomb calorimeter (Model 6200 Isoperibol Calorimeter, Parr Instrument Company, Moline, IL) content at the University of Arkansas. The AA concentrations were analyzed using a high-performance liquid chromatography instrument by a commercial laboratory (ATC scientific, Little Rock, AR).

Titanium dioxide concentration, which was used as an indigestible marker, was determined following the procedures of Short et al. (1996). Apparent ileal digestibility (**AID**) of DM, EE, N, GE, and AA was calculated using the following formula:

$AID, \% = \frac{\left({}^{X}/_{TiO_2}\right)_{diet} - \left({}^{X}/_{TiO_2}\right)_{digesta}}{\left({}^{X}/_{TiO_2}\right)_{diet}} \times 100$, where ${}^{X}/_{TiO_2}$ is the ratio of nutrient or energy concentration (%) to TiO2 (%) in the diet or ileal digesta. Energy AID (%) values obtained from the equation above were multiplied by the GE content of the diet to calculate ileal digestible energy (**DE**) in units of kcal/kg.

Processing Measurements

On d 42, after an overnight feed withdrawal, birds were processed at the University of Arkansas Pilot Processing Plant (Fayetteville, AR). The weight of each processed bird was individually measured before they were subjected to electrical stunning (11 V, 11 mA for 11 s) and exsanguination via a jugular vein cut. After scalding at 53.8°C, feathers were removed with a commercial inline defeatherer (Foodcraft Model 3; Baker international, Dearborn, MI). Necks, heads, and feet were then removed from each bird. Carcasses were mechanically eviscerated, and the weight of the carcass and abdominal fat were recorded. Carcasses were then placed in ice water for a 4 h chilling period. After chilling, carcasses were weighed again. The pectoralis major and minor muscles, wings, and leg quarters were removed from the carcasses and weighed. Total breast meat (**TBM**) was calculated as the sum of the pectoralis major and minor weights, and the yield of each part was determined by dividing the part weight by the individual slaughter weight.

Carcass Part Nutritional Composition

Comparative slaughter technique was performed on the 24 birds selected at d 20 as well as those selected on d 42, using the same processing procedure. The weight of each bird was individually measured before they were subjected to electrical stunning (11 V, 11 mA for 11 s) and exsanguination via a jugular vein cut. After exsanguination, birds were reweighed before scalding at 53.8°C, and feathers were removed using a commercial inline defeatherer (Foodcraft Model 3; Baker international, Dearborn, MI). Birds were dried and reweighed to obtain the blood and feather-free carcass weight. Finally, birds were deboned, and the following carcass parts were individually weighed: small intestine, liver, offal (caeca, large intestine, paws, head, neck, esophagus, gizzard, proventriculus, lungs, trachea, heart, testis, pancreas, kidney, gallbladder),

TBM, and the remaining parts of the carcass (wings, leg quarters, rack, abdominal fat). The carcass parts were passed through a grinder (Lem Big Bite Meat Grinder #32, West Chester, OH), homogenized (Ninja Professional Food Processor Model BN601; SharkNinja, Needham, MA), and frozen for chemical analysis. Abdominal fat was weighed individually before being weighed with the remaining parts of the carcass. Grinder parts and accessories were thoroughly cleaned between each carcass part to prevent cross-contamination. Before analysis, frozen samples were lyophilized and ground using an electric coffee grinder. Moisture, nitrogen, lipid, and GE content were determined for each sample at the University of Arkansas. Moisture, nitrogen, fat, and energy composition were measured for each part of the carcass at both d 20 and d 42.

The nitrogen and energy partitioning models at the whole carcass level are represented in **Figure 5.1** and **Figure 5.2**, along with the calculations used to calculate each parameter. Nitrogen intake (**NI**), digestible NI (**DNI**), N retention (**NR**), N excretion (**NEx**), energy intake (**EI**), digestible EI (**DEI**), energy retention (**ER**), heat production (**HP**), heat increment (**HI**), fasting heat production (**FHP**), net energy (**NE**), and energy excretion (**EEx**) were expressed on a daily metabolic BW basis in $g/kg^{0.70}/d$ or $kcal/kg^{0.70}/d$. Energy retention as protein (**ERPro**) or as fat (**ERFat**) was obtained by multiplying the NR by 6.25 × 5.66kcal/g and the fat retention by 9.37 kcal/g (Swennen et al., 2004). Energy excretion via urinary losses was determined by multiplying the quantity of N excreted via urinary losses by 34.3kJ/g (Van Milgen, 2021). The FHP was estimated using the equation: FHP = 450 kJ/d per $BW^{0.70}$ unit (Noblet et al., 2015, 2022), and daily metabolic BW was estimated by averaging the BW at d 20 and 42. Energy (**EEf**) and nitrogen (**NEf**) efficiency were calculated by dividing the ER or NR by the EI or NI.

Statistical Analysis

In total, there were 12 treatments resulting from the combination of environmental condition and diet arranged in a split-plot design. The 3 environmental conditions were treated as the whole plot and the 4 diets were considered as the sub-plot. Depending on the measurement, the whole (**WP**) and sub plots (**SP**) had different replicate numbers.

For performance measurements, FI and BW were measured on every pen. The WP was based on a completely randomized design, with each environmental condition being replicated 4 times. The WP experimental unit was the house consisting of 16 pens under HS conditions, and half the house consisting of 8 pens under TN-AL and TN-PF conditions. The SP was an unbalanced completely randomized design, with the pen as the experimental unit, and each diet replicated 16 times under HS conditions and 8 times under TN-AL and TN-PF conditions.

For digestibility, processing, and carcass composition measurements, the WP was also based on a completely randomized design, with each environmental condition replicated 4 times. The WP experimental unit was half the house with 8 pens under each of the 3 environmental conditions. However, the SP was based on a balanced completely randomized design, with the pen as experimental unit, and each diet replicated 8 times under TN, HS, and PF conditions.

Data were analyzed by ANOVA based on a split-plot design, and means were separated using a Tukey-Kramer test. The model included fixed effects: environmental condition (n = 3; TN-AL, HS, TN-PF) and dietary condition (n = 4; CTL, MRT+, MRTIV+, AA+), and their interaction. Statistical significance was determined at a significance level of $P < 0.05$, and all analyses were performed using SAS (SAS 9.4).

RESULTS

Temperature Measurements

Temperature recordings in each house and average internal bird temperatures per house are illustrated in **Figure 5.3**. Throughout the experimental period, the average ambient temperature was 24.5°C in TN houses and 32.8°C in HS houses. Average internal temperatures, measured from 33 to 41 d of age, of birds exposed to TN conditions was 41.5°C, versus 42.7°C under HS conditions.

Performance Measurements

Performance results are summarized in **Table 5.2**. From d 20 to 41, FI of HS birds was decreased ($P < 0.001$) compared to TN-AL birds. Similarly, the BW and BW gain (**BWG**) of HS birds were reduced ($P < 0.001$) compared to TN-AL birds. However, PF allowed for equalizing the FI of HS and TN-PF birds, while the BW and BWG of HS birds remained lower than TN-PF birds. Feed conversion ratio was highest ($P < 0.001$) for HS birds, while the FCR of TN-PF birds was intermediate between HS and TN-AL birds. An environmental effect was measured on mortality ($P = 0.039$) with a trend towards higher mortality under HS conditions, although the Tukey test did not yield significant differences.

No diet or environment × diet interaction effects ($P > 0.05$) were observed on bird performance.

Apparent Ileal Digestibility

Nutrient AID and ileal DE results are presented in **Table 5.3**. The environment did not affect AID of nutrients or energy ($P > 0.05$), except for a tendency for HS to reduce DE ($P = 0.088$) and energy AID ($P = 0.094$) compared to TN-PF conditions. Regarding the diet effect, higher ($P = 0.005$) AID of DM was observed with the MRTIV+ and AA+ diets compared to the

MRT+ diet, with an intermediate value for the CTL diet. Additionally, the AA+ diet resulted in lower ($P = 0.004$) energy AID compared to the MRT+ and MRTIV+ diets, and lower ($P < 0.001$) DE compared to other diets.

Essential AA AID results are presented in **Table 5.4**. Birds under HS had lower AID for TSAA ($P = 0.030$), Met ($P = 0.033$), Arg ($P = 0.040$), Ile ($P = 0.023$), Leu ($P=0.002$), Phe ($P = 0.002$), and Trp ($P = 0.017$) compared to TN-PF birds. Similarly, a tendency was also observed for Val AID ($P = 0.059$). This resulted in an overall reduction ($P = 0.031$) of EAA AID in HS birds compared to TN-PF birds. Dietary treatments also influenced EAA AID. The MRTIV+ diet resulted in higher ($P = 0.029$) AID compared to the CTL diet. Specifically, TSAA, Met, Arg, and Thr AID were increased ($P < 0.001$) in birds fed the MRT+ and MRTIV+ diets compared to those fed the CTL diet, and the AA+ diet increased Met and Thr AID. When Ile and Val were supplemented at 135% in the MRTIV+ diet, the AID of Ile ($P < 0.001$) and Val ($P = 0.001$) were increased compared to the CTL and MRT+ diets. Similarly, Ile and Val AID were increased with the AA+ diet compared to the MRT+ diet. Additionally, His AID was increased ($P = 0.012$) in birds fed the AA+ diet compared to the CTL diet. Furthermore, environment × diet interaction (**Figure 5.4**) indicated that the CTL diet reduces TSAA ($P = 0.015$) and Met ($P = 0.008$) AID compared to the other diets under HS conditions only.

Non-essential AA and total AA AID are presented in **Table 5.5**. Under HS conditions, Cys ($P = 0.036$), Glu ($P = 0.007$), and Pro ($P = 0.030$) AID were reduced compared to TN-PF conditions, with TN-AL birds showing intermediate values. Overall, although no significant ($P > 0.05$) environmental effects was observed on NEAA, HS conditions led to a reduction ($P = 0.050$) in total AA AID compared to TN-PF conditions. Dietary treatments also influenced AID of NEAA. Glu AID was lower ($P < 0.001$) with the AA+ diet compared to other dietary

treatments, and Cys AID was reduced ($P < 0.001$) with the AA+ diet compared to the MRT+ and MRTIV+ diet. Moreover, AID of Asp ($P = 0.018$), Gly ($P = 0.011$), Pro ($P = 0.027$), and Ser ($P = 0.027$) were decreased in birds fed the AA+ diet compared to the MRTIV+ diet. Those results contributed to an overall reduced NEAA AID with the AA+ diet compared to the other diets.

Total digestible intake of essential AA per treatment is presented on **Table 5.6**. Each AA supplemented to 135% in birds under HS and TN-PF conditions was similar to the AA intake of birds fed the CTL diet under TN-AL conditions (dietary effect, $P < 0.001$), except for a lower Val intake with the MRTIV+ diet under HS compared to the CTL diet under TN-AL conditions (dietary effect, $P < 0.001$).

Processing Measurements

Live weights and carcass characteristics of processed birds are presented in **Table 5.7**. Consistent with the overall performance data, the live weight of birds selected for processing was lower ($P < 0.001$) under HS, intermediate under TN-PF conditions, and higher under TN-AL conditions. The weights of hot carcasses, fat pads, and chilled carcasses were reduced ($P < 0.001$) under HS and TN-PF conditions compared to TN-AL conditions. Both hot and chilled carcass yields were lower ($P < 0.001$) in TN-PF birds compared to TN-AL and HS birds, while chilled carcass yield was increased ($P < 0.001$) under HS conditions compared to TN-AL conditions. Birds fed the MRT+ and AA+ diets had a higher ($P = 0.003$) hot carcass yield compared to CTL birds, and birds fed the AA+ diet had also a higher ($P = 0.010$) chilled carcass yield compared to CTL birds. Interestingly, an interactive effect was observed ($P < 0.001$) on fat pad yield, as shown on **Figure 5.5**, whereby a reduction in fat pad yield for birds fed the AA+ diet under TN-PF conditions was not observed in TN-AL or HS birds.

Carcass part characteristics are presented in **Table 5.8**. Breast fillet, tenders, TBM, wing, and leg quarter weights were decreased ($P < 0.001$) under HS and TN-PF conditions compared to TN-AL conditions. A similarly trend was observed for breast fillets yields, with higher ($P < 0.001$) yields in TN-AL birds. Wings ($P = 0.018$) and leg quarter ($P < 0.001$) yields were increased under HS compared to TN-AL conditions, and leg quarter yields of TN-PF birds was also lower than that of HS birds. Tender ($P = 0.016$) and leg quarter ($P < 0.001$) yields were increased when birds were fed the MRT+ and MRTIV+ diets compared to the CTL diet. Additionally, the leg quarter yield was also increased with the AA+ diet compared to the CTL diet. A treatment interaction ($P = 0.043$) on TBM yield was found (**Figure 5.5**), characterized by a tendency of yield reduction with the AA+ diet during HS, whereas the yield tended to increase with this diet under TN-AL and TN-PF conditions.

Carcass Part Nutritional Composition

Relative weight gains of blood, feather, and carcass parts are presented in **Table 5.9**. There were no interactive effects on any of these measurements. Blood relative weight gain was decreased ($P < 0.001$) under HS compared to TN-AL and TN-PF conditions. The relative weight gain of the SI of HS birds was reduced ($P < 0.001$) compared to TN-PF birds, which was also lowered compared to TN-AL birds. Liver relative weight gains was reduced ($P = 0.034$) under HS conditions compared to TN-AL conditions, and intermediate in TN-PF birds. In addition, HS birds had a lower ($P = 0.006$) TBM relative weight gains than birds under both TN-AL and TN-PF conditions. A dietary effect was also observed on TBM ($P = 0.049$), but means were not separate with a Tukey test. Finally, abdominal fat relative weight gain of MRT+ fed birds was higher ($P=0.029$) than AA+ birds.

Table 5.10 presents the total carcass retention and carcass composition in DM, N, fat, and energy. A significant interaction (P = 0.015) on DM gain indicated that AA+ fed birds had similar DM gain as birds fed the other diets, while it was reduced in TN-AL conditions and tended to be reduced in TN-PF conditions (**Figure 5.6**). Nitrogen content of carcasses from birds fed the MRT+ and AA+ diet was higher (P = 0.003) than those from birds fed the CTL diet. Carcass composition in fat (P < 0.001) and energy (P = 0.018) was reduced under TN-PF birds compared to HS birds, and intermediate in TN-AL conditions. In addition, fat composition of carcasses was reduced (P < 0.001) in AA+ fed birds compared to the other diets, as well as energy composition (P = 0.004) compared to the CTL and MRTIV+ diet. Total carcass nitrogen retention was lowest (P < 0.001) under HS conditions, intermediate in TN-PF conditions, and the highest in TN-AL birds. Total fat retention was reduced (P < 0.001) under both HS and TN-PF conditions compared to TN-AL birds. Birds fed the AA+ diet retained less (P < 0.001) fat than birds fed with the other diets. Furthermore, total energy retention was lower (P < 0.001) in HS and TN-PF birds than TN-AL birds, and bird fed the AA+ diet had lower (P = 0.007) total energy retention than birds fed the CTL diet.

