The Combined Impact of Lairage Temperatures and Controlled Atmosphere Gas Stunning time on Meat Quality and Blood Metabolites

by

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ABSTRACT

Lairage, a holding area where poultry are kept prior to slaughter and controlled atmosphere stunning (CAS) are two factors associated with poultry processing that can influence both the physiological state of a bird, and the quality of the meat produced. Despite this, the possible impacts of both lairage temperatures and CAS together are widely unexplored. Understanding the potential effects is imperative to improving processing plant efficiency, enhancing meat quality, and optimizing processing conditions. For this study, the effect of lairage temperature and CAS on broiler breast meat quality and blood metabolites was investigated. A 2x2 experimental design was used to evaluate two simulated lairage environments (hot: 28.9°C and cool: 17.3°C) and two stunning durations (4.5 and 6 minutes). Broilers were exposed to the simulated lairage conditions for 1 hour. Blood samples were taken pre-lairage, post-lairage pre-stun, and post-stun. Using an iSTAT Alinity v, 13 blood metabolites were evaluated: PCO_2 (mmHg), pH, PO_2 (mmHg), HCO^{3}- (mEq/L), SO_2 (%), TCO_2 (mEq/L), Na (mEq/L), iCa (mmol/L), glucose (mg/dL), hematocrit (%PVC), and hemoglobin (g/dL). Following lairage birds were stunned using CAS and processed normally. After immersion chilling and deboning, breast fillets were evaluated at 2 and 24 h for pH, color, and drip loss. At 24 h and 7 d lipid oxidation readings were taken. At 2 h postmortem, birds stunned for 4.5 min had higher pH than those stunned for 6 min ($P = 0.027$), and at 24 h, birds from the cooler lairage environment had higher pH than those from the hot environment ($P < 0.0001$). Fillets from hot lairage birds were lighter and more yellow at both 2 h and 24 h postmortem compared to cool birds ($P =0.0008$). No differences were observed for drip loss or lipid oxidation. CAS time significantly affected blood gases (HCO_3^-, pH, PCO_2, SO_2), electrolytes (Na, iCa), and glucose ($P < 0.0001$), with a glucose and hemoglobin interaction observed between lairage temperature and CAS time (P = 0.034, P = 0.046). Hematocrit was higher in the 4.5-minute stunned birds (P = 0.008). This work aims to investigate how different lairage temperatures and CAS can influence broiler meat quality and blood chemistry, ultimately providing valuable insights for improving poultry processing practices.

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War Eagle!

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LIST OF ABBREVIATIONS

PO_2	Partial Pressure of Oxygen
CO_2	Carbon dioxide
PCO_2	Partial Pressure of Carbon Dioxide
$HCO^3{}_-$	Bicarbonate
BE_{ecf}	Base Excess in Extracellular Fluid
SO2	Oxygen Saturation
TCO_2	Total Carbon Dioxide
Na	Sodium
iCa	Ionized Calcium
USDA	United States Department of Agriculture
DOA	Dead on arrival
CAS	Controlled atmosphere stunning

CHAPTER 1: LITERATURE REVIEW

1.1 INTRODUCTION

Poultry has become increasingly popular in both domestic and international markets with 9.16 billion broilers produced in the United States in 2023 (USDA -ERS, 2023). The United States is the top producer of poultry meat, with 21.08 million metric tons produced from 2023 to 2024, contributing significantly to the global supply (USDA-FAS, 2023). Poultry processing is a crucial part of the production of poultry and involves multiple steps to transition a live bird to a marketable poultry product. Typical process flow in the United States includes live bird reception, stunning, slaughter, scalding, picking, evisceration, chilling, and further processing. Each of these processing steps includes various subsections that aid in the transition of live bird to finished product (Marel, 2020). This detailed process creates multiple areas that have the potential to largely impact the final product, and the increasing popularity of poultry products emphasizes the importance of understanding factors associated with poultry processing.

1.2 POULTRY LAIRAGE

Prior to the stunning process to render the bird unconscious, birds are subjected to various operations associated with live haul. Birds are transported to processing facilities by transport trucks and placed in holding areas upon arrival to the plant (Vieira et al., 2011). The place where birds are placed is commonly referred to as lairage. The intended purpose of lairage is to provide an opportunity for birds to recover from the stressors of transport and maintain the efficiency of the processing facility (Bianchi et al., 2005). In the United States, lairage areas are large sheds outside that are typically equipped with misters and fans for temperature control.

Lairage implications on welfare

During transport and lairage, temperature stressors may be experienced by birds (Mitchell and Kettewell, 1998). These stressors may be comprising to a birds' welfare status. Thermal stress during transport and lairage can have detrimental effects on broiler welfare leading to distress, discomfort, or even death. Poultry use the process of thermoregulation to regulate their body temperature and maintain homeostasis (Lee et al. 1945). In response to thermal stress, birds may exhibit different behavioral responses. In hot temperatures, birds may pant or hold their wings away from their body to release heat, promote air circulation, and cool down body temperature. Alternatively, in cold temperatures, behaviors such as huddling with other birds or shivering may be observed to reduce exposure to the cold and to generate heat (Douglas et al., 1945; Hutchinson, 1954).

Stressors associated with lairage and bird stress response

During lairage, potential stressors may include poor ventilation, loud noises, crowding, and changes in temperatures. Relative temperatures at processing facilities can range greatly depending on the location of the plant. Temperature stress occurs when birds are unable to maintain their core body temperature. It can be more specifically described as heat or cold stress dependent on the temperature and conditions birds are exposed to. Heat stress can occur over an extended period, commonly referred to as chronic heat stress, which results from prolonged exposure to elevated temperatures. Alternatively, acute heat stress occurs during shorter durations of exposure to high temperatures, such as during lairage. The introduction of temperature stressors can activate the hypothalamic-pituitary-adrenal (HPA) axis triggering a physiological stress response. Once stressors are present, the hypothalamus releases corticotropin-releasing hormone (CRH) and arginine vasotocin (AVT) which elicits the pituitary gland to secrete adrenocorticotropic hormone (ACTH). This signals the adrenal glands to release corticosterone, the primary stress hormone (Debonne et al., 2008). The release of corticosterone enables the bird to adapt through allostasis. Allostasis is the physiological processes that allow homeostasis to be achieved in changing conditions. Through allostasis birds can mobilize their energy reserves, modifying their immune system, and heightening vigilance to cope with the stressor (Sterling and Eyer, 1988). Although with severe or extensive stress exposure the HPA axis can cause allostatic overload which could cause immune system impairment, metabolic inhibition, and increased mortality (McEwen, 2003).

Thermal environment effects in lairage

Lairage holding areas can create microclimates or small areas that develop atmospheric conditions different from nearby areas. These conditions can be influenced by multiple factors including temperature, humidity, and stocking density (Quinteiro-Filho et al., 2010). High ambient temperatures may lead to an increase of dead on arrival birds (DOA). With such a large number of broilers processed every year, mortality during transport and lairage can result in significant financial losses and presents concern for broiler welfare during live haul (Nijdam et al., 2004). Significant changes in temperature can increase the overall risk of increased mortality. Ritz et al. (2005) noted that high environmental temperatures can lead to heat stress of birds in preslaughter conditions, and that 40% of losses prior to slaughter are related to temperature stress. A study performed in Italy surveyed the DOA birds of 33 poultry processing plants over a 4-year period. Broiler preslaughter mortality was significantly (P£ 0.01) higher in the summer months (0.47%), whereas spring (0.32%) and winter (0.35%) displayed median, and autumn (0.28%) had the lowest DOA percentages. The average temperature and relative humidity observed during that summer period was 16.3 to 27.7°C and 72.7%, respectively (Petracci et al., 2006).

Alternatively, in a study performed in Turkey, the effect of low ambient temperature on the mortality rates of broilers was evaluated during pre-slaughter operations throughout a two-year period. Data from 53 transport vehicles were evaluated and three temperature groups were developed, T1 (10-14°C), T2 (14-19°C), and T3 (19-24°C). The mortality rate was higher for the T1 (10-14°C) temperature group amongst all the groups (Cavusoglu and Riaz, 2021). Another study analyzed the death rates of end-of- lay hens being transported to slaughterhouses during a 7-year period in the Czech Republic. In the Czech Republic, temperatures tend to range lower throughout the year with the highest temperatures being around 16°C. The highest number of deaths during transport operations occurred at temperatures ranging from -6.0 to -3.1°C and -3.0 to -0.1°C. These temperatures being the lowest of the period observed (Vecerkova et al., 2019). These results indicate that fluctuations in temperature (hot or cold) during pre-slaughter operations can drastically impact mortality rates.

1.3 CONTROLLED ATMOSPHERE STUNNING

Controlled atmosphere stunning (CAS) is the modification of the air environment to cause loss of consciousness. CAS involves the use of a multi-stage system exposing birds to increasing levels of carbon dioxide (CO_2) inducing unconsciousness and preventing recovery. Carbon dioxide is commonly used within CAS systems along with oxygen supplementation (Gerritzen et al., 2013). During CAS, birds lose consciousness from a lack of oxygen or hypoxia, and an excess inhalation of CO_2 or hypercapnic hypoxia (Hoen and Lankhaar, 1999). Increased interest in CAS stunning is linked to claims of it being advantageous for animal welfare and meat quality. In the United States, electrical stunning is the most used method in commercial processing facilities, although CAS has gained some popularity as an alternative. CAS has also gained popularity outside of the United States, largely due to legislation related to welfare at the time of slaughter (European Union, 2009).

CAS implications on welfare

CAS has been introduced as an alternative to electrical water-bath stunning systems with claims of it being advantageous for animal welfare. CAS can eliminate welfare concerns associated with electrical water-bath stunning. CAS limits the amount of human interaction during the stunning process, potentially creating a more positive experience. During CAS, birds are shackled once they are already unconscious and are stunned within their transport crates. Another concern with electrical stunning includes the occurrence of pre-stun shocks during electrical stunning. Pre-stun shocks can occur when birds experience premature contact with the water-bath, commonly on their wings (Raj and O'Callaghan, 2004). Pre-stun shock can be influenced by flock uniformity or changes in bird size. A pre-stun shock can lead to reactions such as flapping or raising their head to avoid further contact. In this instance, if birds raise their head in response to a pre-stun shock, they may avoid the water-bath stunner all together, leading to the potential of a live bird receiving a neck cut (Anastasov and Wotton, 2012).

Although, CAS too has its own welfare concerns. Birds are sensitive to CO_2, particularly in high concentrations as CO_2 displaces oxygen in the blood, leading to oxygen deprivation (Gerritzen et al., 2013). With initial introduction or exposure to high concentrations birds may exhibit signs of discomfort and distress, such as headshaking or gasping (Raj, 2006). This has resulted in some mixed responses about welfare implications associated with gas stunning, much of which is associated with system design and application. Poole and Fletcher (1995) analyzed the behavior of broilers exposed to different gas mixtures in a gas delivery system. Gases used included carbon dioxide, argon, nitrogen, or a mix of two of the above gases. Argon (Ar) and Nitrogen (N_2) are not commercially used in stunning systems, although some research articles have experimented with the use of other gases in combination with CO_2. Of the three gases broilers reacted quickest to CO_2, with significantly lower reaction times for Ar and N_2. Although, birds exposed to Ar and N_2 displayed a more severe death struggle with violent wing flapping and muscle contraction (Poole and Fletcher, 1995). McKeegan et al., (2007) performed a study where bird behavior was recorded using video cameras positioned outside of a gas delivery system. Headshaking was exhibited by 52.78% of the birds used in this study, irrespective of the gas or gas mixture used. There appeared to be an initial response to the gas exposure, with the number of head shakes performed by individuals ranging from 1-6 (McKeegan et al., 2007). CAS stunning eliminates a large amount of human interaction during the stunning process, although adverse bird responses to gas exposure can be concerning for animal welfare. Many factors play a role in the welfare of birds in electrical or gas stunning systems, making it difficult to claim one as better for animal welfare leading much up to personal perception.

