ANALYSIS OF WALKING ACTIVITY AS A NON-INVASIVE MEASURE OF TURKEY WELL-BEING

by

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For my dear friends and family

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ABSTRACT

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Animal behavior observation is a widely used method of detecting when animals are ill or injured, but there are limitations to using behavioral observations. Behavioral observations can be labor-intensive, subjective and unreliable. The development of technologies such as accelerometers, which record acceleration and activity-based data in 3D space, enables faster, more accurate and quantitative methods of detecting changes in animal behavior. Previous research has demonstrated the utility of using accelerometers to detect changes in animals' health and well-being. However, limited information is available on the use of accelerometers to detect changes in behavior due to heat stress, which is a major poultry welfare concern, or to detect changes in activity levels of turkeys. The overall objective of this study was to determine whether micro-acceleration data loggers (accelerometers) can be used to detect changes in turkeys' activity levels and to identify changes in turkey behavior that are indicative of changes in turkey well-being. Two trials were conducted. Specific objectives for Trial 1 were to: 1) determine the effects of accelerometers and habituation to accelerometers on turkey gait and welfare, 2) determine age-related changes in gait and welfare, and 3) evaluate the validity of the accelerometers. Thirty-six male commercial turkeys were randomly assigned to one of five groups: accelerometer and habituation period (AH), accelerometer and no habituation (AN), VetRap bandage (no accelerometer) and habituation (VH), bandage (no accelerometer) and no habituation (VN), and nothing on either leg (C). Welfare was assessed prior to video-recording

birds as they walked across a Tekscan® pressure pad at 8, 12 and 16 wk to determine effects of treatment on number of steps, cadence, gait time, gait distance, gait velocity, impulse, gait cycle time, maximum force, peak vertical pressure, single support time, contact time, step length, step time, step velocity, stride length, total double support time, and duty factor. Accelerometer validity and reliability were determined by comparing the number of steps detected with the accelerometer to the number of steps determined from video recordings. Several age-related changes in turkey gait were found regardless of habituation, including a slower cadence at 16 wk, shorter gait distance at 8 wk, and slower gait velocity at 16wk. Habituation to the accelerometer and bandage had limited effects on turkey gait: non-habituated turkeys (VN and AN) spent more time standing on two feet (total double support time) compared to C birds, but did not differ from habituated (VH and AH) birds. Accelerometer validity and reliability were affected by both age and treatment. Validity and reliability were lowest for non-habituated birds (AN). Precision and sensitivity of accelerometers decreased with age but were unaffected by treatment. False discovery rate increased, and accuracy and specificity decreased with age. Results demonstrated that micro-data loggers do not adversely affect turkey welfare, but habituation to wearing accelerometers affects accelerometer reliability and validity. Accelerometer validity and turkey gait are also greatly affected by the age of the turkeys.

The second experiment used the validated accelerometers to assess changes in walking activity when turkeys were under an immune challenge or mild heat stress. Another objective of Experiment 2 was to identify changes in welfare and behavior associated with mild heat stress and a mild immune challenge. A total of 92 tom turkeys (trial 1: 51 turkeys; trial 2: 41 turkeys) were assigned to 3 different treatments in a crossover design: control (C; no heat stress or immune challenge), heat stress (HS), and immune challenge (IC). HS treatment was induced by slowly heating rooms to a peak temperature before slowly returning the rooms to normal temperatures. IC treatment was induced by a live-virus hemorrhagic enteritis vaccine which was added to the drinking water. Video (walking, sitting, standing, eating, drinking, preening, feather pecking, aggression and heat-stress related behavior) and accelerometer (steps/hr) data were recorded for 5 days at 10, 12, and 14 wk of age in order to gather behavior and walking activity data pre and post treatment, which occurred on day 3 of the 5 day period. Steps/hr decreased with age, treatments HS and IC had lower step counts compared to control groups, and each day proved to have a different step count regardless of whether a treatment was imposed. On the day of the imposed treatments, steps/hr were lower for both HS and IC turkeys. Welfare analysis indicated that tail and wing feather condition was worse at 14 wk compared to 10 and 12 wk. Behaviorally, the amount of time spent sitting increased as birds aged. Treatment also affected behavior: HS and IC turkeys performed less standing and walking compared to C birds on the day of the imposed treatment. Turkeys under a heat stress treatment performed more aggressive interactions and were observed performing heat-stress related behavior, including panting and sitting with their wings spread apart.

Results from both trials indicated that accelerometers can be useful tools to assess walking activity of turkeys and that accelerometers have the potential to detect changes in behavior that may be associated with conditions that negatively impact turkey welfare. The process of wearing an accelerometer on the leg did not adversely affect turkey welfare, but habituation is important to ensure that accelerometers are accurately and reliably recording turkeys' steps. In addition, it was determined that changes in behavior, such as decreased walking and standing, can be indicative of potential welfare issues, such as heat stress and an immune challenge in turkeys. Further research is needed to explore the best step threshold for particular turkey ages in order to get the most accurate data in future analysis of walking activity. Furthermore, sex differences were not a factor in these studies as only male turkeys were used which may differ from females in terms of gait and behavior under heat stress and an immune challenge. It may also be beneficial to further explore turkey gait as there were discrepancies in the literature and this study concerning age related differences in gait (duty factor). Future research should focus on the early detection potential accelerometers can provide to the turkey industry for welfare concerns. As shown by our results, walking activity decreases under both a mild heat stress and immune challenge, so future studies should now determine if this decrease in activity level is detectable before overt visual behavioral signs. If accelerometers can detect signs of stress more objectively, accurately, and quicker than visual inspection, then both farmers and researchers could benefit from utilizing these devices to improve animal welfare in the future.

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

Between 1985 and 2015, the amount of turkey meat consumed in the United States has doubled from an estimated 2.8 million pounds of turkey to 5.6 million pounds, respectively (USDA 2015, 1985). With annual poultry meat consumption rising, an increased demand for more product challenged the turkey industry to maximize its efficiency from both a farm management level and a biological level. Today's domesticated commercial turkey is a product of extensive selective breeding, which ultimately created a bird more suited to this increased demand with selected traits for higher growth rates and lower feed intake (Havenstein et al., 2007). Farming facilities have changed over the course of several decades as well. According to the Census of Agriculture (2012), the average farm size continues to increase while the overall number of farms in the United States continues to decrease. It is now common for farms to house hundreds to thousands of turkeys together. Such high flock sizes pose complications from an animal welfare standpoint, and selective breeding has also consequently created issues. Some disadvantages of selectively breeding turkeys for greater efficiency include immune deficiencies and the inability to mate naturally (Rauw et al., 1998). Furthermore, high efficiency management practices such as increasing stocking densities (the number of animals per square foot) can also negatively affect the overall wellbeing of the animals if not managed appropriately. Modern farmers are faced with the challenge of assessing health and welfare in large flocks of turkeys.

An animal's behavioral and physiological responses to stress are often the indicators used to assess animal welfare on farms. However, having farmers use these indicators to assess welfare can lead to visual oversight at high stocking densities and the inability to continuously assess welfare throughout the day and night (Weary et al., 2009). Technology may be a solution to provide a more precise and dependable way to detect behavioral changes in response to stress faster than what is possible by visual inspection. In order to apply practical technologies to farms, such as online monitoring of animals, a management practice known as precision livestock farming has become an emerging advancement in agriculture. Berckmans (2014) defines the purpose of precision livestock farming as “creat(ing) a management system based on continuous automatic real-time monitoring and control of production/reproduction, animal health and welfare, and the environmental impact of livestock production”. From an animal welfare standpoint, precision livestock farming can utilize technology that can be tailored to detecting behavioral changes in response to stress and monitoring environmental factors in barns. Precision livestock farming brings emphases to the importance of continuous monitoring of animals, and technology such as video cameras, microphones, and accelerometers can provide earlier detection of stress as opposed to having to wait for farm personnel to visually inspect during certain working hours of the day (Berckmans et al., 2014). For example, acoustic technology has been used to accurately detect vocalizations associated with heat stress in pigs, and accelerometers can monitor lowered activity levels in chickens for early detection of avian flu (Okada et al., 2010; Ferrari et al., 2013). Most research regarding technology to assess animal behavior has been conducted with chickens and pigs, and not much research has been directed towards the turkey industry despite welfare concerns associated with increased disease susceptibility and heat stress (Deeb, 2002; Rauw et al., 1998). Behavioral signs associated with heat stress and immune stress in turkeys potentially can be detected via technologies but has yet to be researched. The aim of this research project was to study the behavior and wellbeing of turkeys under heat stress and immune stress conditions and micro-accelerometer technology used to detect it as an alternative to visual inspection. Therefore, this literature review will examine common welfare concerns, behavioral responses and physiological responses associated with heat stress and immune stress in poultry. Then, an overview of activity level and how it could be used as an indicator of animal welfare; more specifically, how activity levels are affected by heat stress and immune stress. Finally, there will be a review of the technology currently available to detect activity levels, and how technology can be used to detect welfare concerns.

1.2. Defining Welfare and Stress

Defining the terms ‘welfare’ and ‘stress’ proves to be difficult due to the existence of multiple definitions with each slightly deviating from the other. For this study, stress was defined as “stimulation beyond the capacity for complete adaptation” (Broom and Johnson, 1993) and welfare as “its (an individual’s) state as regards to its attempts to cope with its environment” (Broom, 1986). Stress can also encompass either positive or negative aspects – also referred to as eustress and distress – but for the purposes of this review distress will be used the terms stress and distress interchangeably. Stress and animal welfare often go hand in hand when discussing one or the other. Although animal welfare is difficult to define and hence to measure, stress is a component of welfare that can be measured. Regarding the definitions of stress and animal welfare, when an animal is experiencing a stimulus beyond adaptation (stress), it therefore cannot successfully cope with its environment, and its welfare is negatively affected (animal welfare).

Animal welfare can be thought of in terms of affective state, health and biological functioning, and natural behaviors (Fraser et al., 1997). Ideally, animals will be subject to the Five Freedoms of animal welfare under which each animal will fulfill Fraser et al.’s (1997) “three circle model” for a good quality of life. The Five Freedoms are a widely-used reference to animal welfare which lists five conditions an animal should experience to have an acceptable level of welfare: freedom from hunger or thirst, freedom from discomfort, freedom from pain, freedom to express normal behavior, and freedom from fear (FAWC, 1979). In cases of deviation from any of the Five Freedoms, it is crucial to detect welfare issues as soon as possible to prevent unnecessary stress and/or suffering.

Stress is divided into two categories: acute stress and prolonged/chronic stress. Acute stress and chronic stress can be thought of as short-term and long-term stress respectively (Carroll and Sanchez, 2013). For example, a turkey that is exposed to high temperatures above its thermal neutral zone while being transported to a processing plant would experience acute heat stress; a turkey raised in a room with daily exposure to high temperatures above its thermal neutral zone would experience prolonged or chronic heat stress. Both acute and prolonged stress can cause negative responses as an animal undergoes changes to try to maintain homeostasis. In addition to the duration of stress, there are also many different types or sources of stress. In poultry, some common types of stress involve temperature (heat or cold stress), transportation, noise, feed restriction, restraint, disease, and social stress (Chen et al., 2015). Behavioral changes in response to stress occur quickly and are often the animal's first line of defense in response to a stressor. If certain behaviors are associated with particular stressors, then this knowledge can be utilized and theoretically detected by technology to detect stress non-invasively.

Physiologically, when an animal undergoes stress, the reaction experienced by the animal can be both helpful and hurtful. Hans Selyes first defined a general physiological response to stress as general adaptation syndrome (Selyes, 1936). According to Seyles (1936), an animal undergoing stress would go through a three-step physiological process: an alarm response, a stage of resistance, and a stage of exhaustion. Though Seyles's general adaptation syndrome initially referred to mammals only, most of the responses can be observed in all vertebrates (Faber, 1964). The alarm reaction drew its inspiration from the fight or flight response which was first described in detail by W. B. Cannon (1915). Both the alarm reaction and the fight or flight response details a general response by the sympathetic nervous system secreting various hormones to prepare an animal for escape or for a fight (Cannon, 1915; Seyles, 1936). More specifically, the short-term reaction to stress is ultimately caused by the sympathetic adrenal medullary system (SAM) in comparison to the long-term stress reaction of the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is responsible for the secretion of cortisol (mammals) or corticosterone (birds and reptiles) in the blood. The next step after the alarm response is the stage of resistance. This stage involves the endocrine system in the long-term regulation of stress in the animal. The stage of exhaustion arises if the animal does not recover from the effects of the stressor and the availability of body reserves and hormones are inadequate. Fatigue will set in and potentially could lead to death (Freeman, 1987; Maxwell, 1993).

1.3 Heat Stress

It is estimated that between $128 and $165 million dollars are lost yearly in the poultry industry because of heat related issues (St-Pierre et al., 2003). This economic loss is due to the behavioral, physiological, and nutritional changes birds undergo when in heat stress (Table 1). For instance, chickens that experienced chronic heat stress produced meat that was pale in color, soft in texture, and had poor water holding capacity (PSE) (Hashizawa et al., 2013). In broiler chickens, a higher feed to gain ratio is commonly associated with heat stress, which contributes to more money being spent purchasing more feed. After slaughter, it was found that there was an overall lower nutritional value of the carcass when broilers were exposed to high ambient temperatures compared to those that were not (Tankson et al., 2001). In egg laying chickens, poor egg quality and lower semen quality has been associated with heat stress (Singh et al., 1968). With poultry production rates increasing in locations with warmer climates such as Central America and Africa, heat stress is a growing concern for turkeys as well as other poultry (Daghir, 2008). Moreover, genetic selection for high efficiency turkeys has consequentially made them more susceptible to heat stress (Rauw et al., 1998; Deeb, 2002). Heat stress is especially challenging in facilities that do not provide constant climate-controlled barns and transportation vehicles. With the combination of a higher susceptibility to heat stress and a projected increase in poultry production in warmer climates, turkey producers must be aware of the indicators of heat stress. It is important to be able to detect early signs of heat stress to implement interventions early to avoid unneeded suffering of animals and economic losses.

Stress was previously defined as “stimulation beyond the capacity for complete adaptation”; therefore, heat stress can be thought of as stimulation caused by high ambient temperatures that extends beyond the capacity for complete adaptation to achieve homeostasis (Broom and Johnson, 1993). Additionally, heat stress can also be defined in terms of thermal neutrality. For homoeothermic (ability to maintain stable internal body temperatures regardless of the environment) animals, such as the turkey, thermal neutrality is a range of temperatures that requires no extra metabolic function to maintain homeostasis (Hey, 1975). If high ambient temperatures exceed an animal’s thermal neutral zone, that animal would undergo signs of heat stress. Finding an animal’s thermal neutral zone is dependent on several factors that include species, age, humidity, individual variation in behavioral coping mechanisms, amount of direct sunlight the animal is exposed to, and the ventilation quality of the environment (St-Pierre et al., 2003). Often, behavioral and physiological cues can be used to find an estimated thermal neutral zone.

Birds maintain a stable internal body temperature by balancing the rate of heat production and dissipation. With no sweat glands, poultry must be able to adequately dissipate heat differently than mammals. Heat production is often associated with body weight and amount of feed consumed, while heat dissipation is associated with the environmental temperature (Silanikove, 2002; Daghir, 2008). The higher the environmental temperature, the less heat will dissipate from an individual. If more heat is being produced than dissipated, a negative internal balance will cause different behavioral and physiological responses in order to restore that balance (Lara and Rostagno, 2013). Furthermore, heat loss is maintained in animals via four different mechanisms: convection, conduction, evaporation, and radiation (Mustaf et al., 2009). In regard to poultry, evaporation is limited due to lack of sweat glands, so little heat loss in birds is achieved through this process (Dawson and Whittow, 2000; Wolfenson et al., 2001). Radiation will occur if the bird's environment is at a lower temperature than its skin and body (Shah et al. 2013). Radiation from certain body areas, such as the snood of a turkey, helps regulate the internal body temperature. Conduction is when two independent masses touch and heat is transferred from the warmer mass to the colder mass (Shah et al., 2013). Convection happens within a body system so that moving fluid transfers heat (Shah et al., 2013). All mechanisms of convection, conduction, evaporation, and radiation work together to maintain a state of internal homeostasis.

Because heat dissipation is affected by ambient temperatures, the body will try to compensate by increasing its metabolic rate, and therefore heat production. However, if ambient temperatures are too high, the increased metabolic activity will be dangerous to the body.

Behavioral responses in turkeys can increase heat loss to compensate for increased metabolic function (Daghir, 2008).

Physiological changes in response to heat stress

When poultry are under an environmental heat stress, a variety of physiological measures are activated to help keep themselves cool (Table 1). There are several hormones and proteins associated with heat stress that will initiate behavioral and physiological responses in the body. Many are secreted from the HPA axis in response to stressors. For example, corticosterone is an adrenal hormone that has been associated with increased levels during heat stress in poultry (Edens and Siegel, 1976; Nathan et al., 1976; Halawani et al., 1973). However, it seems there are different hormones associated with acute heat stress and chronic heat stress. In a study researching the relationship in corticosterone levels and heat stress, Edens (1978) discovered that chicks only initially had an increased corticosterone level when subjected to heat stress. Therefore, the corticosterone hormone was associated with acute heat stress only (Edens, 1978). Thyroid hormone is essential for temperature regulation in the body due to its effects on metabolic rate. In previous studies that increased the concentration of thyroid hormone in chickens, an increase in body temperature resulted as well (Ellen and Wentworth, 1958; Singh et al., 1968). Aside from different hormones, heat shock proteins also play a role in the body's response to heat stress. Heat shock proteins are upregulated in times of heat stress to prevent incorrect folding/denaturization of other proteins and cells in the body (Tankson et al., 2001).