Energy partitioning data for the whole carcass expressed on a relative daily metabolic BW basis are presented in **Table 5.11**. Relative EI was increased (P = 0.007) by HS temperatures compared to TN-AL and TN-PF conditions. Additionally, ER was the highest (P = 0.001) under HS, intermediate under TN-AL, and the lowest in TN-PF conditions and birds fed the CTL diet also had a higher (P < 0.001) ER than birds fed with the other diets. Feeding the AA+ diet to birds under TN-AL and TN-PF conditions reduced their ERFat, while this effect was not observed under HS conditions (environment × diet, P = 0.023; **Figure 5.6**). Furthermore, the FHP was increased (P < 0.001) under HS and decreased under TN-AL compared to TN-PF

conditions, and the HI of HS birds relative to the total HP was reduced (P = 0.014) compared to TN-AL birds and intermediate in TN-PF birds. Net energy of MRTIV+ fed birds under HS was higher (P = 0.002) than under TN-AL conditions, and the NE of AA+ fed birds under HS was higher than under TN-AL and TN-PF conditions (**Figure 5.7**). Finally, EEx of birds under HS conditions was increased (P = 0.010) compared to birds reared under TN-AL and TN-PF treatments. Energy partitioning values expressed on a percentage of the EI are available in **Supplementary Table 5.1**.

The nitrogen partitioning of birds at the whole carcass level on a relative daily metabolic BW basis in depicted in **Table 5.12**. Birds reared under HS had a higher (P = 0.006) relative NI than TN-AL and TN-PF birds. The different dietary treatments influenced (P = 0.048) relative NI, although means were not separated by the Tukey test. However, DNI was increased with the MRT+ diet compared to the CTL diet (P = 0.048). The lowest (P = 0.013) NEf observed in HS birds, while TN-AL and TN-PF birds had higher efficiency. Nitrogen partitioning values expressed on a percentage of the NI are available in **Supplementary Table 5.2**.

Lastly, **Table 5.13** presents the results for nitrogen, fat, and energy composition in the small intestine, liver, offal and TBM parts. The observed interactive effect (P = 0.042) on the fat content of the SI indicates that HS birds fed the CTL diet had a higher fat content compared to TN-PF birds fed the MRT+ diet, while intermediate values were found in the other conditions. Liver relative gain in N was reduced (P < 0.001) under HS compared to TN-AL and TN-PF conditions, while its relative fat gain (P < 0.001) was lower in TN-PF birds compared to HS and TN-AL birds. Relative energy gain in the liver was increased (P = 0.015) under HS compared to TN-PF conditions. Offal relative gain in N was reduced (P = 0.003) in HS birds compared to TN-AL and TN-PF birds, and the offal of HS birds had a higher fat relative gain compared to the

offal od TN-PF birds. Distribution of the AA+ diet led to a reduction ($P = 0.044$) in the offal of fat relative gain compared to the CTL diet. In addition, energy relative gain in the offal of TN-PF birds was lower ($P = 0.005$) than in birds from other conditions. Regarding the TBM, while environmental conditions did not affect ($P > 0.05$) the N relative gain, it was reduced ($P = 0.014$) in CTL fed birds compared to AA+ fed birds. The fat relative gain of TN-PF birds was also lower ($P = 0.048$) than that of TN-AL birds, and intermediate with HS birds. The TBM of CTL and MRTIV+ birds also had a higher ($P < 0.001$) fat relative gain compared to the TBM of MRT+ and AA+ fed birds. Finally, the energy relative gain of birds fed with the AA+ diet was lower ($P < 0.001$) than that of birds fed with the other diets.

DISCUSSION

Performance and Internal Temperature

Continuous exposure of broilers to 35˚C markedly reduced performance as evidenced by a 20% reduction in FI, 32% reduction in BWG, and 20% increase in FCR during the challenge period. These performance degradations fall within the low range of what has been observed in previous studies using a constant HS model (De Souza et al., 2016; Awad et al., 2018; Teyssier et al., 2022b). This could be attributed to the actual mean ambient temperature (32.9˚C) in the HS houses being slightly lower than the targeted temperature (35˚C), which may have also prevented the marked increase in mortality observed in other constant HS models. Nonetheless, the observed performance reductions and the higher internal body temperature of HS birds, as reported in other studies (Ruff et al., 2020; Tabler et al., 2020; Flees et al., 2020), suggest that the elevated temperature in the HS houses induced physiological HS. Furthermore, when comparing the performance between TN-PF and HS birds in this experiment, approximately 80% of the BWG degradation under HS was attributed to decreased FI, while the remaining 20% was

directly associated with physiological changes induced by the elevated temperature. These findings align with the previous HS experiment conducted in our laboratory (Teyssier et al., 2022b), and fall within the general range of values reported in other studies, where 60 to 99% of the BWG reduction is explained by the reduced FI (Geraert et al., 1996; Bonnet et al., 1997; Garriga et al., 2006; Lu et al., 2007; Zuo et al., 2015; Zeferino et al., 2016; De Souza et al., 2016; De Antonio et al., 2017; Ma et al., 2021; Emami et al., 2021). In addition to the metabolic changes that will be further discussed below, the performance losses caused by HS *per se* can be attributed to various physiological changes triggered by HS-induced oxidative stress. These changes include alterations in gastrointestinal integrity, hormonal regulation, cardiovascular function, and systemic inflammation and have been previously reviewed (Brugaletta et al., 2022).

In contrast to previous studies that have reported performance improvements with high AA density diets (Dozier et al., 2007; Lilly et al., 2011; Maynard et al., 2019, 2023; Maharjan et al., 2020; Rabello et al., 2021), our experiment utilized isocaloric and isonitrogenous diets. This approach minimized adjustments in feed consumption and resulted in similar performance across all diets. In addition, the use of isonitrogenous diets, with changes in AA density being compensated by Glu inclusion, limited the potential inadequate ammonia detoxification with unbalanced AA diets, which can lead to ammonia toxicity and reduced performance (Stern and Mozdziak, 2019). In contrast to our findings, Nasr and Kheiri (2011) obtained higher BWG in broilers fed high AA levels (with Met and Lys dietary densities increased to 120%, while other AA were increased to 110%) compared to standard AA levels with isonitrogenous and isocaloric diets under TN conditions. In our experiment, the absence of dietary and interaction effects on performance indicates that the CTL diet likely met or exceeded the broiler's requirements for

growth under TN-AL, TN-PF, and HS conditions. This result was expected with TN-AL conditions since the CTL diet met breeder AA recommendations, and the excess AA provided in the other diets would likely be beyond their requirements under homeostatic conditions. However, the lack of beneficial effects with high AA density under TN-PF and HS conditions does not support an increase in essential AA requirements to 135% under HS conditions, and it is possible that the dietary density of certain AA may have been too high and concealed potential benefits at lower levels. Beyond performance results, other parameters measured in this experiment provide further insights into the digestive and metabolic alterations caused by HS, as well as the fate of the supplemental AA.

Apparent Ileal Digestibility

During HS conditions, intestinal integrity is compromised as characterized by reduced gastrointestinal tract blood flow, lower expression and activity of digestive enzymes and macronutrient transporters, and changes in microbiota populations (Brugaletta et al., 2022; Teyssier et al., 2022a). Additionally, the reduced relative weight gain of the small intestine observed in our study indicates that the gastrointestinal tract is affected by HS *per se* and reduced FI, potentially leading to reduced digestibility in broilers. However, in our experiment, we did not observe statistically significant environmental effects on nutrient AID, similar to findings from other experiments for DM (Faria Filho et al., 2007; Seven and Seven, 2008; Attia et al., 2017; Teyssier et al., 2023), and N AID (Laganá et al., 2007; Faria Filho et al., 2007; Habashy et al., 2017b; Ghareeb et al., 2022). Nevertheless, the magnitude of reductions in AID of DM (-2.8%), N (-3.0%), and energy (-3.1%), though not statistically significant, is consistent with what has been observed in other studies (Zuprizal et al., 1993; Bonnet et al., 1997; Laganá et al., 2007; Seven and Seven, 2008; Soleimani et al., 2010; De Souza et al., 2016; Attia et al., 2017; Teyssier

et al., 2023), and the lack of statistical separation in our experiment may be due to the observed variability. Furthermore, even though N digestibility was not significantly reduced, HS resulted in a lower average AID of essential (-5.0%) and total (-4.3%) AA compared to TN-PF conditions, confirming the negative effect of HS *per se* on AA digestibility. Similarly, Soleimani et al. (2010) observed a 5.5% reduction in total AA digestibility between broilers reared under TN or cyclic HS conditions. Regarding individual AA digestibility, our study, as well as others, have reported lower digestibility of Arg, His, Ile, Leu, Lys, Phe, Thr, Val, Ala, Asp, Cys, Glu, Gly, Pro, Ser, or Tyr under HS conditions (Wallis and Balnave, 1984; Soleimani et al., 2010; Teyssier et al., 2023). This reduction could be attributed to the alterations caused by HS on the gastrointestinal tract, as well as lower expression of several AA transporters (Habashy et al., 2017a), although some studies found no effect of HS on AA transporter expression (Sun et al., 2015; Habashy et al., 2017b; Al-Zghoul et al., 2019). Furthermore, birds under TN-PF conditions had the highest digestibility in this experiment, which differs from the results of our precedent experiment where PF birds had intermediate digestibility compared to HS and TN-AL birds (Teyssier et al., 2022b). The higher digestibility of PF birds in this experiment may be explained by better feed distribution throughout the day, with 3 distribution periods per day instead of a single daily allocation used in our previous trial. Indeed, others found variations in digestibility of broilers depending on the frequency of feeding periods during the day (Adeyemo et al., 2017).

Moreover, dietary treatment influenced nutrient digestibility in the current experiment. Interestingly, birds fed the MRT+ diet exhibited lower DM AID compared to birds fed the MRTIV+ and AA+ diets. The imbalanced essential AA profile of the MRT+ diet may be responsible for the reduced digestibility, as feed ingredients with imbalance AA profile have been associated with reduced digestibility (Khalaji et al., 2016; Yan et al., 2017). In the current

study, we observed reduced DE for birds fed the AA+ diet compared to the other diets. This contrasts with other research using non-isonitrogenous diets that did not observe an effect of AA density on apparent metabolizable energy (Rochell et al., 2017; Maharjan et al., 2020; Rabello et al., 2021). However, the lower amount of poultry fat in the AA+ diet, which is an easily digestible source of energy, may explain the observed difference in DE. Furthermore, supplementing AA above the bird's requirements generally resulted in increased AID of those specific AA (except of Leu, Lys, Phe, and Trp) due to the high digestibility of unbound AA (Izquierdo et al., 1988; Chrystal et al., 2020). Additionally, significant interactions between environment and diet were observed for Met and TSAA AID, indicating that over-supplementation of Met in the MRT+, MRTIV+, and AA+ diets increased TSAA and Met AID to a greater extent under HS conditions than under TN-AL or TN-PF conditions. Similar tendencies were also observed for Arg, Thr, Ile, His, Trp, and the average EAA AID. In other words, increasing dietary AA mitigated HS-induced reductions in AA digestibility due to the high digestibility of unbound AA. Interestingly, in contrast with EAA, the AA+ diet resulted in lower NEAA AID compared to the other diets (except for Ala and Ser AID). As different AA have specific transport mechanisms and compete for absorption sites in the small intestine (Hyde et al., 2003), the high digestibility of EAA supplemented in the AA+ diet may have prevented the efficient absorption of bound NEAA, resulting in lower AID.

Overall, the objective of equalizing the amount of digestible EAA intake between HS birds and TN-AL birds was achieved when those AA were supplemented to 135% (except for Val in the AA+ diet) of their concentrations in the CTL diet. Despite the reduced FI, the magnitude of the nutrient AID reduction indicates that digestibility alterations have a limited impact on the performance reduction attributed to HS *per se*. Therefore, to explain the decline in

performance, HS appears to induce important metabolic changes that can be directly measured through carcass characteristics and composition. These measurements will help characterize and quantify the fate of the absorbed nutrient, especially the AA whose digestible intake was equalized between HS and TN conditions.

Processing Measurements

The variation in live weight, hot and chilled carcass weights of processed birds followed a similar trend as performance across the different environmental conditions. The increase in hot and chilled carcass yields of HS broilers compared to TN-AL or TN-PF birds has been reported in other studies (Ain Baziz et al., 1996; Lu et al., 2007; Rosa et al., 2007; Zeferino et al., 2016), and several factors may contribute to this observation. First, HS induces a reduction in relative organ weights (Zeferino et al., 2016), including the small intestine, liver, and offal weights, as observed in our experiment. The lower relative liver weight under HS and TN-PF conditions compared to TN-AL conditions is consistent with the literature (Geraert et al., 1996; Plavnik and Yahav, 1998; Shim et al., 2006; Zeferino et al., 2016; Chen et al., 2020), though Lu et al. (2019b) found opposite results. The reduction in relative liver weight could be attributed to the lower metabolic needs due to reduced FI (Emami et al., 2020). Second, HS reduced the relative blood weight gain, which is likely a consequence of dehydration induced by high temperatures. Third, HS could also lead to a reduction in relative feather weight to improve heat dissipation (Geraert et al., 1993), although no differences on this outcome were observed in our experiment or by others (Geraert et al., 1996). In addition, the reduced carcass yield under TN-PF conditions compared to HS conditions could be explained by the lower fat pad yield, which has also been observed in other studies (Ain Baziz et al., 1996; Lu et al., 2007; Zeferino et al., 2016; Teyssier et al., 2022b). This reduced fat pad yield under HS conditions indicates higher mobilization of fat

as an energy source under TN-PF conditions (Zhan et al., 2007). Moreover, breast fillet yields were reduced under HS and TN-PF conditions, and the relative weight of the TBM was reduced under HS compared to TN conditions. The reduction in breast fillet yield has been observed in other studies (Temim et al., 2000; Zeferino et al., 2016; Emami et al., 2021; El-Tarabany et al., 2021; Teyssier et al., 2022b) and is associated with a relative increase in wing and leg quarter yields under HS conditions. The difference in muscle fibers composition, with oxidative fibers in the legs and glycolytic fibers in the breast, could partly explain the observed difference in yields. Glycolytic fibers rely on glycogen storage, which is depleted following decreased FI in response to HS (Temim et al., 2000; Zeferino et al., 2016). Additionally, breast fillets are a highly selected trait in modern broilers, and as such are highly affected by conditions that degrade performance (Orlowski et al., 2020).

