MEAT QUALITY OF TURKEYS AFFECTED BY HEAT STRESS AND IMMUNE CHALLENGE CONDITIONS, AND THE RELATIONSHIP BETWEEN MEAT QUALITY AND THE SOCIAL INDEX

by

Melissa K. Davis

A Thesis

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Master of Science

Department of Animal Sciences

West Lafayette, Indiana

August 2019

THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. Marisa Erasmus, Chair

Department of Animal Science

Dr. Stacy Zuelly, Chair

Department of Animal Science

Dr. Nicole Olynk Widmr

Department of Agricultural Economics

Approved by:

Dr. Ryan Cabot

Head of the Graduate Program

This is presented as my best work of non-fiction and dedicated to the strongest person I know: me.

ACKNOWLEDGMENTS

I would like to thank everyone that has come and gone in my life during my time here as a graduate student. Every single person has served an integral part in either shaping me into who I am, teaching me valuable life lessons, or being a great friend. I would like to thank my committee members and especially my advisors, Dr. Erasmus and Dr. Zuelly for taking the time to teach me how to be a better researcher and writer. This process was not an easy one and I was certainly challenged both mentally and physically, but you both made sure that I was on track. Thank you for allowing me to teach and participate in extension events, because without those opportunities I wouldn’t have been able to learn so many valuable life skills.

I would like to thank Courtney Bir and Dr. Widmar for guiding us through the other part of my project, and I am looking forward to seeing how all that hard work pays off. I would also like to acknowledge my family for keeping my head above water through this whole process, always wanting to learn more about my research, continuously keeping me fed, and loving me always. Last but certainly not least, I would like to thank my fellow graduate students, especially Emily Ford, for going through these last two years with me, showing me it’s never the wrong time for a mojito, and putting up with my crazy personality; I love you guys.

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LIST OF ABBREVIATIONS

a*	Redness
ASREC	Animal Science Research and Education Center
AOAC	Association of Official Agricultural Chemists
ADHD	Attention-Deficit Hyperactivity Disorder
b*	Yellowness
B	Bottom
C	Celsius
CON	Control
C	Cross
DFD	Dark, Firm, and Dry
d	Day
DNA	Deoxyribonucleic Acid
FAME	Fatty Acid Methyl Esters
FP	Feather Pecking
FRF	Feather Retention Force
FDA	Food and Drug Administration
FSIS	Food Safety Inspection Service
g	Gram
HSP	Heat Shock Protein
HS	Heat Stress
HE	Hemorrhagic Enteritis
HEV	Hemorrhagic Enteritis Virus
hr	Hour
h	Hour
IC	Immune Challenge
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IACUC	Institutional Animal Care and Use Committee
L	Left Wing
LR	Left Wing and Right Wing
L*	Lightness
MRL	Maximum Residue Limit
M	Mean
min	Minutes
NDV	Newcastle Disease Virus
OCD	Obsessive Compulsive Disorder
PSE	Pale, Soft, and Exudative

pH	Potential Hydrogen
ROS	Reactive Oxygen Species
RNA	Ribonucleic Acid
R	Right Wing
SD	Standard Deviation
SE	Standard Error
TNZ	Thermoneutral Zone
T	Top
TB	Top and Bottom
USDA	United States Department of Agriculture
wk	Week
wks	Weeks

ABSTRACT

Author: Davis, Melissa, K. MS
Institution: Purdue University
Degree Received: August 2019
Title: Meat Quality of Turkeys Affected By Heat Stress and Immune Challenge Conditions, and the Relationship Between Meat Quality and the Social Index
Committee Chair: Dr Marisa Erasmus, Dr. Stacy Zuelly

Heat stress (HS) and immune challenges (IC) are just two of the many stressors poultry can experience in commercial settings that can have an effect on bird welfare and final product quality after harvest. Individual animals vary greatly in their responses to stressors, which can further influence product quality. The overall goal of this study was to examine the relationships among stress, behavioral characteristics and meat quality of commercial turkeys. The first objective of this study was to determine if the heat stressed or immune challenged turkeys experienced greater negative effects on overall meat quality compared to a control group. The second objective of the study was to determine if the frequency of nonaggressive pecking behaviors among the birds was related to final meat quality. A total of 92 commercial male, beak-trimmed turkeys were used in two trials (in time replicates) with a total of 15 rooms and 4-7 birds per room. There were two to three rooms experiencing each treatment at a time. The heat stressed (HS) treatment subjected the birds to an ambient temperature of approximately 29 °C, depending on the room's humidity, and lasted 120 minutes before returning to the normal temperature range. The immune challenge (IC) treatment consisted of inoculating the birds with a live vaccine for hemorrhagic enteritis virus. The control (CON) group was not subjected to heat stress or an immune challenge. The birds were rotated every two weeks starting at 10 weeks of age so that each group experienced each of the three treatments in a balanced Latin square design. The last treatment period was at 14 weeks of age. Birds were harvested at the Purdue Boiler Maker Butcher

Block where several meat quality measures (feather retention force, pH, color, fatty acid composition and drip loss, among others) were recorded. There were no significant differences in fatty acid composition ($P > 0.05$) across treatment groups for any of the 38 fatty acids tested. There were also no significant differences in percent protein ($P < 0.05$) among treatment groups. Initial pH values were significantly different between treatment groups ($P < 0.01$), which corresponded with the significant differences in other meat quality attributes such as lightness (L*) and shear force (tenderness) values. Results indicate that even a short heat stress period lasting for 120 min can affect certain aspects of meat quality. Similarly, vaccination with hemorrhagic enteritis vaccine one week prior to slaughter also affects some meat quality measures.

To examine the relationship between non-aggressive pecking behavior and meat quality, turkey behavior was video-recorded at 14 weeks of age prior to any of the treatments that week. Video footage was analyzed to determine the number of aggressive and non-aggressive pecks given and received by each bird in the room. Aggression occurred too infrequently to be able to examine the relationship between aggression and meat quality. Therefore, analyses were only performed using non-aggressive pecking behavior. From these data, turkeys were given a rank that was calculated by dividing the number of non-aggressive pecks given by the number of non-aggressive pecks received. Ranks were standardized for the number of turkeys in each room. A cluster analysis was performed to categorize the birds into low, medium, and high groups based on their frequency of pecking. Clusters were tested to verify that they were significantly different from one another. Once each turkey had been assigned to a cluster, meat quality measures were compared among clusters to determine the relationship between non-aggressive pecking and meat quality. There was a trend ($P < 0.10$) for L* (lightness) and drip loss to differ among clusters; however, post hoc analysis did not reveal any significant differences. There were no significant differences (*P* > 0.05) among clusters for any other meat quality attributes. Therefore, turkeys' tendency to perform and receive non-aggressive pecks does not seem to have an effect on the meat quality attributes tested in this study. Research with other species has indicated a relationship between other behavioral characteristics such as aggressive interactions, fear responses, social rank, body weight, and meat quality; therefore, future research examining other behavioral traits will be valuable in examining factors that can influence turkey meat quality.

Key words: heat stress, immune challenge, vaccine, turkey, meat quality, behavior, pecking

CHAPTER 1. LITERATURE REVIEW

Introduction

With the per capita consumption of turkey meat more than doubling in the United States since 1960, there is an increasing demand from consumers for turkey products all year long (National Chicken Council, 2019). Turkey lunchmeats are also the most popular and "first most often purchased" lunchmeats followed closely by ham, yet consumers are the least concerned for the animal welfare of farmed turkeys compared to other farm animal species (McKendree et al., 2013; Byrd et al., 2017). This growing demand for turkey products requires the attention of commercial turkey farmers to invest in more efficient and welfare friendly farming practices. There have been several studies in the past detailing the effects of stress conditions on meat quality and carcass traits such as feather retention force; however, many of these studies on turkey production, behavior, and meat quality are now more than ten years old and the information is rather outdated for newer genetic lines of turkeys and turkey production methods. There is a significant knowledge gap when it comes to turkey production in general as most poultry research is conducted using broilers or laying hens. Turkeys are very similar to other poultry species, so it is easy to use broiler information to compare; however, turkeys are a different species and respond to stressors in a different way and should be studied just as much as other poultry species. This thesis was designed to study turkey stress conditions and meat quality to fill in the gap of outdated knowledge regarding turkey welfare and meat quality for the growing demand of turkey products.

Animal Welfare and Stress

In 1964, the public changed their opinion on animal welfare in regards to animal agriculture after the release of Animal Machines by Ruth Harrison (Terlouw et al., 2008). Animal welfare refers to the state of an individual in relation to its environment, and both the failure to cope with the environment and the difficulty the individual has in coping are indicators of poor welfare (Broom, 1986). When animals experience extreme cases of poor animal welfare, they can be suffering either physically or mentally, where suffering is defined as something painful or distressing that is inflicted upon them to submit pain, punishment, or even death to the animal (OED, 2019) Animal Welfare is the concern for animal suffering as well as ensuring animal satisfaction (Gregory and Grandin, 1998). Neither suffering nor satisfaction can directly be measured, but their consequences can be compared using various methods. One way to evaluate a cause of suffering is to measure the animal's stress response.

Stress refers to the behavioral, physiological, and emotional status of an animal when exposed to a situation in which it feels threatened with respect to its bodily function or mental well-being (Désiré et al., 2004; Terlouw, 2005; Terlouw et al., 2008). A stressor is defined as an agent that produces stress at any time or in any way (Selye, 1976). Thus, stress is the biological response the animal has to stimuli (i.e., stressors) that disrupt its normal homeostasis. There have been consistent efforts to reduce the amount of stress that animals face in commercial farming in order to improve animal welfare.

Turkeys experience a number of potentially stressful events during their production cycle, including but not limited to handling, catching, transportation, noises, unpredictable events, as well as social stressors (Erasmus, 2018). One of the many animal welfare concerns for food animals is fasting prior to slaughter. Fasting has consistently shown negative effects on animal welfare, and after just two hours of food deprivation there is an increase of corticosterone from metabolic stress and possibly from psychological discomfort (Kannan and Mench, 1996; Nijdam et al., 2005; Terlouw et al., 2008). Fasting reduces glycogen stores in the liver and muscles, which may increase the reactivity of birds to different stress factors during transport and/or slaughter (Sams and Mills, 1993; Kotula and Wang, 1994; Terlouw et al., 2008). Fasting has also shown to have a negative effect on feather plucking and can increase the feather retention force during harvest (This will be discussed in the Feather Retention Force Section) (Levinger, 1975). Not all reasons for suffering are caused by humans. There is no control over weather, although there are ways to protect animals from adverse weather conditions. We also do not have complete control over diseases, which are major causes of suffering in livestock species. Suffering associated with disease is one of the biggest concerns for animal welfare existing today, and in some countries climatic stress is just as common. These two forms of suffering lack the attention needed to properly deal with the stressors (Gregory and Grandin, 1998).

In modern turkey rearing systems, producers are often experiencing economic losses due to increased aggression, feather pecking, cannibalism, injuries, and even the possibility of death (Marchewka et al., 2013). Marchewka (2013) states that the underlying causes of these welfare issues are multifactorial, and could be related to rapid growth, flock size, flock density, poor environmental complexity, or lighting, which may result in improper social and physical environments. There is still little information regarding the effects that stressful living conditions have on turkey welfare; however, this knowledge is needed to improve their quality of life and benefit the industry.