Physiological changes are important to thermoregulation. As previously discussed, rate of evaporation is a priority in heat stress, and because poultry lack sweat glands, increased panting is performed in addition to heat dissipation in combs and wattles in chickens or snoods and caruncles in turkeys. However, increased panting also causes as decrease in carbon dioxide in the body which increases pH in the blood of chickens (Mustaf et al., 2009). Decreased levels of carbon dioxide and high blood pH are also known as respiratory alkalosis (Silanikove, 2002). Alkalosis is thought to be the cause of lameness, fever, and hyperventilation. However, research has provided mixed results on the instances of respiratory alkalosis found in heat stressed birds. For instance, Kohne and Jones, (1975a) found that birds undergoing acute heat stress developed alkalosis, but in another study of chronic heat stress, no instance of alkalosis was found (Kohne and Jonesm, 1975b). Another physiological response to heat stress is the increase in blood circulation around the comb, wattles, and feet of poultry (Whittow et al., 1964). The increased circulation helps the birds dissipate more heat from their bodies. The study of Whittow et al. (1964) also recorded an overall increase in both heart rate and respiratory rate in heat stressed birds.

Behavioral changes in response to heat stress

Heat stress can cause a variety of behavioral responses from poultry (Table 1). In response to heat stress, chickens exhibit decreased activity levels, increased water intake, decreased feed consumption, and increased panting behaviors compared to non-heat stressed chickens (Mack et al., 2013; Li et al., 2015). Higher water consumption and panting can increase an animal's evaporation rate, which aids with heat dissipation, as well as compensates for the increase in water loss (Mench, 1985; Daghir, 2008). Lowering activity level also lowers heat production due to energy expenditure, and decreased feed consumption similarly cuts down on energy expenditure during the digestive process (Daghir, 2008). A decreased social aspect in heat stressed chickens has also been observed, and these chickens displayed behavioral changes that included isolation and lying down with their wings slightly apart to increase surface area (Mench, 1985). Overall, poultry experiencing heat stress display behaviors such as gular flutter (panting while flapping membranes in the throat to increase evaporation), decreased activity level, decreased feed intake, social isolation, and spreading out their wings while lying down to minimize energy expenditure/heat production and maximize evaporation rates (Mench, 1985; Mack et al., 2013; Li et al., 2015).

Table 1. Common physiological, behavioral, and carcass quality changes in poultry as a result of heat stress

Common Physiological Responses to Heat Stress	Common Behavioral Responses to Heat Stress	Meat/Nutritional Changes in Response to Heat Stress
Increased risk of respiratory alikosis	Decreased activity level	PSE-like meat quality
Heat attack	Increased water intake	Lower egg quality
Increased heat rate	Decreased feed consumption	Lower carcass protein
Increased blood circulation	Panting / Gular flutter	Lower carcass weight
Increased respiratory rate	Lying down with wing spread apart	Lower caloric value in meat and eggs

1.4 Immune Stress

Stress can lower the immune response, making animals more susceptible to diseases. From hatch to slaughter, birds rely on their immune system to prevent the contraction of disease. Health may be defined as “an animal’s state as regards to its attempts to cope with pathology” (Broom, 2006). Four types of defensive barriers are involved in the innate immune response, including: anatomical barriers, physiological barriers, phagocytic barriers and inflammatory barriers (Goldsby et al., 2002). Anatomical barriers such as the skin and mucosal membranes prevent pathogens from entering the body. Temperature, pH, and a variety of cell-associated molecules such as lysozyme, interferon, and toll-like receptors all are considered physiological barriers which also contribute to innate immunity. The phagocytic barriers of the innate immune system include phagocytic cells, such as blood monocytes, macrophages and neutrophils. The inflammatory response is also one of the first responses of the immune system to infection or irritation, which include general symptoms such as redness, swelling, heat, pain and possible loss of function of the organs or tissues involved (Goldsby et al., 2002). The APR (acute phase response) is a series of reactions initiated in response to infection, trauma, stress, neoplasia, and inflammation. The APR is a core part of the early defense or innate immune system, which is characterized by a fever, leukocytosis as well as alterations in the metabolism of many organs (Baumann and Gauldie, 1994; Gabay and Kushner, 1999).

Although diseases have specific characteristic symptoms, animals respond to disease with a general response that can be detected through changes in behavior and physiology. In general, when a turkey becomes ill, it will exhibit a general immune response as it tries to cope with the specific disease. Sickness behavior can be thought of as a variety of behaviors associated with an animal that is unwell. During an infection, an animal may display deviations from normal behaviors while other diseases may induce unique behavioral symptoms (Hart, 1988). During all three stages of the general adaptation syndrome discussed earlier, behavioral traits can also be observed, such as fatigue or lower activity level related behaviors. Observing these general behavior responses alerts farmers of when an animal is unwell, and technology has the potential to detect these same responses. Although sickness behavior is beneficial to a sick animal, there are also negative welfare aspects that need to be considered. Altering normal behaviors may cause a sick animal to be targeted by pen-mates if not isolated. Detection of sickness behaviors may also be difficult due to high stocking density and social stress, as animals may hide their symptoms (Millman, 2007). Currently, there are several different methods used in order to research immune response and sickness behavior in poultry. In some studies, a flock is infected with the full live bacterium/virus; others use a vaccination of the live bacterium/virus, and some use LPS (lipopolysaccharide). LPS, a membrane glycolipid of Gram-negative bacteria, is often used to induce systemic inflammation and the acute phase response (APR) for research purposes. Administration of LPS to chickens can cause significant physiological and behavioral changes similar to a bacterial or viral infection (Zhang et al., 1995; Xie et al., 2000; Schaefer et al., 2006). When studying the effects of LPS, one is only observing an animal's response to a bacterial secretion rather than a bacterium itself. Therefore, this could potentially alter a genuine sickness response as the body is not reacting to a true bacterial infection, but rather a byproduct of an infection. It could also be argued that the behavioral changes of an LPS infection may not illicit the same response as an infectious disease. Mild sickness behavior can also be observed from a vaccination in response to the immune challenge. Live-virus vaccines have been shown to create short-term immune responses similar to an LPS general immune response (Roth, 1993). However, using vaccines to study sickness behavior also has several drawbacks. Often, vaccines do not cause an overt behavioral change which could be difficult to detect. There is also not much research detailing subsequent intensity and duration of the side effects.

Studies of changes in animal behavior and physiology have been instrumental in identifying specific changes in animal behavior that may be indicative of disease. The importance of early detection must be enforced when managing livestock; if technology can feasibly detect sickness symptoms accurately and quicker than human visual inspection, then animal welfare problems can be detected earlier and prevented.

1.5 Activity Levels and Accelerometers

As an aspect of sickness behavior, an animal's activity level or change in activity level can be used as a non-invasive indicator of animal welfare. Since animals in pain or stress normally decrease their activity, the use of accelerometer devices that measure movement and activity can be a way to detect welfare concerns. Accelerometers measure gravitational force along different axes and can therefore be used to monitor activity levels and various behaviors and postures, such as standing, walking and lying. These devices are beneficial research tools that can store acceleration data on an individual level. The long-term use of accelerometers could eventually associate certain output to certain behaviors, thus eliminating the need to analyze hours of video footage in some cases. Published research examining the use of accelerometers to identify changes associated with sickness, pain or stress in turkeys is scarce. Only one study (Dalton et al., 2016) has examined the use of accelerometers for detecting turkeys' activity levels, and the focus of this study was to validate accelerometers. However, research with other species, mainly cattle, has demonstrated the value of using accelerometers for identifying animals that are unwell. For example, the amount of time spent lying down is higher in cattle with respiratory disease compared to healthy cattle (reviewed in Theurer et al., 2013) and activity levels are lower and lying time is greater in lame vs. sound cattle (Thorup et al., 2015).

Other studies have examined the utility of accelerometers and body mounted sensors to assess activity levels of laying hens (Quwaider et al., 2010; Buijs et al., 2018; Casey-Trott and Widowski, 2018). Quwaider et al. (2010) used body-mounted sensors in a laying hen facility to accurately assess movement and location in the barn. By comparing video observation to the sensor location output, the technology was shown to have 84% accuracy (Quwaider et al., 2010) In terms of activity recognition and assessment, several transformation equations were proposed, but had yet to be validated in this study (Quwaider et al., 2010). Their accelerometer output (Quwaider et al., 2010) could distinguish between active and inactive birds but did not provide any further assessment of the data. Casey-Trott and Widowski (2018) performed a study both validating a commercially available accelerometer in laying hens and using the output to compare activity levels of laying hens with or without keel bone damage. This study classified behaviors as active (walk, forage, eat, drink, perch, dustbathe, preen, wing-flap) or inactive (sit, sleep, stand). The laying hens with severe keel bone damage spent more time in inactive states compared to slight keel bone damage and no damage. Buijs et al. (2018) researched the behavioral changes in laying hens after applying a body-mounted sensor to the hens' body. Eye temperature, behavioral analysis from video, and weight were compared between birds with and without the sensor continuously attached after 2, 3, 5, and 7 days (Buijs et al., 2018). Group related behavior was also assessed at each time point to account for behaviors of pen-mates targeting birds wearing the sensor (Bujis et al., 2018). It was found that no changes in body weight or aggressive behaviors, but increased preening and higher eye temperature were found in sensor birds (Buijs et al., 2018). Birds wearing a sensor were observed pecking at the sensor during each day (2, 3, 5, and 7), but no other negative effects were documented (Bujis et al., 2018).

1.6 Research Gap

Accelerometers have become more efficient and advanced compared to older models used in animal research. Dalton et al. (2016) previously validated the use of HOBO Pendant® data loggers to detect step counts in turkeys, but the accelerometer model was only able to record for approximately 54 min. The limited battery capacity and memory limited the practicality of measuring activity level changes for turkeys. The size and weight of the HOBO Pendant® data loggers (18 g in weight and a size of 58(h) x 33(l) x 28(w) mm) further limited the use on smaller or younger animals. AXY-3 micro-acceleration data loggers are smaller (9.5 (l) x 15 (h) x 4 (w) mm) and lighter (0.7g) alternatives to the HOBO Pendant® loggers. Micro-acceleration data loggers can record for multiple days at a time, thus recording more activity related data to analyze. However, no studies had explored to use of micro accelerometers as a way to detect activity level changes in turkeys. Furthermore, no research had been conducted on any welfare implications of turkeys wearing an accelerometer. Heat stress and disease are major welfare concerns in the turkey industry, yet no research had been conducted regarding behavioral analysis in response to those stressors. Sickness behavior had been researched in ducks wearing accelerometers to detect lower activity levels caused by an immune challenge (Campbell et al., 2015), but no such study has been explored for turkeys.

1.7 Summary

Modern poultry farming facilities must stay on top of disease prevention on site. The two main methods the poultry industry uses to prevent disease in flocks is through biosecurity and vaccination programs (Pattison, 2008). Heat stress has been linked to a lowered immune response, and chronic high temperatures can create a breeding ground for bacteria and parasites, making disease an even more pressing issue. With poultry meat consumption on the rise, especially in warmer climates where heat stress is a concern, it is increasingly important to detect signs of heat stress and immune stress before animal welfare is compromised. Early detection of these issues can mean the difference between treating one animal for a disease vs treating the entire flock. With the development of new and emerging technologies, it is now possible to detect subtle changes in animal behavior earlier than what had been possible.

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CHAPTER 2. VALIDITY OF MICRO-DATA LOGGERS TO DETERMINE WALKING ACTIVITY OF TURKEYS AND EFFECTS ON TURKEY GAIT

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2.1 Abstract

Accelerometers have the potential to provide objective, non-invasive methods for detecting changes in animal behavior and health. The objectives of this chapter were to: 1) determine the effects of micro-acceleration data loggers (accelerometers) and habituation to accelerometers on turkey gait and welfare, 2) determine age-related changes in gait and welfare, and 3) assess the validity and reliability of the accelerometers. Forty-four male commercial turkeys were randomly assigned to one of five groups: accelerometer and habituation period (AH), accelerometer and no habituation (AN), VetRap bandage (no accelerometer) and habituation (VH), bandage (no accelerometer) and no habituation (VN), and nothing on either leg (C). Turkey welfare was assessed prior to video-recording birds as they walked across a Tekscan® pressure pad at 8, 12 and 16 wk to determine effects of treatment on number of steps, cadence, gait time, gait distance, gait velocity, impulse, gait cycle time, maximum force, peak vertical pressure, single support time, contact time, step length, step time, step velocity, stride length, total double support time, and duty factor. Accelerometer validity and reliability were determined by comparing the number of steps detected with the accelerometer to the number of steps determined from video recordings. Several age-related changes in turkey gait were found regardless of habituation, including a slower cadence at 16 wk, shorter gait distance at 8 wk, and slower gait velocity at 16wk. When comparing bandaged vs. unbandaged limbs, both treatment

and age treatment interactions were reported depending on the gait parameter. Accelerometer validity and reliability were affected by both age and treatment. Precision and sensitivity of accelerometers decreased with age but were unaffected by treatment. False discovery rate increased, and accuracy and specificity decreased with age. Validity and reliability were lowest for non-habituated birds (AN). Results demonstrated that micro-data loggers do not adversely affect turkey welfare, but habituation to wearing accelerometers greatly affects accelerometer reliability and validity. Accelerometer validity and turkey gait are also greatly affected by the age of the turkeys.

2.2 Introduction

The use of wearable sensors is an emerging area of research in the animal behavior and welfare field. The ability to take objective, non-invasive measurements of the behavior of an animal is advantageous compared to visual inspection, which is most frequently used to evaluate animal behavior and welfare. However, on large farms where thousands of birds are housed together, visual inspection can potentially lead to oversight because difficulties could arise when inspecting individual animals housed at high stocking densities. Moreover, visual inspection can only identify an issue after welfare has already been compromised (Dawkins, 2004; Weary et al., 2017).

Wearable sensors have been applied to a variety of species for automated monitoring of animal health and behavior in research. Wearable sensors can record a variety of measures such as internal body temperature, environmental temperature, acceleration, heart rate, and step counts depending on the type used and where on the body it is placed (Neethirajan, 2017). In particular, accelerometers can be a tool to define and record energy expenditure (Lachica and Aguilera, 2005; Miwa et al., 2015), posture (Ito and Keyserlingk, 2009), and locomotor levels

(Ringgenberg et al., 2010; Bloomberg, 2011) that can be early indicators of welfare concerns. Furthermore, acceleration output from activity level sensors can be used to distinguish between different behaviors displayed by an animal. For example, accelerometers attached to a collar can accurately distinguish between grazing, ruminating, and resting behaviors of sheep (Giovanetti et al., 2017). Attached to the leg, accelerometers show potential as an early indicator of lameness in laying hens (Kozak et al., 2016), sheep (Barwick et al., 2018), dairy cattle (Higginson et al., 2010; Beer et al., 2016), and horses (Keegan et al., 2004). Behaviors associated with health status are also detectable using accelerometers or bio-loggers, such as in Pekin ducks to detect lethargy caused by an immune challenge (Marais et al., 2013) and in laying hens as an early detector of avian influenza by assessing decreasing activity levels (Okada et al., 2010). Based on the aforementioned research, accelerometers are useful tools for the automatic, non-invasive monitoring of animal behavior, but limited research has been conducted to evaluate the use of accelerometers to monitor the behavior and welfare of turkeys. One study evaluated the validity and feasibility of using HOBO Pendant® (HPD) loggers for detecting steps of grower turkeys (9-11 wk of age) and finisher turkeys (14 wk) (Dalton et al., 2016a). Their results indicated that HPD loggers are capable of detecting step counts in turkeys. However, the HPD loggers are large (18 g in weight and a size of 58(h) x 33(l) x 28(w) mm), making them cumbersome for measuring activity levels of young turkeys and therefore not suitable for detecting long-term changes in activity levels of growing turkeys. Furthermore, the HPD loggers were only able to record continuously for 54 min. The study observing lethargy in Pekin ducks used a similarly sized sensor (Actical, Mini-Mitter, River Bend, OR, USA) at 17.5g (Marais et al., 2013) while the laying hen study used a prototype accelerometer that is not commercially available (Okada et al., 2010). With recent advances in technology, it has become possible to use micro-data loggers that are much smaller, lighter, and that have a longer memory and battery capacity, enabling changes in animals' activity levels and number of steps taken to be recorded for longer periods of time and for smaller animals. However, no studies have evaluated the feasibility and reliability of using micro-data loggers for measuring activity levels of turkeys.

In addition to the size and weight of the accelerometer, the effect of the accelerometer on animal behavior is another important consideration. The presence of the accelerometer itself can cause changes in an animal's behavior. Habituation is an important concept when introducing novel technology to an animal (Jones, 1996; Stadig et al., 2018). Introducing a novel object can cause fear and affect the validity and reliability of a study (Jones, 1996). Furthermore, the presence of a sensor may cause the animal wearing that sensor to be targeted by pen-mates, leading to further changes in typical behavior and have potential effects on animal welfare. Due to the unfamiliar feeling of wearing an accelerometer, birds may favor the leg with an accelerometer, applying less body weight on that foot (potentially affecting normal walking gait or discourage walking). Video camera footage of broiler chickens processed through a movement-related algorithm have shown potential for detecting bird movement (Aydin, 2017). In many situations, image sensors can be more practical than accelerometers; however, accelerometers have the potential to detect certain aspects image sensors currently cannot. Overhead cameras can only detect movements from the top of the bird as the legs will not be seen in the camera's vision, while accelerometers can be attached to certain body parts of birds to target certain aspects of acceleration.