There were no changes in hot and chilled carcass weights across the different dietary treatments in our study. However, AA+ fed birds had a higher hot and chilled carcass yield compared to CTL fed birds. Unlike the environmental conditions, there were no differences in relative weights of blood, feathers, small intestine, liver, or offal relative weights among diets. Therefore, the higher carcass yield of birds fed the AA+ diets could be mainly attributed to lower fat pad yields and higher TBM yields. Previous research has also observed reduced fat pad yields with high CP or AA levels compared to standard diets (Corzo et al., 2005; Kidd et al., 2005; Dozier et al., 2007; Maharjan et al., 2020; Maynard et al., 2023). However, in our study, a significant environment × diet interaction was observed for both TBM and fat pad yields in processed birds. Under TN-PF conditions, the AA+ diet led to reduced fat pad yields and a tendency to increase TBM yields, but these improvements were not observed under HS conditions. The absence of beneficial effects of increased AA diets on these parameters suggests

that HS *per se* favors fat pad development over TBM development. Similar to our results, Maharjan et al. (2020) did not observe improved breast yields in HS birds fed a high AA density diet (120 vs 100%). However, they did observe a reduction in fat pad yield with the high AA density diet, which differs from our study. This discrepancy could be explained by the lower stress intensity in their HS experiment, with an average temperature of 30°C. In addition, Alhotan et al. (2021) did not find a significant interaction on breast yields between HS and TN conditions with AA density diets ranging from 80 to 110%. However, the lower AA level in their high AA diet (110%) compared to ours (135%), and the lower stress intensity due to the use of a cyclic HS model could explain the absence of interaction. It is worth noting that only main effects were significant in our study when abdominal fat and TBM yields were measured on sampled birds instead of processed birds. The lower statistical power and higher variability of carcass composition data when using one bird per pen as replicate, compared to processing data with the average of processed birds per pen as replicate, could explain this difference. Furthermore, the absence of beneficial effects on chilled carcass yields of the MRT+ and MRTIV+ diets under the different environmental conditions suggests that the relative AA needs of these increased AA were not altered by feed restriction or HS conditions.

The differences obtained on TBM and fat pad yield could be attributed to changes occurring in nutrient partitioning and metabolism. Under TN-PF conditions, energy coming from the excess of AA with the AA+ diet seems to be deposited in the breast instead of fat, while this was not the case under HS conditions. Those results will be further discussed in the light of carcass composition results.

Carcass Part Nutritional Composition

Energy Partitioning. At the whole carcass level, EI relative to metabolic BW was increased under HS compared to both TN-AL and TN-PF conditions, indicating that more energy was ingested to build one metabolic BW unit under HS. This finding aligns with similar studies that have reported increased apparent metabolizable energy intake in HS conditions relative to metabolic BW (Faria Filho et al., 2007; De Souza et al., 2016). Notably, the energy AID was not affected by the environmental conditions, indicating that the higher EEx observed under HS was probably attributed to the higher EI. Interestingly, the total energy retention of HS birds was similar to that of TN-PF birds, consistent with the observation of Geraert et al. (1996). However, when expressed on a metabolic BW basis, the ER of HS birds was increased compared to TN-PF birds. This result contradicts the findings of De Souza et al. (2016), who reported similar ER between PF and constant or cyclic HS birds. In our study, two factors could logically explain the increased ER under HS conditions: either an increase in the carcass nutrient density or a shift in energy storage towards a higher caloric nutrient. Both factors are often interconnected, as protein (4 kcal/g) has a lower caloric content than fat (9 kcal/g) (Hunt and Stubbs, 1975), and proteins are hydrophilic, attracting water, while lipids are hydrophobic and do not interact with water. Results on DM carcass composition support the interaction observed on TBM and fat pad yields. Specifically, birds fed with the CTL diet had similar DM content across the environmental conditions, whereas higher DM retention was observed in carcasses of HS birds fed the AA+ diet. In addition, though the interaction was not significant, the higher fat composition in the carcasses of HS birds contributed to the increased DM and energy content of the carcass, as well as the higher ER per metabolic BW unit.

In terms of energy efficiency, our study did not find any influence of environment, while other studies have reported either no effect or a reduction in energy efficiency under HS conditions (Geraert et al., 1996; Faria Filho et al., 2007; De Souza et al., 2016). These inconsistent findings may be attributed to different stress intensity as De Souza et al. (2016) measured different responses with a cyclic or constant HS challenge. Interestingly, HS increased the FHP, indicating an increase in energy needs for maintenance functions related to thermoregulation (Chowdhury et al., 2021). However, as observed by Geraert et al. (1996) and Faria Filho et al. (2007), the total HP did not differ across the environmental conditions, primarily due to the numerical reduction in HI under HS conditions. Though the reduction in HI was not significant on a metabolic BW basis, data expressed on an energy intake basis indicate that a lower percentage of the EI was used for HI in HS birds compared to TN-AL and TN-PF birds. In addition, relative to total HP, HI of HS birds was reduced compared to TN-AL birds. The reduced HI under HS conditions can be attributed to two factors: a decrease in HP related to the physical activity of the bird (**AHP**), which was not assessed in this study, and a reduction in HP associated with the thermic effect of feed (**TEF**) (Skomiał and Lapierre, 2016). In terms of TEF, the decreased FI seems to account for approximately half of the reduction in HI relative to the total HP. In addition, the shift in energy metabolism to more lipid utilization rather than protein, contributes to the reduction of HP under HS conditions. Conversely, other research has found increased HP under constant HS compared to TN-AL and TN-PF conditions (De Souza et al., 2016), possibly due to a higher increase in FHP to meet thermoregulation needs. The combined effect of increased FHP and ER under HS conditions resulted in a higher NE (+12.5%), which will be further discussed in relation to the ERFat.

Dietary treatments also had a notable impact on energy partitioning in broilers. Similar to the findings of Musigwa et al. (2020) when feeding with low and high CP diets, the energy retained at the whole carcass level (per metabolic BW unit) was reduced in birds fed the AA+ diets compared to those on the CTL diet. As mentioned earlier, the lower energy retention of AA+ fed birds can be attributed to a lower nutrient density and a shift of energy toward low caloric nutrients. Indeed, the DM content of the AA+ fed birds appears to be decreased particularly under TN-PF and TN-AL conditions. This higher water content is probably a consequence of the increase in nitrogen and decrease in fat carcass composition associated with the AA+ diet. In addition, AA density did not change the HP or FHP in our study. While dietary CP is known to contribute to higher metabolic HP due to a higher heat increment than fat and carbohydrates (Morales et al., 2020), feeding iso-nitrogenous diets mitigated this effect in the current study.

Lipid Metabolism and Partitioning. Regarding lipid metabolism, consistent results were obtained between processing and carcass composition data. Although the overall fat content of the carcass did not differ significantly (-11.5%) between TN-PF and HS birds, the fat composition per DMG unit was lower in TN-PF birds compared to HS birds. When expressed on a metabolic BW or intake basis, ERFat was increased in HS conditions compared to TN-PF conditions, which is in line with findings from other studies (Geraert et al., 1996; Faria Filho et al., 2007). In addition, the increased fat retention caused by HS is the main factor contributing to the increased ER discussed above.

Several factors have been proposed to explain the pronounced increase in energy storage in the form of fat during HS periods. First, fat has a lower heat increment compared to carbohydrates and proteins (Fuller and Rendon, 1977; Musharaf and Latshaw, 1999), and the

storage or oxidation of fat, instead of protein, as an energy source during HS periods may help to reduce the bird's HP. Second, fat metabolism also generates more metabolic water than carbohydrate and protein catabolism, which can in turn be utilized for heat dissipation through evaporation (Barboza et al., 2009). Third, lipids are an efficient energy storage source, providing 9 kcal/g, compared to the 4 kcal/g of protein and carbohydrates (Hunt and Stubbs, 1975). Therefore, under HS, where energy availability is limited due to the reduced FI and increased energy requirements for thermoregulation, relying on lipid metabolism could be advantageous.

Physiologically, changes in fat metabolism are coordinated by enzymes involved in lipogenic and lipolytic processes (Jastrebski et al., 2017; Flees et al., 2017; Lu et al., 2019a; b; c; Teyssier et al., 2023). Moreover, increased fat retention could result from hormonal dysregulation, including elevated levels of corticosterone and glucagon (Sands and Smith, 2002; Lu et al., 2019b; Ramiah et al., 2019). Fat deposition during hot periods varies depending on the breed, with heat-tolerant breeds having lesser abdominal fat deposition (Lu et al., 2007). Interestingly, the fat content of liver and offal were increased under HS compared to TN-PF conditions. Higher visceral fat storage under HS, along with the increased abdominal fat, could explain the higher fat retention in the offal. The liver is the main site of *de novo* lipogenesis in avian species (O'Hea and Leveille, 1968; Leveille, 1969), which could explain their higher fat retention. Similar to our study, lower intramuscular fat composition has been previously observed under HS or TN-PF conditions (Lu et al., 2007; De Antonio et al., 2017). However, since the breast and other muscles contribute to a higher proportion of carcass weight, increased lipid content in these areas likely plays a greater role in the overall increase in carcass lipid content compared to the observed increase in fat in vital organs (De Antonio et al., 2017).

In addition, in AA+ fed birds, fat retention was decreased on an absolute, metabolic BW, and intake basis compared to birds fed the other diets. Similarly, lower energy retained as fat in broilers fed high CP diets was observed by Faria Filho et al. (2007) (20 vs 23% CP) and Musigwa et al. (2020) (18.9 vs 23.5% CP) using isocaloric and non-isonitrogenous diets. However, the lower fat retention of high CP diets in these studies was explained by two distinct factors due to different experimental approaches. In Musigwa et al. (2020), the low CP diet based on corn had a higher fat content than the high CP diet based on wheat to obtained isocaloric diets. As dietary fat is more readily metabolized into fat compared to protein (Macleod, 1997), fat deposition is reduced in birds fed high CP diets. In contrast, birds fed the high CP diet had a higher fat content in the study by Faria Filho et al. (2007) because the higher soybean meal content of the high CP diet required a higher added fat inclusion. However, the excess in essential AA provided by the high CP diet resulted in a reduction of the FI, which may explain the lower fat retention. In our study, similar FI was observed across diets and isonitrogenous diets were used, which reduced differences in dietary fat composition. However, Glu, which was used to balance N content, has a lower apparent metabolizable energy compared to the average of the essential AA (NRC, 1994). Consequently, the CTL diets had a higher fat content than the other diets to equalize energy content, which could partly explain the increased fat deposition in CTL fed birds. In our study, the reduced fat content with the AA+ diet was observed for the offal, probably because of the reduced visceral fat, and in the TBM. Difference in fat TBM composition was not observed in other studies with different CP levels (Corzo et al., 2010; Lilly et al., 2011).

Interestingly, we observed similar trends in fat pad yield and ERFat when expressed on a metabolic BW basis. Therefore, HS compromised nutrient utilization, and while diets with a

balanced and high density of AA reduced fat deposition under TN conditions, no reduction in ERFat were observed under HS conditions. Since the FHP was not influenced by dietary treatments, the lower ERFat with AA+ diet in TN-AL and TN-PF conditions resulted in a similar reduction in NE, which was not the case under HS conditions. The reduction in ERFat with the AA+ diet under TN conditions resulted in more energy available for other metabolic pathways. Interestingly, it seems that HS may downregulate these pathways to promote fat deposition.

Protein Metabolism and Partitioning. One plausible explanation for the observed variation in fat deposition could be that feeding high AA diets under TN conditions promotes muscle growth and protein deposition in the carcass, while HS reduces the deposition of energy in the form of protein. In contrast to fat retention, the total N retention of HS birds was reduced compared to TN-PF birds, which is consistent with the findings of Geraert et al. (1996 and De Souza et al. (2016). However, when expressed on a metabolic BW basis, and contrary to Faria Filho et al. (2007), NR as well as ERPro did not differ among environmental conditions. This could seem counterintuitive, but it suggests that the reduction in growth under HS is accompanied by a proportional reduction in total N retention and protein metabolism rate. Several mechanisms have been proposed to explain the reduced deposition of protein under HS beyond that explained by the reduced FI. Molecular markers indicate that a reduction in protein synthesis, via downregulation of the IGF1/mTOR pathways, and an increase in protein breakdown, via upregulation of the ubiquitin–proteasome pathway, lead to lower protein deposition, with some differences among muscle types and HS models (Temim et al., 2000; Zuo et al., 2015; Lu et al., 2019a; Ma et al., 2021; Li et al., 2021; Teyssier et al., 2023).

From a metabolic perspective, though there were no differences in NR in the current study, we observed differences in NI, with higher NI per unit of metabolic BW under HS

conditions. Consequently, NEf was reduced under HS conditions and is in line with findings from other studies (Faria Filho et al., 2007; De Souza et al., 2016). In our study, the same amount of N was deposited per metabolic BW unit under HS or TN conditions, but this required higher relative N intake to form each unit of metabolic BW. Since N AID was not affected in our study, the increased N needs per unit of metabolic BW is probably explained by metabolic changes, further confirming the increased reliance of HS birds on protein as an energy source. In addition, the increased NEx under HS indicates that extra NI was utilized by physiologic or metabolic processes triggered by the heat. First, under HS conditions, more N could be directed toward the synthesis of proteins involved in thermoregulation like heat shock proteins, or inflammation like cytokines, chemokines, and acute-phase proteins (Gabriel et al., 1996; Liu et al., 2014; Xie et al., 2014; Ohtsu et al., 2015; Varasteh et al., 2015; Flees et al., 2017; Alhenaky et al., 2017; Cramer et al., 2018; He et al., 2019; Baxter et al., 2020; Greene et al., 2021, 2022; Alzarah et al., 2021; Emami et al., 2021; Teyssier et al., 2023). Second, free AA, whether absorbed or resulting from protein degradation, could be directed to energetic pathways, such as gluconeogenesis, to meet the increased energy needs associated with thermoregulation during reduced carbohydrate intake associated with reduced FI (Lu et al., 2018; Ma et al., 2018, 2021; Kim et al., 2022a). Therefore, the higher protein catabolism that occurs during constant HS (Ma et al., 2021) could provide AA for gluconeogenesis pathway, even though reduced protein deposition seems to be primarily influenced by a reduction in protein synthesis rather than an increased protein degradation (Temim et al., 2000). In birds, gluconeogenesis occurs in the liver and kidney, which could explain the reduced N content in the liver and offal of HS birds, corresponding with the higher relative fat content due to increased lipogenesis.

Interestingly, similar to the findings of Faria Filho et al. (2007), AA density did not impact N retention, either on a total, metabolic BW, or intake basis. However, N composition on a DMG basis was increased in AA+ fed birds compared to CTL fed birds, and this was driven by the increased N content in the TBM. The conflicting results obtained when data are expressed on a metabolic BW and DMG basis is explained by the higher water retention of AA+ fed birds caused by their higher N composition. Therefore, it seems that the AA+ diet promotes protein synthesis compared to other diets and its lower fat content compared to other diets may have caused those birds to be more dependent on the supplemented AA to satisfy increased energy needs for thermoregulation.