Consumers are becoming increasingly interested in animal welfare associated with animal production systems. Consumer perception is important as it can affect product choice, directly effecting economic value of poultry products. CAS is commonly marketed toward customers as animal welfare friendly. Naald and Cameron (2011) conducted a study evaluating the willingness of consumers to pay more for humanely raised poultry. They concluded that people would be willing to pay about 0.35/lb more for chicken breasts labeled as humanely raised compared to chicken breast labeled as conventionally raised (Naald and Cameron, 2011). De Jonge and Trijp (2013) analyzed consumer perceptions of broiler welfare in production systems. Participants were asked to rate production system profiles based on how animal friendly they viewed them to be. Participants attributed outdoor access, stocking density, and transport duration to be some of the most important aspects to animal friendly production systems of those evaluated (de Jonge and Trijp, 2013). Consumer perception of CAS systems as animal welfare friendly has increased the popularity and implementation of CAS systems as a stunning method within commercial processing facilities, highlighting the importance of understanding the implications of CAS systems on meat quality and potential physiological implications.

1.4 MEAT QUALITY PARAMETERS

Meat quality can be defined as meat attributes that influence consumer preferences and acceptability of the product. Some of the most important for broiler meat include appearance (color), texture, juiciness, wateriness, firmness, tenderness, odor and flavor (Cross et al., 1986; Fletcher, 2002). Consumer preference on meat quality characteristics plays a large role in marketability and the economic success of poultry products. To provide high quality products for consumers, factors such as pre-slaughter stress and its causes should be studied to gain a better understanding and develop strategies to reduce pre-slaughter stressors. Ideally, poultry products should be tender, juicy, with a consistent color, and minimal drip loss.

The conversion of muscle to meat is a complex process that involves multiple biochemical and mechanical processes. After stunning and during bleeding, blood circulation stops and oxygen supply to the muscles is ceased. Without oxygen supply, cells attempt to maintain homeostasis through anaerobic metabolism. Through glycolysis, cells begin to break down glycogen to produce energy. During glycolysis, lactic acid is produced causing the pH to decrease. Muscle cells use up the remainder of the ATP (adenosine triphosphate) present, causing calcium release and bonding between myosin and actin. This locked muscle state is known as rigor mortis (Dransfield and Sosnickci 1999). The occurrence and speed of these processes have major impacts on the quality attributes of meat (Froning et al., 1978). Inability to cope with thermal stressors such as heat or cold stress during lairage can lead to changes in the biochemical processes of muscle to meat (Cassens et al., 1975).

Heat stress has been linked to an increase in PSE-like characteristics in poultry meat. PSE refers to pale, soft, and exudative meat characteristics and occurs mostly in pork. Exposure to high temperatures is noted to expedite postmortem glycolysis resulting in rapid biochemical changes to the muscle and protein denaturation (Briskey et al., 1959). Conversely, cold stress has been linked to DFD- like characteristics (dark, firm, dry) as it can slow glycolysis, resulting in higher ultimate pH levels and reduced lactic acid production (Sung et al., 1976). Tables 1.1 provides an overview of the reviewed literature below.

pH

The stress response can result in a rapid decline of meat pH. Meat pH is an important quality trait as it can impact other quality characteristics including drip loss, color, and lipid oxidation (El Rammouz et al., 2004). The pH of meat is representative of the amount of glycogen stored in the muscle prior to slaughter and the rate at which it is broken down into lactic acid through anaerobic glycolysis. (Melkonian and Schury, 2023). The isoelectric point of meat refers to the point at which the meat proteins have no net charge. At this point the positive and negative charges are equal. This can result in limited attraction of the proteins and water, holding the least amount of moisture and putting water holding capacity at its lowest (Bowker, 2017). These biochemical factors related to pH make it a critical component for meat quality. The normal pH range for chicken breast meat is between 5.7-6.1 (Barbut, 1997; Fletcher et al., 2000).

Temperature impacts on pH

Heat stress conditions are known to produce PSE-like characteristics, while cold stress has been associated with the development of DFD- like characteristics (dark, firm, dry). Wang et al. (2017) performed a study to evaluate the effects of acute heat stress on broiler meat quality and changes in glucose (mg/dL) metabolism. Male broiler chickens were exposed to a climate-controlled room at 36°C for an hour prior to slaughter. This study noted that at 50 min postmortem, birds that were exposed to acute heat stress had increased lactate production and increased rate of pH decline. This study suggested that the exposure to acute heat stress prior to slaughter increased the production of lactate in the muscle causing an increased rate of pH decline, decreasing the breast meat quality leading to more PSE-like meat (Wang et al, 2017). McKee and Sams (1997) evaluated the effect of seasonal heat stress on rigor mortis and the occurrence of PSE turkey meat. Heat stressed birds were exposed to between 32-38°C, while the control group experienced comfortable conditions at 16-24°C. The exposure period lasted 5 weeks, and at 21 weeks of age toms were processed across a three-day period. This heat exposure period is representative of chronic heat stress. Heat stressed birds had significantly lower pH than the controls that continued through 24 h post-mortem (McKee and Sams, 1997). Dadger et al. (2011) exposed birds to temperatures for three hours ranging from -18 to -4°C with a control of +20°C. The results were categorized for DFD- like characteristics by criteria ($pH_u >$ 6.1 and $L^* < 46.0$), and normal (control), ($5.7 < pH_u < 6.1$ and $46.0 < L^* < 53$). Only samples from cold stressed birds were classified as having DFD- like characteristics, and all those samples had a higher pH (Dadger et al., 2011). Dadger et al. (2012) evaluated the effect of cold stress during transport on broiler thigh and breast meat. Birds were exposed to one of 4 treatment groups; a control of (20-24°C) and three cold groups classified as (0 to -8°C), (-8 to -11°C), (-

11°C to -14°C). Thighs had a higher pH from birds exposed to temperatures below -11°C compared to other temperatures. Breast meat had a higher pH for birds in temperatures below -8°C compared to the 0 to -8°C group which was then higher than the control group. Holm and Fletcher (1997) evaluated both cold and hot pre-slaughter holding temperatures, and how they affect broiler meat quality. Broilers were exposed to three different temperature treatment groups (7°C, 18°C, 29±3°C) for 12 hours. For the birds in the 29°C temperature group, pH was significantly lower compared to 7°C and 18°C (Holm and Fletcher, 1997). A study by Schneider et al. (2012), comparing quality characteristics of heat and cold stressed birds with temperature groups of thermoneutral (21°C), heat stressed (30° C), and cool (7°C) found that for pH, birds in the cool group had the highest $pH_{u.}$

Meat Color

Meat color is arguably one of the most important quality traits as outward appearance is one of the first perceptions that consumers develop about a product. There are many factors pre- and post-mortem that can affect poultry meat color. Heme pigments, specifically myoglobin and hemoglobin (g/dL), and the degree of light scattering play a large part in the color of poultry meat (Lawrie and Ledward, 2014). Meat color can be determined by meat lightness (L*), redness (a*), and yellowness (b*) values. The L* value represents the relative lightness of the meat and is represented on a black and white scale with darker pigments being a lower number and lighter pigments being a higher number (0-100). The a* value is on a spectrum that represents red to green, with more positive numbers indicating redness and negative numbers indicating greenness (+100 to -100). The b* values are similar, representing a scale of yellow to blue with positive numbers indicating yellowness and negative numbers indicating blueness (+100 to -100) (Konica-Minolta, 2024). In a study by Lee et al. (2008), they evaluated the average color values of various poultry breast meat samples from multiple grocery stores and found average color values of (L*> 52 < 55), (a*> 8 < 11), and (b*>17< 22). These values give us a basic understanding of color values in commercial poultry meat.

Temperature impacts on meat color

The previous mentioned study by McKee and Sams (1997), that evaluated the effect of heat stress on the occurrence of PSE turkey meat also found differences in meat color. Heat stressed birds (32-38°C) had higher L*(lightness) values up until 24 h post-mortem. Zhang et al. (2012) that analyzed the effects of chronic and acute cyclic heat stress on broiler breast and thigh meat quality also had differences in meat color. Breast and thigh meat of birds exposed to constant high temperatures had a higher L* (lightness), b*(yellowness) and lower a* (redness) values than the control group. Dadger et al. (2011) evaluated the effect of cold stress on meat quality and categorized their samples as DFD-like by by criteria (pH$_u$ > 6.1 and L*< 46.0), and normal (control), (5.7 £ pH$_u$ £ 6.1 and 46.0 £ L*£ 53). Breast samples categorized as DFD-like by the above criteria, were darker (lower L*), redder (higher a*), and less yellow (b*). A similar study by Dadger et al. (2012) that evaluated meat quality of cold stressed birds during transport on breast and thigh meat also found meat color differences. The thigh meat of birds exposed to temperatures below 0°C were darker in color (higher a*), and less yellow (b*) when compared to the control. For breast meat there were similar findings with birds exposed to temperatures below 0°C being darker and redder in color (lower L*, higher a*) (Dadger et al., 2012). A separate study by Holm and Fletcher (1997) that analyzed meat quality impacts of both cold and hot holding temperatures pre-slaughter had color differences. The temperatures had no effect on the L* and a* values, however the 29°C holding temperature elicited more yellow breast fillets (b*).

Another similar study by Schneider et al. (2012) found that birds in thermoneutral (21°C) and heat stressed (30° C) groups had lighter colored breast meat compared to the cool (7°C) group.

Water holding capacity

The water holding capacity of meat is an important functional property associated with poultry products. Water is an important component of poultry meat as it directly associated with the weight of the product. For processors, weight is important for meat products as it impacts the economic value of the product. Water plays a large role in the quality of the product for consumers by impacting palatability traits including juiciness and tenderness. The water present in meat is in three forms: bound, immobilized, and free (Offer et al., 1989). The form of water is highly dependent on the location of the water in the muscle fiber. Immobilized water is mostly found in the myofibrils, free water is in the spaces between the muscle fibers and in the sarcoplasm, while bound water is directly attached to the muscle proteins. Free water is most of the water lost, as it is not tightly bound to the muscle proteins and is easily released (Offer et al., 1989; Bowker, 2017). There are multiple ways that water holding capacity can be evaluated, one of the most common being drip loss. Drip loss refers to the water lost without added force and is an indication of the muscle cell's ability to retain water and a good measurement of water loss that may be experienced by a consumer when buying and cooking a product.

Temperature impacts on water holding capacity

Mckee and Sams (1997) evaluated how seasonal heat stress impacted the occurrence of PSE turkey meat, and along with other meat quality differences found changes in water holding capacity. Heat stressed birds exposed to (32-38°C) had a significantly decreased water holding capacity. Zhang et al. (2012) that analyzed chronic and acute cyclic heat stress on breast meat also found differences in water holding capacity. For this study, to evaluate water holding capacity, this study evaluated cooking loss. Birds that were exposed to the constant high temperatures did have a higher cook loss. In the study Dadgar et al. (2011) that analyzed cold stress impacts on the occurrence of DFD-like characteristics, the samples categorized as DFD-like did have a higher water holding capacity. Holm and Fletcher (1997) that evaluated both cold and hot holding temperatures pre-slaughter and their impact on meat quality also had differences in water holding capacity. A study by Schneider et al. (2012), that analyzed meat quality differences from birds in thermoneutral (21°C), heat stressed (30° C), and cool (7°C) group found that the cool group had the lowest drip loss compared to the heat and thermoneutral group.For the birds in the 29°C temperature group, cook loss was significantly lower compared to 7°C and 18°C, which is different than previous results that noted lower water holding capacity with birds exposed to high temperatures.