Age related changes in turkey gait, although not heavily researched, should be expected regardless of the effect of wearing an accelerometer. A few studies have documented a decrease in overall leg and footpad health in turkeys as they age, and gait was expected to change with age

(Martrenchar et al., 1999; Krautwald et al., 2011; Dalton et al., 2016b). Krautwald-Junghanns et al. (2011) observed increasing severity in footpad dermatitis and lesions from 6 wk to 16 wk of age (Krautwald et al., 2011). Dalton et al. (2016b) observed a worsening gait score as birds aged, but there has been no further research into the gait dynamics of these worsening scores. Turkeys have gone through extensive selective breeding in order to generate a fast-growing bird with a large breast muscle. These changes in body conformation can have effects on how turkeys walk, but limited research has investigated changes in turkey gait. Recently, Kremer et al. (2018) demonstrated that as female turkeys age, certain gait parameters such gait velocity, peak vertical force, and step length increased with age, while other parameters such as gait cycle time were not affected. Similarly, Oviedo-Rondón et. al (2018) demonstrated that in male turkeys, certain gait dynamics change due to leg health and age. Step length was longer in birds without leg abnormalities, peak vertical force and impulse increased as a bird aged, and bipedal cycle time was affected by both leg health and age. These age-related studies indicate that both age and leg health play a crucial role in turkey gait dynamics, so introducing an unfamiliar accelerometer on the leg may further complicate how a bird walks.

The overall goal of this research was to evaluate the feasibility of using micro acceleration data loggers (accelerometers) for detecting steps and changes in activity levels of turkeys at different ages. Specific objectives included: 1) determining the effects of accelerometers and habituation to accelerometers on turkey gait, 2) determining age-related changes in gait, and 3) assessing the validity and reliability of the accelerometers.

2.3 Materials and Methods

2.3.1 Experimental Procedures

This study was carried out in accordance with the recommendations and approval of the Institutional Animal Care and Use Committee of Purdue University.

A total of 44 beak-trimmed tom turkeys (Nicholas Select, Aviagen Turkeys, Lewisburg, West Virginia) were obtained from a commercial hatchery at 1 d of age and housed at the Purdue Animal Sciences Research and Education Center (ASREC). From 1 d to 7 d of age, the poults were housed together in a brooding ring, and then randomly assigned to 8 littered (wood shavings) pens (measuring 2.44 m by 1.52 m) with either 5 or 6 birds per pen. Each pen was supplied with a hanging feeder and bell drinker, providing feed and water ad libitum. Lighting and temperature were maintained according to Aviagen-recommended industry standards (Aviagen Turkeys Inc., 2015). For the first day, poults were provided with 24 h of light, which was gradually adjusted to a final photoperiod of 15 h light: 9 h of darkness by the fourth day. A minimum light intensity of 40 lux was provided. Room temperature was changed weekly as recommended by Aviagen (Aviagen, 2015). Briefly, poults were brooded at a temperature of 30° C, which was gradually decreased to a final temperature of 13°C at 14 wk.

Birds were randomly assigned to one of five groups. Groups differed depending on whether they were habituated to wearing a VetRap^{TM} bandage (with or without an accelerometer) for one week prior to data collection:

Habituated groups (H):

1) AH group: habituated to wearing both a bandage and an accelerometer. Habituation occurred for 1 wk prior to each data collection at 8, 12 and 16 wk (n=7).

2) VH group: habituated to wearing only a bandage. The accelerometer was attached only while data were collected on the Tekscan pressure sensing walkway at 8, 12 and 16 wk. Habituation occurred for 1 wk prior to each data collection at 8, 12 and 16 wk (n=8).

Un-habituated groups (NH):

3) AN group: the bandage and accelerometer were attached only when data were collected on the Tekscan pressure sensing walkway at 8, 12 and 16 wk. No habituation occurred (n=4).
4) VN group: the bandage was attached only when data were collected on the Tekscan pressure sensing walkway at 8, 12 and 16 wk. No accelerometer was attached, and no habituation occurred (n=10).

Control group (C):

5) C group: no bandage or accelerometer were attached at any time during the study (n=6). No habituation occurred.

Sample sizes varied due to the number of birds in each pen and due to mortality of 8 birds over the course of the study. Two turkeys were found dead at 8 d, one at 10 d and one at 14 d, before data collection had started. One turkey from the control (C) group was found dead at 10 wk. One turkey (AN group) was euthanized at 16 wk due to a broken wing. Two turkeys were euthanized due to lameness at 13 (AN group) and 14 (AH group) wk, respectively. The two lame birds' gait data were not used in the analysis of the study at 12 wk. Only complete data sets from 36 birds were used in the final analyses.

In order to attach an accelerometer to the turkey's leg, the accelerometer (AXY-3 Micro Acceleration Data Loggers, TechnoSmArt, Guidonia-Montecelio, Italy) was sealed between two pieces of VetRap bandage, and then secured around the bird’s leg with more bandage. The accelerometer was placed just above the hock, facing outward with the connector pointed towards the ground and the battery in contact with the leg (Figures 1 and 2).

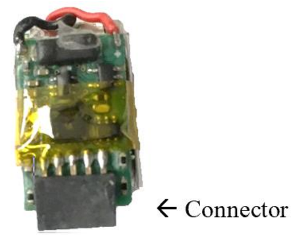

Figure 1: Orientation of the AXY-3 micro accelerometer.

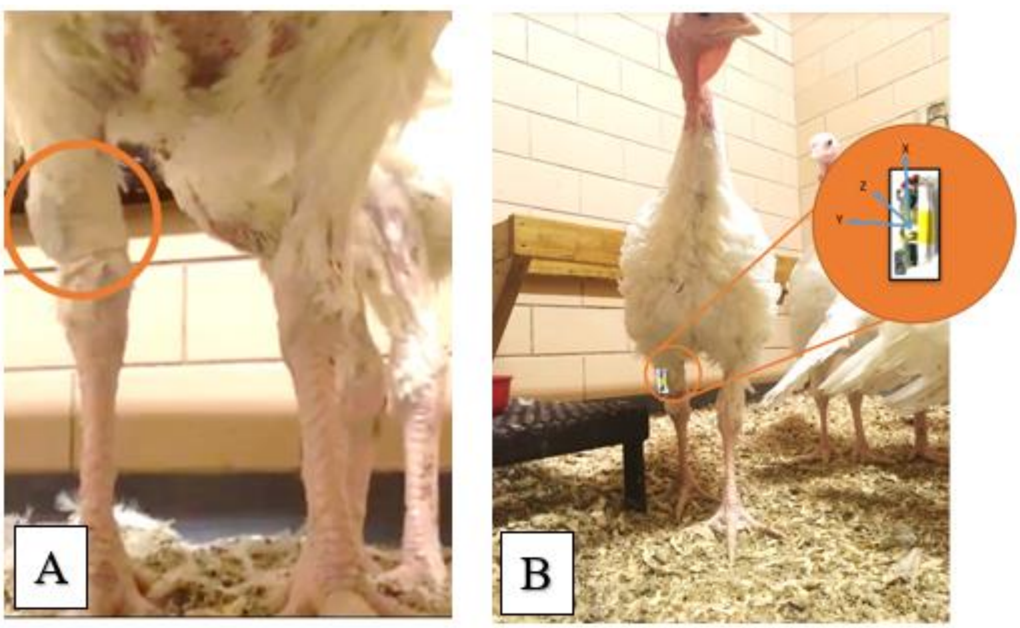

Figure 2: (A) Location of AXY-3 micro accelerometer attached to the leg of the turkey. (B) AXY-3 micro accelerometer axis orientation and placement on each bird.

Accelerometer attachment was balanced for left and right legs across treatment groups so that approximately half of the birds wore the applied treatment on one leg while to other half had the treatment on the other leg. A total of 10 accelerometers were used and set to record at a frequency of 10 Hz. AXY-3 accelerometers had the potential to record for up to 30 days on a single charged battery (TechnoSmArt, 2011). The accelerometers recorded acceleration measurements in 3 planes (X, Y, Z). The accelerometer dimensions are 9.5 (l) x 15 (h) x 4 (w) mm and weight is 0.7g.

This study examined the effects of the accelerometer on turkey gait parameters at 8, 12, and 16 wk using a Tekscan® pressure sensing walkway (Tekscan Inc, South Boston, MA) and by analyzing video recordings of turkeys as they walked across the Tekscan. Some Tekscan gait parameters are calculated by the Tekscan software using both limbs while other measures are calculated for each limb (Table 1). Gait parameters were selected for analysis based on previous leg heath studies with the Tekscan system in turkey hens (Kremer et al., 2018) and Pekin ducks (Campbell et al., 2015).

Table 2. Description of measurements analyzed on the Tekscan pressure sensing walkway (adapted from Tekscan Walkway^{TM} User Manual, 2011) and duty factor (determined using methods of Gatesy and Biewener, 1991; Paxton et al., 2013; Oviedo-Rondón et al. 2018).

Tekscan measure	Definition
Step	The point when all toes of one foot were off the ground while the footpad of the other foot remained in contact with the ground
Cadence (steps/min)	Number of steps taken per minute
Gait time (s)	Time from first contact with the walkway to the last contact with the walkway
Gait distance (cm)	The distance from the heel of the first stance to the heel of the last stance
Gait velocity (cm/s)	Gait distance divided by gait time
Impulse (%)	Amount of force exerted on the walkway over the entire walk (as a percentage of body weight)
Gait cycle time (s)	Average time from the first contact of a foot to the next contact of the same foot

Maximum force (% BW)	Maximum amount of pressure exerted onto the walkway as a percentage of the subject's body weight
Peak pressure (KPa)	The maximum pressure value recorded
Single support time (s)	The time that the foot is in contact with the walkway
Stance time (s)	The average time from when the foot first comes into contact with the walkway to the last time when the foot is in contact with the walkway
Step Length (cm)	The average distance from the heel of the first foot to the heel of the second foot in a single stride
Step time (s)	Elapsed time from the first contact of the foot to the walkway to the first contact of the opposite foot to the walkway
Step velocity (cm/s)	Step length divided by step time
Stride length (cm)	The distance between consecutive footprints of the same foot
Total double support time	A foot's initial double support time (time from first contact of the foot to last contact of the opposing foot's next stance) added to the same foot's terminal double support time (time of first contact of the opposing foot to last contact of the foot under consideration)
Contact time (s)	Total time a foot was in contact with the walkway
Duty factor (s)	Contact time / Gait cycle time; stride data derived from the point where the foot was in contact with the ground

The Tekscan was placed in the aisle between turkeys' pens. A runway was constructed to ensure that turkeys remained on the Tekscan pressure sensing walkway. The runway was the same length as the Tekscan (0.58 m x 1.09 m) and consisted of a piece of clear plexiglass secured by two wooden support boards. Two clear plastic mats were also placed on the floor on either side of the Tekscan so that the birds would walk on the same type of surface to prevent changes in gait as the birds stepped on the Tekscan.

A video camera was positioned 1.23 m from both ends of the mat to record turkeys as they walked across the Tekscan. The camcorders (Sony Camcorders, CX405, Sony Corporation of America, New York, NY) were attached to a tripod at a height of approximately 0.61 m. On data collection days, VN, VH, AN, AH and C birds were tested in random order. During the recording process, one researcher was positioned perpendicular to and approximately 3m from the center of the Tekscan to operate the laptop that controlled the Tekscan. Another researcher removed individual turkeys from their respective pens, applied the determined treatment to the leg, and positioned the bird just before the first plastic mat. Birds were recorded as they walked across the Tekscan in one direction, then back across the Tekscan in the other direction. The bird would walk down the constructed walkway (pass 1), and then the researcher would walk to the other end of the Tekscan to have the bird take another pass through the walkway (pass 2). All birds had been habituated to this setup and process of walking with a researcher present.

Birds were habituated to the Tekscan for the 1 wk period before each data collection. At 6 wk, turkeys were placed on the Tekscan for 30 min daily for 1 wk. However, the 30 min habituation period was shortened to 15 min for the 1 wk prior to 12 and 16 wk because birds began to rest 15 min into the habituation sessions. The habituation procedure included removing an entire pen of turkeys and re-locating the birds to the Tekscan set up. During the 15 min session, birds would be encouraged to walk over the Tekscan several times to get used to the feel of walking on the plastic surface.

Starting at 4 wk of age, turkeys were marked every 2 wk with black non-toxic livestock marker (Prima Tech Marking Stick, Neogen Corp., Lansing, MI USA) for identification purposes and to ensure that markings remained visible. The welfare (feather condition, footpad health, feather cleanliness, body condition, and body weight) of the turkeys was checked and recorded before each day of data collection at 8, 12, and 16 wk. Body feather condition was scored as 0 (little to no missing or broken feathers), 1 (feather loss/damage up to 5 cm in diameter), or 2 (indicated feather loss or damage of 5 cm or greater) (adapted from Bilcik and Keeling, 1999). Feather condition of the wings and tail were scored as 0 (no broken or missing feathers), 1 (less than 25% missing or broken feathers), 2 (between 25% and 50%, missing or broken feathers) or 3 (more than 50% missing or damaged feathers). Feather cleanliness was scored as 0 (no soiling), 1 (moderately soiled) or 2 (severe soiling). Footpad health was scored according to the Global Animal Partnership standards for turkeys (2015) as 0 (no lesions, swelling, or erosion of the footpad, 1 (mild or superficial lesions and/or thickened skin), or 2 (severe lesions, ulcers and/or scabs).

2.3.2 Statistical Analysis

2.3.2.1 Effects of Treatment and Age on Turkey Welfare and Gait

Age related changes in turkey welfare measures were analyzed in SPSS (version 25) for H, C and NH groups using a Friedman test with a post hoc Wilcoxon test and Bonferroni adjustment for multiple pairwise comparisons. Treatment related differences were analyzed in SPSS with a Kruskal-Wallis test. The majority of turkeys received scores of 0 for the various health and body condition measures. Therefore, statistical analyses comparing age and treatment effects were only performed on tail, left wing, and right wing feather condition scores.

Tekscan data were analyzed to determine the effects of age (8, 12 and 16 wk) and treatment group (H, NH and C) on gait parameters. Tekscan data were selected for analysis if several conditions were met:

1) All toes were present on the pressure pad at each step
2) The bird walked continuously during the walk, without stopping, standing or jumping
3) There were at least 4 consecutive steps taken per pass

Under the aforementioned conditions, 95 data files were utilized in the analysis (13 data files did not meet the conditions). In addition to the gait parameters derived from the Tekscan, duty factor was calculated (derived from Gatesy and Biewener, 1991; Paxton et al., 2013) to incorporate previous avian gait dynamics known to change with age (Oviedo-Rondon et al., 2018; Kremer et al., 2018; Gatesy and Biewener, 1991). Duty factor is a measure of the total stride cycle when the foot is in contact with the ground and was calculated by dividing total contact time by gait cycle time (Table 2).

Tekscan parameters and duty factor were analyzed using a repeated measures model (PROC MIXED, SAS 9.4) that included pen as a random effect. The following analyses were conducted:

1) Tekscan measures calculated taking both limbs into consideration (number of steps, cadence, gait time, gait distance and gait velocity):

treatment, age and their interaction were included as factors to determine whether Tekscan parameters changed with age and due to habituation to the VetRap bandage.

2) Tekscan measures calculated for each limb (impulse, gait cycle time, maximum force (% BW), peak pressure, single support time, stance time, step length, step time, step velocity, stride length and total double support time) and duty factor:

Treatment, age, limb (bandaged or not) and all their interactions were included in a model to evaluate whether there were differences between bandaged and unbandaged limbs within treatment groups, and between bandaged or unbandaged limbs among treatment groups and ages. In order to do these comparisons, no differences were determined between left and right limbs of C birds (PROC MIXED, SAS 9.4 with limb and age as factors, individual bird as the repeated measure, pen as a random effect, and body weight as a covariate). Consequently, one limb of each C bird was assigned as the designated limb for comparison so that treatment-related differences could be analyzed.

2.3.2.2 Validity and Reliability of Accelerometers

Accelerometer validity and reliability were analyzed using data from VH, AH and AN groups, groups that had accelerometers attached at the time that birds were walking across the Tekscan walkway. In order to analyze data obtained from the accelerometers, the accelerometer output was transformed and smoothed in LabVIEW (National Instruments, Austin, Texas) using an adapted Pan-Tompkins algorithm (Ying et al., 2007) based on the methods of Dalton et al. (2016a) to determine the number of steps taken by individual birds. Methods of Dalton et al. (2016a), were modified to include all three axes (X, Y, and Z) (derived from Ying et al., 2007). The three axes were combined into a single variable within the Lab VIEW program (Figure 3).

Figure 3: Accelerometer step detection output example after processing through LabVIEW. The top graph depicts the X, Y, and Z axis acceleration while the bottom graph depicts the processed acceleration data where all three axes have been combined. The yellow line on the bottom graph depicts the step threshold; any acceleration peaks above the threshold indicates that a bird is taking a step.