In conclusion, constant HS induces a reduction in performance that is mainly attributed to the reduced FI. From an energetic standpoint, HS increases energy requirements for maintenance functions due to thermoregulation, which is an important challenge for birds with limited heat dissipation capacities. Therefore, birds must adopt mechanisms to limit the HP generated by productive functions (i.e., HI). While reduced FI contributes to about half of the decrease in HI relative to total HP under HS, other factors such as decreased physical activity, lower digestibility, and metabolic alterations also contributed to limit the HP. Physical activity was not measured in our study, and degradation in nutrient AID was limited under HS conditions, which indicates that reduced digestibility had a minor impact on reduced performance and HI caused by HS *per se*. Among macronutrients, essential AA digestibility was more adversely affected, yet supplementation of these AA in the unbound form helped limit this reduction. Further research is needed to explore the potential benefits of low CP diets supplemented with feed-grade AA to mitigate the negative impact of HS on AA digestibility associated with the HP from protein digestion. Regarding metabolic alterations, HS promotes fat storage over protein deposition,

likely due to the lower HI associated with fat metabolism. Interestingly, high dietary density of essential AA did not improve fat and protein deposition, contrary to observations under PF conditions. Therefore, the reduced growth and preference for fat metabolism induced by HS cannot be solely attributed to insufficient intake of digestible AA. Moreover, increasing the digestible density of Met, Arg, and Thr in combination, either with or without addition of Ile and Val, did not have a positive impact under HS. This suggests that these AA are not deficient when feeding recommended dietary concentrations under HS or that high AA supplementation in this study concealed potential benefits at lower supplementation levels. Instead of being deposited, the excess dietary AA could be used for thermoregulation or energy purposes to meet the increased maintenance needs, but further investigation is necessary to quantify the partitioning of among maintenance and productive functions for birds subjected to HS.

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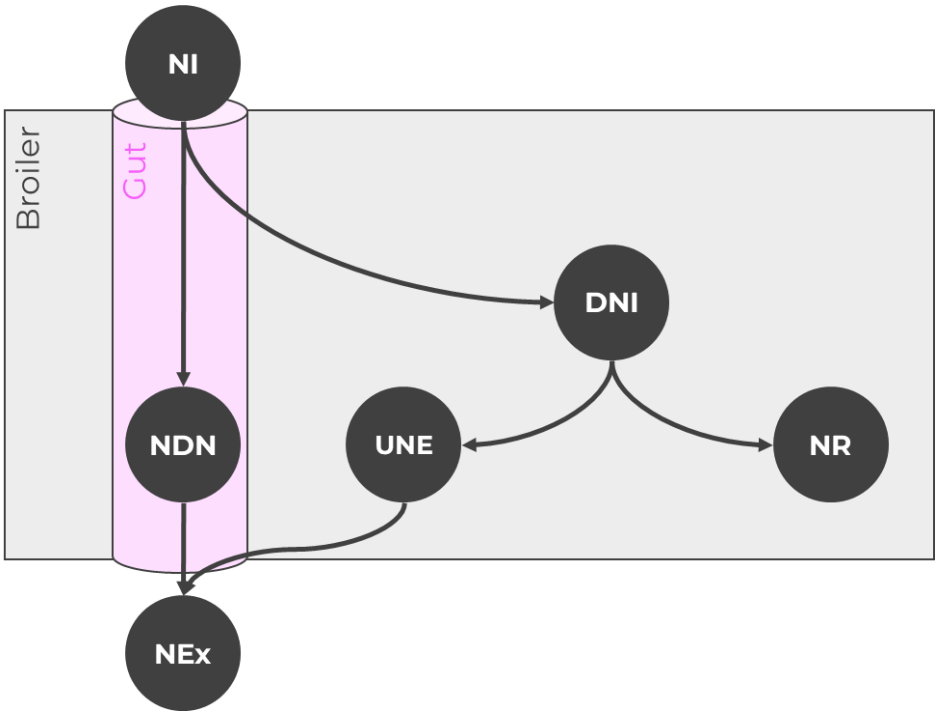
TABLES AND FIGURES

Table 5.1. Diet composition and chemical analysis of the experimental diets

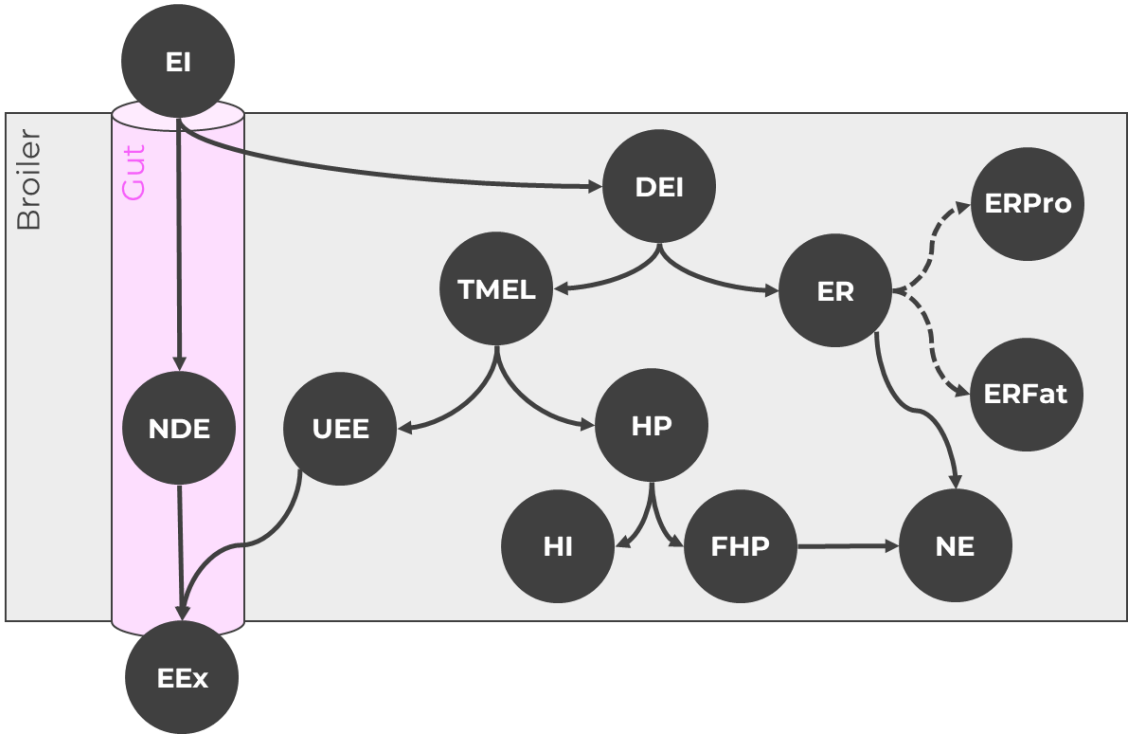
	Starter	Grower	Finisher[1] (d 20 to 42)			
Item, % as-fed	(d 0 to 11)	(d 12 to 19)	CTL	MRT+	MRTIV+	AA+
Corn	58.45	63.05	64.79	64.52	64.97	66.19
Soybean meal (48%)	36.99	32.51	26.28	26.28	26.28	26.28
Poultry Fat	1.28	1.32	2.14	2.02	1.74	1.15
Limestone	1.15	1.11	1.05	1.05	1.05	1.05
Dicalcium phosphate	0.71	0.57	0.43	0.43	0.43	0.42
Salt	0.36	0.24	0.12	0.12	0.12	-
Sodium sulfate	0.10	0.25	0.50	0.50	0.50	0.66
Glutamic acid	-	-	3.34	2.81	2.13	-
Inert Filler	-	-	-	-	-	0.03
L-lysine HCl	0.20	0.21	0.25	0.25	0.25	0.70
DL-methionine	0.35	0.32	0.29	0.58	0.57	0.57
L-threonine	0.11	0.10	0.06	0.29	0.29	0.29
L-valine	-	-	0.02	0.02	0.30	0.30
L-arginine	-	-	0.02	0.40	0.40	0.40
L-Isoleucine	-	-	-	-	0.25	0.24
Leucine	-	-	-	-	-	0.49
L-tryptophan	-	-	-	-	-	0.06
Histidine	-	-	-	-	-	0.16
Phenylalanine	-	-	-	-	-	0.28
Choline chloride (60%)	0.10	0.10	0.03	0.03	0.03	0.03
Enzyme mixture[2]	0.01	0.01	0.01	0.01	0.01	0.01
Vitamin premix[3]	0.05	0.05	0.03	0.03	0.03	0.03
Mineral premix	0.10	0.10	0.10	0.10	0.10	0.10
Titanium Dioxide	-	-	0.50	0.50	0.50	0.50
Coccidiostat[4]	0.05	0.05	0.05	0.05	0.05	0.05
Calculated composition, % unless noted otherwise[5]						
AMEn (kcal/kg)	2,975	3,025	3,100	3,100	3,100	3,100
NE (kcal/kg)[6]	2,404	2,444	2,453	2,452	2,450	2,446
Ether extract	3.25	3.45	4.32	4.19	3.92	3.39
Crude Protein	22.23	20.38	20.63	20.63	20.63	20.63
AID Lys	1.22 (1.00)	1.12 (1.00)	1.02 (1.00)	1.02 (1.00)	1.02 (1.00)	1.38 (1.00)
AID Met	0.63 (0.52)	0.59 (0.53)	0.55 (0.54)	0.83 (0.81)	0.82 (0.81)	0.82 (0.60)
AID TSAA	0.92 (0.75)	0.85 (0.76)	0.80 (0.78)	1.07 (1.05)	1.07 (1.05)	1.07 (0.78)
AID Thr	0.83 (0.68)	0.76 (0.68)	0.66 (0.65)	0.90 (0.88)	0.90 (0.88)	0.90 (0.65)
AID Trp	0.24 (0.19)	0.21 (0.19)	0.18 (0.18)	0.18 (0.18)	0.18 (0.18)	0.25 (0.18)
AID Arg	1.36 (1.12)	1.23 (1.10)	1.09 (1.07)	1.47 (1.44)	1.47 (1.44)	1.47 (1.07)
AID Ile	0.85 (0.69)	0.77 (0.69)	0.69 (0.68)	0.69 (0.68)	0.94 (0.92)	0.94 (0.68)
AID Leu	1.64 (1.34)	1.53 (1.37)	1.45 (1.42)	1.45 (1.42)	1.45 (1.42)	1.96 (1.42)
AID Val	0.92 (0.75)	0.84 (0.75)	0.79 (0.77)	0.79 (0.77)	1.06 (1.04)	1.06 (0.77)
AID His	0.53 (0.43)	0.49 (0.44)	0.46 (0.45)	0.45 (0.44)	0.46 (0.45)	0.62 (0.45)
AID Phe	0.97 (0.80)	0.89 (0.79)	0.80 (0.79)	0.80 (0.79)	0.80 (0.79)	1.09 (0.79)
Total Ca	0.90	0.84	0.76	0.76	0.76	0.76
Total P	0.56	0.52	0.45	0.45	0.45	0.45
Available P	0.45	0.42	0.38	0.38	0.38	0.38
DEB[7] (mEq/kg)	250	250	250	250	250	250

[1] **CTL**: 100% of recommended AA levels. **MRT+**: 135% of recommended levels of TSAA, Arg, Thr. **MRTIV+**: 135% of recommended levels of TSAA, Arg, Thr, Ile, Val. **AA+**: 135% of recommended levels of all 10 essential AA.
[2] Rovabio® AdvancePhy T (Adisseo France S.A.S., Antony France).
[3] Supplied the following per kg of diet: vitamin A 6,173 IU; vitamin D3 4,409 ICU; vitamin E 44 IU; vitamin B12 0.01 mg; menadione 1.20 mg; riboflavin 5.29 mg; d-pantothenic acid 7.94 mg; thiamine 1.23 mg; niacin 30.86 mg; pyridoxine 2.20 mg; folic acid 0.71 mg; biotin 0.07 mg; manganese 24 mg; zinc 14.4 mg; selenium 0.04 mg; copper 0.68 mg; iodine 0.47 mg.
[4] BioCox60 (Huvepharma, INC., USA).
[5] AID: Apparent ileal digestible concentration; the digestible AA:digestible Lys ratio is indicated in parenthesis.
[6] NE: Net energy, predicted using the equation from Wu et al. (2019): NE = 0.808 AMEn (MJ/kg) - 0.017 CP (%) + 0.031 EE (%). All values on DM basis.
[7] DEB: Dietary electrolyte balance.