Lipid Oxidation

Lipid oxidation is the product of complex reactions between fatty acids and oxygen resulting in rancidity and the deterioration of fats. During this process, unsaturated fatty acids react with molecular oxygen, creating products referred to as hydroperoxides. These hydroperoxides produced are unstable, leading them to break down into other secondary compounds. Some of these compounds include hydrocarbons, aldehydes, and ketones which can in turn lead to off flavors and odors in the meat (Dominguez et al., 2019). Lipid oxidation is a major cause of meat spoilage and can result in multiple quality issues including rancidity or off-flavor, color, and texture changes that are undesirable to consumers. Bovine meat is the most vulnerable to lipid oxidation compared to chicken and even pork due to the large amount of myoglobin, intramuscular fat, and iron present in beef (Rhee et al., 1996).

Temperature impacts on lipid oxidation

Heat stress or high ambient temperatures have been suggested to create pro-oxidant that can cause oxidative stress in large amounts (Mujahid et al., 2007). Oxidative stress refers to an excess number of reactive species compared to the antioxidant level of the cell (Halliwell and Whiteman, 2004). Oxidative stress can have a negative effect on meat quality as poultry meat could be affected by oxidative reactions which induce lipid oxidation leading to rancidity and off-odor (Estévez, 2015). One study was performed to analyze flavor changes from heat stress induced lipid oxidation in duck meat (He et al., 2020). This study had significantly higher MDA and LOX levels compared to the control, suggesting that heat stress influenced lipid oxidation in the duck meat. Malondialdehyde (MDA) is a product of lipid oxidation and lipoxygenase (LOX) activity plays a part in the occurrence of lipid oxidation (Jin et al., 2010). In terms of flavor, this study concluded that the heat stressed raw duck meat presented with a more pronounced odor (He et al., 2020). Altan et al. (2003), had similar findings with meat from heat stressed broilers having a significantly higher MDA content. The conditions birds are exposed to in pre-slaughter lairage holding areas can influence the presence of thermal stressors and impact the determinants of poultry meat quality.

Links between CAS and meat quality

Table 1.2 provides key findings from the reviewed literature listed below. There is less peer reviewed literature on the direct effects of CAS on meat quality. As CAS has gained increased interest there have been multiple research studies aimed at trying to understand potential effects of gas stunning on poultry meat quality (Pinto et al., 2012; Riggs et al., 2023; Salwani et al., 2016), One study assessed the differences in meat quality parameters of birds that were not stunned (Halal slaughter), and gas stunned birds (Salwani et al., 2016). Meat from gas stunned birds presented with a lower muscle pH and higher drip and cooking losses. There was no effect on the L* or b* values of any of the breast fillets, but gas stunned birds had a lower a* value (Salwani et al., 2016). It is suggested that the low pH value could be the result of rapid postmortem glycolysis, or that any discomfort of the bird from gas inhalation during the stunning process could lead to the use of energy reserves before exsanguination (Raj, 1998). The low pH could have an impact on the increase in drip and cooking losses, as low pH is commonly linked to decreased water holding capacity with the denaturation of the myofibrillar proteins (Raj, 1999). The lower a* value is similar to other studies (Pinto et al., 2012; Riggs et al., 2023). Although, these studies both reported lower a* values and fillets that were paler in color with higher L* values. Riggs et al. (2023) analyzed meat quality of electrical (ES) and controlled atmosphere sunning systems (CAS). Data was collected from a commercial processing plant, with separate processing lines for ES and CAS. CAS fillets had a lower pH, a high L* value, a lower a* value compared to ES fillets. No differences in in drip loss were present between the ES and CAS fillets. This is different from the previous study where gas stunned birds had increased drip and cooking losses (Salwani et al., 2016). One study focused on how gas concentrations may affect meat quality (Xu et al., 2011a). The results of that study indicated that gas stunning using lower carbon dioxide concentrations enhanced meat quality compared to higher carbon dioxide concentrations. The low CO_2 levels (30-40%) had increased water holding capacity, a higher pH, and lower L* values compared to the use of higher CO_2 concentrations. This range of literature indicates that CAS stunning can have a varying impact on meat quality attributes.

1.5 PHYSIOLOGICAL IMPLICATIONS

Bird respiration as influenced by lairage and CAS

Variations in lairage temperatures and CAS can impact a bird's respiratory rate and function. The function of a bird's respiratory system is gas exchange, specifically delivering oxygen to the bodies tissues and removing carbon dioxide from the tissues. The avian respiratory system is different compared to mammals. Avian anatomy is comprised of small lungs that do not inflate or deflate with respiration, and nine air sacs that create a single flow where air enters and exits (Powell, 2015). Respiration plays an important role in physiological responses to temperature stress and controlled atmosphere stunning. As previously stated, CAS stunning induces unconsciousness with the depletion of oxygen and increase of carbon dioxide, referred to as hypercapnic hypoxia. Respiratory alkalosis is an acid- base imbalance in the blood, when CO_2 levels in the blood become low. At high ambient temperatures, birds may begin to pant to regulate their body temperature. This panting results in evaporative cooling and a loss of blood CO_2 (Teether et al., 1985). Respiratory acidosis is referred to as a state in which there is a buildup of carbon dioxide, and an inability to provide ventilation to the body. Respiratory acidosis results in an increase in PCO_2 (mmHg) levels and a decrease in bicarbonate ($HCO^{3}-$ (mEq/L)) effecting the blood ratio and decreasing the pH of the blood (Patel and Sharma, 2023). These respiratory states have large impacts and physiological implications associated with responses to ambient temperature and CAS stunning.

Temperature impacts on blood metabolites

There are multiple physiological indicators that can create a better understanding of the bird's biological response to stressors, pre-slaughter conditions, and controlled atmosphere stunning. Specific changes in blood metabolites can demonstrate the physiological state of the bird during these conditions. One study evaluated blood metabolites of broilers exposed to either a thermoneutral (24°C) or a cyclic heat stressed environment (24- 35°C) during a 10-day period

(Deyhim and Teeter, 1991). PO_2 (mmHg) increased for the cyclic heat stressed birds, suggesting potential increased blood oxygenation. Corticosterone levels were increased in the heat stressed group compared to the thermoneutral group suggesting those birds were stressed from the thermal conditions (Deyhim and Teeter, 1991).

Another study evaluated how thermal temperatures influenced hemoglobin (g/dL) and hematocrit (%PVC) blood values (Deaton et al., 1969). Birds reared at a temperature of 7.2°C had a higher hemoglobin (g/dL) level compared to birds reared at a temperature of 32.2°C. Hematocrit (%PVC) followed a similar pattern with decreased levels in the high temperature group compared to 7.2°C. The author suggested that metabolic rate could factor into the results. Another study in turkeys analyzed the effect of brooding conditions on the blood chemistry (Crespo and Grimes, 2024). Turkey hens were assigned to 4 treatment groups, control (32°C and 60% RH), cold stress (29°C and 60% RH), heat stress (35°C and 60% RH), and heat stress plus humidity (35°C and 75% RH). The turkeys exposed to heat treatment groups indicated signs of respiratory alkalosis, with a higher blood pH, higher PO_2 (mmHg) and a lower PCO_2 (mmHg). Sodium (Na) levels were lower in the heat treatment groups. This could be attributed to birds increasing their water consumption to cool down. It was also suggested that the small differences in the temperature treatment groups likely attributed to the results still being within normal limits reported by other previous literature (Ripplinger et al., 2023). One study evaluated panting and acid-base changes in heat stressed birds. This study noted an increase in PO_2 (mmHg) in the heat stressed birds and suggested this is from panting which causes an increased demand for oxygen (Marder and Arad, 1989). On the other hand, other literature has described an increase in PO_2 (mmHg) as suggestive of effective transport of oxygen throughout the body (Powell, 2015).

CAS impacts on blood metabolites

Some studies have discussed how CAS has impacted blood metabolites. One study evaluated how gas stunning and electrical stunning impacted blood variables in broilers (Xu et al., 2011b). This study found that gas stunned broilers had consistently decreased blood pH compared to electrically stunned broilers. This study suggested that this decrease in blood pH is likely a result of respiratory acidosis experienced by broilers upon the inhalation of CO_2. Similarly, Gerritzen et al. (2006) performed a study to evaluate how blood gases in ducks and turkeys are impacted by increasing CO_2 concentrations. Ducks and turkeys exhibited similar results with increases in PCO_2 (mmHg), decreases in PO_2 (mmHg) and O_2 saturation, a decrease in blood pH, and a large shift in the acid base equilibrium. This study stated that this is an example of the impact that the inhalation of CO_2 can have on physiological factors in the body, essentially causing a state of acidosis. Along with this, the decreases in PO_2 (mmHg) and O_2 led to hypoxia or inadequate levels of oxygen in the blood. According to the previously mentioned studies, respiratory acidosis plays a large role in the physiological changes of blood metabolites associated with CAS. A few studies in other species have explained the effects of respiratory acidosis on blood variables. A study on the impact of respiratory acidosis on blood gases in rats noted a decrease in Na compared to the controls, suggesting respiratory acidosis directly impacts a decrease in sodium (Carter et al., 1959). Alternatively, another study evaluated plasma variables of broilers exposed to different carbon dioxide conditions, and found limited results as plasma corticosterone and glucose (mg/dL) levels were not affected by the different CO_2 levels (Xu et al., 2011a). These results speak to the potential impact that CAS can have on blood metabolites with the occurrence of respiratory acidosis.

1.6 CLOSING REMARKS

Ultimately there are gaps in the available peer-reviewed published literature evaluating the impacts of thermal stress during lairage conditions and its combined effects with controlled atmosphere stunning on meat quality parameters and blood metabolites. This research gap makes it difficult to have a clear understanding of the physiological implications associated with these operations and subsequent meat quality, leaving much to be desired to draw conclusions on these topics. Lairage temperature and CAS have the potential to have additive impacts on meat quality and evaluating blood metabolites will provide insight into the relationship between the inhalation of carbon dioxide and the resulting change in meat quality. The objective of this thesis is to investigate the combined effects of lairage temperatures and CAS on broiler meat quality and blood metabolites. The findings have the potential to contribute to improved management practices and enhanced bird welfare within lairage environments and CAS systems.

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Table 1.1 Literature on temperature impacts on meat quality

Species	Parameter Measured	Temperature	Main Findings	Reference
Broilers	pH	36°C for an hour prior to slaughter	Lower pH for heat stressed birds	Wang et al., 2017
Turkeys		Heat stressed 32-38°C for 5 weeks	Increased rate of pH declines for acute heat stressed birds	McKee and Sams, 1997
Broilers		Three temperature treatment groups (7°C, 18°C, 29±3°C) for 12 hours	Lower pH for 29°C treatment	Holm and Fletcher, 1997
Broilers	Color	36°C for an hour prior to slaughter	Lower L* for heat stressed birds	Wang et al., 2017
Turkeys		Heat stressed 32-38°C for 5 weeks	Higher L* for acute heat stressed birds	McKee and Sams, 1997
Broilers		Control of (20-24°C) and three cold groups classified as (0 to -8°C), (-8 to -11°C), (-11°C to -14°C) maintained for three hours	Thighs darker and less yellow (higher a*, lower b*) and breasts that were darker and redder in color (lower L*, higher a*) for below 0°C treatment	Dadgar et al., 2012
Broilers		Three temperature treatment groups (7°C, 18°C, 29±3°C) for 12 hours	Yellower fillets (higher b*) for 29°C treatment	Holm and Fletcher, 1997

Broilers	Cooking Loss	36°C for an hour prior to slaughter	Lower cook loss for 29°C treatment	Wang et al., 2017
Turkeys	Rigor Mortis	Heat stressed 32-38°C for 5 weeks	Accelerated rigor (higher R values) for heat stressed birds	McKee and Sams, 1997