To determine the number of steps taken, a step threshold was selected so any acceleration values above the step threshold were considered steps, whereas values below the threshold were not considered steps. The step threshold varied depending on the age of the birds and was set at 0.42g/s for 8 wk, 0.53g/s for 12 wk and 0.66g/s for 16wk (where g represents acceleration due to gravity). The step threshold was examined every 0.01g/s between the range of 0.3 to 0.8g/s and the step threshold level was set to when the cumulative sensitivity for each age group was highest (Dalton et al., 2016a ; Martiskainen et al., 2009).

In this study, a step was defined as the point when all toes of one foot were off the ground while the footpad of the other foot remained in contact with the ground. Video camera step counts were determined by visually counting steps every 0.1s as the bird walked across the Tekscan. A scoring system of 0 and 1 was used to score when the bird took a step (1) or was not stepping (0) (as described in Dalton et al., 2016a; Ringgenberg et al., 2010). A single observer (RS) conducted video analyses to determine when the bird did and did not take a step. The accelerometer step count was compared to the step count determined from video recordings to calculate the sensitivity, accuracy, false discovery rate, specificity and precision of the accelerometers (Dalton et al., 2016a). The accelerometer and video data were both synchronized via a watch and an audio cue on the video so that the researcher verbally stated the moment and the time at which the accelerometer was activated, thus enabling the video time stamp to be matched with the accelerometer time stamp. A true positive was the number of steps detected by the accelerometer that were observed on the video recording, whereas a true negative was the number of non-stepping time points detected by the accelerometer that were non-stepping time points on the video recording. A false positive occurred when the accelerometer detected a step, but no step was observed on the video recording, while a false negative occurred when the video determined the bird was stepping but no step was detected by the accelerometer. The following equations were utilized to determine sensitivity, accuracy, false discovery rate, specificity, precision, and cumulative sensitivity (Dalton et al., 2016a):

$$Sensitivity = True\ Positive\ /\ (True\ Positive + False\ Negative) \times 100$$

$$Accuracy = (True\ Positive + True\ Negative)\ /\ (True\ Positive + True\ Negative + False\ Positive + False\ Negative) \times 100$$

$$False\ discovery\ rate = False\ Positive\ /\ (False\ Positive + True\ Positive) \times 100$$

$$Specificity = True\ Negative\ /\ (True\ Negative + False\ Positive) \times 100$$

$$Precision = True\ Positive\ /\ (True\ Positive + False\ Positive) \times 100$$

2.4 Results

2.4.1 Treatment and Age Effects on Turkey Health and Body Condition

As expected, average body weight increased with age (8 wk: 3.81 ± 1.34kg, 12 wk: 9.24 ± 2.27 kg and 16 wk: 15.17 ± 2.99 kg) but did not differ among treatment groups. Snood wounds were noted on three birds at 12 wk (one each from AN, VH and C with two birds being from the same pen). The large majority of the scores for footpad health, feather cleanliness, and feather condition of the neck, rump, and back were 0, so only tail and wing feather scores were able to be included in further analysis. Wing and tail feather condition scores were not significantly different between VH and AH or between VN and AN groups; therefore, to increase power of the results, treatment groups were combined into habituated (AH, VH; n = 17), non-habituated (AN, VN; n = 10), and control (C; n = 8) groups to analyze age differences.

Both wing and tail feather condition varied due to age for all treatment groups. The habituated (H) birds had tail feather scores (reported as median; 25th quartile, 75th quartile) that were less severe (0; 0,0.5) at 8 wk of age compared to 12 wk (1; 1, 1) and 16 wk (1; 1, 1) ($P < 0.001$). Both left and right wing scores indicated a peak in feather damage severity at 12 wk [left wing: 2 (2, 2); right wing: 2 (2, 2)] compared to 8 wk [left wing: 1 (1, 1); right wing: 1 (1, 1)] and 16 wk [left wing 1 (1, 1); right wing 1 (1, 1)] (left wing P < 0.001; right wing P < 0.001).

Similar to H turkeys, tail feather scores of NH turkeys were less severe at 8 wk (1; 0, 1) compared to 12 wk (1; 1, 1) and 16 wk (1; 1, 1) (P = 0.018). Wing feather damage peaked at 12 wk [left wing: 2 (1.75, 2); right wing: 2 (1, 2)] compared to 8 wk [left wing: 1 (1, 2); right wing: 1 (1, 2)] and 16 wk [left wing: 1 (1, 1); right wing 1 (1, 1)] (left wing P < 0.001; right wing P =0.01).

Turkeys in the control (C) group had the least tail feather damage at 8 wk (0; 0, 1) compared to 12 wk (1; 1, 1) and 16 wk (1; 1,1) (P = 0.007). Wing feather damage of C birds peaked at 12 wk [left wing: 2 (1, 2); right wing 2 (1, 2)] compared to both 8 wk [left wing: 1 (1, 2); right wing 1 (1, 2)] and 16 wk [left wing: 1 (1,2); right wing: 1 (1, 2)] (left wing P = 0.04; right wing P = 0.04).

2.4.2 Treatment and Age Effects on Turkey Gait

Tekscan gait parameter results are presented in Figures 4, 5, 6 and Tables 2, 3, and 4. There was a significant interaction for age and treatment for the number of steps taken, with post hoc comparisons indicating a tendency for the number of steps to be higher for H turkeys than C turkeys at 8 wk (P = 0.06). No other significant differences were found. Cadence generally decreased with age from 8 to 12 to 16 wk (P = 0.04) (Figure 4).

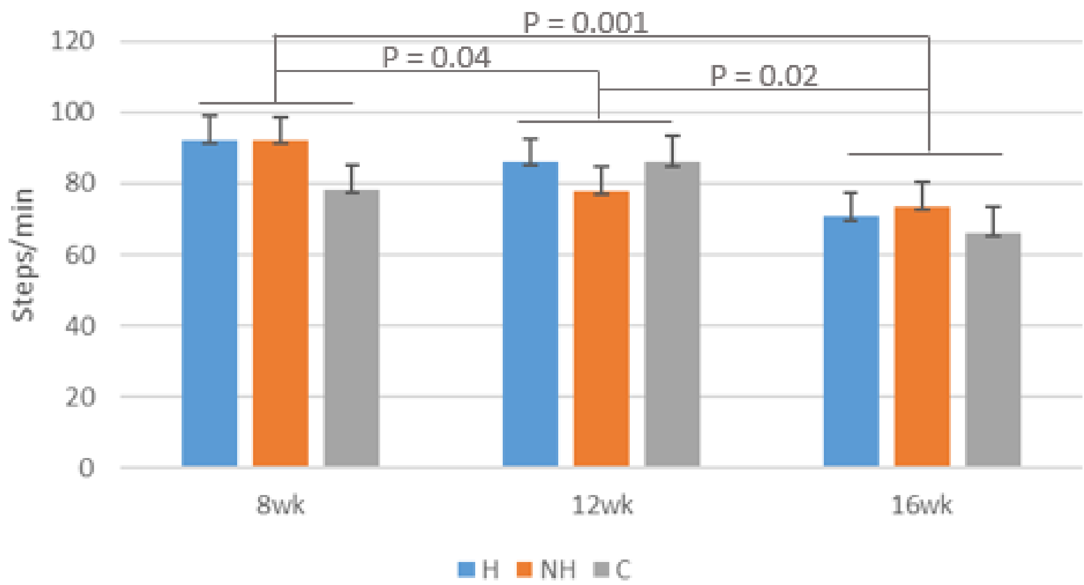

Figure 4. Differences in lsmean (± SE) for the cadence (steps/min) Tekscan gait parameter among turkeys habituated to wearing a VetRap bandage with or without an attached accelerometer (H group), turkeys not habituated to wearing a bandage or accelerometer (NH group) and control turkeys that did not wear any bandage or accelerometer at any time (C group) at 8, 12 and 16 wk of age. Overall changes in cadence at each age are denoted by the P-values above each of the ranges.

No significant effects of age, treatment or their interaction were found for gait time and duty factor. Gait distance was significantly longer at 12 wk compared to 8 wk ($P = 0.03$) and a tendency for turkeys to have a longer step at 12 wk compared to 16 wk ($P = 0.08$). Each treatment group displayed a longer gait distance (Figure 5) at 12 wk compared to each of the same treatment groups at 8 wk (H: $P = 0.02$; NH: $P = 0.02$; C: $P < 0.001$) and 16 wk (H: $P = 0.02$; NH: $P = 0.01$; C: $P < 0.001$).

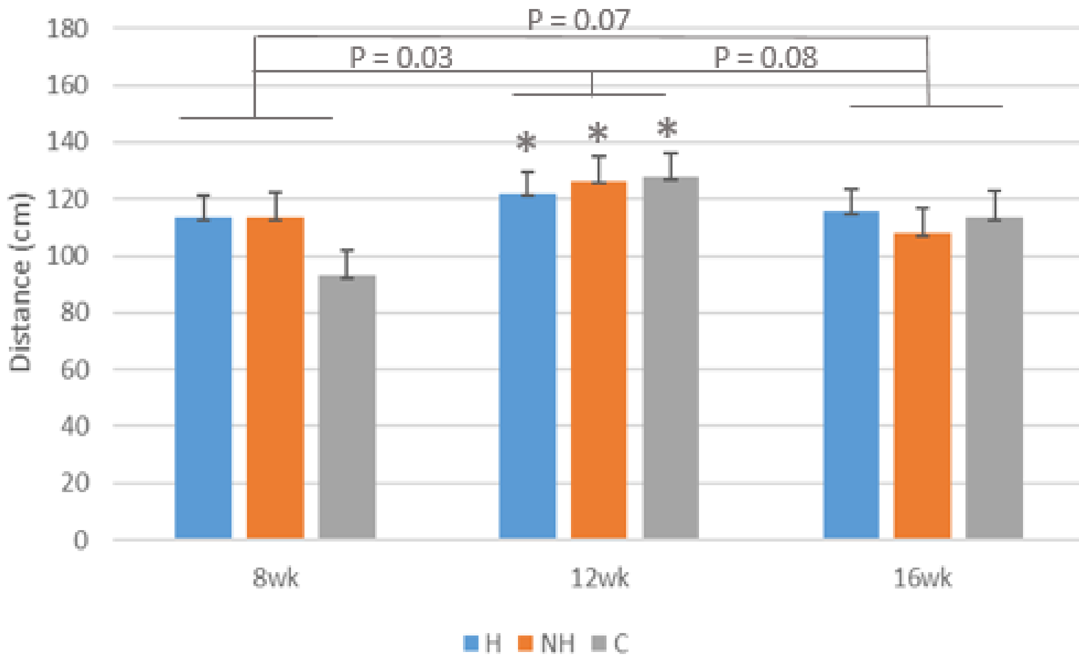

Figure 5. Differences in lsmean (± SE) for the gait distance (cm) Tekscan gait parameter among turkeys habituated to wearing a VetRap bandage with or without an attached accelerometer (H group), turkeys not habituated to wearing a bandage or accelerometer (NH group) and control turkeys that did not wear any bandage or accelerometer at any time (C group) at 8, 12 and 16 wk of age. Overall changes in distance at each age are denoted from the P-values above each of the ranges. Treatment differences at a particular age are denoted with an asterisk (*).

Gait velocity varied with age regardless of treatment group, and birds walked faster at 12 wk than at 8 wk (P = 0.02) and 16 wk (P = 0.01) (Figure 6). Each treatment group walked faster at 12 wk compared to the same treatment groups at 8 wk (H: P = 0.03; NH: P = 0.05; C: P = 0.01) and 16 wk (H: P = 0.001; NH: P = 0.001; C: P < 0.001).

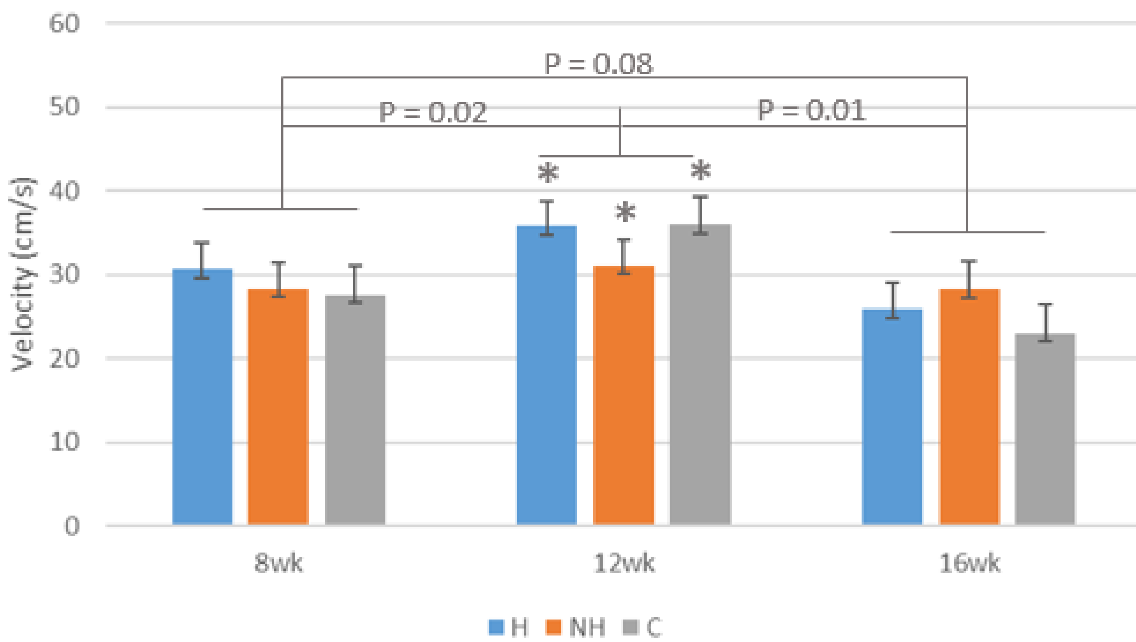

Figure 6. Differences in lsmean (± SE) for the gait velocity (cm/s) Tekscan gait parameter among turkeys habituated to wearing a VetRap bandage with or without an attached accelerometer (H group), turkeys not habituated to wearing a bandage or accelerometer (NH group) and control turkeys that did not wear any bandage or accelerometer at any time (C group) at 8, 12 and 16 wk of age. Overall changes in velocity at each age are denoted by the P-values above each of the ranges. Treatment differences at a particular age are denoted with an asterisk (*).

In order to determine whether the presence of a bandage affected turkey gait, gait parameters were compared among bandaged and unbandaged limbs at each age and for each treatment group. Limbs from control birds were randomly designated as a “bandaged” limb in order to compare gait parameters among treatment groups; previous analyses had indicated that there were no differences in any gait parameters between left and right limbs of control birds. There were no significant differences for any gait parameters between bandaged and unbandaged limbs. Therefore, limb parameters were combined and the effects of age, treatment group and their interaction on gait parameters were recorded (Table 4). The Tekscan software combined left and right foot calculations.

Some parameters (maximum force, peak pressure, and single support time) only had an overall change in gait due to age. No gait changes due to treatment or interactions were determined to be significantly different. Turkeys at 12 wk of age exerted a larger maximum force on the Tekscan compared to both 8 wk and 16 wk of age (P = 0.02). The peak pressure exerted on the Tekscan increased as a bird aged (P = 0.01). Finally, single support time decreased at 12 and 16 wk of age compared to 8 wk (P = 0.04).

Table 3. Differences in lsmean (± SE) Tekscan gait parameters (maximum force, peak pressure, and single support time) at 8, 12 and 16 wk of age. Significant changes across ages are denoted by superscripts ($^{a\ b\ c}$).

Tekscan Parameter	Age			P Value
	8 wk	12 wk	16 wk	
Maximum Force (%)	97.32 ± 1.55^{c}	108.66 ± 1.62^{a}	103.47 ± 1.57^{b}	P = 0.02
Peak Pressure (Kpa)	88.94 ± 2.37^{c}	146.24 ± 2.51^{b}	165.20 ± 2.40^{a}	P = 0.01
Single Support Time (s)	0.45 ± 0.02^{a}	0.34 ± 0.02^{b}	0.35 ± 0.02^{b}	P = 0.04

Impulse, gait cycle time, stance time, step length, step velocity, stride length, and double support time parameters all had an overall age effect along with an interaction of age and treatment (Table 4). Impulse was higher at 16 wk than at 8 wk, whereas gait cycle time and stance time were higher at 16 wk than at 8 and 12 wk. Maximum force (as a percentage of body weight), peak pressure, stride length and total double support time differed among all ages, with peak pressure and total double support time being highest at 16 wk, but maximum force, step length and stride length being highest at 12 wk. Step velocity was lower at 16 wk than at 12 wk, with step velocity at 8 wk being intermediate.

The interaction between age and treatment was significant for impulse, gait cycle time, stance time, step velocity, stride length and total double support time (Tables 4 and 5). Impulse of H and C birds was higher at 16 wk than at 8 wk, while NH turkey's impulse percentages did not change as they aged. Gait cycle time was longer for H birds at 16 wk than at 8 and 12 wk. Stance time of C birds was longer at 16 wk compared to 8 and 12 wk. Step velocity of C birds was higher at 12 wk than at 16 wk, with step velocity at 8 wk being intermediate. Stride length of H birds was longer at 12 wk compared to 8 wk, with stride length at 16 wk being intermediate. Similarly, stride length of C birds was higher at 12 wk compared to both 8 and 16 wk.

Table 4. Differences in lsmean (± SE) Tekscan gait parameters among turkeys habituated to wearing a VetRap bandage with or without an attached accelerometer (H group), turkeys not habituated to wearing a bandage or accelerometer (NH group) and control turkeys that did not wear any bandage or accelerometer at any time (C group) at 8, 12 and 16 wk of age. Significant changes across ages are denoted by superscripts ($^{a\ b\ c}$).