There are many reasons to be concerned for animal welfare, especially when it comes to livestock raised for meat consumption. Poor welfare can lead to poor product quality, and the risk of loss of market share for products that acquire an image of poor welfare (Gregory and Grandin, 1998). Gregory and Grandin (1998) explain that poor welfare can lead to several conditions in meat such as abnormal meat color, pale soft and exudative (PSE) meat, dark firm and dry (DFD) meat, poor shelf life, bruising, torn skin and broken bones. As stated previously, there are many stressors that animals face that can lead to poor meat quality, and any environmental condition that requires body heat generation or dissipation reduces the efficiency of growth, changes the carcass composition, and alters normal physiological processes (Judge, 1989). Judge further explains that any environmental stress factors can result in changes in the metabolites of muscle that are responsible for the differences in the ultimate properties of meat. Poor welfare can affect profitability by harming the product quality; however, relying on profit as the motive to decrease animal welfare problems is not the best strategy. Our concern for animals and our moral responsibilities towards them should be driving us towards better animal welfare. The welfare of poultry is an increasing public concern for both the production and the harvesting processes.

Over the last several years, research showing the effects of animal stress and slaughter conditions on meat quality has increased. In the past, certain papers have looked strictly at meat quality, but researchers are now discussing poor animal welfare and its effects on meat quality (Terlouw et al., 2008).

Meat Quality

The major meat quality attributes for poultry are appearance, texture, juiciness, flavor, and functionality. The most important attributes to consumers are appearance and texture for initial selection and overall product satisfaction (Fletcher, 2002). Fletcher (2002) continues by stating that of all quality attributes, appearance is the most critical as it is the first thing consumers use to determine if they buy the product or not. Appearance can also affect other sensory properties leading to critique of the final product. One of the major components of appearance is color as it has been a major selection criterion for fresh poultry and other meat products. In poultry products, color is important for not only meat, but skin and bones as well. Skin color is most important when marketing fresh whole birds or parts. Bone color can be considered a defect called bone darkening when they are dark or even black in color from being frozen prior to cooking. Meat color is, of course, the most important for deboned and skinless raw meat, as well as for final evaluation of cooked products. Poultry meat color that differs from the expected pale tan to pink in raw meat and tan to grey in cooked meat will cause consumer rejection or dissatisfaction (Fletcher, 2002). Significant variations in raw breast meat color exist, are present at the retail level, and can cause variation in cooked product appearance (Fletcher et al., 2000). Factors affecting poultry meat color have been outlined many times but continue to change and adapt to new problems in the poultry industry. Some of these factors are slaughter conditions, chilling, further processing, and pre-slaughter stress conditions, among others.

Focusing on the pre-slaughter stress conditions, this includes but is not limited to genetics, feed, feed withdrawal, transport, handling, stress, and heat and cold stress (Froning, 1995). Thermal preconditioning and heat shock in chicken has resulted in breast meat that was pale in color, soft, and exudative (PSE), and similar to PSE conditions found in pork (Northcutt et al., 1994). Muscle pH and meat color have consistently shown high correlations, and this is especially true when referring to PSE or DFD meat. Higher pH in muscle is usually associated with darker meat color, and lower pH is associated with lighter meat color. Extreme high pH values are characterized as being DFD-like, while extreme low pH values are classified as PSE-like. Both conditions are related to poor functional properties and product variation. Muscle pH affects the water binding properties of the proteins, which in turn affects the light reflecting and physical properties of the meat. The pH also affects other meat quality attributes such as tenderness, water holding capacity, cook loss, juiciness, and microbial stability also known as shelf-life (Fletcher, 2002). Visual defects can also affect the appearance but are associated with bruising or hemorrhages from physical trauma or blood accumulation. A bruise will initially look red and then darken to blue or black, but as the heme compounds degrade, the bruise will look green or yellow. Stress and many other factors during production or slaughter can cause bruising or hemorrhaging resulting in downgrading of product quality (Fletcher, 2002).

Texture is a critical quality attribute that determines ultimate satisfaction with poultry meat products (Fletcher, 2002). The presence of tougher meat is likely to be caused by adverse conditions during the bird's life, or bad practice in the processing facility (Shrimpton, 1960). Factors such as feed withdrawal, environmental conditions, and stress before slaughter have been shown to affect the amount of glycogen stored in the muscles at the time of slaughter (Mellor et al., 1958). Higher glycogen levels are associated with lower ultimate muscle pH and lower shear force (tenderness) values than birds with lower levels of stored glycogen (Mellor et al., 1958).

Another aspect of carcass and meat quality that is of economic importance to the poultry industry is feather retention force. Feather retention force (FRF) is the force required to remove a feather from the feather follicle, and is reduced by scalding prior to plucking (Buhr et al., 1997). Handling and treatment prior to slaughter can influence the force required to pluck the feathers from their carcasses during harvest (Gregory and Grandin, 1998). Gregory and Grandin (1998) also state that excessive exercise, stress or fasting for longer than 8 hours can also have an impact on feather removal. Withdrawing water from birds for 24 hours has also resulted in a slight tightening of the feather-skin connection (Levinger, 1975).

Concern for feather plucking during slaughter has risen due to three economic forces, including consumer demand for a clean, oven-ready product, the desire for a uniform skin surface with normal appearance, and the need for less expensive feather removal (Pool et al., 1954). Machine plucking of feathers in some cases can toughen meat, and thus there is a need for an understanding of the factors influencing feather retention and release, to develop an improved method of feather removal (Klose et al., 1961).

Efficient plucking is achieved by scalding the carcasses to make feather removal easier, and by ensuring that the plucking machine has good coverage over the carcass and good contact with all feathers. It is widely known in the poultry industry that cold weather prior to harvest can make plucking more difficult. This could be due to the temperature of the scalder, or possibly due to residual activity in the sympathetic nervous system that would cause tightening in the smooth muscle that grips the feather shaft. The easiest way to combat cold birds is to increase the temperature of the scaldwater or increase the force of the plucker, making sure that the meat is not prematurely cooked, and the carcass is not damaged.

Factors Affecting Turkey Welfare and Meat Quality: Heat Stress

A common type of stress known as Heat Stress (HS) occurs after an animal's exposure to a higher ambient temperature beyond the thermal neutral zone (TNZ) for that given species. This thermal neutral zone can be described as a narrow band of body temperature that both birds and mammals comfortably survive in, and is a fundamental and nonadaptive constant in their biological make-up (Scholander et al., 1950). This zone can only be kept constant within certain climates by the physical and chemical regulation of the animal. Heat stress is when the animal exhibits a negative balance between the net amount of energy flowing from its body to the environment and the amount of heat energy that is being produced by the animal. This imbalance can be from a combination of factors in the environment and depend heavily on the animal's individual characteristics (Lara and Rostagno, 2013).

There are two simple methods of determining if an animal is experiencing heat stress or not. The first is to look for obvious signs such as panting or wallowing (e.g. swine); however, some species will stop panting after a certain temperature to conserve water as they reach hyperthermia (Gregory and Grandin, 1998). The second method is to measure rectal temperatures and watch for values exceeding the normal range. Environmental stressors can be detrimental to the animal agriculture industry, and have become a major point of interest to consumers due to recent public awareness and concerns (Nienaber and Hahn, 2007; Nardone et al., 2010; Renaudeau et al., 2012; Lara and Rostagno, 2013). All species are affected by these environmental challenges; however, poultry are more susceptible to temperature changes (Lara and Rostagno, 2013). Truly understanding the impacts of environmental stressors, such as heat stress, on poultry production and meat quality is crucial to understanding how to combat heat stress in more controlled environments.

When subjected to HS conditions, birds will alter their behavior to decrease their body temperature. Birds in HS conditions will spend less time feeding, moving, or walking, and more time drinking, panting, resting, or spreading their wings (Mack et al., 2013). In most cases, HS is usually not the only problem the flock is facing at that time, and can be accompanied by other stressors, such as limited space, poor ventilation, and aggressive social interactions (Hemsworth, 2003; Boissy et al., 2007). The detrimental effects of HS on poultry are very consistent, but stocking density is a compounding factor that increases the risks of HS to both productivity and welfare (Estevez, 2007). These stressful conditions can result in lower body weights and a higher risk of mortality. Several studies have shown impaired growth performance in poultry species that were subjected to heat stress (Deeb and Cahaner, 2002; Niu et al., 2009; Attia et al., 2011; Imik et al., 2012; Ghazi et al., 2012). In addition to affecting growth performance, heat stress has measurable effects on meat quality.

Heat stress has been shown to accelerate the process of glycogen depletion increasing the rate of pH decline, and ultimately resulting in tougher meat (Simpson and Goodwin, 1975; Lee et al., 1976; Babji et al., 1982). A previous study reported that chronic exposure to heat conditions will negatively affect fat deposition and meat quality in broilers; the severity, however, is highly dependent on breed and species (Lu et al., 2007). Chronic HS has also been shown to decrease the size of breast muscle while increasing the size of thigh muscles in broilers. A previous study also showed that protein concentrations were lower and fat deposition was higher for HS birds (Zhang et al., 2012).

Heat Stress Effects on Animal Health and Welfare

Stress has a direct and negative consequence on turkey welfare, and can cause further detrimental effects on the immune response, leading to increase susceptibility to bacterial infections (Huff et al., 2007). Several studies in the past few years have investigated HS effects on the immune response in poultry. Generally, all studies have showed heat stress to have an immunosuppressing effect on poultry species. For example, Thaxton et al. (1968) demonstrated that high environmental temperatures (44.4 to 47.8 °C) affect the development of specific immune responses in young chickens. Other studies have also reported reduced lymphoid organ weights, liver, spleen, and thymus weights, antibodies, IgM and IgG levels in poultry (Bartlett & Smith, 2003; Felver-Gant, Mack, Dennis, Eicher, & Cheng, 2012; Ghazi, Habibian, Moeini, &

Abdolmohammadi, 2012; Niu, Liu, Yan, & Li, 2009; Quinteiro-Filho et al., 2010). Reduced antibody response as well as reduced phagocytic ability of macrophages have also been reported in broilers under HS conditions (Bartlett and Smith, 2003; Niu et al., 2009).

When environmental stressors occur, a bird's body will attempt to maintain homeostasis, but levels of reactive oxygen species (ROS) will increase with time. A build up of ROS result in damage to DNA, RNA, and other proteins, and can even lead to cell death. The body will then enter oxidative stress and release heat shock proteins (HSP) to combat the ROS (National Cancer Institute, n.d.; Dröge, 2002). It has also been reported that effects on immune responses may depend on the length and intensity of the heat exposure (Keith W. Kelley, 1983). There are very few studies that address the effects of heat stress on production, immune response, and meat quality, and they are primarily on swine and broiler chickens. (Mashaly et al., 2004). Thus, there is a need to detail the effects of heat stress and immune stress on meat quality of turkeys.

Vaccinations, Immune Stress, and Withdrawal Periods

The animal agriculture industry has significantly improved production efficiency by shifting to an in integrated system. The danger of this system is the ability of diseases to spread quickly among the animals (Carroll et al., 2014). The effects of diseases and illness are usually a decrease in the overall performance of the livestock and even sometimes total loss of the animal. Consumer and producer concern for the health and well-being of animals drives the industry to discover and treat illnesses early in development to lower mortality rates and widespread diseases. Birds raised under commercial conditions are vulnerable to the environment and the potential diseases that may come with it. Thus, diseases can be prevented through the use of vaccines as an integral part of flock health management protocols (Sharma, 1999a). The use of live vaccines for immunizations is the current industry standard, and routinely used vaccines in turkeys include NDV (Newcastle disease virus) and HEV (Hemorrhagic enteritis virus) vaccines. Birds respond to vaccines by developing humoral and cellular immune responses, which can result in them feeling fatigue and ill for several days following the vaccine. This response will eventually wear off, but leaves behind an immunologic memory that can be boosted when exposed to the same agent again (Sharma, 1999b).