Parameter	Definition	Determination
NI	Nitrogen intake	Measured
DNI	Digestible nitrogen intake	Calculated from apparent ileal digestibility
NDE	Non-digestible nitrogen	NDN = NI – DNI
NR	Nitrogen retention	Measured by carcass slaughter technique
UNE	Urinary nitrogen excretion	UNE = DNI – NR
NEx	Nitrogen excretion	NEx = NDN + UNE

Figure 5.1. Nitrogen partitioning calculations at the whole carcass level



Parameter	Definition	Determination
EI	Energy intake	Measured
DEI	Digestible energy intake	Calculated from apparent ileal digestibility
NDE	Non-digestible energy	NDE = EI – DEI
ER	Energy retention	Measured by carcass slaughter technique
ERPro	Energy retained as protein	ERPro = N retention (g) × 6.25 × 5.66 (kcal/g)
ERFat	Energy retained as fat	ERFat = Fat retention (g) × 9.37 (kcal/g)
TMEL	Total metabolic energy losses	TMEL = DEI – ER
UEE	Urinary energy excretion	Estimated from N content of urinary losses (UNE) UEE = UNE × 8.20 (kcal/g)
EEx	Energy excretion	EEx = NDE + UEE
HP	Heat production	HP = TMEL – UEE
FHP	Fasting heat production	FHP = 106.1 (kcal) × daily $BW^{0.70}$ ×22 (d), with daily $BW^{0.70}$ estimated by: $\left(\frac{BW_{d20}+BW_{d42}}{2}\right)^{0.70}$
HI	Heat increment	HI = HP – FHP
NE	Net energy	NE = ER + FHP

Figure 5.2. Energy partitioning calculations at the whole carcass level

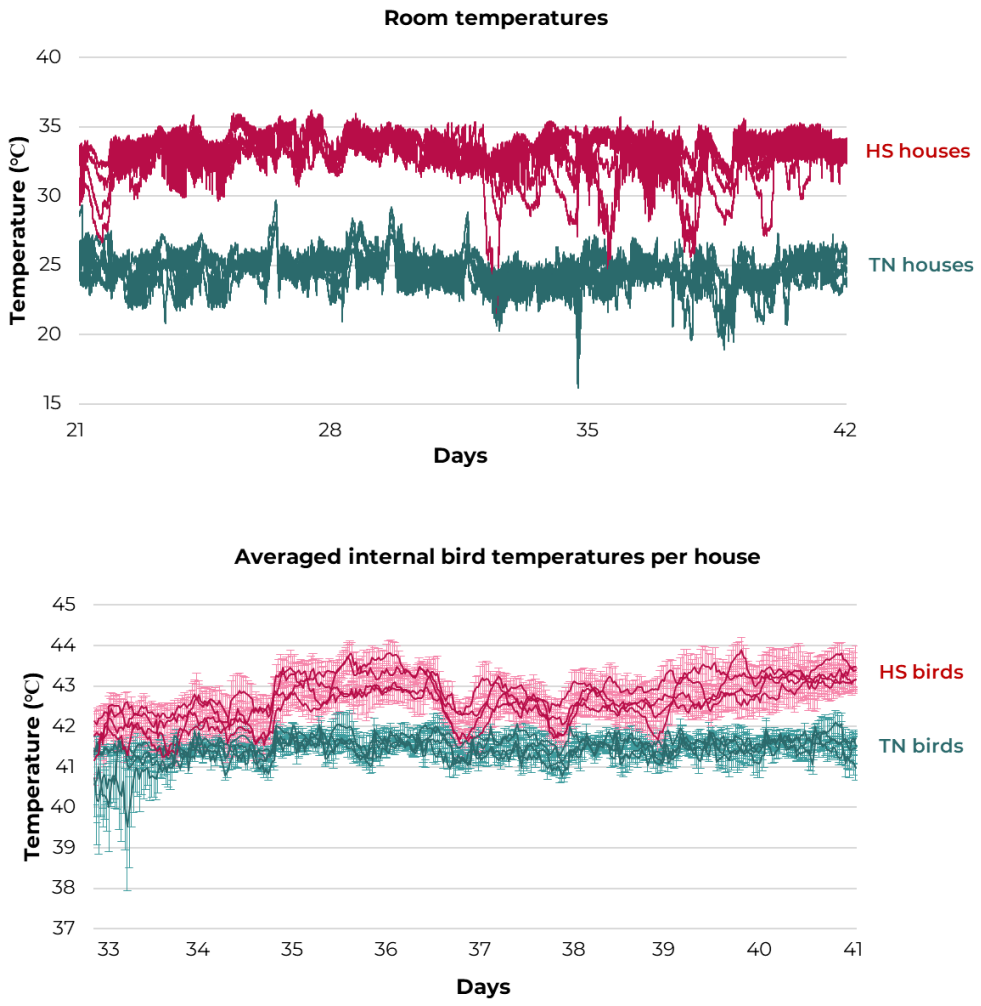


Figure 5.3. House and averaged internal temperature recorded during the experiment.

TN: Houses set to a continuous 24°C. **HS**: Houses set to a continuous 35°C
Error bars in the second graph represent the standard error of the mean of the 4 birds per house.

Table 5.2. Effect of dietary amino acid density on performance of broilers reared under thermoneutral, heat stress, and pair-feeding conditions from d 20 to 41.

Item	20 d BW, kg	41 d BW, kg	20-41 d FI, kg	20-41 d BWG, kg	20-41 d FCR, kg:kg	20-41 d Mortality, %
Main effect of environment[1]						
TN-AL	0.993	3.522[a]	3.910[a]	2.529[a]	1.549[c]	1.042[a]
HS	0.999	2.716[c]	3.124[b]	1.716[c]	1.852[a]	3.056[a]
TN-PF	0.999	2.881[b]	3.098[b]	1.882[b]	1.655[b]	1.326[a]
SEM[3]	0.0074	0.0371	0.0299	0.0406	0.0277	0.7178
Main effect of diet[2]						
CTL	0.998	3.023	3.409	2.026	1.718	1.782
MRT+	0.999	3.046	3.375	2.046	1.675	1.435
MRTIV+	0.995	3.024	3.366	2.028	1.692	2.361
AA+	0.997	3.066	3.359	2.069	1.657	1.652
SEM[3]	0.0048	0.0386	0.0325	0.0400	0.0257	0.7617
P-values						
Environment	0.796	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.039**
Diet	0.703	0.836	0.701	0.855	0.318	0.847
Environment × Diet	0.205	0.854	0.982	0.829	0.572	0.787

[1] **TN-AL**: Birds reared under continuous 24°C and ad libitum feeding. **HS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-PF**: Birds reared under continuous 24°C and pair-fed to the HS treatment.

[2] **CTL**: 100% of recommended AA levels. **MRT+**: 135% of recommended levels of TSAA, Arg, Thr. **MRTIV+**: 135% of recommended levels of TSAA, Arg, Thr, Ile, Val. **AA+**: 135% of recommended levels of all 10 essential AA.

[3] SEM: Pooled standard error of the mean.

[a-b] Means within row without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey-Kramer comparison test.

Table 5.3. Effect of dietary amino acid density on nutrient apparent ileal digestibility (AID) and digestible energy (DE) on broilers reared under thermoneutral, heat stress, and pair-feeding conditions from d 20 to 41.

	Dry Matter	Energy		Nitrogen	Fat
Item	AID, %	AID, %	DE, kcal/kg	AID, %	AID, %
Main effect of environment[1]					
TN-AL	73.7	75.3	2,941	84.5	95.8
HS	71.6	73.0	2,862	82.0	95.1
TN-PF	76.0	77.3	3,033	85.8	96.3
SEM[3]	1.27	1.20	47.9	1.13	1.04
Main effect of diet[2]					
CTL	73.1[ab]	75.5[ab]	2,967[a]	83.4	95.4
MRT+	72.1[b]	75.6[a]	2,962[a]	84.9	95.5
MRTIV+	74.6[a]	76.4[a]	3,002[a]	84.6	96.3
AA+	75.3[a]	73.2[b]	2,851[b]	83.4	95.9
SEM[3]	0.92	0.89	35.6	0.79	0.72
P-values					
Environment	0.104	0.088	0.094	0.105	0.721
Diet	**0.005**	**0.004**	**<0.001**	0.071	0.498
Environment × Diet	0.576	0.439	0.766	0.606	0.971

[1] **TN-AL**: Birds reared under continuous 24°C and ad libitum feeding. **HS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-PF**: Birds reared under continuous 24°C and pair-fed to the HS treatment.

[2] **CTL**: 100% of recommended AA levels. **MRT+**: 135% of recommended levels of TSAA, Arg, Thr. **MRTIV+**: 135% of recommended levels of TSAA, Arg, Thr, Ile, Val. **AA+**: 135% of recommended levels of all 10 essential AA.

[3] SEM: Pooled standard error of the mean.

[a-b] Means within row without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey-Kramer comparison test.

Table 5.4. Effect of dietary amino acid density on apparent ileal digestibility (AID) of total sulfur amino acids (TSAA) and essential amino acids (EAA) of broilers reared under thermoneutral, heat stress, and pair-feeding conditions from d 20 to 41.

Item	TSAA	Met	Arg	Thr	Ile	Val	His	Leu	Lys	Phe	Trp	EAA
Main effect of environment[1]												
TN-AL	88.9^{ab}	94.3^{ab}	93.8^{ab}	81.7	87.9^{ab}	86.0	87.7	85.1^{ab}	90.0	88.0^{ab}	86.4^{ab}	88.1^{ab}
HS	85.7^{b}	92.4^{b}	91.9^{b}	79.2	85.2^{b}	83.3	85.4	81.6^{b}	88.2	85.0^{b}	84.7^{b}	85.8^{b}
TN-PF	89.8^{a}	95.3^{a}	94.2^{a}	83.3	89.9^{a}	87.5	88.5	88.6^{a}	90.7	90.9^{a}	88.3^{a}	89.7^{a}
SEM[3]	0.94	0.66	0.56	1.48	0.97	1.06	1.16	0.94	0.89	0.79	0.69	0.87
Main effect of diet[2]												
CTL	85.8^{b}	92.3^{c}	92.2^{c}	78.0^{c}	86.4^{bc}	84.7^{bc}	86.3^{b}	84.5	89.2	87.3	86.2	86.7^{b}
MRT+	89.6^{a}	94.9^{ab}	93.9^{ab}	82.3^{ab}	86.1^{c}	84.3^{c}	87.0^{ab}	84.3	89.5	87.5	87.1	87.7^{ab}
MRTIV+	89.7^{a}	95.1^{a}	94.3^{a}	84.2^{a}	89.9^{a}	87.0^{a}	87.0^{ab}	85.2	89.5	88.4	86.2	88.7^{a}
AA+	87.4^{b}	93.8^{b}	92.9^{bc}	81.1^{b}	88.2^{ab}	86.5^{ab}	88.4^{a}	86.4	90.3	88.7	86.3	88.3^{ab}
SEM[3]	0.69	0.48	0.43	1.03	0.74	0.78	0.78	0.80	0.64	0.68	0.66	0.66
P-values												
Environment	**0.030**	**0.033**	**0.040**	0.210	**0.023**	0.059	0.207	**0.002**	0.169	**0.002**	**0.017**	**0.031**
Diet	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**0.012**	0.085	0.233	0.205	0.664	**0.029**
Environment × Diet	**0.015**	**0.008**	0.069	0.075	0.096	0.146	0.098	0.196	0.116	0.161	0.080	0.076

[1] **TN-AL**: Birds reared under continuous 24°C and ad libitum feeding. **HS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-PF**: Birds reared under continuous 24°C and pair-fed to the HS treatment.

[2] **CTL**: 100% of recommended AA levels. **MRT+**: 135% of recommended levels of TSAA, Arg, Thr. **MRTIV+**: 135% of recommended levels of TSAA, Arg, Thr, Ile, Val. **AA+**: 135% of recommended levels of all 10 essential AA.

[3] SEM: Pooled standard error of the mean.

[a-b] Means within row without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey-Kramer comparison test.

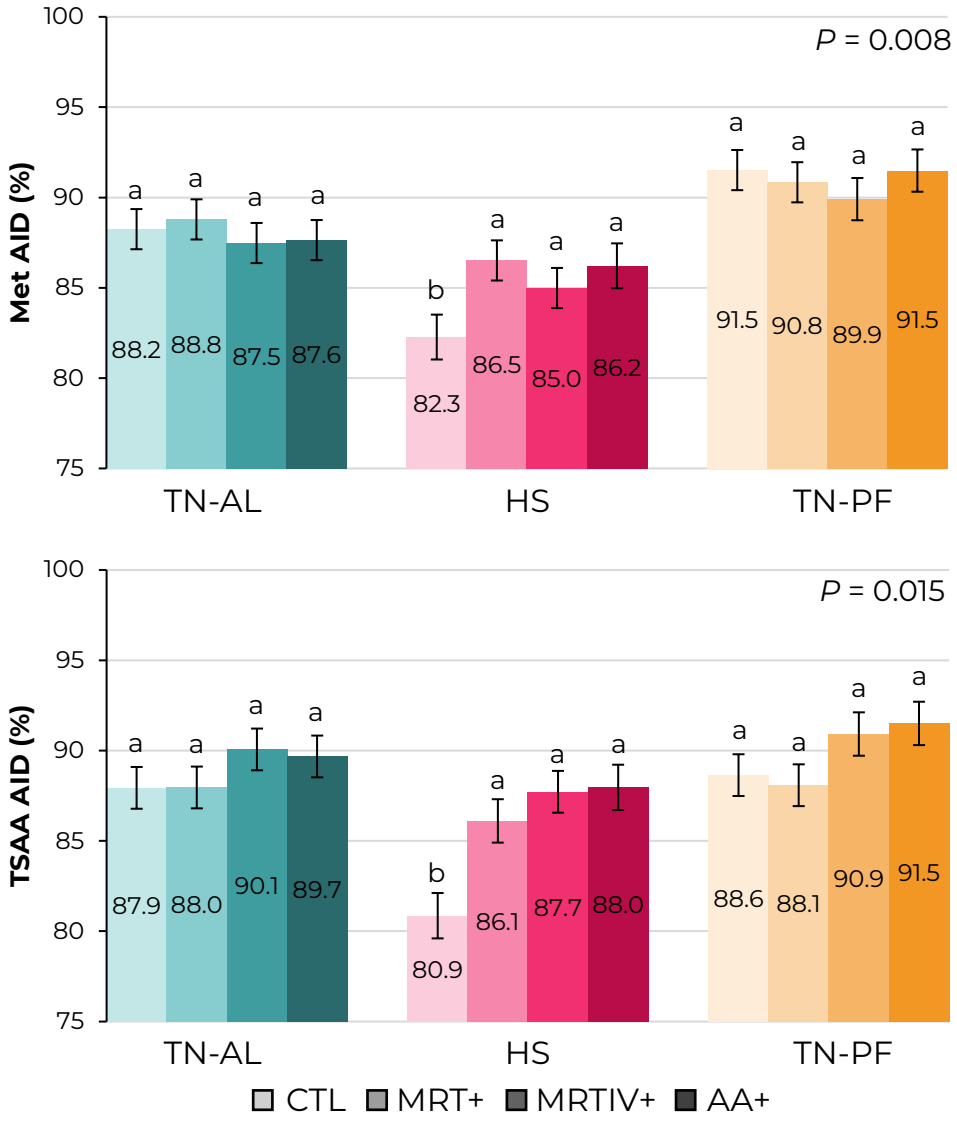


Figure 5.4. Interactive effects of environment and dietary amino acid density on total sulfur amino acids (TSAA) and Met apparent ileal digestibility (AID) of broilers reared under thermoneutral, heat stress, and pair-feeding conditions from d 20 to 41.

Environments: **TN-AL**: Birds reared under continuous 24°C and ad libitum feeding. **HS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-PF**: Birds reared under continuous 24°C and pair-fed to the HS treatment.
Diets: **CTL**: 100% of recommended AA levels. **MRT+**: 135% of recommended levels of TSAA, Arg, Thr. **MRTIV+**: 135% of recommended levels of TSAA, Arg, Thr, Ile, Val. **AA+**: 135% of recommended levels of all 10 essential AA.
Error bars represent the pooled standard error of the mean.
[a-b] Means without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey-Kramer comparison test.

Table 5.5. Effect of dietary amino acid density on apparent ileal digestibility (AID) of non-essential amino acids (NEAA) and total amino acids (TAA) of broilers reared under thermoneutral, heat stress, and pair-feeding conditions from d 20 to 41.