Gait Parameter	Treatment group	Age			P Value
		8 wk	12 wk	16 wk	
Impulse	H	56.76 ± 4.98^{b}	72.57 ± 5.01^{ab}	82.21 ± 5.26^{a}	P = 0.01
	NH	71.37 ± 5.93	80.62 ± 6.15	77.09 ± 5.65	P = 0.72
	C	68.58 ± 5.60^{b}	70.0 ± 6.12^{ab}	91.75 ± 5.85^{a}	P < 0.001
Gait cycle time	H	1.35 ± 0.12^{b}	1.42 ± 0.12^{b}	1.91 ± 0.13^{a}	P = 0.02
	NH	1.60 ± 0.15	1.75 ± 0.15	1.64 ± 0.14	P = 0.79
	C	1.68 ± 0.14	1.42 ± 0.15	1.88 ± 0.15	P = 0.08
Stance time	H	0.95 ± 0.10	1.07 ± 0.01	1.36 ± 0.10	P = 0.44
	NH	1.22 ± 0.11	1.21 ± 0.12	1.21 ± 0.11	P = 0.91
	C	1.19 ± 0.11^{b}	1.13 ± 0.12^{b}	1.58 ± 0.11^{a}	P = 0.04
Step length	H	19.75 ± 0.68^{c}	24.30 ± 0.68^{a}	22.05 ± 0.73^{b}	P = 0.05
	NH	19.69 ± 0.84	23.02 ±0.90	23.02 ± 0.80	P = 0.09
	C	20.39 ± 0.80^{b}	24.71 ± 0.89^{a}	20.78 ± 0.84^{ab}	P = 0.03
Step velocity	H	32.62 ± 2.79	37.09 ± 2.82	27.17 ± 2.96	P = 0.86
	NH	30.15 ± 3.35	29.78 ± 3.48	31.36 ± 3.19	P = 0.78
	C	28.63 ± 3.18^{ab}	36.48 ± 3.47^{a}	22.73 ± 3.32^{b}	P = 0.03
Stride length	H	38.82 ± 1.34^{b}	48.40 ± 1.35^{a}	43.48 ± 1.43^{ab}	P = 0.03
	NH	38.57 ± 1.64^{b}	44.73 ± 1.74^{ab}	45.05 ± 1.57^{a}	P = 0.01
	C	40.44 ± 1.59^{b}	48.55 ± 1.75^{a}	41.85 ± 1.67^{b}	P = 0.01

The only significant treatment effect that was found was for total double support time (Table 5), which was longer for NH birds than for C birds, with H birds being intermediate but not different from the other two groups at both 8 wk and 16 wk ($P < 0.001$). At 8 wk of age, turkeys in the C group walked with a higher total double support time compared to both H and NH groups ($P = 0.001$). At 16 wk of age, turkeys in the C and H group had a higher total double support time compared to NH groups ($P = 0.03$). Total double support time of H and C birds was higher at 16 wk than at 8 and 12 wk ($P = 0.01$), whereas NH birds had a lower total double support time at 8 wk only ($P = 0.02$).

Table 5. Differences in lsmean (± SE) total double support time among turkeys habituated to wearing a VetRap bandage with or without an attached accelerometer (H group), turkeys not habituated to wearing a bandage or accelerometer (NH group) and control turkeys that did not wear any bandage or accelerometer at any time (C group) at 8, 12 and 16 wk of age. Significant changes across ages are denoted by superscripts ($^{a\ b\ c}$). Significant changes across treatment are denoted by superscripts ($^{X\ Y}$).

Gait Parameter	Treatment group	Age			P- Value (Age)
		8 wk	12 wk	16 wk	
Total double support time	H	0.54 ± 0.08^{bY}	0.74 ± 0.08^{b}	1.08 ± 0.08^{aX}	P = 0.01
	NH	0.49 ± 0.10^{bY}	0.80 ± 0.10^{ab}	0.85 ± 0.09^{aY}	P = 0.02
	C	0.87 ± 0.09^{bX}	0.79 ± 0.10^{b}	1.23 ± 0.09^{aX}	P = 0.01
P Value (Trt)		P = 0.001	P = 0.08	P = 0.03	

2.4.3 Accelerometer Reliability and Validity

The sensitivity, accuracy, false discovery rate, specificity, and precision of the accelerometers were calculated by comparing the number of steps determined from the accelerometers to the number of steps determined from video recordings (Table 6). Accelerometers detected fewer steps and had false positives and negatives compared to video observations (Table 6). Age and treatment affected accelerometer precision (age only), sensitivity (age only), accuracy (age and treatment), specificity (age and treatment), and false discovery rate (age and treatment) (Table 7). No age-treatment interactions were found for any of the accelerometer parameters.

Table 6. Total number of steps recorded from video and accelerometers for the three accelerometer-wearing treatment groups. True positive, true negative, false positive, and false negative were determined from comparing accelerometer output to the true number of steps from the video output. Birds were assigned to treatment groups: AH = habituated to wearing an accelerometer; AN = not habituated, but wearing an accelerometer; VH = habituated to wearing a bandage

Treatment	Video Step Counts (Total)	Accelerometer Step Counts (Total)	True Positive	True Negative	False Positive	False Negative
AH	89	87	84	82	3	14
AN	69	86	59	58	35	10
VH	116	107	101	89	6	15

Table 7. Mean percent (± SE) false discovery rate, sensitivity, accuracy, specificity and precision of the accelerometers relative to video observations of step counts at 8, 12 and 16 wk for each treatment group. [a, b, c, d] Different means within each variable differ statistically. [XY] Means within columns that have different letters are significantly different. Birds were assigned to treatment groups: AH = habituated to wearing an accelerometer; AN = not habituated but wearing an accelerometer.

Variable	Treatment	8 wk	12 wk	16 wk	P Value
Precision	AH	84.54 ± 0.50^{ab}	85.48 ± 0.54^{a}	83.25 ± 0.49^{b}	P = 0.01
	AN	85.73 ± 0.56^{a}	85.94 ± 0.52^{a}	78.19 ± 0.52^{b}	P < 0.001
	VH	86.18 ± 0.47^{a}	85.40 ± 0.45^{a}	80.44 ± 0.46^{b}	P < 0.001
	P Value	P = 0.47	P = 0.56	P = 0.08	
Sensitivity	AH	88.53 ± 0.52^{a}	87.49 ± 0.56^{a}	81.72 ± 0.55^{b}	P = 0.01
	AN	88.93 ± 0.60^{a}	87.41 ± 0.56^{a}	82.14 ± 0.55^{b}	P = 0.03
	VH	88.36 ± 0.49^{a}	86.54 ± 0.49^{a}	82.84 ± 0.52^{b}	P = 0.01
	P Value	P = 0.83	P = 0.46	P = 0.29	
Accuracy	AH	88.55 ± 0.26^{aY}	88.02 ± 0.28^{aY}	84.46 ± 0.28^{b}	P = 0.01
	AN	84.65 ± 0.30^{bX}	83.73 ± 0.28^{bX}	84.60 ± 0.28^{b}	P = 0.09
	VH	87.98 ± 0.25^{aY}	87.29 ± 0.25^{aY}	84.43 ± 0.26^{b}	P = 0.01
	P Value	P = 0.03	P = 0.04	P = 0.75	
Specificity	AH	84.00 ± 0.24^{a}	85.72 ± 0.28^{aX}	82.15 ± 0.27^{b}	P = 0.01
	AN	84.30 ± 0.28^{a}	83.19 ± 0.26^{aY}	79.42 ± 0.26^{b}	P = 0.01
	VH	83.86 ± 0.23^{c}	86.38 ± 0.23^{bX}	82.17 ± 0.24^{c}	P = 0.05
	P Value	P = 0.33	P = 0.05	P = 0.61	
False Discovery Rate	AH	11.69 ± 0.47^{aX}	11.45 ± 0.50^{aX}	21.18 ± 0.50^{bX}	P < 0.001
	AN	15.34 ± 0.54^{aY}	10.73 ± 0.50^{aY}	25.35 ± 0.50^{bY}	P < 0.001
	VH	12.08 ± 0.44^{aX}	12.10 ± 0.44^{aX}	22.00 ± 0.47^{bX}	P < 0.001
	P Value	P = 0.001	P = 0.03	P = 0.04	

2.5 Discussion

This study examined changes in turkey welfare immediately prior to data collection at 8, 12, and 16 wk to determine any changes due to age or treatment and to assess whether a bird was healthy to include in the gait analysis. No differences in welfare due treatment were detected. Therefore, it was inferred that despite the changes in gait due to wearing an unfamiliar accelerometer, no negative side effects were observed in terms of feather condition, body weight, feather cleanliness or footpad health up to 16 wk of age. When accelerometers were first placed on each bird, birds pecked at the VetRap intermittently for several minutes (this study did not systematically collect data to examine this behavior). Some birds were also observed to shake and kick the leg that had the bandage, but this behavior was only seen when the bandage was first applied. There were two instances of the birds successfully tearing off the bandage during a habituation period at 12 wk and then another at 16 wk. The bandage and accelerometer were then re-applied the same day, and accelerometers did not appear to shift during any other incidents. The other pen mates did not appear to be interested in the birds' leg that had a bandage applied, and no instances of other birds in the pen pecking at bandages were observed. No long term behavioral or health issues were observed by the researchers. In terms of welfare changes due to age, feather damage of both left and right wing feathers peaked in severity at 12 wk, indicating that feather damage from feather pecking was highest at 12 wk of age. Tail feathers had the highest scores at both 12 and 16 weeks of age. The welfare scores were to be expected as several studies have demonstrated an increase in injurious feather pecking as turkeys age (Busayi et al., 2006; Duggan et al., 2014; Dalton et al., 2016b).

As a turkey ages, gait variables would be expected to change due to physical and morphological changes, such as increased body weight and leg length. In addition, overall foot and leg health has been shown to decline with age in domestic turkeys, resulting in poorer gait scores in older birds (Martrenchar et al., 1999; Krautwald et al., 2011; Dalton et al., 2016b). There were several variables for which age affected how a bird walked, including cadence, gait distance, and gait velocity while number of steps and gait time remained unchanged (Table 3). The results of this study determined that by the time a turkey reaches 16 wk of age gait parameters change when walking so that they take fewer steps per minute (cadence), spend more time on both feet (single and total double support time) and exert more pressure on the ground (peak pressure and maximum force) compared to 8 wk and 12 wk old turkeys. At 12 wk of age, several gait parameters peaked compared to 8 wk and 16 wk turkeys. Generally, 12 wk old turkeys walked faster (gait velocity) and took longer steps (step length and stride length) compared to the other ages. The number of significant differences among parameters were similar to recent studies on turkey gait (Kremer et al., 2018; Oviedo-Rondon et al., 2018). However, some age-related changes were not observed in their results. Cadence is the number of steps taken per minute (Tekscan Walkway™, 2011) which was lower at 16 wk of age compared to 8 and 12 wk. Cadence had not been previously shown to decrease with age in turkey hens (Kremer et al., 2018). Male vs. female gait changes may be a factor as hens displayed a longer step length with age (Kremer et al., 2018), while males showed no change (Oviedo-Rondon et al., 2018). It could have been possible that continued exposure to the Tekscan over time resulted in a lower cadence by 16 wk in our study. Alternatively, the birds could have walked at a slower pace due to an increase in body size or leg health. This study also observed a longer gait distance and slower gait velocity at both 12 and 16 weeks of age, further confirming that cadence would also be lower if the bird is taking a slower and longer stride (Table 3). Although Kremer et al. (2018) did not see a significant difference in cadence as turkey hens age, they did see a slower gait velocity with age, similar to our findings and those of Oviedo-Rondon et al. (2018). Oviedo-Rondon et al. (2018) also reported that step length of male turkeys increased with age (13, 15, and 20 wk), which is similar to the results of our study; however, step length was at its highest at 12 weeks and then decreased at 16 weeks (Oviedo-Rondon et al., 2018).

Using the gait parameters that compared bandaged vs. unbandaged limbs, almost all were affected by age (Table 4). More specifically, total double support time, stride length, step velocity, step length, stance time, single support time, peak vertical pressure, maximum force, gait cycle time, and impulse were all found to have changes associated with age. Although it was initially, anecdotally, observed that non-habituated birds displayed behaviors of discomfort, such as kicking or pecking at the bandage, many gait parameters seemed unaffected by treatment group. Perhaps heavier birds are unable to maintain balance using a longer stride relative to leg length unlike younger lighter birds. The overall gait dynamic of swing could also have changed causing more medial-lateral swing rather than a straight-line path (indicated by the changes in double support time, single support time, and gait time changes in age). Previous research comparing broiler and laying hen gait showed that laying hens walk a straight line path while broilers possess greater body movements (Waiblinger et al., 2006). It may be that turkeys, similar to broilers, have more body oscillations, but due to weight, not necessarily a longer step as they age. Similar to Kremer et al. (2018), single support time decreased with age. It seems that most speed related parameters tend to decrease as turkeys get older after peaking at a certain age. Surprisingly, duty factor was unchanged throughout the study. In male turkeys it had been found that duty factor decreased with age much like the other gait parameters analyzed. However, there is an inconsistency as Kremer et al. (2018) reported an overall decrease in duty factor as turkey hens aged. The male turkey study analyzed gait over a longer time period (13, 15, and 20 wk), so in addition to sex differences, turkey gait may have been affected differently depending on the ages observed (Oviedo-Rondon et al., 2018). In our study, many parameters also had age and treatment interactions including total double support time, stride length, step velocity, step time, stance time, gait cycle time, and impulse. An age and treatment interaction was observed for step length, and maximum force as well. This indicates that treatment may have more or less of an effect depending on the age of the bird. For all gait parameters, the foot the treatment was applied to did not significantly affect gait.

The validity of accelerometers is determined by both accuracy and specificity, while reliability refers to the precision and sensitivity (Martin and Bateson, 1993; Waiblinger et al., 2006). Validity reflects how well the accelerometers measure the true step counts. In contrast, the reliability of the accelerometers reflects how consistent accelerometers are in determining step counts. Relative to previous studies utilizing accelerometers, the AXY-3 Data Loggers used in this study were comparable in terms of accuracy, but had a higher false discovery rate (Martiskainen et al., 2009; Moreau et al., 2009; Ringgenberg et al., 2010; Dalton et al., 2016a). Dalton et al. (2016a) used HPD loggers with an average false discovery rate of 12.10 ± 5.82%, which is similar to AXY-3 false discovery rates at 8 wk (11.93 ± 0.28%) and 12 wk (12.04 ± 0.48%) for habituated birds. However, at 16 wk AXY-3 false discovery rate rose to an average of 21.48 ± 0.93% which was higher than the HPD loggers. The false discovery rate increased as birds aged with the highest values reported at 16 wk. These high false discovery rates could be due to the increased variation among the individual birds as they aged or alternatively, the processing method used to smooth the data in LabVIEW. One method to reduce the false discovery rate would be to adjust the step threshold to each individual turkey rather than using an average step threshold for all birds of a certain age. However, this would not be feasible for large numbers of birds. The validity and reliability of these accelerometers was also greatly affected by non-habituated birds shaking and pecking at the accelerometers on their legs during data collection. When examining data from habituated and non-habituated turkeys, 35 out of 44 false positives were attributed to the non-habituated birds, further demonstrating the importance of habituation when using accelerometers to detect the stepping activity of turkeys.

Accelerometers have been shown to provide a use for both scientific studies and commercial uses in animals. By showing the potential validity of AXY-3 Data Loggers, steps can be taken to study how stepping behavior and activity level changes can be indicators of welfare issues such as lameness.

Although the sample size was small, which had the potential to greatly affect the results of this study, results are still helpful in contributing to the lack of research regarding uses of micro accelerometers for poultry. Balancing the treatment between left and right foot also provided an extra factor to consider in the already low power of the results. Further research should be conducted on the long term uses of micro-accelerometers to detect behaviors and welfare concerns.

Conclusions

Based on the results, AXY-3 Micro Accelerometers are effective tools for recording the stepping activity of turkeys, but the reliability and validity of these accelerometers varied by bird age and prior habituation to the bandages used to secure the accelerometers. Most gait parameters in turkeys are sensitive to age effects, and unhabituated birds were shown to have an additional age-treatment interaction further affecting gait. Turkey health status and body condition scores were affected by age and not by treatment group, with feather condition worsening as birds aged. Based on our results, a one-week habituation period using only a bandage is effective in habituating turkeys to wearing micro-data loggers. Further steps should be taken to assess the uses of activity level related behaviors in turkeys and to determine if stepping behavior can be used as a proxy for changes in behavior. Furthermore, future studies should look into age-related gait dynamics and male and female gait changes.

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CHAPTER 3: ANALYSIS OF WALKING ACTIVITY AS A NON-INVASIVE MEASURE OF TURKEY WELL-BEING UNDER MILD HEAT STRESS AND AN IMMUNE CHALLENGE

3.1 Introduction

Heat stress and disease are major concerns within the commercial turkey industry (Rauw et al., 1998; Deeb, 2002; Appleby et al. 2004). It is estimated that between $128 and $165 million dollars are lost yearly in the poultry industry because of heat related issues. Diseases such as hemorrhagic enteritis can spread to an entire farm's flock within the span of a few days (St-Pierre et al., 2003), and diseases such as avian influenza can necessitate entire flocks of birds to be culled. The earlier farmers can detect birds that are unwell, the less potential for these issues to become more severe. Therefore, early detection of health problems, deviations from normal behavior, and environmental stressors are key to ensuring animal welfare on farms. Animal behavior is a common indicator farmers use to identify changes in the overall health and welfare of an animal, but visually inspecting large groups of animals for changes in behavior is labor-intensive and often unreliable. For example, a sick turkey could go undetected in a flock of thousands of birds, which could lead to prolonged suffering and potential spread of disease. Moreover, problems that do not cause overt, visually detectable changes in behavior may be even more difficult to detect until the problem has worsened to a more obvious deviation in normal behavior and body condition.