[1] WHC- water holding capacity
[2] HS- heat stress
[3] AHS- acute heat stress

Table 1.2 Literature on CAS impacts on meat quality

Species	Parameter Measured	Stun Type	Gas Concentration	Main Findings	Reference
Broilers	pH	None	40% CO_2[1], 30% O_2, and 30% N	Lower muscle pH for gas	Salwani et al., 2016
		Electrical & Gas	20% to 85% over the course of 5 min with O_2 added to 21%	Lower pH for gas	Riggs et al., 2023
		Gas	Low CO_2 levels (30-40%) High CO_2 levels (60%+)	Higher pH for low CO_2 birds	Xu et al., 2011a
		Electrical & Gas	30% CO_2, 60% argon	Lower pH for gas	Raj et al., 1997
Broilers	Color	None	40% CO_2, 30% O_2, and 30% N	Lower a* for gas	Salwani et al., 2016
		Electrical & Gas	20% to 85% over the course of 5 min with O_2 added to 21%	Higher L* and lower a* for gas	Riggs et al., 2023
		Gas	Low CO_2 levels (30-40%) High CO_2 levels (60%+)	Lower L* for low CO_2 birds	Xu et al., 2011a
Broilers	Drip Loss	None	40% CO_2, 30% O_2, and 30% N	Higher for gas	Salwani et al., 2016
		Gas	Low CO_2 levels (30-40%) High CO_2 levels (60%+)	Increased WHC[2] for low CO_2 birds	Xu et al., 2011a

Broilers	Cooking Loss	None	40% CO_2, 30% O_2, and 30% N	Higher for gas	Salwani et al., 2016
Broilers	Texture	Electrical & Gas	30% CO_2, 60% argon	More tender for gas	Raj et al., 1997

[1] CO_2- Carbon Dioxide

[2] WHC- Water Holding Capacity

CHAPTER 2: COMBINED IMPACT OF LAIRAGE TEMPERATURES AND CONTROLLED ATMOSPHERE STUNNING TIME ON BROILER MEAT QUALITY PARAMETERS

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2.1 ABSTRACT

During lairage, birds are exposed to a variety of temperatures dependent on the location and time of year. Carbon dioxide-controlled atmosphere stunning (CAS) is becoming an increasingly utilized method to render poultry unconscious prior to slaughter. The objective of this study was to analyze the effect of lairage temperatures and CAS time on broiler meat quality. This study utilized a 2x2 design evaluating two lairage temperatures (hot and cool) and two stunning times (4.5 and 6 min). In simulated lairage environments, hot birds were exposed to an average temperature of 28.9°C and cool birds were exposed to 17.3°C for 1 h. Following lairage, birds were stunned using CAS and processed. After immersion chilling and deboning, breast fillets were evaluated at 2 h and 24 h postmortem for pH, color, and drip loss. Lipid oxidation was evaluated at 24 h and 7 d postmortem. At 2 h postmortem birds stunned for 4.5 min had a higher pH (6.41) than the 6 min stunned (6.32; P=0.027). At 24 h birds in the cooler lairage environment had a higher pH (5.75) than those in the hot lairage environment (5.70; P<0.0001). Fillets from hot lairage birds were lighter (43.82), and more yellow at (5.58) than the cool birds at 2 h postmortem (42.81, 5.24; P=0.0002, P=0.001) At 24h this persisted, with hot birds being lighter (46.80) and more yellow (5.31) than cool birds (46.19, 4.96; P=0.0008, P=0.0002). There were no significant differences observed for drip loss or lipid oxidation.

2.2 INTRODUCTION

According to the USDA, consumption of poultry meat is considerably higher compared to beef and pork (USDA-FAS, 2023). This increased consumption is related to the accessibility and convenience of purchasing and cooking poultry meat products, which emphasizes the importance of the poultry industry being able to provide high quality poultry meat to its customers and consumers. Common characteristics associated with meat quality include meat color, texture, pH, and drip loss. Appearance is important for consumer selection of poultry products (Grzybowska-Brzezińska et al., 2023). Conditions associated with poultry processing may impact the ability to provide consumers with the poultry meat products they desire.

After arriving to the processing plant, broilers are placed in a designated holding area known as lairage. Lairage serves multiple functions by allowing birds a period of rest and recovery from stressors experienced during transport and supporting processing plant efficiency by ensuring a consistent flow of birds from reception onto the processing line. This allows the plant to maintain optimal processing speed and product quality (Vieira et al., 2011). In the United States, lairage areas are typically outside under large sheds that transport trucks can park under after arriving to the processing plant. These holding areas are commonly equipped with fans, or misters for temperature management.

The first step after lairage and reception is typically stunning. In most cases broilers are stunned prior to exsanguination. Stunning is the process of rendering an animal unconscious and insensible to pain prior to slaughter. This is important to ensure animal welfare by preventing pain during mechanical neck cut. Stunning also facilitates more efficient processing by immobilizing the animal to improve accuracy and automation on the processing line. In the United States, electrical water bath stunning is the most used stunning method in poultry processing facilities (Berg and Raj, 2015). However, the use of CAS systems has become more frequent. CAS involves using inert gases in increasing concentrations to render birds unconscious. Within a CAS system, multiple phases are used that gradually increase CO_2 concentrations to induce irreversible unconsciousness (Berg and Raj, 2015). This method is often suggested to be advantageous for animal and worker welfare (Gerritzen et al., 2013). CAS systems provide less direct human to bird contact by removing the process of live shackling as birds are shackled while unconscious. Removing the process of live shackling can have an added welfare benefit as shackling can be a painful process for birds. However, CAS stunning can have potential negative impacts on meat quality such as breast fillets that are lower in pH and paler in color. Pinto et al. (2016) compared the meat quality attributes of CAS and electrically stunned poultry and found the fillets from gas stunned birds were lighter and less red in color than those of the electrically stunned birds. Other studies reported similar changes in meat quality characteristics, with meat that was paler in color and that was linked to higher drip loss and a lower pH (Woelfel et al., 2002; Salwani et al., 2016; Riggs et al., 2023).

Heat exposure during lairage can cause acute heat stress to birds and is a prominent issue within the poultry industry. Previous work has shown that lairage conditions influence bird stress responses and mortality. Due to typical lairage conditions in the southeastern United States, birds often experience high temperatures during lairage. Heat stress has been linked to pale, soft, exudative (PSE) like characteristics with reported increases in lightness and drip loss of poultry (McKee and Sams, 1997; Wang et al., 2017). Respiration may change in response to changes in the temperature, particularly in hot conditions. Krusic (1928) reported on the importance of panting in birds. Panting is a method of heat loss caused by increased water evaporation in the respiratory tract. This type of respiration can cause a physiological change from closed mouth breathing to open mouth breathing and higher respiration rates with more water evaporation from the lungs due to the increased intake of air.

In CO_2 stunning systems, an increase in respiration rate occurring with hot lairage temperature could cause the birds to intake larger amounts of CO_2, potentially impacting their response to carbon dioxide gas stunning. This also poses the question of how an increased intake of carbon dioxide may affect subsequent meat quality. The objective of this study was to evaluate the impact of two lairage temperatures and two CAS exposure times on meat quality attributes including color, pH, drip loss, and lipid oxidation.

2.3 MATERIALS AND METHODS

Experimental Design

A 2x2 factorial experimental design was used to evaluate two lairage temperature conditions and two controlled atmosphere stunning times. The experiment was conducted at the Charles C. Miller Jr. Poultry Research Center at Auburn University. There was a total of 3 repetitions, with 130 broilers processed in repetition one, 57 for repetition two, and 57 for repetition three. In this experiment, two variables were tested, lairage temperature (cool and hot) and stunning time (4.5 and 6 min). These variables resulted in four treatment groups: cool/4.5 min, cool/6 min, hot/4.5 min, and hot/6 min, which were conducted in duplicate for a total of 8 runs for each of the three repetitions. Following stunning, birds were bled, scalded, picked, eviscerated, chilled and deboned. Meat quality attributes color, pH, and drip loss were measured at 2 and 24 h postmortem. Lipid oxidation measurements were taken at 24 h and 7 d postmortem. This experiment was in accordance with the Auburn University Institutional Animal Care and Use Committee PRN 2023-5300.

Bird Source

For each repetition, Ross 708 broiler chickens with a target weight of 1.91 kg were sourced from live haul at a commercial processing plant. The first and second repetition occurred in March, while the third repetition occurred in April of 2024. Average temperatures and relative humidity for the day of each repetition were: repetition 1:(17.2°C, 92.6%), repetition 2:(12.8°C, 51.9%), repetition 3:(18.3°C, 76.8%). Birds were manually loaded into transport modules at the processing facility and transported 45 minutes to the Charles C. Miller Poultry Research Center on a flatbed trailer.

Lairage conditions

To simulate two separate lairage environments, two industrial tents were utilized to maintain a "hot" or "cool" temperature (Canopy Tent 11C540, Grainger). A space heater or air conditioning unit were used within each tent to create the desired conditions. One BAADER transport module with drawers was placed inside each tent (Uniload, SU502). The tents were brought to the desired temperature prior to the arrival of birds. Upon arrival, birds were immediately leg banded and placed in the drawers inside for a minimum of 60 min to simulate commercial processing lairage time periods. The drawers used within the transport modules were the Uniload large drawers that have an inner area of 13,620 cm^2, and can hold up to 85 kg, if bird weights are between 1.6-3.0 kg. Figure 2.3 shows the below listed information about bird location and stocking densities within each tent. For repetition 1, in the hot tent there were four drawers with birds, one containing 17 birds, two containing 16 birds and one containing 15 birds. For the drawer with 17 birds, the stocking density was 23.84 kg/m^2. The drawers with 16 birds had a stocking density of 22.43 kg/m^2, and the drawer with 15 birds had a stocking density of 21.03 kg/m^2. In the cold tent, there were four drawers, two containing 17 birds, and two containing 16 birds. For the drawers with 17 birds, the stocking density was 23.84 kg/m^2 and the drawers with 16 birds has a stocking density of 22.43 kg/m^2. For repetition 2, in the hot tent there were two drawers with birds, one with 12 birds and one with 15 birds. In the drawer with 12 birds, the stocking density was 16.83 kg/m^2, and in the drawer with 15 birds the stocking density was 21.03 kg/m^2. In the cold tent, there were two drawers with 15 birds per drawer, and the stocking density was 21.03 kg/m^2. In repetition 3, in the hot tent there were two drawers with 14 birds per drawer, and the stocking density was 19.64 kg/m^2. In the cold tent, there were two drawers, one with 15 birds and one with 14 birds. In the drawer with 15 birds, the stocking density was 21.03 kg/m^2. In the drawer with 14 birds the stocking density was 19.64 kg/m^2. The National Chicken Council (NCC) recommends a stocking density of 32 to 44 kg/m^2, while the Global Animal Partnership (GAP) recommends a lower stocking density of 27−29 kg/m^2. HOBO data loggers were placed on the transport containers to record the temperatures within each tent (MX1101, HOBO, Bourne, MA) and temperatures were averaged over the entire hour. In the hot tent for each of the three repetitions mean temperatures were 29.3°C ± 0.5, 28.4°C ± 1.7, and 29.1°C ± 1.4, respectively. In the cool tent mean temperatures were 17.3°C ± 0.4, 17.4°C ± 0.6, and 17.2°C ± 0.6, respectively.

Processing

A prototype five stage BAADER CAS system was used to render the bird's unconscious prior to mechanical neck cut. Carbon dioxide gas concentrations were recorded using a Felix Instruments F-920 Check It! gas analyzer. The analyzer was placed within the crate with the birds for the duration of stunning. Each of the eight runs from each repetition was recorded and means calculated. For the 4.5 min stunning time, stage one through five had the following CO_2 gas concentrations and durations; (2-28%; 39 s), (29-43%; 55 s), (44-73%; 61 s), (74-89%; 56 s), and (90-95%; 48 s), resulting in an applied stunning time of 259 s (4.32 min). For the 6 min stunning time, stage one through five had the following CO_2 gas concentrations and durations; (2-26%; 53 s); (27-34%; 75 s); (35-67%; 82 s); (68-89%; 75 s); (90-93%; 64 s), resulting in an applied stunning time of 349 s (5.81 min). Temperatures within the gas stunning system were averaged over the entire stunning period for each repetition. The average temperatures were repetition 1: 25.45°C, repetition 2: 27.39°C, and repetition 3: 28.21°C. Following stunning, transport drawers were conveyed to the shackle line and broiler chickens were removed from the drawer and manually hung. Birds were mechanically neck cut, bled for 262 s, scalded at 53°C for 143 s, and picked for 35 s. Carcasses were mechanically eviscerated, immersion chilled in tap water with ice and air agitation for 90 min, then hand deboned removing the right breast filet for meat quality measurements.