Consumers expect the food they eat to be clean and not contaminated with chemicals that could cause them to become ill. Most of the foods that we consume come from livestock species purposely raised for human consumption. Current practice in animal agriculture relies on the use of pharmacologically active drugs to keep animal health and well-being a top priority. However, the use of these drugs is associated with human health effects (National Research Council, 1999). To eliminate the risk of humans ingesting any residue from these drugs, withdrawal periods are determined for each drug and on each species to ensure the drug is below the maximum residue limit (MRL) before it is used for human consumption. The National Turkey Federation Chemical Residue Avoidance Program ensures that the tissue of turkeys produced and slaughtered in the United States will not contain any chemical residues as established by the U.S. Environmental Protection Agency, the FDA, and the USDA-FSIS (National Research Council, 1999). Thus, several studies have discussed the impacts of hormones and antibiotics on carcass quality of farm animal species, but few have detailed fresh meat quality of turkeys that were still within the withdrawal period. One study evaluated vaccination stress on boiler performance and carcass quality, and concluded that the vaccination may not have affected flavor, tenderness or juiciness, but that it can have a detrimental economic effect by decreasing growth performance and grade (Quarles and Kling, 1974). Therefore, this indicates that the use of vaccines can also have an economic impact on the industry if the birds are not given ample amount of time to recover before harvesting.

Factors Affecting Animal Welfare and Meat Quality: Social Behavior

Dominance

There are several definitions of the term dominance that can be divided into structural and function ones. Structural definitions describe the pattern of the interactions, whereas a functional definition would give the reasoning behind the action. A possible function of dominance is to control resources and allow reproductive success by using the minimum amount of energy expenditure through the use of displays to control the reactions of the receiver (Vessey, 1981; Drews, 1993). Social dominance was a term coined by Schjelderup-Ebbe in 1922 as he was the first person to investigate a social organization in flocks of chickens (Schjelderup-Ebbe, 1922; Syme and Syme, 1979). Relationships among dominant and subordinate individuals are how group life is managed, as 'rules' are created to control social encounters (Keeling and Gonyou, 2001). Keeling and Gonyou (2001) stated that the term 'dominance' refers to the predictable relationship between a pair of conspecifics, where one animal has learned to dominate another, resulting in a subordinate that will avoid confrontations. This relationship is learned and relies on the animals being able to recognize one another and remember previous social interactions. The summation of the dominance relationships in a group makes up the dominance hierarchy, or 'peck order' (Keiper and Sambraus, 1986). Thus, the rank represents an animal's relative position with respect to the other animals in the group. It is important to note, however, that the dominance rank is unique to each specific group and when individuals are added or removed, the equilibrium of the group will be temporarily upset until a new rank has been established (Keiper and Sambraus, 1986).

The dominance hierarchy remains stable through the combination of the dominant's aggression and the subordinate's submission (Keeling and Gonyou, 2001), which implies that the dominant individual is not aggressive when the subordinate is acting appropriately. If the subordinate were to respond inappropriately, then the dominant individual may inflict injury towards to the subordinate, or even force them to leave the group. Animals also exhibit an avoidance behavior where more subordinate group members will avoid conflict or provocation by turning or walking away. The advantage of being a dominant or higher-ranking individual is the ability to gain and maintain priority access to resources over their subordinate (Keeling and Gonyou, 2001). Although a dominance order does help in locating and gathering resources, those resources are not equally distributed depending on the dominance rank (Craig, 1986), meaning there are plenty more resources for the top-ranking individual compared to the rest. Higher-ranking hens have better egg production than the lower-ranked birds in a cage due to their greater access to feed (Cunningham and Van Tienhoven, 1983). Cunningham and Van Tienhoven (1983) further demonstrated that an alternative hierarchy feeding method developed, where a majority of the flock had a relatively equal amount of feed, but the lowest-ranking hens were allowed very little food, which further explains the idea of resources not being equally distributed amongst flock members.

Studies have also discovered that smaller group sizes allow birds to establish a more stable dominance hierarchy compared to slightly larger group sizes that exhibit more complex relationships and have more changes in rank (Keeling and Gonyou, 2001). However, if the group is very large, such as in intensive poultry housing systems, aggression is lower (Keeling and

Gonyou, 2001), which indicates that there could be a threshold beyond which no attempts to form hierarchies are made and a non-intervention strategy is adopted. Keeling and Gonyou (2001) also noted that combative interactions in established flocks are subtler, but there can still be pecking, chasing and even fighting among them. This aggressive behavior is more noticeable between males, but it can occur between females as well, and if these aggressive interactions persist or become more severe they can result in injuries or wounds. Male turkeys are particularly aggressive towards one another, with severe pecks towards the head leading to death in certain cases (Sherwin and Kelland, 1998).

Aggression

There are several reasons that animals will act aggressively towards one another. Aggression can be brought about by maternal instincts to protect their young, dominance over other animals, unbearable pain causing them to lash out, fear resulting in the fight response and even protection of territory (Gregory and Grandin, 1998). Aggression is a normal behavior exhibited by all species, and is sometimes heightened by an outside stimulus. Although aggression is a normal behavior, sometimes it can be abnormal in severity, duration, or intensity, and result in significant welfare concerns such injury or cannibalism (Sherwin and Kelland, 1998). Injurious pecking behaviors that stem from aggression in turkeys are a significant welfare concern in commercial facilities (reviewed in Dalton et al., 2013).

Research suggests that outbreaks of aggression and injurious pecking behaviors are primarily due to a lack of a suitable environment in commercial settings that hinders the performance of the turkey’s normal behavior patterns (Hughes and Grigor, 1996; Sherwin et al., 1999). Other problems with farm management such as poor ventilation, extreme temperature fluctuations, ectoparasites, and the presence of dead or injured birds may also heighten stress conditions and lead to damaging pecking behaviors within a flock (Hughes and Duncan, 1972; Jendral and Robinson, 2004). Both female and male turkeys perform injurious pecking behaviors after environmental disturbances in order to restore the pre-existing dominance hierarchy, maintain the stability of the group, and settle the flock (Gill and Leighton, 1984; Cunningham et al., 1992; Buchwalder and Huber-Eicher, 2003).

Several changes to management and environment can help control the amount of aggression that animals display during their lifetime. Some possible methods for controlling aggressive behaviors are to change the environmental design of the area the animals are held in, surgical removal or shortening of claws or beaks, castration to decrease testosterone levels (non-poultry species), avoiding mixing unfamiliar animals together, and management interventions during mixing (Fraser and Rushen, 1987). Injurious behaviors are currently managed by practices such as beak-trimming, snood removal, toe-clipping, and reduction of light intensity that may also create other welfare concerns (Sherwin and Kelland, 1998). Intensive genetic selection and dimly lit environments have been used to reduce aggressive behaviors in turkeys, however, it is still an issue decreasing production efficiency (Marchewka et al., 2013). Due to modern advances in livestock production, humans now control all aspects of the animal's environment and genetic make-up. We are now placing animals in situations that were never anticipated during their evolution such as sudden mixing of unfamiliar animals together, and environments that don't allow escape from attacking individuals. To ensure both productivity and animal welfare, there is an urgent need to be able to understand the fundamentals of aggressive animals and the impact that behavior has on meat quality, so that that aggression can be better managed. However, before attempting to reduce injurious pecking in turkeys and other poultry species, causation of the behavior needs to be better understood (Dalton et al., 2013).

There is a need to redefine the reason for aggression in domestic species for the purpose of improving animal welfare and controlling or limiting aggression (Schaefer et al., 1990). An idea originally described by Lorenz (1966) was to redirect an attack into harmless channels in order to avoid injury of the animal itself or of other members of the species. It may be worthwhile exploring the possibility of diverting the behavior towards less injurious targets such as different types of food (Hughes and Grigor, 1996). Some experiments have even been done testing the use of enrichments on several species altering the animal's behavior to improve social well-being and animal welfare. An experiment with pigs proved that a car tire as an enrichment was capable of altering some behavior traits and improving growth rates (Schaefer et al., 1990). Schaefer's (1990) second experiment with a mineral block enrichment was effective in reducing total aggression and improving animal growth rates. It is possible to dramatically change an animal's behavioral and physiological capabilities through manipulation of their environment (Jones, 2001).

Pecking Behavior

Investigative behaviors typically involve the use of the beak to peck at the litter or walls of the pen, at the feeding troughs and the drinkers, and pecking at other birds (Hughes and Grigor, 1996). Aggressive interactions are typically defined as bouts of repeated, forceful pecking directed at the head or body of another bird, whereas feather pecking (FP) is typically defined as repeated plucking or pecking of feathers of another bird (Sherwin and Kelland, 1998). This Indicates that FP is not motivated by aggression and is different from aggressive interactions, however feather pecking can still be used to determine certain aspects of social status or rank (Cordiner and Savory, 2001). FP and cannibalism are major welfare concerns for intensively housed turkeys (Savory and Hughes, 1993). Injurious pecking affects millions of turkeys annually and is a common behavior exhibited in most commercial turkey flocks (reviewed in Erasmus, 2018). The exact reason as to why turkeys are motivated to peck each other is still unknown, but the idea of beak related behaviors such as pecking for food is a clue towards the damaging pecking behaviors (Hughes and Grigor, 1996).

Feather pecking (FP), although not an aggressive behavior, can be painful as the feathers are pulled out and damaged, and the recipient birds can be wounded (Brunberg et al., 2011). Severe forms of feather pecking can have a serious effect on bird welfare and flock productivity as it is painful for the recipient, and victims often react adversely by squawking and moving away from the instigator (Nicol, 2018). Although the exact purpose is not yet known, several studies have shown that FP is a redirected pecking behavior related to the birds' motivation to forage (Hubereicher and Wechsler, 1998; Klein et al., 2000; Dixon et al., 2008). Regardless of environmental influences on FP behavior, it is only performed by a certain number of individual birds in the group (Bilčík and Keeling, 2000), suggesting that differences on an individual level contributes to the development of this behavior. A study by Brunberg et al. (2011) suggested that genes had different expression patterns in FP birds than in birds not performing FP behaviors.

Studies have also shown positive correlations between FP behaviors and immune responses (Buitenhuis et al., 2004), and stimulating the humoral immune response leads to more feather damage. Immune mechanisms have also been suggested to be implicated in disorders such as obsessive compulsive disorder (OCD) and attention-deficit hyperactivity disorder (ADHD) in humans (da Rocha et al., 2008; Pelsser et al., 2009), and it has been suggested that feather pecking is possibly a similar OCD or hyperactivity disorder in poultry species (van Hierden et al., 2004; Kjaer, 2009). Considering the negative effects of pecking behaviors on bird welfare, it is not surprising that the alternative damage-limitation strategies, such as beak trimming, have been developed (Nicol, 2018).

Social Rank and Meat Quality

In all species, dominance-related interactions can cause stress, bruises, and physical injuries (Fordyce et al., 2002). For example, the stressors cattle face being in group housing systems can have negative effects on carcass and meat quality, and a growing body of evidence suggests that social stress not only affects muscle color, firmness, and water-holding capacity, but can also reduce tenderness (Andrighetto et al., 1999). Specifically for fresh meat quality attributes, stress results in reduced product yield and a reduction in sales from pale colored, dry, or bruised meat (Albright et al., 1997). Previous studies investigating “dark cutters” in cattle indicated that they were often either the lightest or the heaviest animals in the pen (Grandin, 1978), which indicated the social hierarchy was related to stress since the heaviest animals were usually the most dominant, and the cause of dark cutters in lighter cattle was most likely due to their constant battling with heavier animals. Although dark cutting beef is often more tender, it comes at the cost of decreased flavor (Grandin, 1971). Social rank can also be reflected in some physiological indicators of welfare as well when animals experience social stress, limited access to feed, and possible injury it can lead to poor meat quality and carcass composition (Miranda-de la Lama et al., 2013). This behavior is expressed in many species in animal agriculture resulting in poor carcass and meat quality and would most likely show similar effects in poultry species if more studies had been done. Craig (1992) discusses the social behaviors of poultry and possible effects that dominance hierarchies could have on animal well-being and production characteristics such as meat quality. Considering there is not enough research thus far on this subject matter, the possible correlation between social status or rank and meat quality needs to be researched.