Item	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Tyr	NEAA	TAA
Main effect of environment[1]										
TN-AL	81.9	84.4	76.4^{ab}	92.0^{ab}	81.4	80.8^{ab}	81.8	81.5	82.5	85.6^{ab}
HS	78.7	81.6	70.1^{b}	90.3^{b}	78.5	77.2^{b}	78.6	78.9	79.2	82.9^{b}
TN-PF	85.0	85.7	77.2^{a}	93.6^{a}	81.9	83.3^{a}	83.9	83.4	84.2	87.3^{a}
SEM[3]	1.76	1.22	1.73	0.54	1.11	1.34	1.49	2.5	1.40	1.09
Main effect of diet[2]										
CTL	82.3	84.3^{ab}	74.5^{ab}	93.8^{a}	81.1^{ab}	80.4^{ab}	82.2^{ab}	81.5	82.5^{a}	84.8
MRT+	81.6	84.3^{ab}	75.9^{a}	93.4^{a}	81.0^{ab}	80.9^{ab}	81.6^{ab}	81.0	82.5^{a}	85.4
MRTIV+	83.3	84.9^{a}	76.8^{a}	93.2^{a}	81.6^{a}	81.9^{a}	82.4^{a}	83.0	83.4^{a}	86.3
AA+	80.3	82.2^{b}	71.0^{b}	87.4^{b}	78.7^{b}	78.5^{b}	79.5^{b}	79.7	79.6^{b}	84.5
SEM[3]	1.26	0.90	1.30	0.45	0.87	1.06	1.10	1.74	1.03	0.81
P-values										
Environment	0.093	0.102	**0.036**	**0.007**	0.117	**0.030**	0.091	0.472	0.082	**0.050**
Diet	0.082	**0.018**	**<0.001**	**<0.001**	**0.011**	**0.027**	**0.027**	0.194	**0.002**	0.122
Environment × Diet	0.488	0.385	0.195	0.694	0.282	0.516	0.350	0.597	0.426	0.205

[1] **TN-AL**: Birds reared under continuous 24°C and ad libitum feeding. **HS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-PF**: Birds reared under continuous 24°C and pair-fed to the HS treatment.
[2] **CTL**: 100% of recommended AA levels. **MRT+**: 135% of recommended levels of TSAA, Arg, Thr. **MRTIV+**: 135% of recommended levels of TSAA, Arg, Thr, Ile, Val. **AA+**: 135% of recommended levels of all 10 essential AA.
[3] SEM: Pooled standard error of the mean.
[a-b] Means within row without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey-Kramer comparison test.

Table 5.6. Effect of dietary amino acid density on digestible total sulfur amino acids (TSAA) and essential amino acid intakes (g) of broilers reared under thermoneutral, heat stress, and pair-feeding conditions from d 20 to 41.

Intake (g)		TSAA	Met	Arg	Thr	Ile	Val	His	Leu	Lys	Phe	Trp
TN-AL	CTL	32.0^{bc}	21.6^{e}	41.5^{b}	21.5^{b}	23.3^{b}	29.2^{c}	17.1^{b}	52.1^{b}	39.5^{b}	28.7^{b}	8.9^{b}
	MRT+	42.9^{a}	33.0^{ab}	54.3^{a}	28.3^{a}	22.9^{b}	26.7^{cd}	17.5^{b}	50.5^{b}	39.4^{b}	28.3^{b}	8.7^{b}
	MRTIV+	43.6^{a}	33.9^{a}	53.3^{a}	27.9^{a}	29.6^{a}	33.7^{b}	16.6^{b}	49.0^{b}	37.1^{b}	27.5^{b}	8.7^{b}
	AA+	40.8^{a}	31.2^{b}	52.5^{a}	27.7^{a}	29.5^{a}	37.2^{a}	21.9^{a}	66.0^{a}	51.5^{a}	36.7^{a}	10.5^{a}
HS	CTL	23.8^{d}	16.7^{f}	32.2^{c}	15.8^{c}	17.4^{c}	21.9^{f}	12.9^{c}	38.3^{c}	30.4^{c}	21.4^{c}	6.8^{c}
	MRT+	33.4^{bc}	26.0^{cd}	42.8^{b}	22.3^{b}	17.8^{c}	20.7^{f}	13.8^{c}	39.0^{c}	31.0^{c}	22.0^{c}	6.9^{c}
	MRTIV+	32.4^{bc}	25.7^{cd}	40.0^{b}	21.5^{b}	22.1^{b}	25.1^{de}	12.6^{c}	37.0^{c}	28.4^{c}	20.9^{c}	6.7^{c}
	AA+	31.7^{c}	24.6^{d}	41.0^{b}	21.7^{b}	23.1^{b}	29.2^{c}	17.1^{b}	51.6^{b}	40.7^{b}	28.5^{b}	8.3^{b}
TN-PF	CTL	25.3^{d}	17.2^{f}	32.9^{c}	17.3^{c}	18.8^{c}	23.5^{ef}	13.6^{c}	42.5^{c}	31.3^{c}	23.4^{c}	7.2^{c}
	MRT+	34.8^{bc}	26.8^{cd}	43.7^{b}	22.9^{b}	18.7^{c}	21.7^{f}	14.2^{c}	42.1^{c}	31.8^{c}	23.4^{c}	7.0^{c}
	MRTIV+	35.4^{b}	27.4^{c}	42.9^{b}	23.1^{b}	24.4^{b}	27.8^{cd}	13.5^{c}	41.3^{c}	30.1^{c}	22.8^{c}	7.2^{c}
	AA+	32.1^{c}	24.7^{d}	41.0^{b}	21.9^{b}	23.5^{b}	29.4^{c}	17.1^{b}	53.4^{b}	40.3^{b}	29.5^{b}	8.3^{b}
SEM[3]		0.78	0.51	0.87	0.62	0.54	0.66	0.37	1.20	0.77	0.63	0.18
P-values												
Environment		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Diet		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Environment × Diet		0.312	**0.024**	0.179	0.729	0.141	0.104	0.180	0.347	0.115	0.326	0.984

[1] **TN-AL**: Birds reared under continuous 24°C and ad libitum feeding. **HS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-PF**: Birds reared under continuous 24°C and pair-fed to the HS treatment.

[2] **CTL**: 100% of recommended AA levels. **MRT+**: 135% of recommended levels of TSAA, Arg, Thr. **MRTIV+**: 135% of recommended levels of TSAA, Arg, Thr, Ile, Val. **AA+**: 135% of recommended levels of all 10 essential AA.

[3] SEM: Pooled standard error of the mean.

Gray cells: Diets formulated to equalize intake of highlighted AA with that of the TN-AL birds fed the CTL diet.

[a-b] Means within row without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey-Kramer comparison test.

Table 5.7. Effect of dietary amino acid density on live weight and carcass characteristics of broilers reared under thermoneutral, heat stress, and pair-feeding conditions from d 20 to 42.

Item	Live weight, kg	Hot carcass		Fat pad		Chilled carcass	
		Weight, kg	Yield[4], %	Weight, kg	Yield, %	Weight, kg	Yield, %
Main effect of environment[1]							
TN-AL	3.488[a]	2.649[a]	76.1[a]	0.028[a]	0.82[b]	2.709[a]	77.7[b]
HS	2.639[c]	2.013[b]	76.4[a]	0.024[b]	0.90[a]	2.068[b]	78.5[a]
TN-PF	2.868[b]	2.132[b]	74.4[b]	0.022[b]	0.76[b]	2.187[b]	76.3[c]
SEM[3]	0.0527	0.0384	0.12	0.0007	0.020	0.0398	0.13
Main effect of diet[2]							
CTL	3.019	2.270	75.3[b]	0.026[a]	0.87[a]	2.327	77.2[b]
MRT+	3.000	2.273	75.8[a]	0.026[a]	0.85[a]	2.328	77.6[ab]
MRTIV+	2.949	2.228	75.5[ab]	0.024[ab]	0.84[a]	2.285	77.4[ab]
AA+	3.026	2.288	75.9[a]	0.023[b]	0.75[b]	2.346	77.8[a]
SEM[3]	0.0462	0.0343	0.12	0.0008	0.023	0.0350	0.13
P-values							
Environment	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Diet	0.533	0.540	**0.003**	**0.011**	**0.003**	0.547	**0.010**
Environment × Diet	0.982	0.871	0.202	0.167	**<0.001**	0.867	0.191

[1] **TN-AL**: Birds reared under continuous 24°C and ad libitum feeding. **HS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-PF**: Birds reared under continuous 24°C and pair-fed to the HS treatment.
[2] **CTL**: 100% of recommended AA levels. **MRT+**: 135% of recommended levels of TSAA, Arg, Thr. **MRTIV+**: 135% of recommended levels of TSAA, Arg, Thr, Ile, Val. **AA+**: 135% of recommended levels of all 10 essential AA.
[3] SEM: Pooled standard error of the mean.
[4] Yield relative to live weight.
[a-b] Means within row without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey-Kramer comparison test.

Table 5.8. Effect of dietary amino acid density on carcass part characteristics of broilers reared under thermoneutral, heat stress, and pair-feeding conditions from d 20 to 42.

	Breast fillets		Tenders		Total breast meat		Wings		Leg quarters	
Item	Weight, kg	Yield[4], %	Weight, kg	Yield, %	Weight, kg	Yield, %	Weight, kg	Yield, %	Weight, kg	Yield, %
Main effect of environment[1]										
TN-AL	0.769[a]	22.0[a]	0.136[a]	3.9	0.905[a]	25.9[a]	0.272[a]	7.9[b]	0.803[a]	23.1[b]
HS	0.538[b]	20.3[b]	0.104[b]	4.0	0.642[b]	24.2[b]	0.216[b]	8.2[a]	0.645[b]	24.4[a]
TN-PF	0.572[b]	20.0[b]	0.112[b]	3.9	0.683[b]	23.9[b]	0.228[b]	8.0[ab]	0.677[b]	23.6[b]
SEM[3]	0.0160	0.21	0.0022	0.03	0.0181	0.22	0.0031	0.07	0.0098	0.14
Main effect of diet[2]										
CTL	0.634	20.8	0.116	3.8[b]	0.750	24.7	0.242	8.1	0.700	23.2[b]
MRT+	0.630	20.8	0.119	4.0[a]	0.749	24.8	0.238	8.0	0.712	23.8[a]
MRTIV+	0.605	20.5	0.116	4.0[a]	0.721	24.4	0.235	8.0	0.702	23.9[a]
AA+	0.635	21.0	0.119	3.9[ab]	0.754	24.9	0.240	8.0	0.718	23.9[a]
SEM[3]	0.0132	0.19	0.0021	0.05	0.0150	0.20	0.0030	0.05	0.0102	0.13
P-values										
Environment	**<0.001**	**<0.001**	**<0.001**	0.411	**<0.001**	**<0.001**	**<0.001**	**0.018**	**<0.001**	**<0.001**
Diet	0.167	0.182	0.467	**0.016**	0.224	0.203	0.416	0.684	0.538	**<0.001**
Environment × Diet	0.420	0.055	0.557	0.294	0.436	**0.043**	0.787	0.740	0.822	0.669

[1] **TN-AL**: Birds reared under continuous 24°C and ad libitum feeding. **HS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-PF**: Birds reared under continuous 24°C and pair-fed to the HS treatment.

[2] **CTL**: 100% of recommended AA levels. **MRT+**: 135% of recommended levels of TSAA, Arg, Thr. **MRTIV+**: 135% of recommended levels of TSAA, Arg, Thr, Ile, Val. **AA+**: 135% of recommended levels of all 10 essential AA.

[3] SEM: Pooled standard error of the mean.

[4] Yield relative to live weight.

[a-b] Means within row without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey-Kramer comparison test.

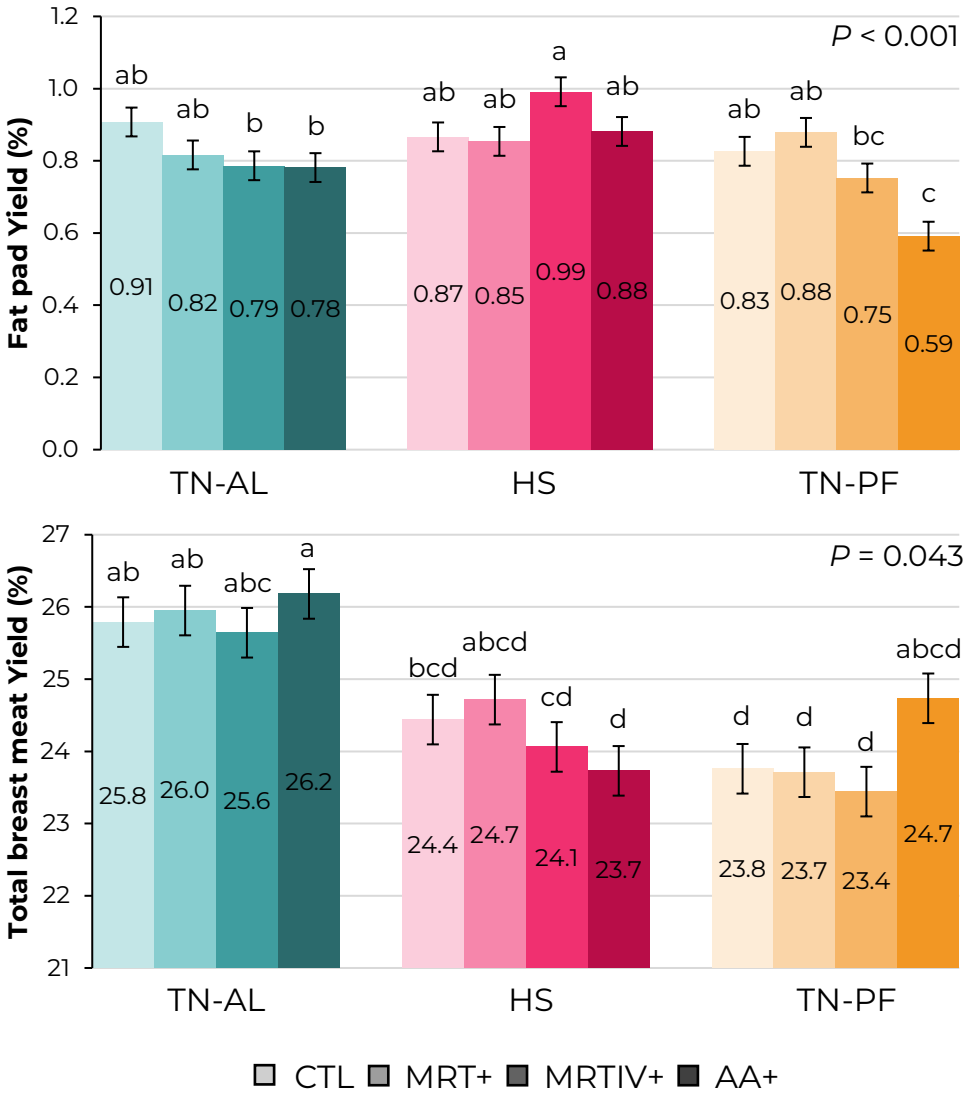


Figure 5.5. Interactive effects of environment and dietary amino acid density on fat pad and total breast meat yields of broilers reared under thermoneutral, heat stress, and pair-feeding conditions from d 20 to 42.