Meat quality evaluation

For repetition one, 130 breast fillets were manually deboned and for repetitions two and three, 57 breast fillets per repetition were manually deboned, with an n= 59 to 62 fillets per treatment. Differences in the number of fillets per treatment group are because some carcasses were not able to be used for meat quality evaluation because they were damaged by the picker. These breast fillets were evaluated for color, pH, drip loss, and lipid oxidation. Color and pH were measured at 2 and 24 h post-mortem. Readings were taken at 2 h post-mortem as this is a typical deboning time in a commercial processing facility and gives an idea of pH decline while 24 h post-mortem is the ultimate pH and rigor is fully resolved. The three-color measurements were recorded and averaged from the dorsal side of the fillet, top, middle, bottom, for L*, a*, and b* color values (Konica Minolta Chroma Meter CR-400). Fillet pH was recorded by inserting a meat piercing probe in the cranial end of the breast fillet (H198161, HANNA Instruments, Woonsocket, RI). Fillets were weighed at 2 h postmortem, placed in a zip top bag, and kept on ice in a cooler, which was transported back to the laboratory. At 24 h measurements for color, pH, and drip loss followed the same protocol. For drip loss, fillets were allowed 30 min to drip before weighing. Drip loss was calculated by subtracting the 24 h fillet weight from the initial weight, dividing the difference by the initial weight and multiplying by 100.

Lipid oxidation was evaluated at 24 h and 7 d as previously described by Manjankattil et al. (2025). For repetition one, 24 meat samples (6 per treatment) were evaluated, and for repetitions two and three 57 samples per repetition were evaluated for lipid oxidation. For repetition 2, there were 15 samples per treatment, except for cool/5 min that had 12 samples. For repetition 3, there were 15 samples per treatment, except for cool/5 min and hot/5 min which had 13 and 14 samples. The differences in samples per treatment are because during processing there were some carcasses that had to be removed because breast fillets were torn by the picker. Briefly, a 2 g meat sample was cut from the breast fillet, and combined in a 50 mL tube with 8 mL of 5% trichloroacetic acid, and vortexed for 90 s. This solution was then filtered with Whatman no. 1 filter paper into a clean 50 mL tube. Next, 5% trichloroacetic acid was added to reach 10 mL of filtrate. Then 5 mL of this filtrate along with 5 mL of 20 mM thiobarbituric acid (TBA) solution was transferred to another 50 mL sample tube. This solution was vortexed for 30 s, kept in the dark for 20 h at room temperature then OD was measured by a spectrophotometer at 532 nm.

Statistical Analysis

A three-way ANOVA was conducted using Proc Glimmix with lairage temperature, CAS time, and sampling time as fixed effects. Gamma distribution was used for a*, b*, pH, and drip loss. Gaussian and poisson distributions were used for L* and lipid oxidation. Stocking density was included in the model as a covariate to control for its potential impact. There were no significant interactions so lairage temperature, CAS time, and sampling time main effects are presented independently. The means for color, pH, drip loss, and lipid oxidation were separated by Tukey's HSD with significance determined at P < 0.05.

2.4 RESULTS

pH

When evaluating pH at 2 h postmortem CAS time main effects were significant. At 2 h post-mortem, the 4.5-minute stunned birds had a higher pH (6.41), compared to 6-minute stunned birds (6.32; P=0.027). When evaluating pH at 24 h postmortem, lairage temperature was significant. At 24 h postmortem, birds exposed to the cool lairage environment had a higher pH (5.75). compared to the birds in the hot lairage environment (5.70; P<0.0001). A significant effect of stocking density was observed for pH (P<0.0001). Overall means for pH at 2 and 24 h indicated that pH values at 2 h (6.36) were higher compared to pH values at 24 h (5.73; P<0.0001).

Color

The L* values (Table 1) indicated lighter breast fillets in the hot treatment groups at 2 h postmortem, with hot birds exhibiting a mean of 43.82 compared to cool birds with a mean of 42.81 (P=0.0002). This persisted through 24 h postmortem with hot birds having a mean of 46.80 compared to cool birds 46.19 (P=0.0008). When a* value was evaluated at 2 and 24 h postmortem there were no significant differences for lairage temperature or CAS time main effects. When b* values were evaluated at 2 h postmortem lairage temperature was significant. The b* values indicated yellower breast fillets in the hot treatment groups, with hot birds presenting with a mean of 5.58, compared to cool birds with a mean of 5.24 (P=0.001). At 24 h this persisted with yellower breast fillets in hot treatment groups, with hot birds having yellower fillets with a mean of 5.31 compared to cool birds with a mean of 4.96 (P=0.0002).

When evaluating the overall means for 2 and 24 h, L* was higher at 24 h postmortem at 46.48 compared to 2 h postmortem at 43.3 (P<0.0001). Alternatively, overall means for a* and b* values were higher at 2 h at 2.33 and 5.41, respectively, when compared to 24 h at 1.79 and 5.14 (P<0.0001; P=0.009). Stocking density had a significant effect on L* and b* values (P P<0.0001).

Drip loss

There were no significant differences for lairage temperature or CAS time for drip loss. Breast fillet drip loss values ranged between 2.30% to 2.59%. Stocking density had a significant effect on drip loss (P<0.0004).

Lipid Oxidation

There were no significant differences for lairage temperature or CAS time for lipid oxidation at either 24 h or after 7 d of refrigerated storage. However, 7 d lipid oxidation readings (0.02) were higher than the 24 h readings (0.00) indicating an increase in lipid oxidation with increased storage time (P<0.0001).

2.5 DISCUSSION

Lairage temperature impact on meat quality

Breast fillets from the cool lairage treatment group displayed a higher pH compared to the hot lairage birds at 24 h postmortem. This is similar to previous literature, where chickens exposed to between 32 and 38°C presented with PSE- like characteristics such as pale color, low pH, and higher drip loss (McKee and Sams, 1997; Sandercock et al., 2001). These results have even been seen in other livestock species. In a study where pigs were processed during hot summer temperatures (33°C), they presented with the lowest pH values compared to the other seasons (Čobanović et al., 2020).

It has been shown that pH is one of the main determinants of water holding capacity (Offer et al., 1989). When pH reaches the isoelectric point (5.2), there is limited attraction of protein and water, decreasing water holding capacity (Bowker, 2017). As pH becomes more acidic, meat quality properties can change, specifically meat may present with more PSE-like characteristics such as decreased water holding capacity, soft texture, and protein denaturation which can change the light scattering of meat proteins leading to a paler meat color. If the pH becomes more alkaline, the opposite can occur with meat that may present with more DFD-like characteristics such as increased water holding capacity tight within the muscle causing a drier firmer texture, and changes in myoglobin production and a darker appearance to the meat (Dadger et al., 2011; Mckee and Sams, 1997). In this study, there were no significant differences in drip loss values between the two temperature groups. These results are similar to Wang et al., (2017), who reported no significant differences in drip loss values when evaluating the effects of acute heat stress on broiler breast meat quality. Although, other studies have reported decreased water holding capacity of birds exposed to heat stress prior to slaughter. Tang et al. (2013) evaluated the effect of heat stress on meat quality with different exposure times and reported a significant increase in cooking loss and expressible moisture after 3 h of heat stress exposure and lower pH values after 1-2 h of heat exposure. Similarly, another study found increased drip loss and lower pH values for birds exposed to heat stress conditions compared to those that were not heat stressed (Zhu et al., 2011). It is possible that if birds were exposed to longer durations of temperature exposure in this study, the results may have more notable differences. An assessment of water binding capacity of the breast fillets could have given further insight on if there were any differences in the fillets water holding capacity.

Birds exposed to the hot lairage treatment had paler breast fillets at 2 and 24 h postmortem. It is suggested that lower pH associated with heat stress could be a result of a stress response from heat exposure. This stress response can result in rapid decline of pH, which has been previously shown to impact other quality characteristics such as water holding capacity and color (Papinaho et al., 1995). Another study found that turkeys subjected to preslaughter heat stress had lighter breast fillets compared to the control and birds exposed to cold pre-slaughter conditions (Babji et al., 1982). This study observed higher b* values of breast fillets from the hot treatment groups at 2 and 24 h postmortem compared to the cool lairage treatment. This is different than previous literature that has reported a decrease in b* values of breast fillets of broilers exposed to acute heat stress (Pertracci et al., 2004; McCurdy et al., 1996). Zhang et al. (2012) did report increased yellowness of the thighs of birds exposed to heat stress conditions repeatedly, although the possible cause of this increase in yellowness was unclear. As previously mentioned, it is plausible that greater differences in meat quality parameters could have been observed with increased time birds were exposed to the conditions in simulated lairage environments, or with more extreme temperatures. we chose the lairage duration of 1 h as that is representative of commercial processing lairage times.

CAS impact on meat quality

Carbon dioxide gas stunning has also been noted to produce paler breast fillets that are less red in color. Riggs et al. (2023) evaluated the meat quality of birds exposed to carbon dioxide gas stunning compared to electrical stunning and noted that fillets from gas stunned poultry had a higher L* value and a lower a* value, suggesting gas stunning led to changes in the color of the fillets. Another study had similar results between gas killing and electrical stunning with lighter fillets that were less red in color (Pinto et al., 2016). In this study we did find differences in the color of fillets as birds exposed to the hot lairage environments had paler and yellower breast fillets.

Riggs et al. (2023), when comparing meat quality of electrical and CAS stunned birds, did note that CAS breast fillets presented with a lower pH. At 2 h postmortem, 4.5-minute stunned birds had a higher pH than those stunned for 6 minutes. This could be a result of the inhalation of CO_2 during these two different stun times. One study evaluated meat quality parameters on broilers stunned with different CO_2 concentrations. When evaluating pH of the pectoralis major, pH at 24 h postmortem was higher with lower CO_2 concentrations and the lowest pH at 24 h postmortem was in high CO_2 concentrations (Xu et al., 2011). Similar results were seen in swine when one study evaluated the pH of the ham and observed a higher initial pH with pigs exposed to lower gas concentrations (60-80%) compared to higher (85-90%) (Troeger and Woltersdorf, 1991). This study suggested that a possible reason for increased lactate could be that in the higher CO_2 concentrations pigs may have struggled more and subsequently had more convulsions which led to more muscle movement, and hence more lactate production. An increase in lactate could result in a rapid decline of pH making the meat more acidic. Alternatively, a more simplified explanation could be that the drop in pH is a result of the inhalation of CO_2 and could explain why in both studies higher CO_2 concentrations resulted in a lower pH and lower CO_2 concentrations had a high pH (Troeger and Woltersdorf, 1991; Xu et al., 2011).

Previous work has noted increasing lipid oxidation with increased days of storage (Manjankattil et al., 2025). Similar results were found in this study with increased lipid oxidation values with prolonged storage from 24 h to 7 d. Previous studies have attempted to draw conclusions on heat stress and oxidation implications on meat, suggesting that the excessive formation of reactive oxygen species during heat stress can lead to the degradation of proteins and cell membranes, leading to increases in lipid oxidation (Bruskov et al., 2002). Another study on the impacts of heat stress on lipid oxidation of meat found similar findings to this study with no differences for broilers in either the heat stress or thermoneutral group (Altan et al., 2003). It is plausible that extending the lairage time that birds are exposed to the desired temperature conditions or adding seasonal ambient and transport temperature could have led to an increase in the meat quality effects seen.