Methods for Determining Social/Dominance Ratios or Ranks

Rushen (1984) explains that peck orders of chickens have been measured in many ways, but the comparability of these methods have been questioned. Staging paired contests between birds is the best way to determine intrinsic traits that underlie dominance relationships and has been used in many studies such as the paired-tests in Leonard and Weatherhead's (1996) study investigating the dominance rank in domestic fowl. However, placing birds in a situation where they are to compete may in turn create a dominance relationship rather than simply measure one that was already established (Rowell, 1974). Rushen (1984) observed that priority access to limited resources such as food and water can also be a method in determining dominance ranking amongst birds, but it is not often used as a measure of dominance in poultry species.

Another ranking method used is similar to a “dominance ratio” or “social tension index”, which appears to be suitable for characterizing agonistic behaviors of individual birds in a flock, without determining the actual pecking order of the birds (Biswas and Craig, 1971). The social tension index was also found to be highly correlated with the actual pecking order of laying hens in one study (Lee et al., 1982). It is assumed that the ratios in this type of method reflect relative propensities of individual birds to give or receive different sorts of pecks, and to approach or avoid other birds, to thus indicate their status in different contexts (Cordiner and Savory, 2001).

Summary

Different environmental and social stressors can have detrimental effects on the carcass characteristics, meat quality, and behavior patterns of all species, including turkeys. Social dominance and social index ratios may also have a direct correlation with meat quality attributes in poultry species, but it has yet to be fully investigated. Thus, the research conducted herein strived to determine the effects of heat stress and immune stress on carcass traits such as feather retention force, and meat quality of commercial male turkeys, and to determine a relationship between these attributes and their social index. Collectively, the experiments conducted allow an in-depth comparison of the consequences of heat stress versus immune stress on behavior and meat quality, and a possible relationship with social index.

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CHAPTER 2. RELATIONSHIPS AMONG STRESS, SOCIAL BEHAVIOR AND MEAT QUALITY OF TURKEYS

Abstract

There are many stressors that poultry species can face at commercial farming facilities and during slaughter that can affect bird welfare and final product quality. Heat stress (HS), immune challenges (IC), and social stress are just a few of the many stressors. The objective of this study was to determine the effects of these stressors on meat quality of turkeys. A total of 92 male, beak-trimmed turkeys were used in two trials (in time replicates). The turkeys were housed in 15 rooms with 4-7 birds per room. Turkeys were subjected to heat stress (HS) and vaccination (immune challenge, IC) between 10 and 15 weeks of age. The HS treatment subjected the birds to an ambient temperature of approximately 29 °C depending on the room's humidity and lasted 120 minutes before returning to normal. The IC treatment consisted of vaccinating turkeys for hemorrhagic enteritis virus. Turkeys in the control (CON) group were not subjected to HS or IC. The birds rotated every two weeks starting at 10 weeks of age so that each group experienced each of the three treatments in a balanced Latin square design. The turkeys were harvested at Purdue University's Boiler Maker Butcher Block and breast meat samples were taken for further meat quality analyses. PROC MIXED in SAS 9.4 was used to test for differences in carcass traits and meat quality attributes among treatment groups. There were no significant differences in fatty acid composition ($P > 0.05$) across treatment groups. There were significant differences in percent protein ($P < 0.05$) between the treatment groups with the HS group having the lowest percent protein and highest moisture content. Initial pH values were significantly different between treatment groups ($P < 0.01$), which corresponded with the significant differences in other meat quality attributes such as L* and shear force values. Results indicated that a short HS period lasting

for 120 min can affect certain aspects of meat quality. Similarly, vaccination with hemorrhagic enteritis vaccine one week prior to slaughter also affects meat quality.

Another goal of this study was to examine the relationships among stress, behavioral characteristics and meat quality of commercial turkeys. This objective was to determine if the frequency of nonaggressive pecking behaviors among the birds was related to final meat quality. To examine the relationship between non-aggressive pecking behavior and meat quality, turkey behavior was video-recorded at 14 weeks of age prior to HS or IC that week. Aggressive interactions occurred too infrequently to be able to examine the relationship between aggression and meat quality. Therefore, analyses were only performed using non-aggressive pecking behavior. From these data, turkeys were given a rank that was calculated by dividing the number of non-aggressive pecks given by the number of non-aggressive pecks received. Ranks were standardized for the number of turkeys in each room. A cluster analysis was performed using PROC FASTCLUC to categorize the birds into low, medium, and high groups based on their frequency of pecking. Clusters were then tested using PROC GLM to verify that they were significantly different from one another. Once each turkey had been assigned to a cluster, meat quality measures were compared among clusters to determine the relationship between non-aggressive pecking and meat quality using PROC MIXED. There were no significant differences ($P > 0.05$) among clusters for any carcass traits or meat quality attributes. Therefore, turkeys' tendency to perform and receive non-aggressive pecks does not appear to have an effect on carcass traits or meat quality attributes in the present study. Research with other species has indicated a relationship between other behavioral characteristics such as aggressive interactions, fear responses, social rank, body weight, and meat quality; therefore, future research examining other behavioral traits will be valuable in examining factors that can influence turkey meat quality.

Key words: heat stress, immune, cluster, behavior, turkey, meat quality

Introduction

There are many stressors that affect final product quality of commercially raised food animal species. A stressor is an agent that produces stress for an animals and stress is the animal's individual biological response to that stressor that disrupts the animal's normal behavioral activities (Selye, 1976). Environmental stressors such as heat stress or disease have become major points of interest to consumers in recent years, and poultry species are very susceptible to these types of stress (Nienaber and Hahn, 2007; Nardone et al., 2010; Renaudeau et al., 2012; Lara and Rostagno, 2013).

Another stressor that many species face is the idea of social stress in group living situations. Stress in groups can result from aggressive or non-aggressive interactions. Non-aggressive interactions, such as feather pecking, can lead to pain, feather loss and damage and in some cases, lead to cannibalism, feather pecking is the repeated plucking of feathers of another individual and can be categorized as an investigative or modified foraging behavior (Hughes and Grigor, 1996; Sherwin and Kelland, 1998). Aggressive interactions, on the other hand, are used to maintain the social dominance hierarchy, but can also lead to stress and decreases in animal welfare. Social hierarchies are designed so that each member understands its role or place within the group relative to the other members, and then "rules" are created to control social encounters with one another (Keeling and Gonyou, 2001). This social hierarchy is also unique to each group, and will change with the addition or removal of an individual (Keiper and Sambraus, 1986). Male turkeys are particularly aggressive towards one another, and the advantage of being the highest-ranking individual in the group is priority access to resources (Craig, 1986; Sherwin and Kelland, 1998).

Aggressive interactions among turkeys are typically defined as forceful, repeated pecking bouts directed at the head or body of another individual (Hughes and Grigor, 1996; Sherwin and Kelland, 1998). Craig (1992) discussed that the behaviors that poultry species exhibit within their social hierarchies could have an effect on the well-being and production characteristics such as meat quality. Considering there is very little research on this subject, especially in turkeys, the possibility of a relationship between social status and meat quality should be assessed. Studies since the early 1920s have shown positive relationships between social rank and productions traits, specifically in egg quantity and quality (Schjelderup-Ebbe, 1922; Sanctuary, 1932; Tindell and Craig, 1959; Cunningham and Van Tienhoven, 1983). However, these studies have only gone as far as to measure weight gain and final body weight, and we are unaware of any research on the possible relationship between the social ratio of turkeys and their fresh meat quality attributes since most studies similar to this have been done on chickens.

Over the last several years, research has increased on the effects of stress conditions on meat quality (Terlouw et al., 2008). Meat quality is defined by the compositional quality and the palatability factors such as appearance, tenderness, flavor, and juiciness (FAO, 2019). Poor animal welfare can lead to poor product quality and several conditions in meat such as abnormal meat color, pale soft and exudative meat (PSE), dark firm and dry meat (DFD), poor shelf life, bruising, torn skin and broken bones (Gregory and Grandin, 1998). Several studies in the last few years have shown impaired growth performance in poultry species subjected to periods of heat stress (Deeb and Cahaner, 2002; Niu et al., 2009; Attia et al., 2011; Imik et al., 2012; Ghazi et al., 2012), however we don’t know as much about turkey welfare and meat quality as we do in other poultry species. Furthermore, environmental stress factors can result in changes in the metabolites of muscle that are responsible for the differences in the ultimate properties of meat (Judge, 1989).

Another quality factor that has been a problem in the industry is feather retention force, or the force required to remove a feather from the feather follicle. Handling and treatment prior to slaughter can influence the force required to pluck the feathers from their carcasses during harvest (Gregory and Grandin, 1998). Carcasses with difficult feathers to pluck will slow down production lines and ultimately cause a decrease in economic output. There is little research on stressors affecting feather retention force; however, it is important to know what factors affect it in order to prevent it.

Currently, there have been several studies examining the impact of heat stress on meat quality and immune function in other species; however, there have been no studies with a combination of heat stress and vaccinated immune stress at different age points in turkeys. We hypothesize that heat stressed, social stressed, and vaccinated turkeys would show negative effects on fresh meat quality and feather retention force (FRF) compared to turkeys in the control group. Therefore, the aim of the present study was to determine if the heat stressed birds or immune stressed birds showed greater negative effects on overall meat quality compared to a control group, and to understand how stimulation of a turkey's immune response through vaccination may influence meat quality. We also wanted to determine if individual differences in pecking frequency influences turkey fresh meat quality attributes.

Materials and Methods

Animals and Housing

This study was part of a larger study that examined the effects of heat stress and immune challenge conditions on the behavior of male turkeys (Stevenson, 2019). Therefore, pre-slaughter procedures were the same as reported in Stevenson (2019). Tables 2.1 – 2.4 include information presented in Stevenson (2019). The data presented here were collected at the conclusion of Stevenson's (2019) study and examine meat quality attributes of the turkeys after turkeys were slaughtered at 15 weeks of age.

All procedures of this study were approved by the Institutional Animal Care and Use Committee (IACUC) of Purdue University. This study involved two trials (in-time replicates) with a total of 92 turkeys (Trial 1: 50 turkeys, Trial 2: 42 turkeys). The commercial male beak trimmed turkeys (Nicholas Select, Aviagen Turkeys, Lewisburg, West Virginia) were housed at the Purdue Animal Sciences Research Center (ASREC) once they were received from a commercial hatchery at 1 d of age. From 1 d to 7 d of age, the turkey poults were housed together in a brooding ring, and then randomly assigned to 8 littered (wood shavings) pens (measuring 2.44m by 1.52m) in trial 1 and 7 different pens in trial 2 with 4 to 7 birds per pen.

Each pen included a hanging feeder and bell drinker to provide feed and water ad libitum. Room temperature and lighting were maintained according to industry standards (Aviagen, 2015). For 1 d, poults were given 24 h of light that was gradually adjusted to 15 h light:9 h darkness by the fourth day. A minimum light intensity of 40 lux was maintained, and room temperature was changed weekly as recommended by Aviagen (Aviagen, 2015). Poults were brooded at a temperature of 30° C, which was gradually adjusted to a final temperature of 13° C by 14 wk. At 7 wk, the turkeys were moved to the Purdue University Veterinary Animal Isolation Building (VA2). Birds that were housed together previously continued to be housed together in the new building. Each room had its own separate temperature and lighting controls, but each room was set to a lighting schedule of 0700 to 2100 with an average temperature (± SD) of 17.9 ± 1.9° C. At

13 wk of trial 2, there was a power outage that caused the lighting schedule of one room to change lights off from 2100 to 1800.

At 7 wk of age, the turkeys were individually marked with a black non-toxic livestock marker (Prima Tech Marking Stick, Neogen Corp., Lansing, MI USA) to be able to identify each bird in each room. There were seven unique marking locations: left wing (L), right wing (R), base of the neck or top (T), start of tail feathers or bottom (B), center of the back and across the shoulders (C), both T and B (TB) and both L and R (LR). The livestock marker was reapplied every two weeks until harvest to ensure the markings did not fade.