The utilization of technology to identify deviations in animal behavior has become an emerging topic of research in the field of animal welfare for its potential to be more practical, non-invasive, objective, and accurate compared to visual inspection (Weary et al., 2009). With the use of technology, behavior can also be quantified. For example, accelerometers allow data to be collected in a 3D plane and have been used to measure activity level and step counts in many farm species such as dairy cattle (Thorup et al., 2015; Stewart et al. 2018), sheep (Alvarenga et al., 2016; Barwick et al., 2018), and laying hens (Kozak et al., 2016). Changes in animal health and welfare can be detected using these types of technologies as well. Decreased walking activity has been previously associated with heat stress and as part of the general immune response in poultry (Hart, 1988; Quinteiro-Filho et al., 2010; Mahmoud et al., 2015; Li et al., 2015). A previous study validated the use of bio-loggers to detect lethargy in Pekin ducks brought on by an immune challenge (Maraia et al. 2013), while another study used accelerometers as a proposed early detector of avian influenza from decreased activity levels in laying hens (Okada et al., 2010). Therefore, accelerometers may prove to be a useful tool for early indications of activity related changes in turkeys.

Chapter 2 validated AXY-3 micro accelerometers (TechnoSmArt, Guidonia-Montecelio, Italy) as an accurate measure of tom turkey step counts at different ages. The main goal of this project was to evaluate the use of accelerometers as non-invasive, objective and quantitative measures of turkey welfare, using mild heat stress and an immune challenge as stressors. Another objective was to identify changes in behavior associated with these stressors.

3.2 Materials and Methods

3.2.1 Animals and Housing

All procedures were approved by the Institutional Animal Care and Use Committee of Purdue University. This study consisted of two trials (in-time replicates). A total of 92 (trial 1: 51 turkeys; trial 2: 41 turkeys) commercial male beak trimmed turkeys (Nicholas Select, Aviagen Turkeys, Lewisburg, West Virginia) were obtained from a commercial hatchery at 1d of age and housed at the Purdue Animal Sciences Research and Education Center (ASREC) until 7wk of age. Animal care and housing were the same as outlined in Chapter 2. Poults were randomly assigned to 8 and 7 pens (trial 1 and trail 2, respectively) so that there were between 6 and 7 birds per pen (2.44 m x 1.52 m). Room temperature and lighting used in this study were based on industry guidelines (Aviagen, 2015). At 7 wk, birds were transferred to the Purdue University Veterinary Animal Isolation Building (VA2) where birds from the same pen continued to be housed together. Each room had separate temperature and lighting controls, and each room was set to a lighting schedule of 0700 to 2100 with an average temperature (± SD) of 17.9 ± 1.9 °C. However, during the second trial, starting at week 13 a power outage caused the lighting schedule of one room to change from lights off at 2100 to 1800.

Starting at 7 wk of age, turkeys were marked with a black non-toxic livestock marker (Prima Tech Marking Stick, Neogen Corp., Lansing, MI USA) for identification purposes and to ensure that markings remained visible. Each bird was randomly marked on one of 7 unique locations: left wing (L), right wing (C), base of neck (T), tail feathers (B), center of back (C), both T and B (TB), and both L and R (LR). Livestock marker was reapplied every 2 wk after the initial application to prevent fading.

3.2.2. Treatments

In a crossover design, each room experienced each of 3 treatments at 10, 12, and 14 wk of age, including mild heat stress (HS), an immune challenge (IC), and control conditions (CON; no heat stress or immune challenge). A total of 5 rooms were assigned to each treatment order (Table 8) (n = 5).

Table 8. Schedule and order in which turkeys in each room experienced mild heat stress (HS), an immune challenge (IC) or neither (CON).

	Trial 1: Rooms 5, 7, 11 Trial 2: Rooms 4, 11	Trial 1: Rooms 4, 6, 10 Trial 2: Rooms 5, 10	Trial 1: Rooms 8, 9 Trial 2: Rooms 6, 8, 9
10 wk	IC	CON	HS
12 wk	CON	HS	IC
14 wk	HS	IC	CON

The heat stress treatment was designed to induce a mild heat stress to turkeys in the assigned HS rooms. During the treatment days at 10, 12, and 14 wk, the room temperature was gradually increased to a specific peak temperature range dependent on each room's humidity (Table 9). To determine the correct peak temperature range, a heat index chart created for hen turkeys was referenced from Xin and Harmon (1998).

Table 9. Averages (± SD) of humidity and temperature of each heat stress treatment room 10 min before heating the rooms at each age (10 wk, 12 wk, and 14 wk). Target temperature ranges to impose a heat stress were derived from Xin and Harmon, 1998.

Trial Number, Age	Average humidity before heat stress (%)	Average temperature before heat stress (°C)	Target heat stress temperature range (°C)
1, 10 wk	55.0 ± 1.0	17.4 ± 0.1	27.8 – 29.4
1, 12 wk	16.5 ± 1.5	17.9 ± 0.2	29.4 – 30.6
1, 14 wk	24.5 ± 1.5	17.7 ± 0.1	29.4 – 30.6
2, 10 wk	15.9 ± 0.9	18.0 ± 0.3	29.4 – 30.6
2, 12 wk	51.5 ± 0.4	17.3 ± 0.1	27.8 – 29.4
2, 14 wk	20.0 ± 1.3	18.3 ± 0.1	29.4 – 30.6

This heat index chart (Xin and Harmon, 1998) was used to identify temperatures in the range defined as "danger" to induce a mild heat stress rather than the "extreme" heat stress range. The humidity of the HS rooms was recorded and applied to the reference chart to determine the peak temperature range. In order to verify that turkeys experienced heat stress, cloacal body temperature was recorded for two turkeys in each room (Table 10).

Table 10. Cloacal temperatures (average ± SD) of turkeys at each age (10 wk, 12 wk, and 14 wk) 10 min before (pre-heat stress treatment), after birds had experienced peak temperatures for 2 h (during peak heating), and 2 h after the imposed heat stress (post-heat stress treatment).

Age	Pre-heat stress treatment (°C)	During peak heating (°C)	Post-heat stress treatment (°C)
10 wk	40.6 ± 0.3	41.1 ± 0.3	40.6 ± 0.4
12 wk	40.6 ± 0.1	41.4 ± 0.3	40.6 ± 0.3
14 wk	40.0 ± 0.4	41.4 ± 0.4	40.0 ± 0.4

Rooms took approximately 140 min to reach the peak temperature and were held at this temperature for 120 min. Thereafter, it took approximately another 140 min to cool back down to original room temperatures. During heating and cooling of HS rooms, an observer recorded temperature and humidity levels every 10 min to determine when peak heat stress temperature was reached.

Immune challenge treatments were induced using a live-virus hemorrhagic enteritis vaccine (Oralvax HE®, Merck Animal Health) that was administered to turkeys in their drinking water. The vaccine was prepared according to manufacturer’s directions. The entire re-hydrated vaccine created 80 gallons of prepared drinking water for 2000 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 the room, only 4 to 7 doses were needed for this study, and the amount of vaccine water prepared was calculated by multiplying the number of birds in each room by 0.04 gallons. On the treatment day, water was removed from IC rooms for 2 h prior to vaccine administration to ensure that all birds would drink the vaccine water. The water containing the vaccine was provided after the 2 h deprivation period and remained in the rooms for 2 h. Fresh, clean water was provided to each of the rooms afterwards. Birds in CON groups were not subjected to heat stress or an immune challenge.

3.2.3 Welfare Assessments

Body weights were recorded and welfare was assessed for every bird 2 d before heat stress and immune challenge were imposed at 10, 12 and 14 wk. Welfare assessments were conducted as described in Chapter 2 and included assessments of feather condition, feather cleanliness, footpad health, snood wounds, and head and neck wounds.

3.2.4 Accelerometers and Other Technology

Several different technologies were installed in each of the rooms. Temperature and humidity sensors were placed at the height of the turkeys. An overhead camcorder (Sony Camcorders, CX405, Sony Corporation of America, New York, NY) was installed in each room to monitor turkey behavior. Video was continuously recorded each week of data collection so that 5 d of video data were recorded (2 d before the imposed treatment, the day of imposed treatment, and 2 d after the imposed treatment). Camera SD cards were replaced once daily in order to continuously record from 0800 until 2100. Accelerometers (AXY-3 Micro Acceleration Data Loggers, TechnoSmArt, Guidonia-Montecelio, Italy) were attached to 2 birds per room using a Vetwrap bandage on their leg as described in Chapter 2 to record bird walking activity during the same time period that behavior was recorded. Turkeys assigned to wear an accelerometer were habituated to wearing the Vetwrap bandage 1 wk prior to data collection.

3.2.5 Turkey Behavior

Behavior (Table 11) of turkeys was analyzed from the video recordings during the 5d period. Scan and focal sampling were used. Four focal birds were selected, including 2 birds each wearing an accelerometer and 2 randomly selected birds that did not wear an accelerometer. Focal observations consisted of 30 min continuous observations of all 4 birds at 0900, 1100, 1300, and 1700. Time points were chosen to encompass both treatments' time points (Table 12). Scan sampling was conducted at 10-minute intervals for an hour at 0830, 1300, and 1730; behavior of all birds in the room were recorded at each 10 min time point.

Table 11. Ethogram of turkey behavior

Behavior	**Description**
Sitting/sleeping	Lying down with breast in contact with the ground, sitting with the head up/sleeping with the head lowered or tucked under a wing
Standing	Standing up but not doing any other activity
Walking	Two or more steps. Relatively low speed motion; placing one foot in front of the other.
Feeding	Pecking at or eating food, or standing with the head over the rim of the feeder
Drinking	Dipping beak into the water, or standing at drinker and swallowing, or standing with the head over the rim of the drinker
Feather pecking	Repeated pecking at another bird's feathers (at least 3 pecks in succession).
Preening own feathers	Using the beak to manipulate its own feathers on the wings, back or breast.
Aggressive interaction	Repeated forceful pecking directed by one bird at the head or face of another bird. Birds may be flapping and kicking or standing with the head and neck raised and gaze directed at the other bird. Birds may be pushing and circling each other. Birds may peck at and/or grab on to the neck and snood of one another. One bird may be chasing another bird.
Missing/other	Not possible to record bird behavior because the bird is not visible, or its head is not visible. Or bird is performing a behavior not classified anywhere else in the ethogram.
Panting / gular flutter	Birds visibly panting so that the beak is open and tissues surrounding the neck are in motion.
Sitting with wings splayed out	Bird has breast in contact with the ground, sitting with the head up. Both wings are held away from the body so that a gap is noticeable between the body and wing.

Table 12. Timeline of imposed heat stress and immune challenge treatments during the day of the imposed stressor.

	Time of Day				
	0700	0900	1100	1300	1500
Heat Stressed Rooms		Start heating up to peak temperature range	Peak temperatures were achieved	Temperature began declining back to normal	Normal temperature post heat stress
Immune Challenge Rooms	Water deprivation period began	Vaccine was added to drinking water	Vaccine water was removed, and normal water was restored		

3.2.6 Statistical Analysis

3.2.6.1 Welfare Assessments

Age related changes in turkey welfare measures were analyzed in SPSS (v.25) for all treatment groups using a Friedman test with a post hoc Wilcoxon test and Bonferroni adjustment for multiple pairwise comparisons. Treatment related differences were analyzed in SPSS with a Kruskal-Wallis test. Because the majority of turkeys received scores of 0 for the various health and body condition measures, statistical analyses comparing age and treatment effects were only performed on footpad, tail, left wing, and right wing feather condition scores.

3.2.6.2 Accelerometer Step Counts

The total number of steps for each bird was determined using LabVIEW 2013 (National Instruments, Austin, Texas). For each turkey, the number of steps was divided by the number of light hours to obtain the number of steps per hour (steps/hr). For the first day (2 days before treatment at 10 wk) the accelerometers were placed on the birds before 1000. Therefore, the total number of steps/hr was determined from 1000 to 2100. For all other days, the total number of steps/hr was determined from 0700 to 2100 (lights on to lights off). The effects of treatment, age, day of treatment and their interactions on the number of steps/hr were analyzed using a repeated measures model with body weight as a covariate (PROC MIXED, SAS 9.4). Room nested within repetition was included as a random effect and bird was included as the subject in the repeated statement.

3.2.6.3 Behavior

The effects of age on the number of animals performing each behavior (scan sampling) were analyzed using a Friedman test with a post hoc Wilcoxon test and Bonferroni adjustment for multiple comparisons. For scan data, the number of birds performing each behavior was divided by the total number of birds in the room to obtain a proportion. Median, 25th and 75th interquartile ranges were reported from the Friedman analysis. The effects of treatment on proportion of animals (scan sampling) was done by performing a Kruskal-Wallis test in SPSS. Frequency of behavior was analyzed using a repeated measures model (PROC GLIMMIX, SAS 9.4) with a negative binomial distribution and the log link function. Room nested within repetition was included as a random effect. The individual bird was included as the subject in the random statement to account for repeated measures, and the results were back transformed using the ilink function in SAS. The behaviors "eating” and “drinking” were combined to form one “eating and drinking” category. Similarly, aggression and feather pecking behaviors were combined into one category for analysis. The effects of treatment, age, day of treatment, whether a turkey was wearing an accelerometer, and their interactions on the durations of behaviors (focal sampling) were analyzed using a repeated measures model (PROC GLIMMIX, SAS 9.4). Room nested within repetition was included as a random effect and bird was included as the subject in the repeated statement.

3.3 Results

3.3.1 Welfare Assessments

As expected, body weight increased with age; turkeys weighed on average (± SD) 2.23 ± 1.13 kg, 3.22 ± 0.26 kg, and 4.54 ± 0.36 kg at 10, 12, and 14 wk, respectively. In terms of age-related differences, the large majority of turkeys received scores of 0 for several welfare measures; therefore, most measures were not analyzed. At both 10 and 12 wk, 3 birds had received a snood wound score of 1 (CON and HS) while all other birds received a score of 0. Head wounds consisted mostly of 0 scores; however, there was some variation throughout the course of the study. At 10 wk, 4 birds (2 HS, 2 IC) received a score of 1 for head wounds (CON), at 12 wk 7 birds received a score of 1 (4 IC, 2 HS, 2 CON) and 3 birds received scores of 2 (1 HS, 1 IC, 1 CON). At 14 wk, 2 birds received a score of 1(1 IC, 1 HS) and 1 bird received a score of 2 (HS) for head wounds. Similarly, skin wounds on the head were again all mostly 0, but at 14 wk 4 birds received a score of 1 (2 CON, 1 IC, 1 HS) and 5 birds received a score of 2 (2 HS, 2 IC, 1 CON). During trial 1, two birds were euthanized due to poor leg health; one at 12 wk and one at 14 wk of age. Across treatment groups (C, IC and HS), feather condition of the tail and wings (median (25^{th} quartile, 75^{th} quartile) scores) varied due to age (Table 13).

Table 13. Tail feather, left wing, and right wing feather condition scores (median (25^{th} quartile, 75^{th} quartile) for turkeys experiencing a mild heat stress (HS), immune challenge (IC) or neither (CON) at 10, 12 and 14 weeks of age. Significant changes in feather condition across ages are denoted as superscripts ($^{a\ b}$).

Welfare Parameter	Treatment group	Age			P Value
		10 wk	12 wk	14 wk	
Tail Feathers	CON	2 (2, 3)^a	2 (2, 3)^a	3 (2, 3)^b	P < 0.001
	HS	2 (2, 3)^a	2 (2, 3)^a	3 (2, 3)^b	P < 0.001
	IC	2 (2, 3)^a	2 (2, 3)^a	3 (2, 3)^b	P < 0.001
Left Wing Feathers	CON	3(2,3)^a	3(2,3)^a	3(3,3)^b	P = 0.01
	HS	3(2,3)^a	3(2,3)^a	3(3,3)^b	P = 0.01
	IC	3(2,3)^a	3(2,3)^a	3(3,3)^b	P < 0.001
Right Wing Feathers	CON	3(2,3)^a	3(2,3)^a	3(3,3)^b	P = 0.02
	HS	3(2,3)^a	3(2,3)^a	3(3,3)^b	P = 0.01
	IC	3(2,3)^a	3(2,3)^a	3(3,3)^b	P < 0.001

For C birds, feather condition of the tail was worse at 14 wk compared to both 10 and 12 wk (10 wk: 2 (2, 3), 12 wk: 2 (2, 3), and 14 wk: 3 (2, 3), P < 0.001). Feather condition of both the left wing and right wing of C birds? also varied due to age at 10 wk 3 (2, 3), 12 wk 3 (2, 3)) and 14 wk: 3 (3, 3) (left wing: P < 0.001; right wing: 0.02). HS and IC treatment birds had the same welfare score outcomes as C birds (Table 13). Footpad scores of both left and right feet had medians and quartiles of 0 at all ages; however, post hoc analysis revealed differences among ages because the number of birds with scores of 1 increased with age (P < 0.001).