Links between CAS and lairage temperature

In this study all meat quality measurements were performed on fully processed breast fillets. The combination of both CAS and heat exposure prior to slaughter may have led to results that were not necessarily expected. This study did not find any differences in drip loss measurements, while previous literature has found that birds exposed to chronic or acute heat stress have exhibited increased drip loss (Zhu et al., 2011; Tang et al., 2013). It could be possible that the use of CAS could have led to this unexpected result.

2.6 CLOSING REMARKS

These results provide similar and conflicting results compared to previous literature and emphasize how short-term exposure to thermal stress in lairage and CAS can impact meat quality characteristics. Our results show that lairage conditions can impact meat quality characteristics, potentially increasing undesirable effects such as lower pH and lighter breast fillets. The simulated lairage environments may not be completely representative of the thermal conditions dependent on the location of the processing plant as some locations may have far colder or hotter weather extremes. Reproducing this study in a commercial setting in different seasons might be more insightful towards meat quality impacts. If this study were repeated, I would like to change the lairage temperature groups by having a hot group of 30-35°C, a comfortable group at 21°C, and a cold group at 40-50°C. I would also like to have multiple lairage exposure times, to see if increases in lairage duration could have potential effects. These temperature groups could give us more insight into potential impacts on meat quality and could give us a wider range of applicability to temperatures throughout different areas and seasons. Moving forward it will be important to investigate how lairage conditions may potentially impact subsequent meat quality with the results of this study to compare how and why meat quality may change. From these results, my current recommendation to the industry would be for processing facilities to be aware of potential temperatures experienced by birds during transport and in lairage environments, and to ensure that interventions are in place to help buffer the adverse temperatures birds may be subjected to, to maximize welfare and meat quality.

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Table 2.1 Impact of lairage temperature and CAS stunning on pH, color, and drip loss

	pH	L	a	b	Drip loss
2 h					
Hot	6.41 ± 0.032^{a}	43.82 ± 0.264^{a}	2.44 ± 0.078^{a}	5.58 ± 0.107^{a}	
Cool	6.32 ± 0.029^{a}	42.81 ± 0.259^{b}	2.22 ± 0.067^{a}	5.24 ± 0.092^{b}	
P-value	0.779	0.0002	0.224	0.001	
CAS 4.5 min	6.41 ± 0.027^{a}	42.95 ± 0.290^{a}	2.34 ± 0.073^{a}	5.38 ± 0.101^{a}	
CAS 6 min	6.32 ± 0.032^{b}	43.67 ± 0.232^{b}	2.32 ± 0.073^{a}	5.43 ± 0.101^{a}	
P-value	0.027	0.066	0.691	0.772	
24 h					
Hot	5.70 ± 0.046^{b}	46.80 ± 0.261^{a}	1.81 ± 0.076^{a}	5.31 ± 0.120^{a}	2.29 ± 0.239^{b}
Cool	5.75 ± 0.044^{a}	46.19 ± 0.324^{b}	1.78 ± 0.069^{a}	4.96 ± 0.103^{b}	2.59 ± 0.188^{a}
P-value	<0.001	0.0008	0.728	0.0002	0.4639
CAS 4.5 min	5.76 ± 0.045^{a}	46.22 ± 0.291^{a}	1.79 ± 0.073^{a}	5.11 ± 0.109^{a}	2.59 ± 0.204^{a}
CAS 6 min	5.69 ± 0.045^{a}	46.74 ± 0.301^{a}	1.80 ± 0.073^{a}	5.16 ± 0.117^{a}	2.30 ± 0.225^{a}
P-value	0.139	0.2060	0.897	0.698	0.7259
2 h average	6.36 ± 0.027^{a}	43.30 ± 0.196^{b}	2.33 ±0.052^{a}	5.41 ± 0.075^{a}	
24 h average	5.73 ± 0.027^{b}	46.48 ± 0.197^{a}	1.79 ± 0.052^{b}	5.14 ± 0.076^{b}	2.44 ± 0.152
P-value	P<0.0001	P<0.0001	P<0.0001	P= 0.009	

$^{a-d}$Values within a column with different superscripts are significantly different ($P \leq 0.05$)

Hot = exposure to hot lairage environment (rep 1: 29.3°C ± 0.5, rep 2: 28.4°C ± 1.7, rep 3: 29.1°C ± 1.4)

Cool = exposure to cool lairage environment (rep 1: 17.3°C ± 0.4, rep 2: 17.4°C ± 0.6, rep 3: 17.2°C ± 0.6)

L*, a*, and b* are color values

Table 2.2 Impact of lairage temperature and CAS stunning on lipid oxidation

	Lipid Oxidation
24 h	
CAS 4.5 min	0.02 ± 0.003^{a}
CAS 6 min	0.01 ± 0.002^{a}
P-value	0.877
Hot	0.02 ± 0.002^{a}
Cool	0.01 ± 0.002^{a}
P-value	0.825
7 d	
CAS 4.5 min	0.11 ± 0.021^{a}
CAS 6 min	0.10 ± 0.022^{a}
P-value	0.865
Hot	0.09 ± 0.021^{a}
Cool	0.12 ± 0.022^{a}
P-value	0.187
24 h average	0.00 ± 0.001
7 d average	0.02 ± 0.015
P-value	P<0.0001

$^{a\text{-}d}$Values within a column with different superscripts are significantly different ($P \leq 0.05$)
Hot = exposure to hot lairage environment (rep 1: 29.3°C ± 0.5, rep 2: 28.4°C ± 1.7, rep 3: 29.1°C ± 1.4)
Cool = exposure to cool lairage environment (rep 1: 17.3°C ± 0.4, rep 2: 17.4°C ± 0.6, rep 3: 17.2°C ± 0.6)

Figure 2.3 Diagram of simulated lairage tents with bird location and stocking densities

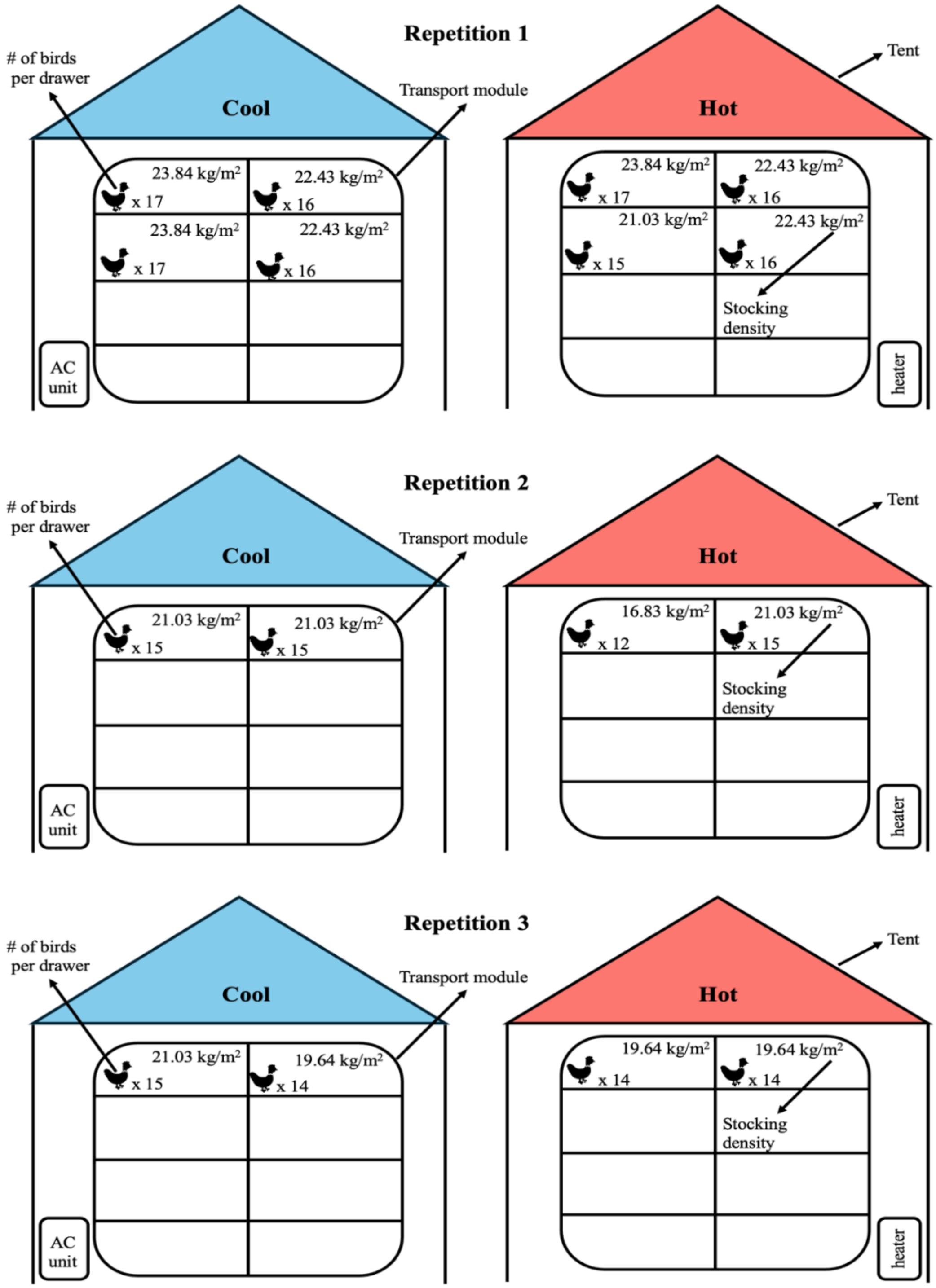
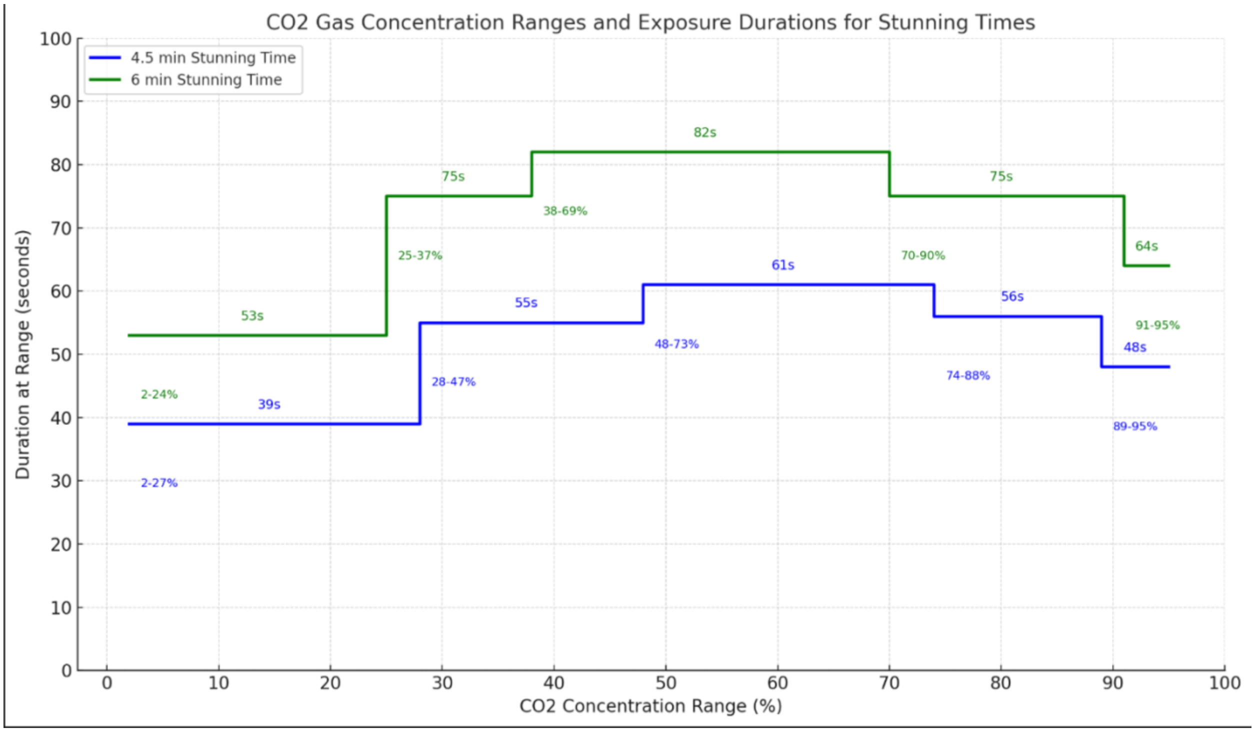