Environments: **TN-AL**: Birds reared under continuous 24°C and ad libitum feeding. **HS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-PF**: Birds reared under continuous 24°C and pair-fed to the HS treatment.
Diets: **CTL**: 100% of recommended AA levels. **MRT+**: 135% of recommended levels of TSAA, Arg, Thr. **MRTIV+**: 135% of recommended levels of TSAA, Arg, Thr, Ile, Val. **AA+**: 135% of recommended levels of all 10 essential AA.
Error bars represent the pooled standard error of the mean.
[a-b] Means without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey-Kramer comparison test.

Table 5.9. Effect of dietary amino acid density on blood, feather and carcass part relative weights of broilers reared under thermoneutral, heat stress, and pair-feeding conditions from d 20 to 42.

	Blood	Feather	Small Intestine	Liver	Offal	TBM[5]	Abdominal fat
Item	g/100g live WG[4]		g/100g carcass WG				
Main effect of environment[1]							
TN-AL	3.18[a]	5.53	0.72[a]	1.43[a]	12.6[ab]	29.3[a]	1.62
HS	1.04[b]	5.84	0.08[c]	1.06[b]	11.7[b]	26.7[b]	1.64
TN-PF	2.88[a]	6.04	0.41[b]	1.09[ab]	13.1[a]	28.8[a]	1.32
SEM[3]	0.213	0.295	0.073	0.093	0.290	0.44	0.120
Main effect of diet[2]							
CTL	2.50	6.04	0.49	1.24	12.4	28.8	1.59[ab]
MRT+	2.28	5.87	0.33	1.24	12.6	27.9	1.63[a]
MRTIV+	2.53	5.62	0.39	1.21	12.4	27.4	1.60[ab]
AA+	2.16	5.70	0.40	1.09	12.6	28.9	1.28[b]
SEM[3]	0.202	0.306	0.086	0.070	0.269	0.46	0.107
P-values							
Environment	**<0.001**	0.489	**<0.001**	**0.034**	**0.026**	**0.006**	0.164
Diet	0.411	0.749	0.552	0.125	0.907	**0.049**	**0.029**
Environment × Diet	0.315	0.311	0.156	0.245	0.111	0.497	0.850

[1] **TN-AL**: Birds reared under continuous 24°C and ad libitum feeding. **HS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-PF**: Birds reared under continuous 24°C and pair-fed to the HS treatment.
[2] **CTL**: 100% of recommended AA levels. **MRT+**: 135% of recommended levels of TSAA, Arg, Thr. **MRTIV+**: 135% of recommended levels of TSAA, Arg, Thr, Ile, Val. **AA+**: 135% of recommended levels of all 10 essential AA.
[3] SEM: Pooled standard error of the mean.
[4] WG: Weight gain.
[5] TBM: Total breast meat.
[a-b] Means within row without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey-Kramer comparison test.

Table 5.10. Effect of dietary amino acid density on carcass nutrient retention and composition in broilers reared under thermoneutral, heat stress, and pair-feeding conditions from d 20 to 42.

	Carcass composition				Total carcass retention		
	DM	Nitrogen	Fat	Energy	Nitrogen	Fat	Energy
Item	g/100g WG[4]	g or kcal/100g DM gain			g or kcal/bird		
Main effect of environment[1]							
TN-AL	31.4[b]	19.4	35.3[b]	623[ab]	62.4[a]	246.4[a]	4317[a]
HS	33.4[a]	20.6	37.9[a]	630[a]	43.9[c]	196.7[b]	3264[b]
TN-PF	32.2[ab]	19.6	32.1[c]	606[b]	49.7[b]	173.9[b]	3254[b]
SEM[3]	0.32	0.43	0.69	4.8	1.35	7.25	96.3
Main effect of diet[2]							
CTL	33.4	18.5[b]	37.5[a]	630[a]	53.7	234.3[a]	3918[a]
MRT+	32.5	20.5[a]	35.6[a]	619[ab]	51.0	204.9[a]	3526[ab]
MRTIV+	32.4	19.7[ab]	36.2[a]	624[a]	50.5	211.1[a]	3630[ab]
AA+	31.0	20.9[a]	31.1[b]	608[b]	52.7	172.3[b]	3372[b]
SEM[3]	0.34	0.47	0.80	4.7	1.45	8.43	111.9
P-value							
Environment	**0.005**	0.193	**<0.001**	**0.018**	**<0.001**	**<0.001**	**<0.001**
Diet	**<0.001**	**0.003**	**<0.001**	**0.004**	0.334	**<0.001**	**0.007**
Environment × Diet	**0.015**	0.355	0.182	0.080	0.232	0.611	0.191

[1] **TN-AL**: Birds reared under continuous 24°C and ad libitum feeding. **HS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-PF**: Birds reared under continuous 24°C and pair-fed to the HS treatment.

[2] **CTL**: 100% of recommended AA levels. **MRT+**: 135% of recommended levels of TSAA, Arg, Thr. **MRTIV+**: 135% of recommended levels of TSAA, Arg, Thr, Ile, Val. **AA+**: 135% of recommended levels of all 10 essential AA.

[3] SEM: Pooled standard error of the mean.

[4] WG: Weight gain.

[a-b] Means within row without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey-Kramer comparison test.

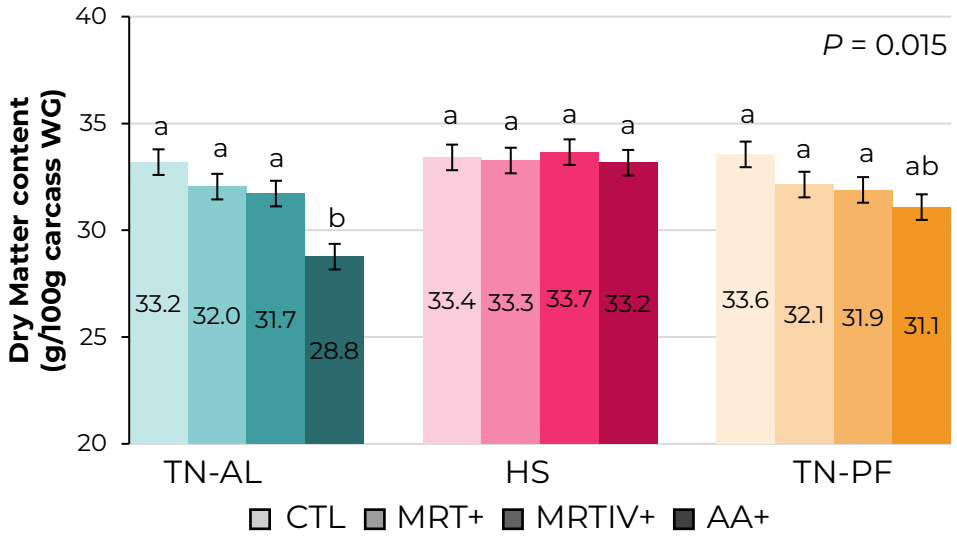


Figure 5.6. Interactive effects of environment and dietary amino acid density on carcass dry matter content of broilers reared under thermoneutral, heat stress, and pair-feeding conditions from d 20 to 42.

Environments: **TN-AL**: Birds reared under continuous 24°C and ad libitum feeding. **HS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-PF**: Birds reared under continuous 24°C and pair-fed to the HS treatment.
Diets: **CTL**: 100% of recommended AA levels. **MRT+**: 135% of recommended levels of TSAA, Arg, Thr. **MRTIV+**: 135% of recommended levels of TSAA, Arg, Thr, Ile, Val. **AA+**: 135% of recommended levels of all 10 essential AA.
Error bars represent the pooled standard error of the mean.
[a-b] Means without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey-Kramer comparison test.

Table 5.11. Effect of dietary amino acid density on energy partitioning (expressed on a metabolic BW basis) of broilers reared under thermoneutral, heat stress, and pair-feeding conditions from d 20 to 42.

Item	EI	DEI	ER	ERPro	ERFat	HP	HI	FHP	NE	EEx	EEf	HI:HP
	kcal/kg$^{0.70}$/d										%	%
Main effect of environment[1]												
TN-AL	496^{b}	369	139^{ab}	70.9	74.6^{a}	218	85.8	134^{c}	272^{c}	140^{b}	27.9	38.2^{a}
HS	550^{a}	393	143^{a}	70.1	79.2^{a}	233	67.5	167^{a}	306^{a}	173^{a}	26.4	28.7^{b}
TN-PF	503^{b}	385	132^{b}	71.8	66.3^{b}	234	80.6	155^{b}	287^{b}	130^{b}	26.9	33.7^{ab}
SEM[3]	9.8	8.3	2.1	0.94	1.90	7.0	5.83	2.8	3.8	7.9	0.95	1.83
Main effect of diet[2]												
CTL	512	382	148^{a}	71.5	81.7^{a}	223	73.8	149	293	143	38.5	33.0
MRT+	527	392	137^{b}	70.6	74.6^{a}	234	82.3	153	290	148	36.1	35.4
MRTIV+	514	387	137^{b}	69.7	74.0^{a}	234	83.9	152	287	145	36.3	34.3
AA+	512	368	131^{b}	71.9	63.2^{b}	222	70.1	154	285	156	35.6	31.5
SEM[3]	9.8	8.3	2.5	0.99	2.19	7.7	6.20	2.8	3.5	6.3	1.09	1.88
P-values												
Environment	**0.007**	0.157	**0.001**	0.503	**<0.001**	0.215	0.148	**<0.001**	**<0.001**	**0.010**	0.255	**0.014**
Diet	0.584	0.145	**<0.001**	0.352	**<0.001**	0.502	0.341	0.610	0.276	0.296	0.197	0.443
Environment × Diet	0.151	0.206	0.068	0.720	**0.023**	0.127	0.112	0.276	**0.002**	0.844	0.092	0.081

Abbreviations - **EI**: Energy intake, **DEI**: Digestible energy intake, **ER**: Energy retention, **ERPro**: Energy retention as protein, **ERFat**: Energy retention as fat, **HP**: Heat production, **HI**: Heat increment, **FHP**: Fasting heat production, **NE**: Net energy, **EEx**: Energy excretion, **EEf**: Energy efficiency.

[1] **TN-AL**: Birds reared under continuous 24°C and ad libitum feeding. **HS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-PF**: Birds reared under continuous 24°C and pair-fed to the HS treatment.

[2] **CTL**: 100% of recommended AA levels. **MRT+**: 135% of recommended levels of TSAA, Arg, Thr. **MRTIV+**: 135% of recommended levels of TSAA, Arg, Thr, Ile, Val. **AA+**: 135% of recommended levels of all 10 essential AA.

[3] SEM: Pooled standard error of the mean.

[a-b] Means within row without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey-Kramer comparison test.

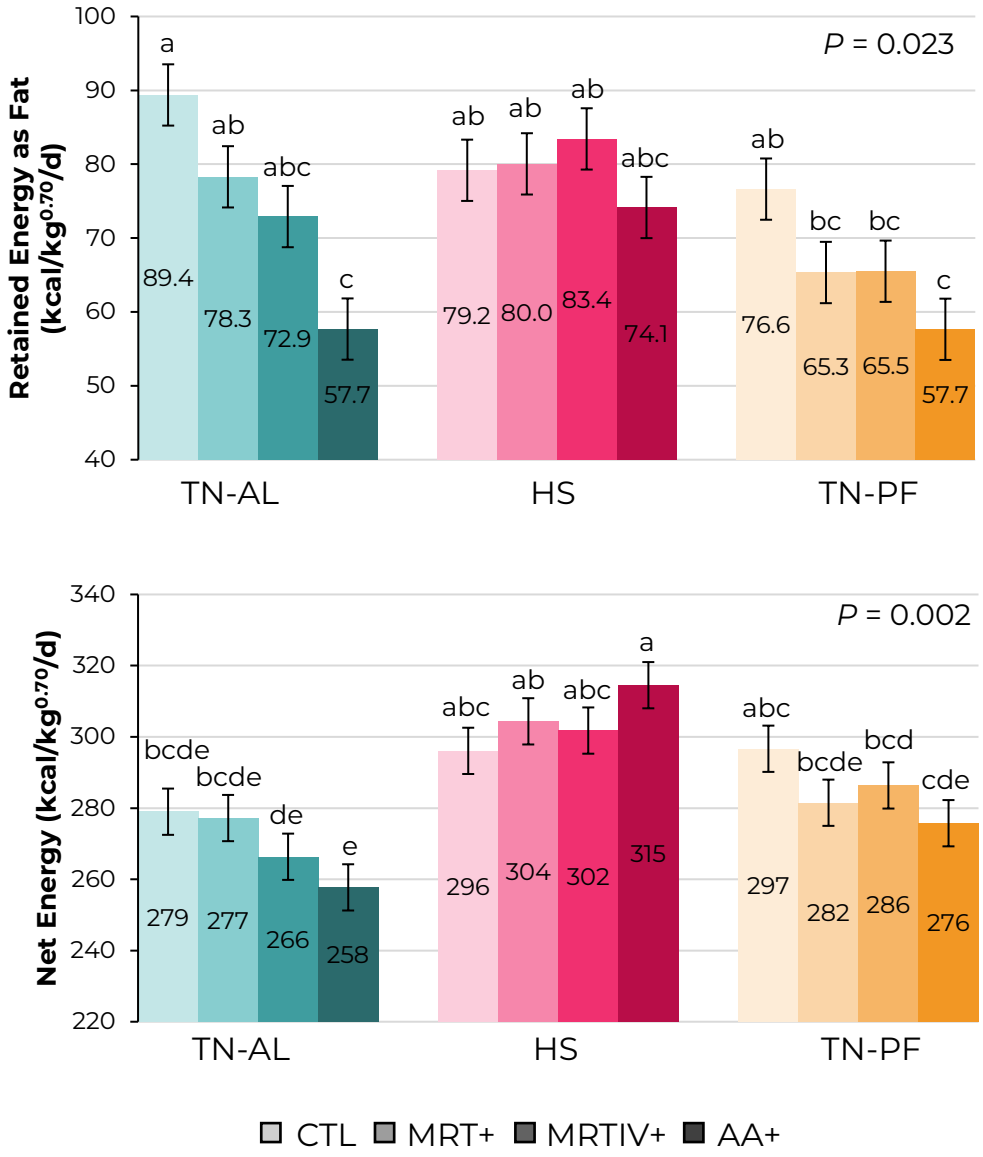


Figure 5.7. Interaction effects of environment and of dietary amino acid density on retained energy as fat and net energy of broilers reared under thermoneutral, heat stress, and pair-feeding conditions from d 20 to 41.