Treatment related differences were similar to the age-related differences as most birds received scores of 0, aside from the feather condition of wings and tail. No treatment differences were found for feather condition (P = 0.93 tail; P = 0.88 left wing; P = 0.92 right wing).

3.3.2 Accelerometer Walking Activity Analysis

Many differences in steps/hr were present depending on the turkeys' age, the treatment applied, and the day relative to treatment (Figure 7). As turkeys aged, the number of steps taken per hour decreased (P < 0.001). HS- and IC-treated birds took fewer steps compared to CON groups (P < 0.001). Number of steps/hr differed among all days relative to treatment (P = 0.002), except between day -1 and day 2.

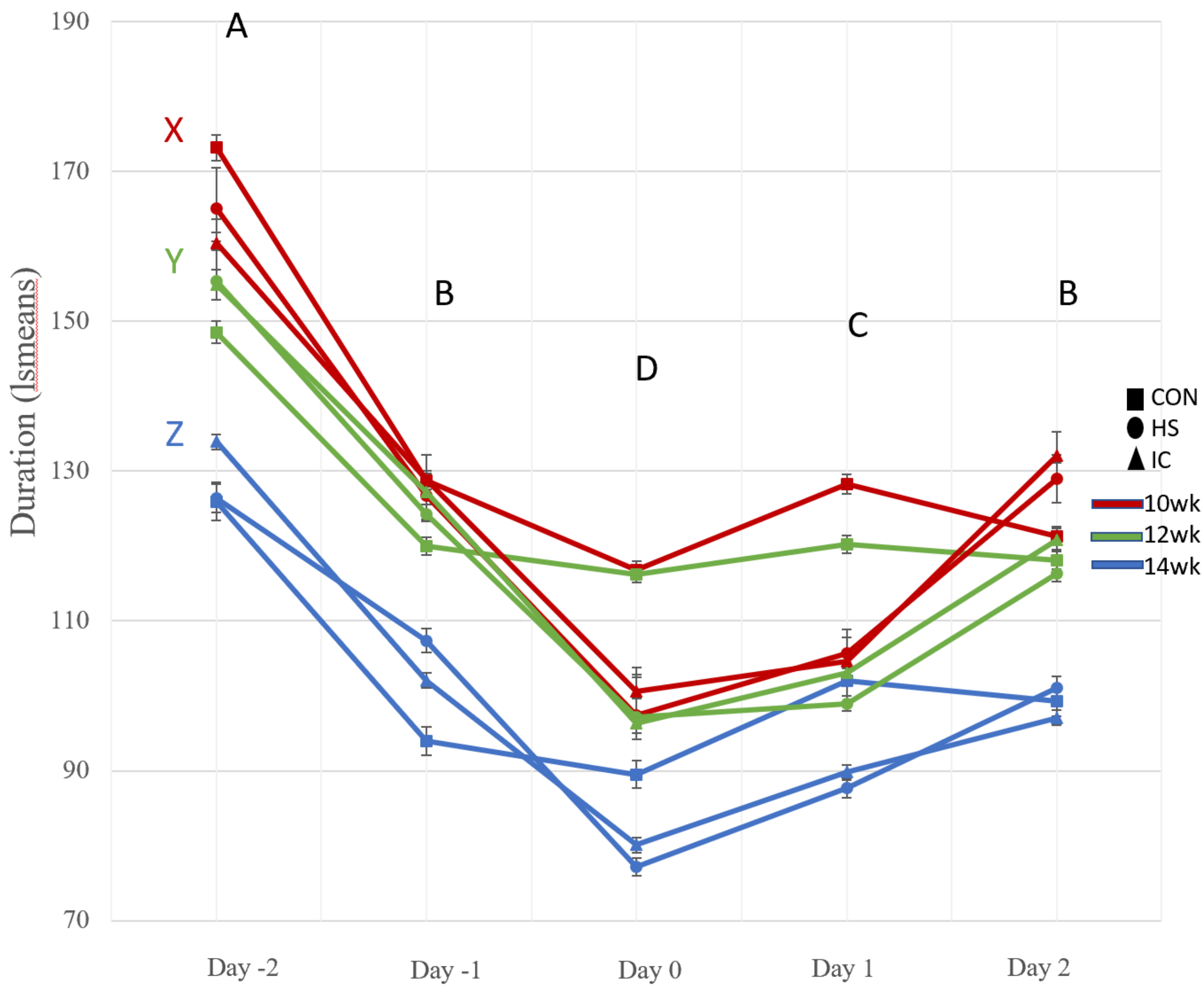

Figure 7. Mean number of steps/hr (lsmean ± SE) for turkeys experiencing a mild heat stress (HS), an immune challenge (IC) or no treatment (C) two days before treatment (day -2), one day before treatment (day -1), the day of treatment (day 0), one day after treatment (day +1) and two days after treatment (day +2) at 10, 12, and 14 weeks of age. Overall changes in steps/hr at each age are denoted by X, Y, and Z. Overall changes in duration due to treatment are denoted by A, B, C, and D.

3.3.3 Behavioral Scans

Age-related differences in the proportions of birds performing each behavior occurred irrespective of treatment group (Table 14). The majority of behaviors (standing, eating, drinking, walking, panting while standing, wing splay, and missing) did not change due to age, but the proportion of turkeys sitting and panting while standing did. Compared to weeks 10 and 12, a greater proportion of birds performed sitting behavior at 14 wk ($P = 0.01$). There was also a difference in panting while standing, with the proportion of birds that panted while standing was lowest at 14 wk ($P = 0.03$). When analyzing age-related differences by treatment group, a few differences were found (Appendix, Table 1). Sitting behavior was performed by a larger proportion of birds in HS and IC groups compared to the CON treatment ($P < 0.001$) at all ages, but the highest proportions occurred at 12 and 14 wk. Standing and walking behaviors were higher for CON groups compared to HS and IC treatments ($P= 0.01$) at all ages with these behaviors occurring the most at 10 wk. The HS treatment showed an increase in panting while sitting, panting while standing, wing splay, aggressive interactions, and feather pecking behaviors compared to both IC and CON treatments ($P= 0.001$) at each age, with week 10 being when the greatest proportion of birds performed these behaviors. Although HS birds performed most of the panting and wing splay behaviors, sometimes IC and CON would perform these behaviors, but at lower proportions. At 12 wk, the proportion of birds performing sitting and walking behaviors was higher for CON birds compared to both IC and HS at 12 wk ($P < 0.001$).

Day also effected the proportion of birds performing certain behaviors (Appendix, Table 2). The proportion of animals showing wing splay, panting, and aggressive interaction behaviors was higher for HS birds at each age compared to CON and IC treatments at each age only on day 0 (day of imposed treatment) ($P < 0.001$). Sitting, walking, and standing were also performed in a higher proportion of turkeys in HS and IC groups on day 0 and day 1 compared to all other days ($P < 0.001$).

On day -1, the proportion of CON birds performing behavior categorized as other was higher than for the other days and treatments ($P < 0.001$).

Table 14. Changes in the proportion of sitting and panting while sitting behaviors (median (25th quartile, 75th quartile) for turkeys at 10, 12 and 14 weeks of age. Significant changes in behavior proportions across ages are denoted as superscripts ([a b]).

Behavior	Age			P Value
	10 wk	12 wk	14 wk	
Sitting	0.17 (0.0, 0.4)[a]	0.17 (0.0, 0.4)[a]	0.2 (0.0, 0.5)[b]	P = 0.01
Panting while standing	0.0 (0.0, 0.0)[a]	0.0 (0.0, 0.0)[a]	0.0 (0.0, 0.0)[b]	P = 0.03

3.3.4 Behavior of Focal Turkeys (Frequencies and Durations))

Differences in the frequency of behaviors are presented in Tables 15 and 16. Sitting, aggressive interactions and feather pecking, and other behaviors were performed at different frequencies depending on turkeys' age. At 14 wk, turkeys performed sitting behaviors more frequently compared to both 10 and 12 wk ($P < 0.001$). Frequencies of aggressive interactions and feather pecking were higher at 12 and 14 wk of age compared to 10 wk ($P = 0.04$). Finally, the frequency with which turkeys performed behavior classified as other was highest at 14 wk of age ($P = 0.01$). HS and IC groups sat more frequently compared to CON groups, with HS turkeys sitting the most frequently ($P = 0.03$) (Table 15). No other treatment related differences in frequencies of behaviors were found.

Table 15. Frequency (lsmean ± SE) of performance of behaviors (sitting, standing, walking, eating and drinking, preening, aggressive interaction and feather pecking, and missing/other) at 10, 12, and 14 wk of age. Significant differences among ages denoted with $^{a\ b\ c\ d}$.

Behavior	Age			P Value
	10 wk	12 wk	14 wk	
Sitting	14.04 ± 0.23^{b}	14.89 ± 0.20^{b}	19.65 ± 0.22^{a}	P < 0.001
Standing	18.02 ± 0.16	17.94 ± 0.14	18.06 ± 0.14	P = 0.82
Walking	32.42 ± 0.24	32.51 ± 0.21	32.31 ± 0.22	P = 0.81
Eating and Drinking	10.80 ± 0.15	10.93 ± 0.13	11.05 ± 0.13	P = 0.40
Preening	6.66 ± 0.87	6.44 ± 0.84	6.80 ± 0.88	P = 0.09
Aggressive Interaction and Feather Pecking	4.70 ± 0.08^{a}	4.47 ± 0.06^{b}	4.49 ± 0.06^{b}	P = 0.04
Missing/other	12.97 ± 0.29^{b}	12.97 ± 0.24^{b}	13.97 ± 0.25^{a}	P = 0.01

Table 16. Average (lsmean ±SE) Frequency of behaviors in turkeys performing behaviors (sitting, standing, walking, eating and drinking, preening, aggressive interaction and feather pecking, and missing/other) during a mild heat stress (HS), immune challenge (IC), or neither (CON). Significance differences across treatments denoted with $^{a\ b\ c\ d}$.

Behavior	Treatment			P Value
	CON	HS	IC	
Sitting	15.18 ± 0.22^{b}	16.55± 0.22^{a}	16.18 ± 0.22^{b}	P = 0.03
Standing	17.92 ± 0.15	18.04 ± 0.15	18.07 ± 0.15	P = 0.69
Walking	32.79 ± 0.22	32.44 ± 0.21	32.32 ± 0.22	P = 0.26
Eating and Drinking	10.93 ± 0.14	10.92 ± 0.14	10.92 ± 0.14	P = 0.98
Preening	6.78 ± 0.88	6.58 ± 0.87	6.54 ± 0.85	P = 0.34
Aggressive Interaction and Feather Pecking	4.60 ± 0.07	4.64 ± 0.07	4.52 ± 0.07	P = 0.25
Missing/other	13.64 ± 0.27	12.86 ± 0.25	13.40 ± 0.27	P = 0.09

In some instances, the presence of a bandage and accelerometer appeared to affect certain behaviors (walking and other behavior). In particular, the interaction between the presence of an accelerometer, age, treatment and day relative to treatment was significant in the model (P = 0.01) examining effects on walking behavior, but after adjusting for multiple comparisons no significance was found between turkeys wearing vs. not wearing an accelerometer. A significant difference was found for other behavior between turkeys in the CON group that wore an accelerometer and turkeys in the HS group that wore an accelerometer (P = 0.004), but no other differences due to the presence of an accelerometer were found.

The duration of turkeys sitting changed due to treatment, age, and the day of the imposed treatment (Figure 8). Turkeys sat for longer periods of time at 14 wk compared to 10 and 12 wk of age (P < 0.001). Overall, HS and IC birds sat for longer periods of time compared to CON birds (P =0.001). Turkeys during Day -2 and -1 sat for the least amount of time before the duration of sitting increased on Day 0 (P < 0.001). Turkeys sat less on Day 1 and 2 but still more compared to Day -2 and -1 (P < 0.001). During several instances, the treatment, treatment day, and age affected how long a turkey would sit for. At 10 wk, CON turkeys sat for a shorter period of time compared to Day -2 (P = 0.004) and Day -1 (0.004). At 12 wk, CON turkeys sat for shorter periods of time on Day 0 compared to Day -2 (P =0.001) and Day -1 (P = 0.02). At 10 wk, HS turkeys on Day -1 sat less than Day 0 (P = 0.01). At 12 wk, HS turkeys sat for longer durations on Day 0 and Day 1 compared to Day -2 (Day 0 P < 0.001; Day 1 P = 0.02) and Day -1 (Day 0 P = 0.001; Day -1 P = 0.005). At 14 wk, HS turkeys sat less on Day 2 compared to Day 1 (P = 0.01). At 12 wk, IC turkeys sat more on Day 0 compared Day -2 (P < 0.001), Day -1 (P < 0.001) and Day 1 (P < 0.001).At 14 wk, IC turkeys sat the most during Day 1 compared to all other days (all P < 0.001).

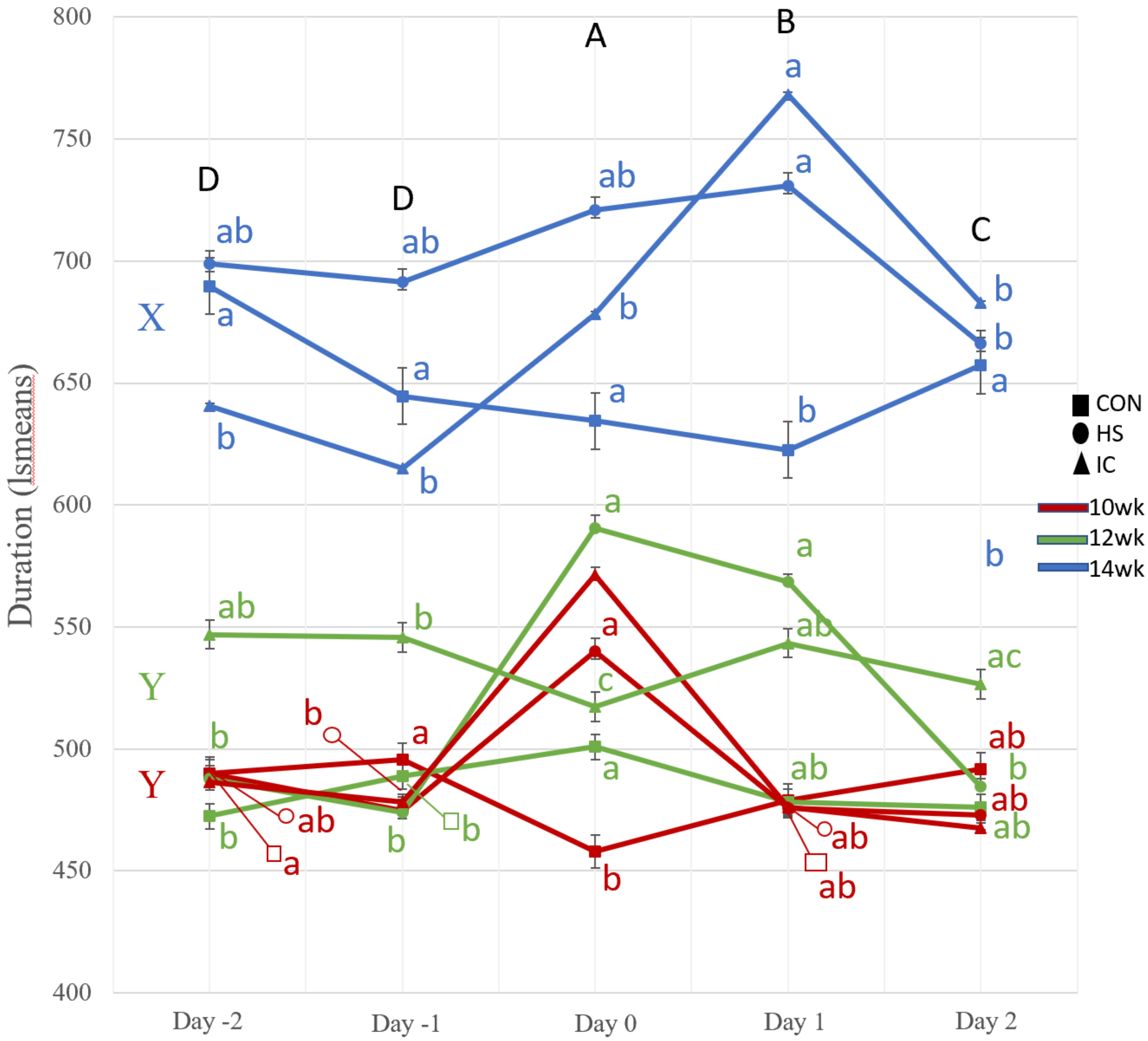

Figure 8. Duration of sitting behavior (lsmean ± SE) for turkeys experiencing a mild heat stress (HS), an immune challenge (IC) or no treatment (C) two days before treatment (day -2), one day before treatment (day -1), the day of treatment (day 0), one day after treatment (day +1) and two days after treatment (day +2) at 10, 12 and 14 weeks of age. Overall changes in duration at each age are denoted by X, Y, and Z. Overall changes in duration at each day in relation to the imposed treatment are denoted by A, B, C, and D. Changes in duration at each day within a treatment group and age range (i.e. CON at 10 wk) are denoted by a, b, c, and d.

Changes in the duration of turkeys standing varied by age and the day of imposed treatment (Figure 9). Turkeys spent less time standing at 14 wk compared to 10 and 12 wk of age ($P < 0.001$). CON turkeys generally stood more compared to IC birds ($P = 0.03$) while no differences were found between HS treatment birds and the other treatments. Turkeys on Day 1 stood less often compared to Day -2 (P = 0.01), Day -1 (P = 0.01), and Day 2 (P = 0.002). At 14 wk, turkeys spent less time standing on Day 0 and Day 1 compared to all other days.