Figure 2.4 CO_2 gas concentration ranges and exposure durations



CHAPTER 3: COMBINED IMPACT OF LAIRAGE TEMPERATURES AND CARBON DIOXIDE CONTROLLED ATMOSPHERE STUNNING TIME ON BROILER BLOOD METABOLITES

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3.1 ABSTRACT

CAS is becoming increasingly popular to render poultry unconscious prior to slaughter. Lairage is the holding area where birds are placed upon arrival to the processing facility. Depending on the location and time of year, temperatures within lairage settings can range between hot to cold. Little is known about the combined impact of lairage conditions and CAS on poultry blood metabolites. Birds were placed in a simulated lairage environment at two different temperatures (17.3 C or 28.9 C) for one hour, then stunned using a CAS system at two different stunning times, 4.5 or 6 minutes. Blood was taken from birds pre-lairage, post-lairage pre-stun, and post-stun. PCO_2 (mmHg), pH, PO_2 (mmHg), $HCO^{3}-$ (mEq/L), BE_{ecf} (mEq/L), SO_2(%), TCO_2 (mEq/L), Na (mEq/L), iCa (mmol/L), glucose (mg/dL), hematocrit (%PVC), and hemoglobin (g/dL) were measured using an iSTAT Alinity v blood analyzer. Data were analyzed by General Linear Models procedure through SAS 9.4 University Edition with significance determined at $P \leq 0.05$ and means separated by Tukey's HSD. Blood gases associated with acid-base balance (HCO_3^-, pH, PCO_2, SO_2) were significantly affected by CAS time, with 4.5-minute stunned birds showing higher HCO_3^- and pH, while 6-minute stunned birds had higher PCO_2 and SO_2 ($P < 0.0001$). Electrolytes and metabolites (Na, iCa, glucose) were also influenced by CAS time, with 4.5-minute stunned birds having higher Na and iCa, and 6-minute stunned birds

showing elevated glucose levels ($P < 0.0001$); glucose also had a lairage temperature CAS time interaction ($P = 0.034$). Among blood components, hematocrit was higher in 4.5-minute stunned birds ($P = 0.008$), while hemoglobin showed a significant lairage temperature CAS time interaction ($P = 0.046$). Understanding the implications of these results can lead to a better understanding of how lairage conditions and CAS can impact poultry physiology and broiler blood metabolites potentially improving lairage environments and CAS systems to optimize broiler welfare.

3.2 INTRODUCTION

Understanding the physiological responses of broilers is important for developing a deeper understanding of how the physiological status of broilers may change depending on responses to stressors and environmental factors. Blood metabolites can serve as a key indicator into a bird's physiological state, as these biochemical markers can describe a bird's metabolic function (Jiang et al., 2023).

Lairage refers to the holding area where birds are placed after they arrive at the processing facility. The intended purpose of lairage is to provide broilers an opportunity to recover from the stress of transport and maintain efficiency within the processing plant (Warriss, 1987). Prior to exsanguination, birds are stunned which is the process of rendering an animal unconscious and insensitive to pain. This step is crucial for both animal welfare and processing plant efficiency by preventing birds from experiencing pain at neck cut and allowing accuracy and automation within the processing facility (Berg and Raj, 2015). The use of controlled atmosphere stunning (CAS) systems has become increasingly popular in poultry processing as a method of stunning (Berg and Raj, 2015, Gerritzen et al., 2013). CAS systems use multiple phases of increasing CO_2 concentrations to induce unconsciousness (Hoen and Lankhaar, 1999).

Some suggest that CAS stunning is beneficial for animal and worker welfare, with less bird and human contact. During CAS birds are stunned within their transport crates minimizing live handling, and birds are shackled unconscious reducing physical struggle and pain from shackling.

Temperature conditions in lairage environments and CAS stunning can have a physiological impact on avian respiration. In lairage environments with high ambient temperatures if birds begin to pant to regulate their body temperature, the panting increases the bird's respiratory rate and can cause a decrease in blood CO_2 levels (Teeter et al., 1985). If CO_2 levels continue to deplete, this can lead to respiratory alkalosis an acid base imbalance in the blood. Exposure to CAS can initially increase bird respiratory rate; however continued exposure can suppress respiratory function and potentially lead to the opposite physiological response, referred to respiratory acidosis. Respiratory acidosis refers to the buildup of carbon dioxide, when the lungs are unable to provide ventilation to the body (Patel et al., 2023).

The measurement and analysis of blood metabolites offer an objective view into the physiological condition of an animal (Harr, 2006). Some studies have implemented the use of broiler blood metabolites to investigate how blood chemistry may be impacted by transport stress. Zhang et al. (2009) investigated the impact of transport stress on blood metabolism, suggesting that transport stress led to increased release of plasma CORT. Zheng et al. (2020) noted that simulated transport stress did cause physiological changes stating that at 2 h it altered the release of hormones and the biochemical characteristic of blood. Both studies also mentioned that the changes in blood chemistry could potentially impact meat quality characteristics (Zhang et al., 2009; Zheng et al., 2020). These are just a few of the available studies, however, little peer-reviewed literature is available regarding how broiler blood metabolites may be affected by lairage environment and CAS. The objective of this study was to explore the impact of lairage temperatures and CAS on resulting broiler blood metabolites prior to and following carbon dioxide gas stunning. We hypothesized that the combined impacts of lairage temperature and CAS would lead to physiological changes in the blood. More specifically, we thought that the birds exposed to the warmer lairage temperature may show signs of respiratory alkalosis, while birds that were control atmosphere stunned may show signs of respiratory acidosis.

3.3 MATERIALS AND METHODS

Experimental design

This study was performed at the Charles C. Miller Jr. Poultry Research Center at Auburn University. The design of the experiment was a 2x2 factorial that evaluated two lairage temperature conditions (hot and cool) and two controlled atmosphere stunning times (4.5 and 6 minutes). There were two total repetitions with blood samples collected from 57 broiler chickens at each repetition. This created 4 treatment groups for the birds exposed to CAS: cool/ 4.5 min, cool/ 6 min, hot/ 4.5 min, hot/ 6 min. Blood was collected at three timepoints: pre-lairage, post-lairage pre-stunning, and post-stunning. This study was in accordance with the Auburn University IACUC *Institutional Animal Care and Use Committee* (2023-5300).

Bird source

Ross 708 broiler chickens were obtained from live haul at a commercial processing plant in the United States with a target weight of 1.91 kg. The first and second repetition occurred in March, while the third repetition occurred in April of 2024. Average temperatures and relative humidity for the day of each repetition were: repetition 2:(12.8°C, 51.9%), repetition 3:(18.3°C, 76.8%). At the commercial processing facility, the birds were manually moved into transport modules and transported 45 minutes by a flatbed trailer to the Charles C. Miller Poultry Research Center.

Lairage conditions

To create two simulated lairage environments, two industrial tents were used to develop “hot” and “cool” temperature environments (Canopy Tent 11C540, Grainger). A space heater and an air conditioning unit were used within each of the tents to maintain the desired temperatures. Inside each tent was one BAADER transport module with drawers (Uniload, SU502). Each tent was brought to the desired temperature prior to the arrival of the birds. Upon arrival broilers were leg banded and put into the tents for a minimum of 60 min to simulate commercial processing lairage time periods. The drawers used within the transport modules were the Uniload large drawers that have an inner area of 13,620 cm^2, and can hold up to 85 kg, if bird weights are between 1.6-3.0 kg. Figure 2.3 details the below listed information about bird location and stocking densities within each tent. For repetition 2, in the hot tent there were two drawers with birds, one with 12 birds and one with 15 birds. In the drawer with 12 birds, the stocking density was 16.83 kg/m^2, and in the drawer with 15 birds the stocking density was 21.03 kg/m^2. In the cold tent, there were two drawers with 15 birds per drawer, and the stocking density was 21.03 kg/m^2. In repetition 3, in the hot tent there were two drawers with 14 birds per drawer, and the stocking density was 19.64 kg/m^2. In the cold tent, there were two drawers, one with 15 birds and one with 14 birds. In the drawer with 15 birds, the stocking density was 21.03 kg/m^2. In the drawer with 14 birds the stocking density was 19.64 kg/m^2 . The National Chicken Council (NCC) recommends a stocking density of 32 to 44 kg/m^2, while the Global Animal Partnership (GAP) recommends a lower stocking density of 27−29 kg/m^2 . HOBO data loggers were used inside each of the tents to record temperatures within each tent and the temperatures were averaged over the entire hour (MX1101, HOBO, Bourne, MA). Inside the hot tent for each of the two repetitions mean temperatures were 28.4°C ± 1.7, and 29.1°C ± 1.4, respectively. In the cool tent mean temperatures were 17.4°C ± 0.6, and 17.2°C ± 0.6, respectively.

Stunning

A prototype five-stage BAADER Controlled Atmosphere stunning (CAS) stunning system was utilized to render the broiler unconscious prior to mechanical neck cut. This system exposes birds to increasing CO_2 concentrations throughout the five stages. A Felix Instruments F-920 Check It! gas analyzer was placed inside the crates with the birds for the duration of stunning to record the carbon dioxide gas concentrations. Birds were run through the stunner in two series with eight separate runs by treatment: hot/4.5 min, hot/6 min, cool/ 4.5 min, cool/6 min. The eight runs from each of the two series were recorded and the means were calculated. For the 4.5 min stunning time, stages one through five had the following CO_2 gas concentrations and durations; (2-27%; 39 s), (28-47%; 55 s), (48-73%; 61 s), (74-88%; 56 s), and (89-95%; 48 s). The 6 min stunning time stages one through five had the following CO_2 gas concentrations and durations; (2-24%; 53 s); (25-37%; 75 s); (38-69%; 82 s); (70-90%; 75 s); (91-95%; 64 s). Temperatures within the gas stunning system were averaged over the entire stunning period for each repetition. The average temperatures were repetition 2: 27.39°C, and repetition 3: 28.21°C. Following stunning, birds were then manually shackled and exsanguinated via mechanical neck cut.

Blood collection

Blood was collected at three time points: pre-lairage, post-lairage pre-stunning, and post-stunning. For samples taken prior to or after lairage, broiler chickens were individually removed from their transport containers and cervically dislocated. Immediately following cervical dislocation, the head was removed, and blood was collected. For blood samples following stunning, blood was collected on the shackle line at mechanical neck cut. There was roughly two minutes between the birds exiting the gas stunner and arrival at the neck cut where the samples were obtained. All blood samples were collected in 10 mL sodium heparinized tubes (BD Vacutainer). For repetition 1, a total of 44 blood samples were collected. 5 samples were collected before lairage and 10 from after lairage but before stunning with n=5 from the "hot" tent and n=5 from the "cool" tent. There were also 24 post-stunning samples collected with n=6 obtained from each treatment (cool/4 min, cool/ 6 min, hot/4 min, hot/6 min). For repetition 2, a total of 30 blood samples were collected, 5 pre-lairage, 10 post-lairage pre-stunning with 5 from the "hot" tent and 5 from the "cool" tent, and 20 post-stunning with 5 from each treatment. Immediately following collection, blood samples were analyzed using the ISTAT Alinity v. blood analyzer. With a 1 cc pre-heparinized syringe, 95 μL of blood was placed in a CG8+ test cartridge and then inserted into the iSTAT Alinity v. blood analyzer. The iSTAT Alinity v. blood analyzer takes approximately 2 minutes to read each blood sample. A total of 13 blood metabolites were measured: partial pressure of carbon dioxide PCO_2 (mmHg), pH, partial pressure of oxygen PO_2 (mmHg), bicarbonate (HCO^3- (mEq/L), base excess in the extracellular fluid (BE_{ecf} (mEq/L), oxygen saturation SO_2 (%), total carbon dioxide TCO_2 (mEq/L), sodium Na (mEq/L), ionized calcium iCa (mmol/L), glucose (mg/dL), hematocrit (%PVC) and hemoglobin (g/dL).