Experimental Design

The design of the study was a crossover design where each room experienced each treatment, but treatments were applied in different orders at 10, 12 and 14 wk of age (Table 2.1). The treatment groups included a heat stress (HS), an immune challenge (IC) and a control condition where no other treatment was given (CON). Between the two trials, five total rooms (n = 5) were randomly assigned to each of the three treatment orders (Table 2.1).

Table 2.1. Schedule and order of treatments imposed on turkeys in each room: heat stress(HS), immune challenge (IC), or neither HS or IC (CON). Revised table from original study by Stevenson (2019).

Trial 1: 3 rooms Trial 2: 2 rooms	Trial 1: 3 rooms Trial 2: 2 rooms	Trial 1: 2 rooms Trial 2: 3 rooms	Bird Age
IC	CON	HS	10 wks
CON	HS	IC	12 wks
HS	IC	CON	14 wks

During the HS treatment days for each time point, the room temperature gradually increased until the rooms reached a peak temperature range that depended on each room’s humidity level, and each room was heated to about 30 ºC. In order to determine the correct temperature range to use, a heat index chart that was created for hen turkeys was referenced from Xin and Harmon (1998). The heat index chart (Xin and Harmon, 1998) was used to ensure that the temperature range chosen and defined as “danger” was used instead of an “extreme” heat stress range so that a mild heat stress was imposed. The humidity of the rooms was also recorded and applied to the reference chart to help determine the temperature range. The humidity and temperatures were checked before starting the heat stress treatment and the detailed information is provided in Stevenson (2019). To verify that the turkeys experienced heat stress, cloacal body temperatures were recorded for two turkeys in each of the HS rooms. Average cloacal temperatures pre-treatment was 40.6 ºC, and average cloacal temperatures during peak heating was 41.3 ºC. Detailed information can be found in Stevenson (2019).

Each of the rooms took approximately 140 min to reach the peak temperature range and then the temperature was held there for 120 min. Then, the rooms took approximately 140 min to cool back down to the temperature the room was originally at. During the heating and cooling process for each HS room, an observer was recording the temperature and humidity levels every 10 min to determine when the peak temperature range for the heat stress was reached.

The immune challenge treatments were given using a live-virus hemorrhagic enteritis vaccine (Oralvax HE®, Merck Animal Health) that was administered via their drinking water. The vaccine was prepared according to the manufacturer’s directions. The re-hydrated vaccine can create 80 gallons worth of prepared vaccine for a total of 2,000 doses, or 0.04 gallons per one dose. The vaccine was prepared in a 1-gallon jug by pipetting out 0.375 mL of the rehydrated vaccine into the jug. Depending on how many birds were in each room, only 4 to 7 doses were needed, and the amount of vaccine water prepared was calculated by multiplying the number of birds in each room by 0.04 gallons. On the day of the IC treatment, the water jugs were removed from the rooms

2 h prior to the vaccine administration to ensure that all birds would drink the vaccine water when it was returned. The water containing the vaccine was provided after the 2 h deprivation period and then was left in the rooms for 2 h before it was replaced with fresh, clean water. Turkeys in CON treatment groups were not subjected to any heat stress or an immune challenge.

Temperature and humidity sensors were placed in the room at the height of the turkeys for an accurate reading. To examine activity levels of turkeys, accelerometers (AXY-3 Micro Acceleration Data Loggers, TechnoSmArt, Guidonia Montecelio, Italy) were attached to one leg of two birds per room using a Vet Wrap bandage. Turkeys assigned to wearing the accelerometers were familiarized to the Vet Wrap wrapped around their leg 1 wk prior to the data collection week. Accelerometer data are presented in Stevenson (2019) and will not be discussed here.

Turkey Behavior

An overhead camcorder (Sony Camcorders, CX405, Sony Corporation of America, New York, NY) was installed into each room in order to monitor the turkey behavior. The roles (Table 2.2) and behaviors (Table 2.3) of the turkeys were analyzed from the video recording two days prior to treatment at 14 weeks of age. Each turkey was observed continuously for 15 min at 1300 and 1600 hours two days before the treatments were imposed.

Table 2.2. Bird role in behavior

Bird Role	**Description**
Instigator	The bird who is responsible for starting the interaction. The first bird to peck, chase, threat, etc. another bird
Recipient	The bird who is receiving the action that the instigator bird started

Table 2.3. Ethogram of turkey behavior

Behavior	
Aggressive Peck	Pecking at another bird on the face, head or neck repeatedly. Usually a forceful downward peck that results in the recipient moving away. Pecking or grabbing on to the neck or snood of another bird may be exhibited
Feather Pecking	Using beak and extending neck to peck at the feathers of another bird. Feathers are sometimes pulled, but usually not pulled out
Beak Pecking	Using beak to gently peck at another bird's beak, neck, or face
Chase	Running (or chasing) towards another bird in an aggressive manner (neck and head are stretched out in a threatening posture and feathers are erect)
Threat	Head is raised in front of another bird and is sometimes accompanied with raising of the feathers of the neck (neck and head are stretched out in a threatening posture). The bird that is doing the threatening action is considered the instigator
Avoidance	Walking or running away from another bird. Also includes moving out of the way of another bird. Usually accompanied with lowering of the head. The bird that is doing the avoiding is considered the recipient

Nonaggressive Pecking Frequency Determination

Methods for determining the social ratio amongst the birds was completed using the outcome of all agonistic interactions between pairs of birds. A bird was considered the instigator if observed to be giving the pecking, and the recipient if that bird was receiving the pecking. The values were determined for the birds according to which other birds they dominated within the periods of time observed (adapted from Leonard and Weatherhead, 1995). A calculation for each bird's behavior was used to determine an aggressive (aggressive pecks), nonaggressive (feather pecking or beak pecking) and approach/avoidance (chase, threat, avoidance) values to compare to the other birds in the same room (adapted from Cordiner and Savory, 2000). This ranking method is similar to a "dominance ratio" or "social tension index" that is used for characterizing agnostic behaviors of birds in flocks without actually calculating the pecking order, and this has been found to be highly correlated with peck order in a previous study with laying hens (Cordiner and Savory, 2001). Cordiner and Savory (2001) explained that the ranking orders developed using this method reflect the relative propensities of individual birds to give or receive different sorts of pecks, and thus indicate their status in different contexts. Calculations were done this way instead of using pair-wise "fights" since placing birds in pair-wise encounters may in turn create a dominance relationship rather than us measuring one already established in the group (Rowell, 1974). We also chose to use this method since the footage was taken of the whole group of birds in each room ahead of time, leaving us with the only option of measuring group behaviors. Calculations were as follows:

$$\text{For Aggressive Interaction Value Per Bird} = \frac{All\ aggressive\ pecks\ given + 1}{All\ aggressive\ pecks\ received + 1}$$

$$\text{For Nonaggressive Interaction Value Per Bird} = \frac{All\ nonaggressive\ pecks\ given + 1}{All\ nonaggressive\ pecks\ received + 1}$$

$$\text{For Approach/Avoidance Interaction Value Per Bird} = \frac{All\ approaches + 1}{All\ avoids + 1}$$

One (1) was added to both the numerator and denominator values to ensure that the resulting values were never zero. If a bird resulted in a value of 1, then they received as many pecks or approaches as they gave. If a bird had a value of <1, then they received more pecks or approaches than they gave, and a value of >1 meant they would give more pecks and approaches than they received.

A descriptive bar graph of the behaviors showed us calculated interactions per bird per hour to determine if there was sufficient data in all behavior categories (non aggressive, aggressive, and approach/avoidance) to use them as indicators of social dominance (Figure 2.1). Due to instances of aggressive interactions and approach/avoidance interactions not occurring often enough, only values for nonaggressive interactions such as feather pecking and beak pecking were used to determine a nonaggressive pecking ratio. The values were then divided by the number of birds in each room to standardize each turkey's ratio for the number of turkeys in the room. Using the FASTCLUS procedure in SAS (SAS version 9.4; SAS Institute Inc., Cary, NC, USA), turkeys were grouped into three groups based on their non-aggressive peck ratios, including a high pecking group, a moderate pecking group and a low pecking group. This was done for every room on the 14th week of age prior to any treatment. Further statistical analyses were conducted to verify that pecking activity differed significantly among clusters. Fresh meat quality attributes were then compared among clusters to assess possible relationships between meat quality attributes and a turkeys' peck ratio.

Harvesting Preparation

Room numbers were used to assign the rooms at random for processing except for rooms that were given the vaccine last, and which were not yet through the withdrawal period and had to be harvested last to avoid any cross contamination with other birds. To determine the processing order for the birds in each room, each bird was marked with a color (red, orange, yellow, green, blue, purple or pink) using a non-toxic livestock marker (Prima Tech Marking Stick, Neogen Corp., Lansing, MI USA) the day before harvesting. Red or orange colors were given to the two birds that wore accelerometers in each room and the rest of the birds in each room were randomly assigned a color. At 12 hr prior to harvesting, feed was taken from each room to ensure that the birds were fasted before evisceration. Drinking water was left in the rooms and was provided up until harvest.

Harvesting

At 15 wk of age, turkeys were transported to the Purdue Boilermaker Butcher Block for harvesting and sample processing. Birds were first individually weighed, and then slaughtered under standard conditions of electrical stunning, bleeding for 120 s, scalding at 60° C and feather removal in a rotary drum plucker. Eviscerated carcasses were weighed, and initial pH measurements were taken before carcasses were air-chilled in a 2° C carcass cooler breast-side up for 24 hr. After carcasses were chilled, ultimate pH measurements were taken (specific procedures described below). Breast meat (*M. Pectoralis major*) was cut from the right side of each carcass and weighed. The right breast was sliced into different sections which were used to determine water-holding capacity, cook loss, Warner-Bratzler shear force, color, proximate analysis and fatty acid composition. Following sample collection, slices were individually vacuum packaged and then frozen at -40° C until further use.

Feather Retention Force Procedure

Prior to feather removal in the plucker, the feather release force was taken for each bird after scalding. Three mature feathers from the tail were pulled out individually with a hemostat attached to a FG-3008 digital force gauge (Nidec-Shimpo Corporation, Glandale Heights, IL, USA) to measure the force required to pull out each feather (adapted from Pool, 1954). The three recorded measures were then averaged for each bird.

pH Measurements

pH values were measured in duplicate from two randomly selected locations at the top half of the right breast of each carcass approximately 20 minutes post-mortem. These same locations were used for the ultimate pH measurement taken after 24 hr of air chilling at 2° C. Measurements were taken using a calibrated meat pH probe that was directly inserted into the muscle tissue (HANNA HI 99163, Hanna Instrument, Inc., Warner, NH, USA).

Instrumental Color Measurements

After the right breast muscle had been sliced into different sections at 1 d postmortem, the slices for Warner-Bratzler shear force for each bird were placed on a table for approximately 1 hr before surface color was measured in three randomly selected locations using a Hunter MiniScan EZ colorimeter (Hunter, Reston, VA, USA). The setting for the illuminant was A source, and the observer was at the standard 10°. CIE lightness (L*), redness (a*) and yellowness (b*) values were recorded. Following color measurements, samples were frozen at -40 °C until further use.

Water-holding Capacity

Drip loss was measured according to the Honikel drip loss protocol (Honikel, 1998) as described by Kim et al. (2017) and is expressed as percent difference between the initial and 24 hr weight, and initial and 48 hr weight after hanging in a plastic storage container at 4° C.

Cook Loss and Warner-Bratzler Shear Force

To determine cook loss, samples were individually weighed prior to cooking to measure initial weight. Samples were then cooked in an 80° C water bath until internal temperatures reached 71° C, which was monitored using a T-type thermocouple (Omega Engineering, Stamford, CT, USA) connected to an OctTemp 2000 data logger (Madge Tech, Inc., Warner, NH, USA). Following cooking, the samples were cooled, then weighed. Cook loss was expressed as the percent change between the initial and final weight of the samples.