Environments: **TN-AL**: Birds reared under continuous 24°C and ad libitum feeding. **HS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-PF**: Birds reared under continuous 24°C and pair-fed to the HS treatment.
Diets: **CTL**: 100% of recommended AA levels. **MRT+**: 135% of recommended levels of TSAA, Arg, Thr. **MRTIV+**: 135% of recommended levels of TSAA, Arg, Thr, Ile, Val. **AA+**: 135% of recommended levels of all 10 essential AA.
Error bars represent the pooled standard error of the mean.
[a-b] Means without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey-Kramer comparison test.

Table 5.12. Effect of dietary amino acid density on nitrogen partitioning (expressed on a metabolic BW basis) of broilers reared under thermoneutral, heat stress, and pair-feeding conditions from d 20 to 42.

	NI	DNI	NR	NEx	NEf
Item	g/kg$^{0.70}$/d				%
Main effect of environment[1]					
TN-AL	4.24[b]	3.58	2.00	2.25[a]	47.2[a]
HS	4.70[a]	3.85	1.98	2.68[a]	42.2[b]
TN-PF	4.29[b]	3.67	2.03	2.26[a]	47.5[a]
SEM[3]	0.084	0.080	0.027	0.112	1.11
Main effect of diet[2]					
CTL	4.26[a]	3.55[b]	2.02	2.25	47.5
MRT+	4.54[a]	3.83[a]	2.00	2.54	44.3
MRTIV+	4.36[a]	3.70[ab]	1.97	2.33	45.2
AA+	4.49[a]	3.72[ab]	2.03	2.46	45.5
SEM[3]	0.084	0.078	0.028	0.099	1.10
P-values					
Environment	**0.006**	0.106	0.500	**0.040**	**0.013**
Diet	**0.048**	**0.048**	0.360	0.064	0.163
Environment × Diet	0.128	0.123	0.701	0.092	0.161

Abbreviations – **NI**: Nitrogen intake, **DNI**: Digestible nitrogen intake, **NR**: Nitrogen retention, **NEx**: Nitrogen Excretion, **NEf**: Nitrogen efficiency.
[1] **TN-AL**: Birds reared under continuous 24°C and ad libitum feeding. **HS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-PF**: Birds reared under continuous 24°C and pair-fed to the HS treatment.
[2] **CTL**: 100% of recommended AA levels. **MRT+**: 135% of recommended levels of TSAA, Arg, Thr. **MRTIV+**: 135% of recommended levels of TSAA, Arg, Thr, Ile, Val. **AA+**: 135% of recommended levels of all 10 essential AA.
[3] SEM: Pooled standard error of the mean.
[a-b] Means within row without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey-Kramer comparison test.

Table 5.13. Effect of dietary amino acid density on liver, offal, and total breast meat composition in broilers reared under thermoneutral, heat stress, and pair-feeding conditions from d 20 to 42.

	Small Intestine			Liver			Offal			Total Breast Meat		
	Nitrogen	Fat	Energy	Nitrogen	Fat	Energy	Nitrogen	Fat	Energy	Nitrogen	Fat	Energy
Item	g or kcal/100g DM gain			g or kcal/100g DM gain			g or kcal/100g DM gain			g or kcal/100g DM gain		
Main effect of environment[1]												
TN-AL	12.0	8.7	514	11.6^{a}	14.8^{a}	578^{ab}	7.9^{a}	34.6^{ab}	605^{a}	13.4	11.9^{a}	572
HS	9.6	32.7	660	10.4^{b}	17.4^{a}	586^{a}	6.9^{b}	38.4^{a}	618^{a}	13.8	10.4^{ab}	569
TN-PF	14.3	11.0	616	12.1^{a}	10.6^{b}	558^{b}	7.9^{a}	31.7^{b}	576^{b}	13.8	9.0^{b}	567
SEM[3]	1.83	11.02	55.3	0.29	1.13	5.7	0.17	1.00	6.6	0.11	0.71	3.1
Main effect of diet[2]												
CTL	9.6	41.4^{a}	668	11.5	14.8	577	7.5	36.4^{a}	605	13.5^{b}	11.5^{a}	572^{a}
MRT+	13.5	-0.1^{b}	555	11.3	13.5	570	7.7	35.5^{ab}	603	13.8^{ab}	9.5^{b}	570^{a}
MRTIV+	12.0	12.5^{ab}	552	11.5	14.6	583	7.4	35.1^{ab}	600	13.5^{ab}	11.8^{a}	575^{a}
AA+	12.8	16.1^{ab}	611	11.1	14.1	565	7.9	32.6^{b}	591	13.9^{a}	8.8^{b}	559^{b}
SEM[3]	2.00	10.98	56.1	0.33	1.29	6.4	0.19	0.56	7.3	0.12	0.61	3.1
P-values												
Environment	0.246	0.297	0.209	**<0.001**	**<0.001**	**0.015**	**0.003**	**0.003**	**0.005**	0.055	**0.048**	0.411
Diet	0.492	**0.034**	0.330	0.718	0.879	0.200	0.300	**0.044**	0.517	**0.014**	**<0.001**	**<0.001**
Environment × Diet	0.148	**0.042**	0.060	0.726	0.170	0.062	0.060	0.712	0.665	0.579	0.783	0.068

[1] **TN-AL**: Birds reared under continuous 24°C and ad libitum feeding. **HS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-PF**: Birds reared under continuous 24°C and pair-fed to the HS treatment.

[2] **CTL**: 100% of recommended AA levels. **MRT+**: 135% of recommended levels of TSAA, Arg, Thr. **MRTIV+**: 135% of recommended levels of TSAA, Arg, Thr, Ile, Val. **AA+**: 135% of recommended levels of all 10 essential AA.

[3] SEM: Pooled standard error of the mean.

[a-b] Means within row without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey-Kramer comparison test.

Supplemental Table 5.1. Effect of dietary amino acid density on energy partitioning (expressed as a percentage of the energy intake) of broilers reared under thermoneutral, heat stress, and pair-feeding conditions from d 20 to 42.

	EI	DEI	ER	ERPro	ERFat	HP	HI	FHP	NE	EEx
Item					%					
Main effect of environment[1]										
TN-AL	100	74.4	27.9	14.3[a]	14.9[a]	43.9[ab]	16.8[a]	27.1[c]	55.0[b]	28.2
HS	100	72.4	26.4	12.7[b]	14.9[a]	42.4[b]	12.6[b]	29.7[b]	56.2[ab]	30.5
TN-PF	100	76.7	26.9	14.4[a]	13.3[a]	47.3[a]	16.4[a]	30.8[a]	57.8[a]	26.1
SEM[3]	-	1.21	0.95	0.33	0.49	1.18	1.14	0.19	0.74	1.16
Main effect of diet[2]										
CTL	100	75.2	38.5	14.0	16.1[a]	43.5	14.4	29.1	57.9	27.3[b]
MRT+	100	74.8	36.1	13.5	14.3[ab]	45.6	16.5	29.0	55.8	28.1[ab]
MRTIV+	100	75.5	36.3	13.6	14.8[a]	45.1	15.9	29.2	56.3	27.4[b]
AA+	100	72.5	35.6	14.1	12.3[b]	44.0	14.3	29.5	55.3	30.3[a]
SEM[3]	-	0.91	1.09	0.33	0.56	1.11	1.18	0.22	0.84	0.89
P-values										
Environment	-	0.094	0.255	**0.012**	**0.027**	**0.041**	**0.049**	**<0.001**	**0.029**	0.071
Diet	-	**0.008**	0.197	0.393	**<0.001**	0.415	0.384	0.336	0.149	**0.004**
Environment × Diet	-	0.766	0.092	0.129	0.175	0.189	0.150	0.069	0.095	0.754

Abbreviations - **EI**: Energy intake, **DEI**: Digestible energy intake, **ER**: Energy retention, **ERPro**: Energy retention as protein, **ERFat**: Energy retention as fat, **HP**: Heat production, **HI**: Heat increment, **FHP**: Fasting heat production, **NE**: Net energy, **EEx**: Energy excretion.

[1] **TN-AL**: Birds reared under continuous 24°C and ad libitum feeding. **HS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-PF**: Birds reared under continuous 24°C and pair-fed to the HS treatment.

[2] **CTL**: 100% of recommended AA levels. **MRT+**: 135% of recommended levels of TSAA, Arg, Thr. **MRTIV+**: 135% of recommended levels of TSAA, Arg, Thr, Ile, Val. **AA+**: 135% of recommended levels of all 10 essential AA.

[3] SEM: Pooled standard error of the mean.

[a-b] Means within row without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey-Kramer comparison test.

Supplementary Table 5.2. Effect of dietary amino acid density on nitrogen partitioning (expressed as a percentage of the nitrogen intake) of broilers reared under thermoneutral, heat stress, and pair-feeding conditions from d 20 to 42.

	NI	DNI	NR	NEx
Item		%		
Main effect of environment[1]				
TN-AL	100	84.5	47.2[a]	52.8[b]
HS	100	82.0	42.2[b]	57.8[a]
TN-PF	100	85.8	47.5[a]	52.5[b]
SEM[3]	-	1.10	1.11	1.13
Main effect of diet[2]				
CTL	100	83.4	47.5	52.6
MRT+	100	84.6	44.3	55.8
MRTIV+	100	84.9	45.2	54.7
AA+	100	83.4	45.5	54.5
SEM[3]	-	0.79	1.10	1.13
P-values				
Environment	-	0.105	**0.013**	**0.013**
Diet	-	0.071	0.163	0.171
Environment × Diet	-	0.606	0.161	0.173

Abbreviations – **NI**: Nitrogen intake, **DNI**: Digestible nitrogen intake, **NR**: Nitrogen retention, **NEx**: Nitrogen Excretion.

[1] **TN-AL**: Birds reared under continuous 24°C and ad libitum feeding. **HS**: Birds reared under continuous 35°C and ad libitum feeding. **TN-PF**: Birds reared under continuous 24°C and pair-fed to the HS treatment.

[2] **CTL**: 100% of recommended AA levels. **MRT+**: 135% of recommended levels of TSAA, Arg, Thr. **MRTIV+**: 135% of recommended levels of TSAA, Arg, Thr, Ile, Val. **AA+**: 135% of recommended levels of all 10 essential AA.

[3] SEM: Pooled standard error of the mean.

[a-b] Means within row without a common superscript were determined to be significantly different ($P < 0.05$) by a Tukey-Kramer comparison test.

CHAPTER VI - GENERAL CONCLUSIONS

The comparison of cyclic and constant HS exposure on performance and carcass characteristic data indicated that the response of birds to HS depends on the stress intensity (Chapter III). Cooler periods under cyclic HS exposure allow the birds to mitigate the adverse effects of increased temperatures. Under constant HS, the degradation of performance is primarily caused by the HS-induced reduction in feed intake (**FI**) in an attempt to limit the heat production (**HP**) resulting from the thermic effect of the feed associated with nutrient digestion, absorption, and metabolism. Additionally, HS affects carcass characteristics, leading to a reduction in breast yield and increase in abdominal fat yield.

Investigations conducted in Chapter IV confirmed that constant HS exposure causes higher physiological stress on birds than cyclic HS. However, comparison of acute and cyclic HS birds, both before and after the elevation in temperatures, did not reveal physiological adaptation or recovery mechanisms to high temperatures during cyclic HS. Therefore, the lower overall stress intensity of cyclic HS exposure might be the reason for the limited performance degradation, rather than an adaptation or overnight recovery. In addition, HS *per se* was responsible for the degradation in nutrient digestibility observed in constant HS birds, but the magnitude of reduction in digestibility indicates that it has a limited contribution to the impaired feed efficiency of HS birds. Interestingly, constant HS exposure considerably affected the regulatory mechanisms of protein and lipid metabolism, although further research is necessary as the regulation of lipid metabolism markers did not correlate with the increased abdominal fat deposition. Regarding protein metabolism, HS-induced downregulation in protein synthesis pathways seems to be mainly explained by the reduced FI, while the upregulation of protein degradation pathways solely resulted from the heat *per se*.

The use of the comparative slaughter technique in Chapter V indicated that constant HS increases energy requirements and HP related to maintenance functions. With limited heat dissipation at high temperatures, birds need to limit the HP related to productive functions to avoid the heat overload. In addition to the reduced physical activity (which was not measured in our experiment), the decreased FI and alterations in bird metabolism contribute to limiting the HP. Specifically, HS promotes fat over protein deposition due to the lower heat increment of fat. In an attempt to stimulate muscle protein deposition, we tested diets with increased density of some or all essential amino acids (**AA**). However, the absence of beneficial response with these diets suggests that reduced protein deposition is not related to a lack of digestible AA intake. Instead, it appeared that the additional essential AA, supplied above the standard requirements, were directed toward non-productive functions such as energy production to support the increased energy needs for maintenance or the production of proteins involved in thermoregulation processes.

Collectively, these data provide valuable insights into the detrimental impact of HS on performance, digestibility, and nutrient metabolism, in relation to the HS-induced reduction in FI. Even though no beneficial effects of high AA density were observed in our last experiment, further investigation is required on individual AA supplementation, using different inclusion levels and AA combinations, to better define AA requirements under HS conditions. Moreover, the reduced protein deposition observed under HS raises some questions regarding the metabolic fate of AA. Determining the carcass part AA composition through the comparative slaughter technique, or the utilization of tracing techniques like radiolabeled AA, could enhance our understanding of the partitioning and utilization of essential AA. Even though stimulating protein deposition and preventing fat deposition during constant HS conditions might be

challenging, such mechanistic insights may reveal successful feeding strategies in less intensive stress conditions. Indeed, feeding different AA and energy profiles during and after a period of HS could possibly convert the energy stored as fat under HS into energy that participates in protein deposition. Additionally, a better definition of AA requirements under HS would contribute to the successful supplementation of low crude protein diets under HS conditions. Nonetheless, evaluation of feeding strategies under HS conditions should consider carcass characteristics to ensure that any mitigation of the adverse effects of HS on performance improves lean protein deposition and is not solely attributed to fat deposition.

APPENDIX

UNIVERSITY OF ARKANSAS

Office of Research Compliance

To:	Samuel Rochell
Fr:	Billy Hargis
Date:	September 5th, 2019
Subject:	IACUC Approval
Expiration Date:	August 29th, 2022

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # **20020:** *Characterization of heat stress models on performance, carcass characteristics, and nutrient digestibility and metabolism in broiler chickens*.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond August 29th, 2022 you must submit a newly drafted protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: Sam Rochell, Chuanmin Ruan, Jean-Remi Teyssier, Brooke Bodle, Kenia Mitre, Derrell Tre Lee, Alyson Gautier, Valeria Cuadros Bloch, Valeria Cuadros Bloch, Cole Crumpacker, Ethan Collins, Lacy Barrett, and Sami Dridi. Please submit personnel additions to this protocol via the modification form prior to their start of work.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

BMH/tmp