Statistical Analysis

A two-way ANOVA procedure using Proc Glimmix was used to evaluate lairage temperature, CAS time, and their interactions. Gamma distribution was used for all blood metabolites. Stocking density was included in the model as a covariate to control for its potential influence, although there were no significant differences for stocking density, so it is not reported below. The means were separated using Tukey's HSD and significance was determined at $P\leq 0.05$. This data analysis was performed using SAS OnDemand for Academics (SAS Institute Inc).

3.4 RESULTS

Blood gases and acid-base balance

HCO^{3}_{-}(mEq/L) , pH, PCO_2 (mmHg),, SO_2 (%), PO_2 (mmHg),, and TCO_2 (mEq/L), were grouped together as blood gases associated with acid-base balance. HCO^{3}_{-} and pH was significant for CAS time, with 4.5 min (16.58, 6.89) stunned birds having higher means compared to 6 min stunned birds (16.13, 6.83; $P<0.0001$). PCO_2 and SO_2 were also significant for CAS time with 6 min stunned birds (99.96, 57.53) having higher means than 4.5 min stunned birds (91.52, 53.81; $P<0.0001$). There were no significant differences for lairage temperature for HCO^{3}_{-} , pH, PCO_2, SO_2, and no significant differences for lairage temperature or CAS time for PO_2 and TCO_2.

Electrolytes and metabolites

Na (mEq/L), iCa, and glucose (mg/dL) were grouped together as electrolytes and metabolites. Na and iCa were significant for CAS time with 4.5 min stunned (135.18, 1.41) birds having higher means compared to the 6 min stunned birds (131.77, 1.37; $P<0.0001$). Glucose was also significant for CAS time with 6 min stunned birds (315.32) having higher means compared to 4.5-minute stunned birds (283.23; P<0.0001). There were no significant differences for lairage temperature for Na, iCa, or glucose. Glucose did have a significant lairage temperature and CAS time interaction (P=0.034).

Blood components

Hemoglobin (g/dL) and hematocrit (%PVC) were grouped together as blood components. Hematocrit was significant for CAS time with 4.5 min stunned birds (19.86) having a higher mean compared to 6 min stunned birds (18.41; P=0.008). Hematocrit was not significant for lairage temperature, and hemoglobin was not significant for CAS time or lairage temperature. Although, hemoglobin did have a significant lairage temperature and CAS time interaction (P=0.046).

3.5 DISCUSSION

Blood gases and acid-base balance

Significant effects of CAS time were observed for HCO_3^- (mEq/L), pH, PCO_2 (mmHg), and SO_2 (%), with 4.5-minute stunned birds showing higher HCO_3^- and pH, while 6-minute stunned birds had elevated PCO_2 and SO_2. These could be indicative of respiratory acidosis a condition characterized by increased blood CO_2 levels and corresponding drops in pH and bicarbonate buffering capacity (Bayliss & Till, 2009). The lower pH and HCO_3^- observed in 6-minute stunned birds could reflect this acidic shift, potentially due to extended CO_2 exposure. A reduced buffering ability would impair the body's capacity to neutralize accumulating carbonic acid, further contributing to acidosis (Marder & Arad, 1989).TCO_2 did not significantly differ by CAS time or lairage temperature, TCO_2 reflects total carbon dioxide including dissolved CO_2 and bicarbonate (Kim et al., 2015; Burger & Schaller, 2023). The observed elevation in PCO_2 in 6-

minute stunned birds is likely the result of longer CO_2 exposure that increased dissolved CO_2 in blood (Becerril-Herrera et al., 2008). SO_2 (%), a measure of hemoglobin oxygen saturation, was lower for the 4.5 min stunned birds, while PO_2 showed no significant differences, which may indicate the beginning of oxygen displacement by CO_2—another sign of respiratory acidosis (Patel & Sharma, 2023). Although, there was no significance for PO_2 or the amount of oxygen dissolved in the blood which could suggest that the CO_2 inhalation comprised oxygen availability but had a limited effect on the amount of dissolved oxygen.

Electrolytes and metabolites

Na and iCa levels were significantly higher in birds stunned for 4.5 minutes than for 6 minutes. The elevation of iCa could be attributed to a decrease in blood pH, which reduces the binding of calcium to plasma proteins, resulting in higher free calcium levels (Alexander et al., 2016). Glucose was significantly higher in 6-minute stunned birds, and could be suggestive of insulin resistance created from the increase in the acidity of the blood. Insulin is crucial to transport glucose (mg/dL) that is needed for energy, and without it an accumulation of glucose (mg/dL) can occur (Olanrewaju et al, 2007).

Blood components

Hematocrit (%PVC) was significantly higher in 4.5-minute stunned birds compared to the 6 min stunned birds. This difference in hematocrit could be from fluid shifts from the CO_2 exposure and inhalation. Hemoglobin (g/dL) did not differ significantly for CAS time or lairage temperature, though a significant interaction suggests that its response may depend on the specific conditions experienced. Although the direction of hemoglobin changes was inconsistent, this variability may be attributed to sample size limitations or variation.

It is important to note that this study has several limitations. Increasing the sample size would be more accurate to capture the full range of variability that may be associated with the factors of this study. We may have seen more differences in the results with increased time that the birds were exposed to the lairage temperatures, although we chose the lairage duration of an hour to replicate commercial processing lairage durations. The simulated lairage environments are also not fully representative of real commercial processing conditions and the temperatures did not fully account for changes in ambient temperatures or seasonal changes that may be associated with certain areas. The blood samples from pre-lairage, and post-lairage pre-stun birds was collected from birds that were cervically dislocated. It is possible that cervically dislocating the birds could have impacted our baseline results, and blood samples from live birds at those timepoints may have been more accurate. These results display how large of an impact lairage temperatures and CAS can have on bird physiology and emphasize the importance of further research on how these changes may be associated with bird welfare in lairage environments and CAS systems.

3.6 CLOSING REMARKS

This work highlights the implications of lairage temperatures and carbon dioxide gas stunning on broiler blood metabolites. Generally, CAS induced respiratory acidosis which resulted in changes to multiple blood parameters. Analyzing blood metabolites in different lairage environments could help develop a better understanding of the physiological status of birds as this study had limitations with low sample size and short lairage duration. Additionally, research into behaviors observed in different lairage temperatures and during CAS could be insightful to understanding how birds physiologically respond in these settings.

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Table 3.1 Impact of lairage temperature and CAS on broiler blood metabolites

	pH	PCO$_2$	PO$_2$	HCO$_3^-$	SO$_2$	TCO$_2$	Na	iCa	Glu	Hct	Hb
Temp											
Hot	7.04^a	73.13^a	56.47^a	17.30^a	62.45^a	19.48^a	138.16^a	1.36^a	272.06^a	18.91^a	6.81^a
Cool	7.05^a	75.8^a	72.97^a	17.79^a	72.03^a	19.79^a	137.63^a	1.35^a	244.34^a	20.91^a	7.17^a
P-value	0.544	0.636	0.278	0.715	0.614	0.720	0.314	0.545	0.566	0.969	0.349
SEM	± 0.036	± 4.41	± 4.36	± 0.430	± 3.85	± 0.407	± 0.910	± 0.012	± 10.96	± 0.436	± 0.138
Stun											
CAS 4.5 min	6.89^a	91.52^b	63^a	16.58^a	53.81^b	19.29^a	135.18^a	1.41^a	283.23^b	19.86^a	7.06^a
CAS 6 min	6.83^b	99.96^a	65.59^a	16.13^b	57.53^a	18.89^a	131.77^b	1.37^b	315.32^a	18.41^b	6.58^a
P-value	<0.0001	<0.0001	0.981	<0.0001	<0.0001	0.083	<0.0001	<0.0001	<0.0001	0.008	0.069
SEM	± 0.013	± 7.36	± 4.54	± 0.775	± 0.734	± 3.96	± 2.06	± 0.025	± 28.23	± 1.47	± 0.511
Temp x Stun											
Hot 4.5 min	6.87^b	89.76^a	62.36^a	15.93^b	52^{ab}	18.73^a	134.91^b	1.42^{ab}	324.54^{ab}	17.27^a	6.29^a
Cool 4.5 min	6.90^b	93.27^a	63.63^a	17.3^{ab}	55.8^c	19.9^a	135.45^b	1.4^a	241.91^{bc}	22.45^a	7.63^a
Hot 6 min	6.84^b	97.75^a	57.54^a	15.97^b	50.2^{bc}	18.8^a	132.45^b	1.35^{abc}	308.64^{ab}	18.09^a	6.66^a
Cool 6 min	6.83^b	102.15^a	73.63^a	16.3^{ab}	65.67^{ab}	19^a	131.09^b	1.39^{ab}	322^a	18.73^a	6.52^a
Hot	7.47^a	27.76^b	48.8^a	20.15^a	86.2^a	21^a	148^a	1.29^{bc}	174.10^{cd}	21.6^a	7.36^a
Cool	7.47^a	27.58^b	82.5^a	19.61^{ab}	94^{ab}	20.4^a	147.20^a	1.25^c	161.60^d	21.6^a	7.34^a
P-value	0.546	0.967	0.403	0.446	0.361	0.594	0.681	0.169	0.034	0.022	0.046
SEM	± 0.013	± 7.36	± 4.54	± 0.775	± 0.734	± 3.96	± 1.02	± 0.025	± 28.13	± 1.48	± 0.511

$^{a\text{-}d}$Values within a column with different superscripts are significantly different (P£0.05)
Hot = exposure to hot lairage environment (rep 2: 28.4°C ± 1.7, rep 3: 29.1°C ± 1.4)
Cool = exposure to cool lairage environment (rep 2: 17.4°C ± 0.6, rep 3: 17.2°C ± 0.6)
*- post-lairage pre-stunning

CHAPTER 4: THESIS CONCLUSION AND FUTURE IMPLICATIONS

Currently, there is a lack of peer-reviewed literature addressing the combined effects of thermal stress during lairage and CAS on meat quality and blood metabolites. This research gap complicates the understanding of how these factors influence bird physiology and meat characteristics, leaving many questions unanswered. This study attempted to explore these effects and help to bridge the knowledge gap within the literature. The findings of this study both align with and contrast previous literature, highlighting the significant impact of short-term exposure to different lairage temperatures and CAS on meat quality. Specifically, the results suggest that lairage conditions can affect meat quality, potentially leading to outcomes such as lower pH and lighter breast fillets. However, the simulated lairage environments in this study may not fully capture the range of thermal conditions found in real-world processing plants, where extreme temperature variations can occur depending on the location. Repeating this study in a commercial setting throughout different seasons could offer a clearer picture of the seasonal impacts on meat quality. These results also show that CAS induces respiratory acidosis, which in turn alters several blood parameters. While analyzing blood metabolites in different lairage environments could deepen our understanding of the birds' physiological status, this study's limitations, such as a small sample size and short lairage duration restrict the conclusions that can be drawn. Further research into the behaviors of birds under varying lairage temperatures and during CAS would be valuable in understanding their physiological responses in these settings. Overall, the study suggests that both lairage temperature and CAS can impact broiler meat quality and blood metabolites.