Once completely cooled, six 1 cm x 1 cm slices were cut from each sample parallel to fiber direction to be used for Warner-Bratzler shear force. Slices were then sheared perpendicular to fiber direction using a TA-XT Plus Texture Analyzer (Stable Micro System Ltd., UK) with the

Warner-Bratzler shear attachment. Test speeds were set at 2 mm/sec and peak shear force in Newtons per slice was then averaged to calculate an average shear force value for each sample.

Fatty Acid Composition

Intramuscular lipids were extracted in duplicate from powdered samples using the method described by Folch et al. (1957) with modifications described by Shin and Ajuwon (2018). Only two birds from each pen that were closest to pen average were chosen to sample for this portion. Fatty acid methyl esters (FAME) were prepared from the extracted lipids by adding sodium methoxide to methanol. The FAME were analyzed using a gas chromatograph (Varian CP 3900) equipped with a 105 m Rtx-2330 (Restek) fused silica capillary CG column (0.22 mm ID and 0.20 µm d_f). Helium was used as the carrier gas with a flow rate of 40 ml/min. Injector and detector temperatures were at 260 °C. Injection volume was set at 1 µL with a 50:1 split injection. The column oven temperatures were increased from 140 °C to 180 °C at a rate of 8 °C/min, from 180 °C to 260 °C at a rate of 5 °C/min, and then held at 260 °C for 15 minutes. The fatty acids were identified by comparing them to a retention time of a known standard (Supelco 37 components FAME Mix, Sigma-Aldrich, USA) and the peak area of the fatty acid detected was expressed as a percent of the total peak area.

Proximate Analysis

Proximate Analysis of breast meat samples was conducted using the AOAC guidelines (AOAC, 2006). Only two birds from each pen that were closest to the pen average body weight were chosen for sampling. Moisture was determined in triplicate measurements using the oven air-drying method at 105 °C and weighing the samples before and after drying. Ash was measured in triplicate by combusting dried samples in a 580 °C muffle furnace and weighing the samples before and after ashing. Nitrogen was measured in duplicate using the Dumas combustion method and then multiplied by 6.25 to determine crude protein concentration (Leco, St. Joseph, MI, USA). Carbohydrate composition was assumed to be approximately 0%; thus, lipid concentration was determined as 100% - (% moisture + % protein + % ash).

Statistical Analysis

Treatment (HS, CON, IC) effects on meat quality were analyzed using PROC MIXED (SAS version 9.4; SAS Institute Inc., Cary, NC, USA) for all data except shear force. Body weight was included as a covariate; room nested within trial was included as a random effect. Tukey's test for multiple comparisons was used to determine post-hoc differences among treatment groups. Normality of data were verified by examining qq plots and plots of studentized residuals. Shear force values were log-transformed to meet normality assumptions and were analyzed using PROC GLIMMIX, using the link=log function and ilink option. Body weight was included as a covariate; room nested within trial was included as a random effect. Tukey's test for multiple comparisons was used to determine post-hoc differences among treatment groups.

To examine the relationship between social tension based on non-aggressive pecking behavior and meat quality, the FASTCLUS procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) was used to separate the birds into three groups based on their nonaggressive pecking frequency value. The GLM procedure was then used to verify that the pecking values were different among clusters. All analyses used post hoc Tukey tests for multiple comparisons, and body weight was included as a covariate. The MIXED procedure was used to compare the meat quality parameters among the resulting clusters and room and trial were included as random effects. Significance was set at $P < 0.05$ for all analyses.

Results

Fatty acid composition (g/100g) of intramuscular lipids in the turkey meat did not differ among treatment groups for all 38 fatty acids tested and for the percentage of total saturated, monounsaturated, polyunsaturated, and the ratio of saturated to unsaturated ($P > 0.05$; Table 2.4). However, Caproic (C6:0) and Heptadecanoic (C17:0) tended to differ ($P < 0.10$; Table 2.4) among treatment groups. The most abundant fatty acid detected was Linoleic (C18:2n6c).

Treatment had a significant effect on percent moisture per sample ($P = 0.04$); however, post hoc analyses did not reveal any significant differences among treatment groups (Table 2.5). Treatment also had a significant effect on percent protein ($P = 0.03$); birds subjected to HS in the week before slaughter had lower percent protein compared to CON ($P = 0.04$, Figure 2.2). IC and CON birds did not differ ($P = 0.92$), and HS and IC did not differ ($P = 0.09$). Percent ash and fat were not different among treatment groups ($P > 0.05$, respectively; Table 2.5).

Treatment had a significant effect on initial pH values ($P = 0.009$); birds subjected to IC had higher pH values compared to CON ($P = 0.006$). CON and HS groups did not differ ($P = 0.35$), and HS and IC groups did not differ from each other either ($P = 0.18$, Table 2.6, Figure 2.3). Treatment also had a significant effect on L*, or lightness ($P = 0.04$; however, post hoc analyses did not reveal any significant differences among treatment groups (Table 2.6). Lastly, treatment had a significant effect on shear force ($P = 0.03$); birds subjected to IC before slaughter had higher shear force values compared to CON ($P = 0.05$, Table 2.6, Figure 2.4).

Nonaggressive pecking frequencies were categorized into three clusters of either high (cluster 3) with a mean and standard deviation of 0.92 ± 0.09, medium (cluster 1; 0.56 ± 0.11), or low (cluster 2; 0.23 ± 0.08). The higher the value, the more pecking given to others rather than received. Instances of aggressive pecking behaviors were so infrequent that there was not enough data to use it as a means for comparing their behavior to their meat quality, thus we only used nonaggressive pecking behaviors. None of the meat quality parameters we measured, including feather retention force, differed among clusters (P-value > 0.05 respectively; Table 2.7.).

Discussion

Some aspects of turkey meat quality were affected by a mild heat stress and vaccination before slaughter. Specifically, turkeys in the HS group had lower percentages of protein, higher pH and shear force values compared to the control group, and turkeys in the IC group had higher pH and higher shear force values compared to the control and heat stressed groups. A limitation to this study was that the birds were subjected to all three treatment conditions which may have led to possible carryover effects, however, the two weeks in between treatments should have helped in reducing any possible carryover effects in final meat quality.

When comparing turkey meat quality to that reported in other studies, the main fatty acids found in the breast muscles were Palmitic (C16:0), Stearic (C18:0), Oleic (C18:1n9c), Linoleic (C18:2n6c), and Arachidonic (C20:4n6) acid, which is consistent with the findings of Baggio et al. (2002) of the most abundant fatty acids being C18:2n6, C18:1n9, C16:0, C18:0, and C20:4n6. We were unable to find any similar studies of fatty acid composition of heat stress or immune challenged turkeys to compare to. Wong et al. (1993) reported higher amounts of Palmitic acid (C16:0) and total percent of polyunsaturated fatty acids, but very similar amounts of Linoleic acid

(C18:2n6c) in their raw turkey composite for light meat compared to the results in the present study. Polyunsaturated fatty acids for all three treatment groups present in this study were similar to those found in a study by Wong et al. (1993).

The IC and CON birds were very similar in proximate composition, with only the heat stressed group being significantly different for protein and moisture content. Protein content was significantly lower for the HS group which correlated well with the higher moisture content for that group of birds. Very similar amounts of percent moisture were found in other studies of turkey meat by Wong et al., (1993) and Paleari et al., (1998) with 74.4% and 74.8%, respectively, which are most similar to the HS group of birds, and much higher than the percent moisture found in the CON and IC birds. A study on broiler meat by Zhang et al. (2012) also reported higher moisture content and lower protein content in the breast muscle, similar to what we report here. Higher ambient temperatures significantly decrease body protein content by changing protein metabolism, decreasing protein synthesis, and increasing protein breakdown (Geraert et al., 1996; Yunianto et al., 1997; Zhang et al., 2012). It has also been demonstrated that heat stress lowers protein synthesis by changing ribosomal gene transcription (Jacob, 1995; Temim et al., 1998). Therefore, the decreased protein content in the HS birds can be attributed to lower ribosomal capacity and a decreased rate of protein synthesis resulting in the reduction of protein deposition.

The amount of water and how well it is distributed within muscles may affect the visual appearance of meat, but also affects the tenderness and juiciness. A similar study with heat stressed and control groups of turkeys by McKee and Sams (1997) showed L* values at 53, which is much higher than any of the mean L* values for all three treatments in the present study. However, McKee and Sams (1997) reported lower L* values for their control group compared to our CON and HS groups, but very similar to our IC group. The study by McKee and Sams (1997) presented their birds with a much longer heat stress period, and their turkeys were 17 weeks of age compared to ours at 15 weeks of age. McKee and Sams (1997) also reported that acute and chronic heat stress can cause poor water-holding properties in meat. Stress conditions cause a high metabolic rate during rigor mortis that causes more protein denaturation that affects the protein's ability to bind water as well (Klont et al., 1994). The turkey breasts sampled did not exhibit any typical PSE conditions for any of the treatments here that are usually found with porcine muscle under similar conditions. Findings by Barbut (1998) and Owens et al. (2000) suggested that higher L* values (> 51-53) were associated with PSE meats and had paler coloring, a changed texture, and poor water holding capacity. However, in the present study, the HS and IC groups with the higher pH values and lower L* values showed poorer water holding capacity in drip loss and tougher texture measured by shear force. Cook loss values did show a trend of poor water holding capacity with paler colored meat. The CON group and heat stressed group had higher L* values of 51.5 and lower pH values indicating that it was the start of PSE-like conditions, but not enough to cause a significant enough issue with final product quality. It is interesting to note that the CON birds had lower overall pH values compared to the two stress treatments since lower pH values are usually associated with PSE-like conditions in swine and poultry (Fletcher, 2002). A study by Çelen et al. (2016) showed that the initial pH of normal turkey breast muscle is about 6.20, whereas the pH in our CON group was as low as 6.1 and our IC group as high as 6.25. Shear force values, or tenderness, was observed to be significantly higher for the HS and IC groups. The increased shear force values under the stress conditions is closely related to results reported in several studies with other food animal species.

The higher pH values and shear force values for the IC birds correspond with results of a study on broiler meat by Mellor et al. (1958) where they found birds with a higher muscle glycogen content at slaughter to have lower final muscle pH and lower shear force values than the other birds. In the present study, our IC birds likely had less glycogen content at slaughter and therefore had a higher final muscle pH and higher shear force values. Weary et al. (2009) concluded that animals who are consuming less feed with displaying sickness behaviors will use up stored glycogen for energy when stressed, leaving less glycogen to be converted into lactic acid during the muscle to meat conversion and thus higher pH and shear force values. Another explanation for the increased shear force values in stressed turkeys could be due to the excessive amounts of reactive oxygen species (ROS) that the body can generate that will lead to oxidation of the sarcoplasmic and myofibrillar proteins, and ultimately reduce the proteins' solubility and ability to bind water (Wang et al., 2009). Heat stress has been suggested to be an environmental factors that increases the production of ROS (Belhadj Slimen et al., 2014). This inability to bind water leads to increases in drip loss, cook loss, and reduces the water-holding capacity, juiciness and tenderness of meat (Wang et al., 2009).

Feather retention force was variable but did not differ among treatment groups. The force required to pull out the feathers is also highly dependent on scald tank temperature and time in the tank, with scald temperature being the most critical component (Pool et al., 1954). Pool et al. (1954) also noted that higher temperatures make the feathers easier to pull out, but also risk the possibility of prematurely cooking the meat or dehydrating it. The scald tank temperature in this study was kept at 60 ºC, which is consistent with the standard methods of poultry slaughter. To the best of our knowledge, there are no published studies examining feather retention force of turkeys.

Our results did not support our hypothesis that individual pecking behavior influenced turkey meat quality. Due to low frequency of aggressive interactions, we were unable to examine the relationship between aggression and meat quality and used the frequency of non-aggressive pecking instead. We did not examine whether pecking behavior was consistent among turkeys, and further research is needed to fully examine the characteristics of non-aggressive pecking of turkeys. However, research with laying hens demonstrated that some birds remained consistent in feather pecking behavior (Daigle et al., 2015) and that consistent behavioral patterns can be related to certain aspects of meat quality (e.g. bulls) (Partida et al., 2007; Miranda-de la Lama et al., 2013). Considering none of the traits or meat quality parameters measured were statistically significant between cluster groups and the fact that there is very little information comparing poultry pecking behaviors and meat quality, there is limited discussion possible on this subject. However, there is enough information on this topic in cattle and some poultry species that we can still compare results. A similar study analyzing the approach-avoidance behaviors in humans also used a cluster analysis to categorize people into three qualitatively different groups based on their behaviors (Kashdan et al., 2008). This method of cluster analysis can reveal statistically reliable and distinct groups which is why we decided using a cluster analysis on this present study was the best way to categorize the birds into three distinct groups based on pecking frequency. Living animals form dominance hierarchies as a result of a number of dyadic dominance relationships and social interactions (Forkman and Haskell, 2004). There are several hypotheses as to how these relationships are developed; however, pecking behaviors (aggressive or not) in poultry species are a good indicator of the possible social hierarchy at large (Cordiner and Savory, 2001).

There were no significant differences among clusters for carcass traits; however, there was a noticeable value differences between cluster 1 and the other two for feather retention force. Birds in the middle of the social index had higher feather retention scores indicating some possible signs of stress with tightening of the smooth muscle that surrounds the hair follicle (Gregory and Grandin, 1998). No significant differences in hot carcass weight indicated that there was no noticeable difference in size between the clustered groups and this did not have similar results to studies with cattle where the lightest and heaviest in the pens were experienced the most social stress (Grandin, 1978). The difference with these birds could be due to their young age and ample amount of room in the pen to feed without having to compete for resources.

A previous study by Andrighetto et al. (1999) revealed that social stress not only affects muscle color, firmness, and water holding capacity, but also tenderness. Considering none of our meat quality parameters, including pH, color, water holding capacity, and tenderness, were significantly different among clusters, we can say that the social stress related to non-aggressive pecking was not significant enough to influence meat quality. However, prior to post hoc analysis, there was a trend for significant differences (P-values < 0.10) for L* (lightness) and drip loss which would be similar to the results of Andrighetto's study.

It appears that stress conditions prior to slaughter have an important role in subsequent turkey meat quality characteristics. Better control of the environment of the birds prior to slaughter and better ways of dealing with behavioral changes and individual responses, including sickness behaviors, after vaccines should be considered in order to minimize color and toughness problems. Considering there are no previous studies on the impacts of the hemorrhagic enteritis vaccine on meat quality, it is difficult to compare our results to others. However, immune stressing birds prior to slaughter may possibly be causing them to exhibit sickness behaviors and use up their glycogen energy stores, which can have detrimental effects on meat quality (Weary et al., 2009).

Poultry scientists are interested in knowing how dominant and social interactions are associated with well-being and production characteristics such as meat quality (Craig, 1992). Thus, detailing these relationships and measuring the outcomes needs to be tested to understand the associations between these characteristics. Then, multiple factors such as genetics, management systems, and physical and social environments are open to scrutiny to determine whether it is behavioral or other issues that are causing the differences in production outcomes. Further research examining turkey behavioral traits and welfare will be valuable in identifying factors that influence turkey meat quality. Also, detailing the behavioral traits of turkeys at an older age and a denser population may be more applicable to large scale production, and offer a better insight as to the relationship between individual behavioral characteristics and meat quality.

Conclusion

The heat stress treatment did not appear to have as detrimental of an effect as expected on meat quality; however, the immune challenged birds did show more of an effect. This supports the use of an appropriate recovery time after the vaccine was given before the birds were slaughtered, and that withdrawal periods are important not just for the safety of humans, but also for better quality meat products. Studies with a more extreme heat stress may cause more of an effect on meat quality and other carcass traits such as feather retention force.

Turkeys' tendency to perform and receive non-aggressive pecks does not seem to influence the carcass traits and meat quality attributes tested in this study. Research with other species has indicated a relationship between behavioral traits such as pecking and aggressive social interactions on meat quality.

Table 2.4. Fatty Acid Composition (g/100g) of Intramuscular Lipids in Turkey Meat Subjected to mild Heat Stress (HS), Immune Challenge (IC), and a Control Group (CON)

Fatty Acid		CON (M ± SE)*	HS (M ± SE)*	IC (M ± SE)*	*P*-value
C4:0	Butyric	1.91 ± 0.29	1.67 ± 0.29	1.67 ± 0.30	0.80
C6:0	Caproic	0.54 ± 0.11	0.89 ± 0.11	0.74 ± 0.11	0.10
C8:0	Caprylic	0.30 ± 0.03	0.27 ± 0.03	0.22 ± 0.03	0.27
C10:0	Capric	0.12 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.47
C11:0	Undecanoic	0.04 ± 0.008	0.04 ± 0.008	0.05 ± 0.009	0.45
C12:0	Lauric	2.07 ± 0.42	1.51 ± 0.41	1.34 ± 0.43	0.46
C13:0	Tridecanoic	0.05 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.72
C14:0	Myristic	0.41 ± 0.03	0.46 ± 0.03	0.45 ± 0.03	0.45
C14:1	Myristoleic	0.08 ± 0.03	0.10 ± 0.03	0.17 ± 0.03	0.17
C15:0	Pentadecanoic	0.18 ± 0.03	0.18 ± 0.03	0.20 ± 0.03	0.95
C15:1	cis-10-Pentadecanoic	0.07 ± 0.01	0.09 ± 0.01	0.05 ± 0.02	0.23
C16:0	Palmitic	16.6 ± 0.31	16.9 ± 0.30	17.3 ± 0.31	0.30
C16:1	Palmitoleic	0.88 ± 0.12	1.17 ± 0.11	1.10 ± 0.12	0.21
C17:0	Heptadecanoic	0.20 ± 0.01	0.22 ± 0.01	0.18 ± 0.01	0.07
C17:1	cis-10-Heptadecanoic	0.07 ± 0.04	0.17 ± 0.04	0.14 ± 0.04	0.28
C18:0	Stearic	10.1 ± 0.40	9.57 ± 0.39	10.2 ± 0.41	0.50
C18:1n9t	Elaidic	0.14 ± 0.04	0.14 ± 0.04	0.15 ± 0.04	0.96
C18:1n9c	Oleic	13.3 ± 0.78	14.7 ± 0.77	14.8 ± 0.80	0.33
C18:2n6t	Linolelaidic	0.04 ± 0.03	0.07 ± 0.02	0.09 ± 0.03	0.34
C18:2n6c	Linoleic	23.3 ± 1.12	23.4 ± 1.11	23.2 ± 1.14	0.99
C20:0	Arachidic	0.07 ± 0.009	0.07 ± 0.009	0.06 ± 0.01	0.63
C18:3n6	γ-Linoleic	0.24 ± 0.02	0.27 ± 0.02	0.25 ± 0.02	0.57
C20:1n9	cis-11-Eicosenoic	0.12 ± 0.03	0.14 ± 0.03	0.15 ± 0.03	0.70
C18:3n3	α-Linoleic	1.32 ± 0.12	1.41 ± 0.12	1.31 ± 0.12	0.82
C21:0	Heneicosanoic	0.17 ± 0.05	0.14 ± 0.05	0.08 ± 0.05	0.45
C20:2	cis-11,14-Eicosadienoic	0.53 ± 0.03	0.50 ± 0.03	0.49 ± 0.03	0.50
C22:0	Behenic	0.11 ± 0.02	0.08 ± 0.02	0.07 ± 0.02	0.21
C20:3n6	cis-8,11,14-Eicosatrienoic	0.71 ± 0.47	0.51 ± 0.47	1.64 ± 0.47	0.23
C22:1n9	Erucic	1.31 ± 0.42	0.88 ± 0.42	0.37 ± 0.43	0.33
C20:3n3	cis-11,14,17-Eicosatrienoic	1.19 ± 0.51	1.85 ± 0.50	1.12 ± 0.52	0.23
C23:0	Tricosanoic	0.09 ± 0.19	0.32 ± 0.19	0.02 ± 0.19	0.29
C20:4n6	Arachidonic	7.76 ± 0.55	6.52 ± 0.55	7.47 ± 0.56	0.28
C22:2	cis-13,16-Docosadienoic	0.05 ± 0.01	0.06 ± 0.01	0.03 ± 0.01	0.33
C24:0	Lignoceric	0.04 ± 0.006	0.03 ± 0.006	0.02 ± 0.006	0.14
C20:5n3	cis-5,8,11,14,17-Eicosapentaenoic	0.24 ± 0.02	0.19 ± 0.02	0.24 ± 0.02	0.10
C24:1n9	Nervonic	0.03 ± 0.005	0.03 ± 0.004	0.03 ± 0.005	0.74
C22:6n3	cis-4,7,10,13,16,19-Docosahexaenoic	0.90 ± 0.09	0.68 ± 0.09	0.85 ± 0.09	0.21
	Saturated (%)	33.8 ± 0.86	32.8 ± 0.85	32.7 ± 0.88	0.62
	Monounsaturated (%)	15.9 ± 0.84	17.4 ± 0.83	17.1 ± 0.86	0.45
	Polyunsaturated (%)	35.6 ± 1.18	35.2 ± 1.16	36.8 ± 1.21	0.64
	Saturated:Unsaturated	0.66 ± 0.03	0.63 ± 0.03	0.61 ± 0.04	0.59

* Mean and standard error of each duplicate sample ran in duplicate.
No means in this table were significantly different from one another across treatment groups at the 0.05 level.

Table 2.5. Proximate Composition of Turkey Meat Subjected to mild Heat Stress (HS), an Immune Challenge (IC), and a Control Group (CON)

Proximate Composition	CON (M ± SE)*	HS (M ± SE)*	IC (M ± SE)*	*P*-value
% Moisture	72.1 ± 0.93	75.2 ± 0.92	71.9 ± 0.95	0.04
% Ash	4.18 ± 0.16	4.00 ± 0.16	4.55 ± 0.17	0.10
% Protein	22.0[a] ± 0.80	18.9[b] ± 0.79	21.6[ab] ± 0.82	0.03
% Fat	1.72 ± 0.31	1.89 ± 0.30	2.00 ± 0.31	0.82

* Mean and standard error of triplicate measurements of moisture and ash, and duplicate measurements of protein; fat was calculated as the remaining value to reach 100%.

[ab] Values in the same line with the same letters are not significantly different from one another at the 0.05 level.

Table 2.6. Harvesting and Meat Quality Attributes of Turkey Meat Subjected to mild Heat Stress (HS), an Immune Challenge (IC), and a Control Group (CON)

Trait	CON (M ± SE)*	HS (M ± SE)*	IC (M ± SE)*	P-value
Carcass traits				
Feather Retention Force, N	21.9 ± 3.26	25.9 ± 3.26	21.4 ± 3.28	0.57
Hot Carcass Weight, kg	8.57 ± 0.05	8.57 ± 0.05	8.53 ± 0.05	0.73
pH, color, water holding capacity and shear force				
pH_{20min}	6.10^{a} ± 0.03	6.17^{ab} ± 0.03	6.25^{b} ± 0.03	0.009
pH_{24h}	5.65 ± 0.08	5.61 ± 0.08	5.81 ± 0.08	0.16
CIE L* (lightness)	51.5 ± 0.41	51.5 ± 0.41	50.2 ± 0.41	0.04
CIE a* (redness)	12.1 ± 0.35	12.2 ± 0.35	12.8 ± 0.36	0.34
CIE b* (yellowness)	11.7 ± 0.69	12.0 ± 0.69	12.4 ± 0.69	0.75
Drip $Loss_{24h}$, %	0.52 ± 0.12	0.57 ± 0.12	0.59 ± 0.12	0.92
Drip $Loss_{48h}$, %	0.92 ± 0.16	0.94 ± 0.16	0.98 ± 0.17	0.97
Cook Loss, %	11.8 ± 0.32	12.5 ± 0.32	11.5 ± 0.32	0.07
Shear Force, N	17.7^{a} ± 1.11	18.3^{ab} ± 1.13	21.6^{b} ± 1.13	0.03

* Mean and standard error of measurements; CON=control, HS=heat stress, IC=immune challenged.

[ab] Values in the same line with the same letters are not significantly different from one another at the 0.05 level.

Figure 2.1.

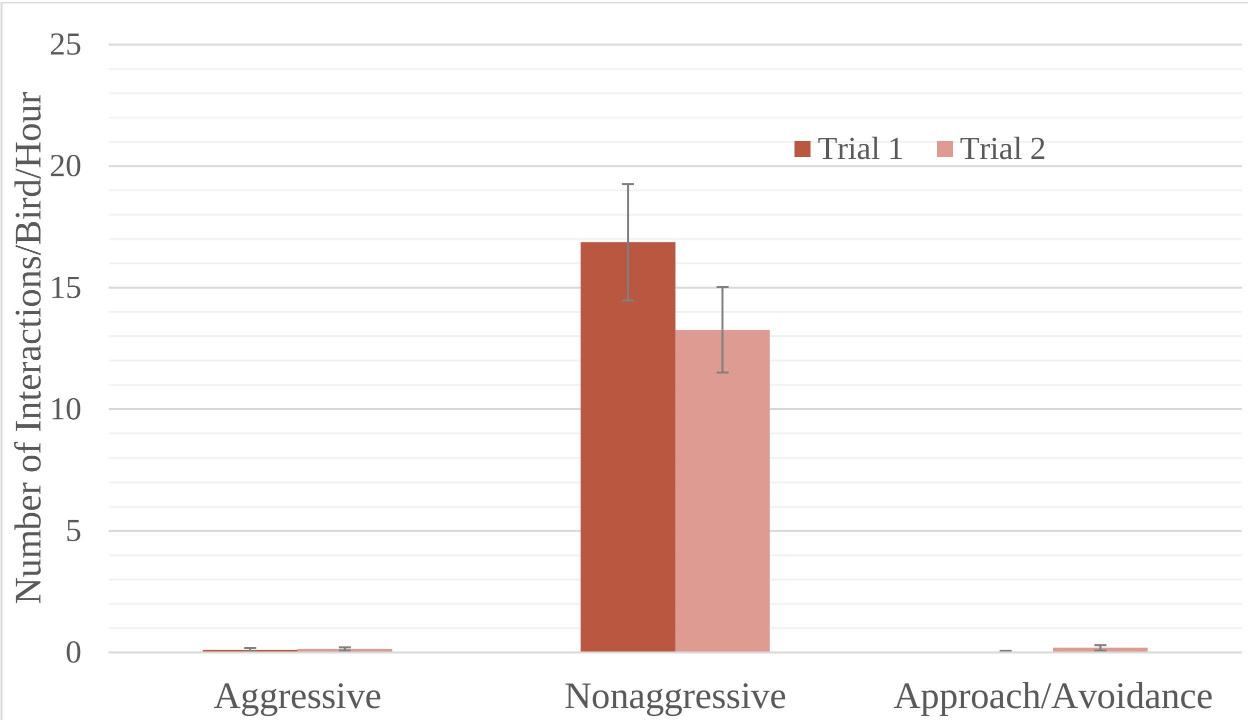

Figure 2.1. Descriptive bar graph of turkey behaviors categorized into aggressive, nonaggressive, and approach/avoidance interactions per bird, per hour for trials 1 and 2.

Figure 2.2.

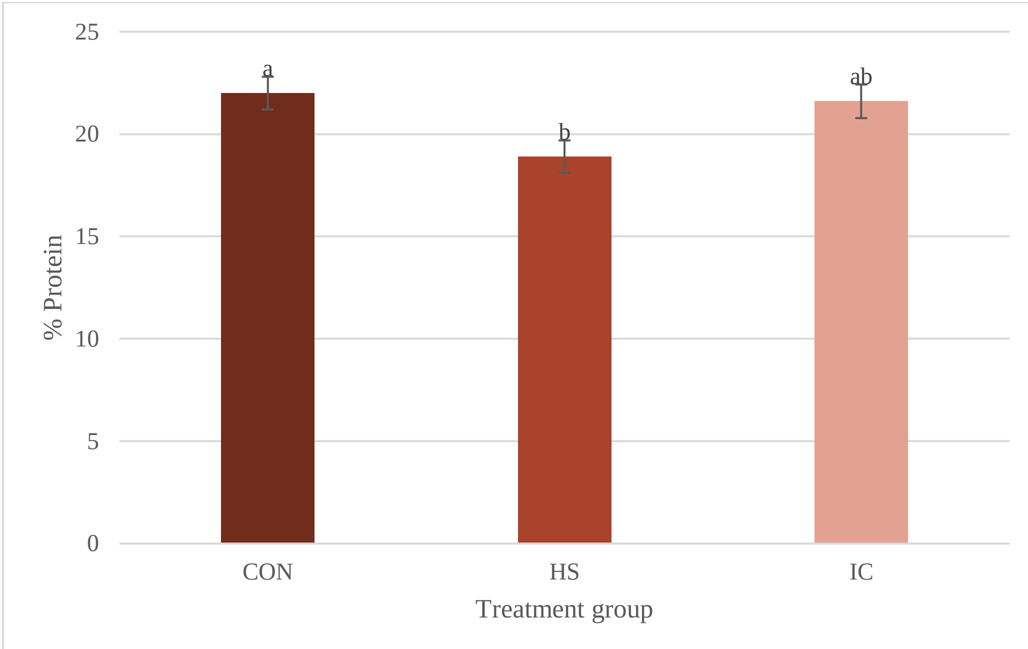

Figure 2.2. Percent protein in turkey breast meat samples from control (CON), heat stress (HS) and immune challenge (IC) treatment groups. Means reported as least square means ± SE.

[a,b] Different letters indicate significant difference between treatment groups (P < 0.05)

Figure 2.3.

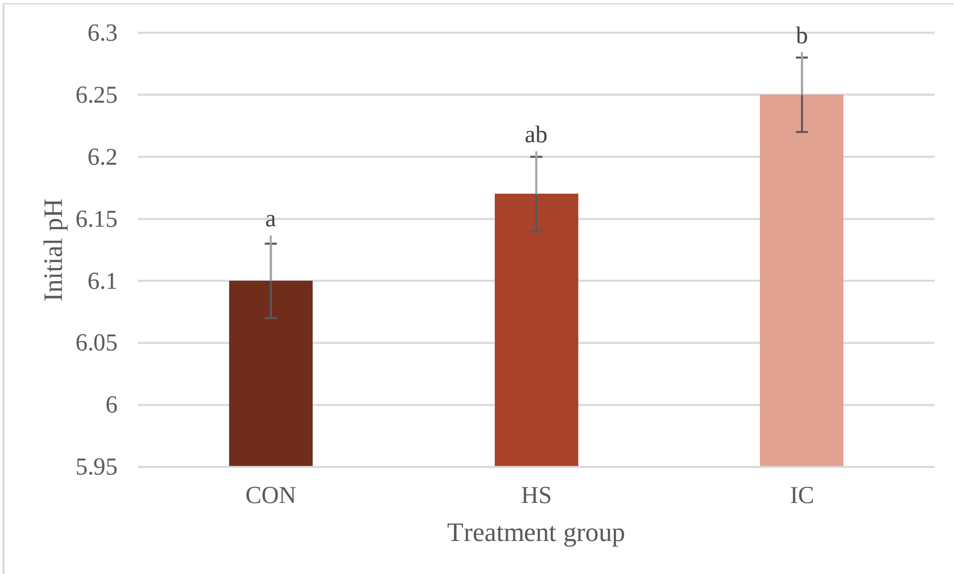

Figure 2.3.Initial pH values of turkey breast muscle approximately 20 minutes post-mortem from control (CON), heat stress (HS) and immune challenge (IC) treatment groups. Means reported as least square means ± SE.

[a,b] Different letters indicate significant difference between treatment groups ($P < 0.05$).

Figure 2.4.

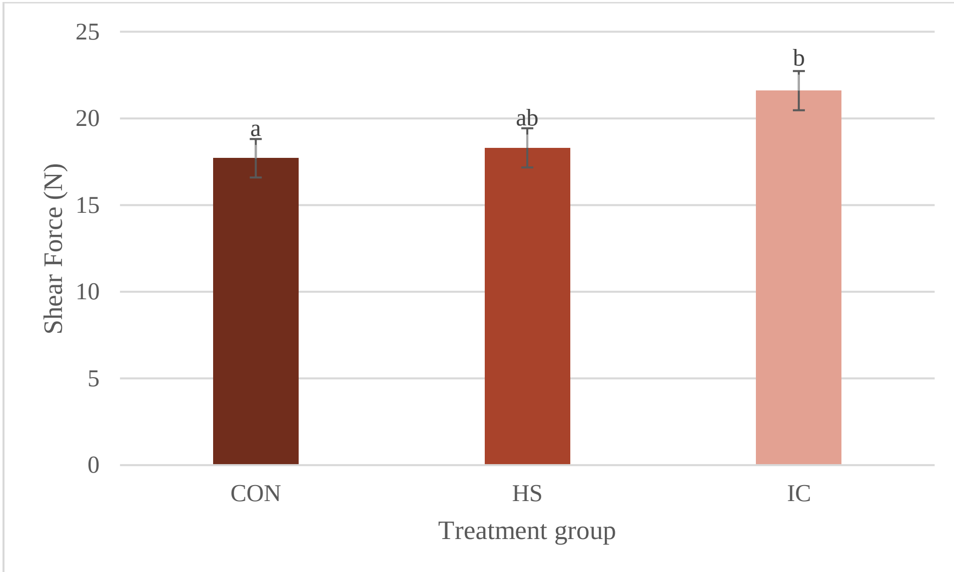

Figure 2.4. Shear force (N) values of cooked turkey meat samples from control (CON), heat stress (HS) and immune challenge (IC) treatment groups. Means reported as least square means ± SE.

[a,b] Different letters indicate significant difference between treatment groups (*P* < 0.05).

Table 2.7. Meat Quality Measurements (Mean ± SE) of Turkeys Divided into Clusters Based on Nonaggressive Pecking Frequency

	Cluster			
Trait	1	2	3	*P*-value
Carcass traits				
Feather Retention Force, N	24.6 ± 2.22	21.6 ± 2.29	21.6 ± 2.24	0.17
Hot Carcass Weight, lb	18.5 ± 0.21	19.0 ± 0.24	19.0 ± 0.22	0.13
pH, color, water-holding capacity and shear force				
pH_{20min}	6.19 ± 0.02	6.17 ± 0.02	6.18 ± 0.02	0.61
pH_{24h}	5.72 ± 0.04	5.70 ± 0.04	5.72 ± 0.04	0.63
CIE L*	50.9 ± 0.30	50.9 ± 0.33	51.0 ± 0.31	0.93
CIE a*	12.3 ± 0.28	12.5 ± 0.29	12.3 ± 0.28	0.65
CIE b*	11.8 ± 0.44	12.0 ± 0.45	12.1 ± 0.45	0.42
Drip Loss, g	0.35 ± 0.03	0.39 ± 0.04	0.35 ± 0.03	0.65
Cook Loss, g	17.5 ± 0.73	18.0 ± 0.80	17.7 ± 0.75	0.90
Shear Force, N	18.9 ± 1.12	20.9 ± 1.24	18.3 ± 1.16	0.31

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