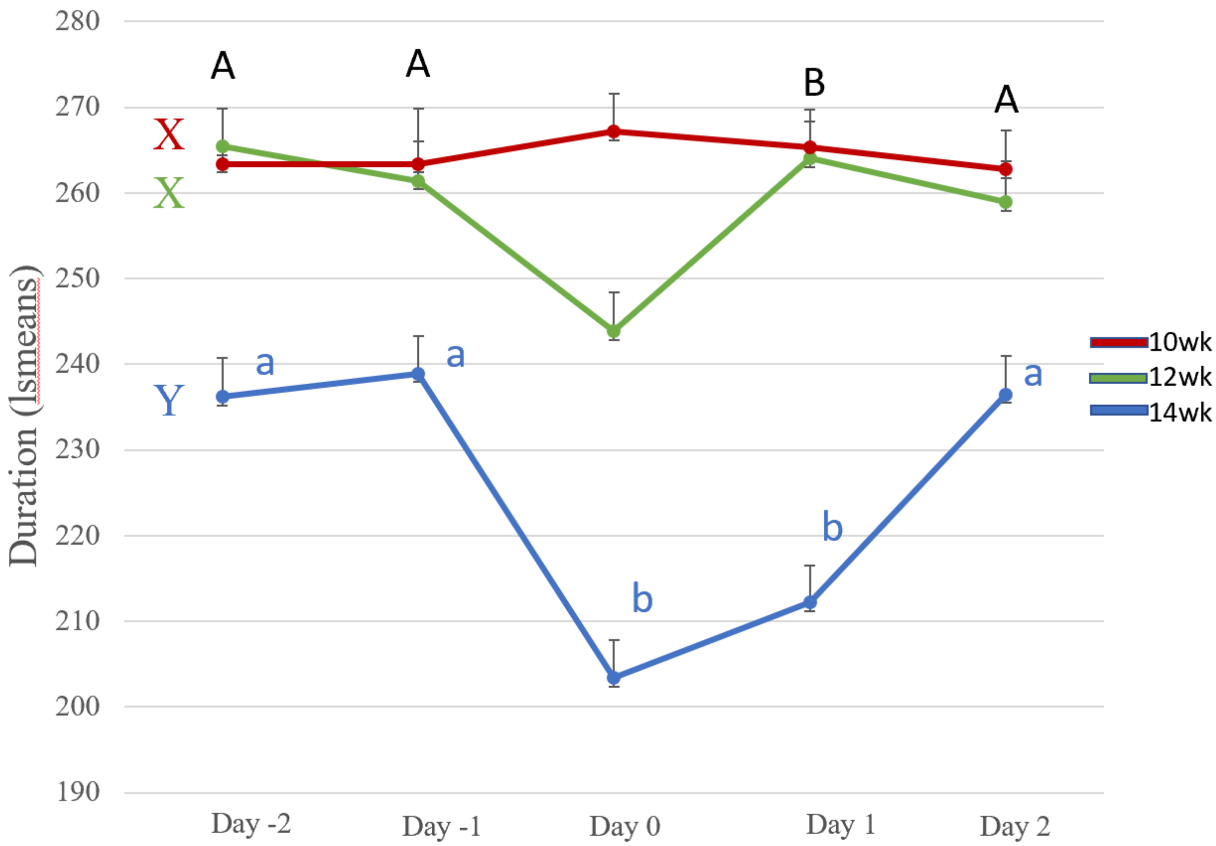

Figure 9. Mean duration of standing behavior (lsmean ± SE) for turkeys two days before an imposed treatment (day -2), one day before treatment (day -1), the day of treatment (day 0), one day after treatment (day +1) and two days after treatment (day +2) at 10, 12 and 14 weeks of age. Overall changes in duration at each age are denoted by a, b, and c. Day interactions are denoted with an asterisk (*).

The duration of turkeys eating and drinking changed due to age and day of imposed treatment (Figure 10). The duration of turkeys performing eating and drinking behaviors decreased at 14 wk of age (P < 0.001). CON turkeys generally ate and drank more compared to IC turkeys (P = 0.03). Turkeys on Day 1 performed lower duration of eating and drinking behavior compared to Day -2 (P = 0.01), Day -1 (P =0.004), and Day 2 (P < 0.001). At 14 wk, turkeys ate and drank less compared at all other days (all P < 0.001). Turkeys on Day 0 also performed less eating drinking behaviors, but only in comparison to Day 2 (P = 0.02).

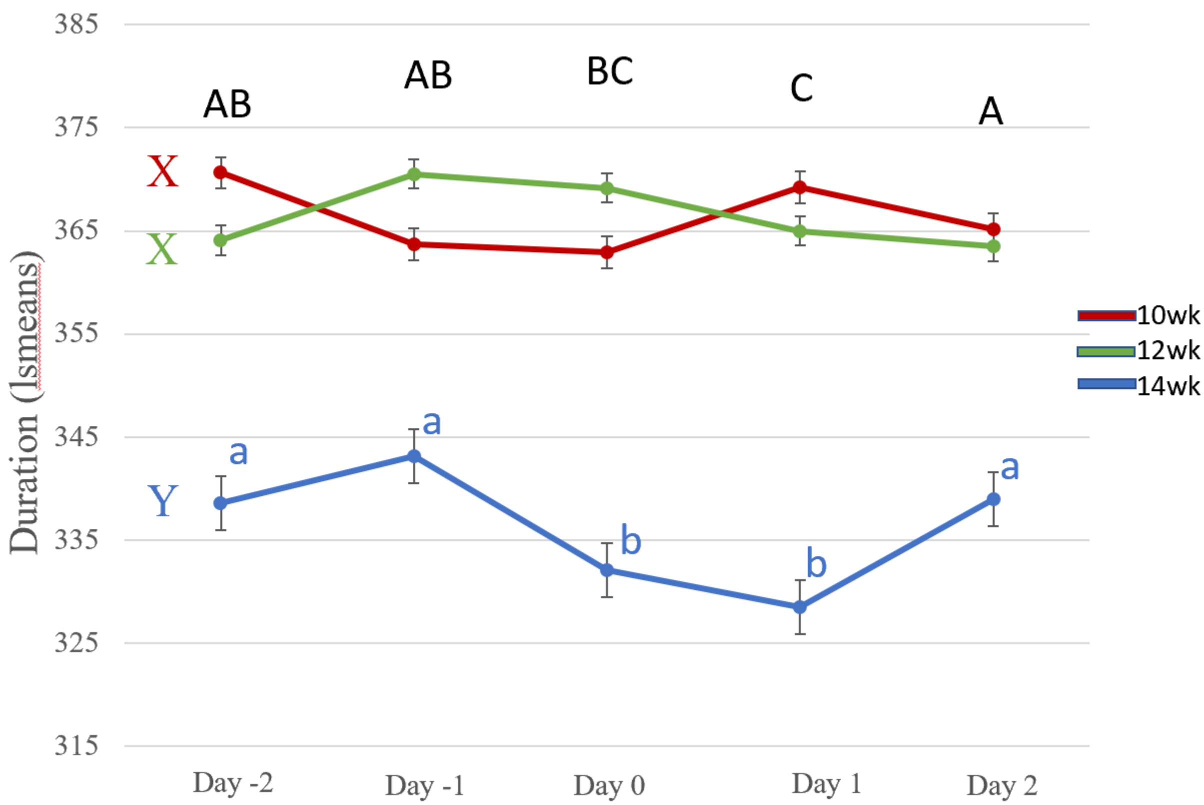

Figure 10. Mean duration of eating and drinking behavior (lsmean ± SE) for turkeys two days before an imposed treatment (day -2), one day before treatment (day -1), the day of treatment (day 0), one day after treatment (day +1) and two days after treatment (day +2) at 10, 12 and 14 weeks of age. Overall changes in duration at each age are denoted by a, b, and c. Day interactions are denoted with an asterisk (*).

Changes in a turkey's duration of walking behavior varied due to age, treatment, and day of imposed treatment (Figure 11). Turkeys at 10 and 12 wk of age generally walked more compared to turkeys at 14 wk of age (P < 0.001). HS and IC birds walked less overall compared to CON birds (P < 0.001). Turkeys on Day 0 and Day 1 performed walking behaviors less often compared to all other days. At 14 wk, CON turkeys performed walking behaviors longer compared to all other days (all P < 0.001). At 14 wk, HS turkeys performed walking behaviors longer on Day -2 and -1 compared to Day 0 and Day 1 ($P < 0.001$). At 14 wk, IC turkeys performed longer walking behaviors on Day 0 and Day 1 compared to all other days (all $P < 0.001$).

Turkeys performed preening behavior more at 12 wk of age compared to 14 wk ($P = 0.03$) (Figure 12). The duration of preening was not different at 10 wk compared to all other ages. Treatment and the day of imposed treatment seemingly had no effect on how long turkeys performed preening behavior.

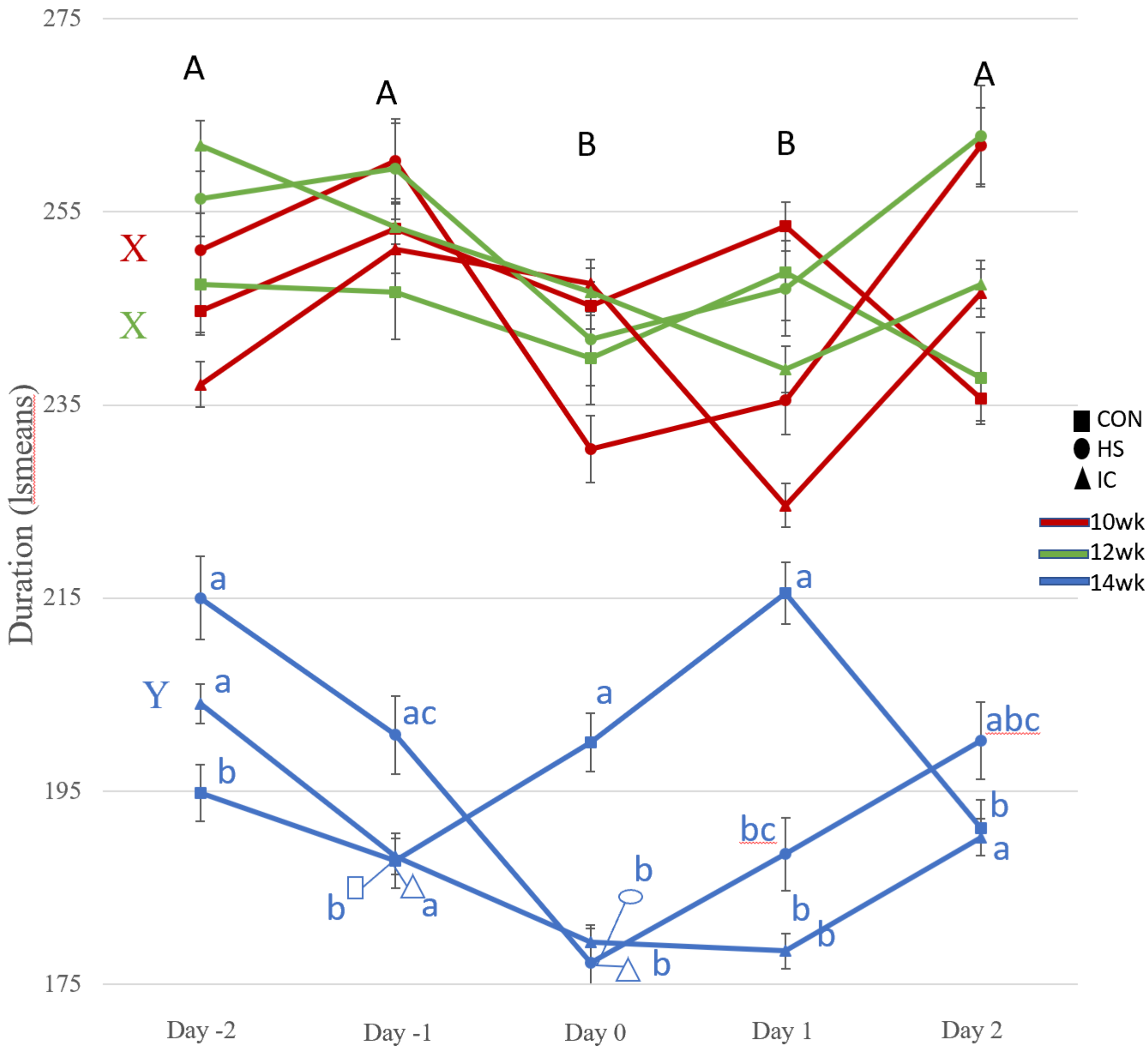

Figure 11. Duration of walking behavior (lsmean ± SE) for turkeys experiencing a mild heat stress (HS), an immune challenge (IC) or no treatment (C) two days before treatment (day -2), one day before treatment (day -1), the day of treatment (day 0), one day after treatment (day +1) and two days after treatment (day +2) at 10, 12 and 14 weeks of age. Overall changes in duration at each age are denoted by X, Y, and Z. Overall changes in duration at each day in relation to the imposed treatment are denoted by A, B, C, and D. Changes in duration at each day within a treatment group and age range (i.e. CON at 10 wk) are denoted by a, b, c, and d.

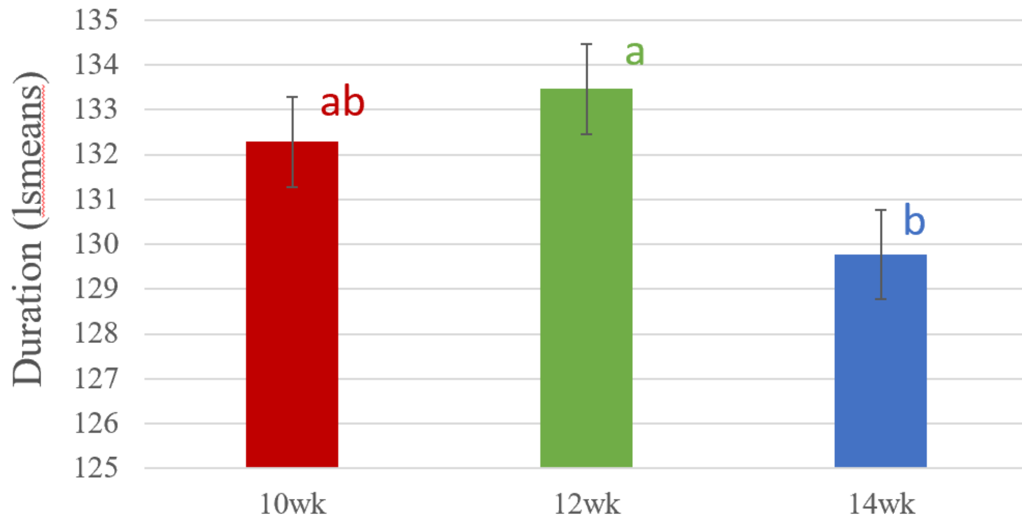

Figure 12. Mean duration of preening behavior (lsmean ± SE) for at 10, 12 and 14 weeks of age. Overall changes in duration at each age are denoted by a, b, and c.

The time spent performing other behaviors varied due to age, treatment, and the day of imposed treatment (Figure 13). Birds at 10 and 12 wk of age performed behavior categorized as other for longer durations compared to birds at 14 wk ($P < 0.001$). HS and IC turkeys performed other behaviors longer compared to CON groups ($P < 0.001$). Turkeys on Day 0 performed less behaviors classified as other compared to all other days aside from Day 2 (all $P < 0.001$). Day 2 behavioral durations were no difference than any of the days. At 10 wk, CON turkeys performed more other behavior on Day -1 compared to Day 0. At 10 wk, HS turkeys on Day 0 and Day 1 were significantly performing less other behavior compared to all other days (all $P < 0.001$). At 10 wk, IC turkeys performed more other behaviors on Day 0 compared to all other days (all $P < 0.001$). At 12 wk, CON turkeys performed less other behavior on Day 0, 1, and 2 compared to Day -2 ($P = 0.02$). At 12 wk, HS turkeys performed less other behavior on Day 0 compared to all other days (all $P < 0.001$) aside from Day -1. At 12 wk, IC turkeys performed less other behavior on Day 0, 1, and 2 (all $P < 0.001$) with Day 1 being the day the duration was performed the least.

At 14 wk, CON and IC turkeys performed other behavior more on Day 1 compared to all other days (all $P < 0.001$). At 14 wk, HS turkeys performed other behaviors the most on Day 1 and 2 (all $P < 0.001$).

Lastly, turkeys performed feather pecking and aggressive interactions varied due to age, treatment, and the day of imposed treatment (Figure 14). Birds at 14 wk of age performed feather pecking and aggressive interaction more compared to 10 and 12 wk of age ($P < 0.001$). HS turkeys performed the highest amount of feather pecking and aggressive interactions compared to CON and IC birds ($P < 0.001$). Turkeys on Day 0 performed more feather pecking behavior compared to all other days ($P < 0.001$). Turkeys on Day 1 performed the least instances of feather pecking and aggressive interactions. At 14 wk, HS turkeys performed more feather pecking and aggressive interactions on Day 0 compared to all other days (all $P < 0.001$) while Day 1 had the least instances of feather pecking ($P = 0.001$). At 14 wk, CON turkeys performed more feather pecking on Day 0 and Day 1 compared to all other days (all $P < 0.001$).

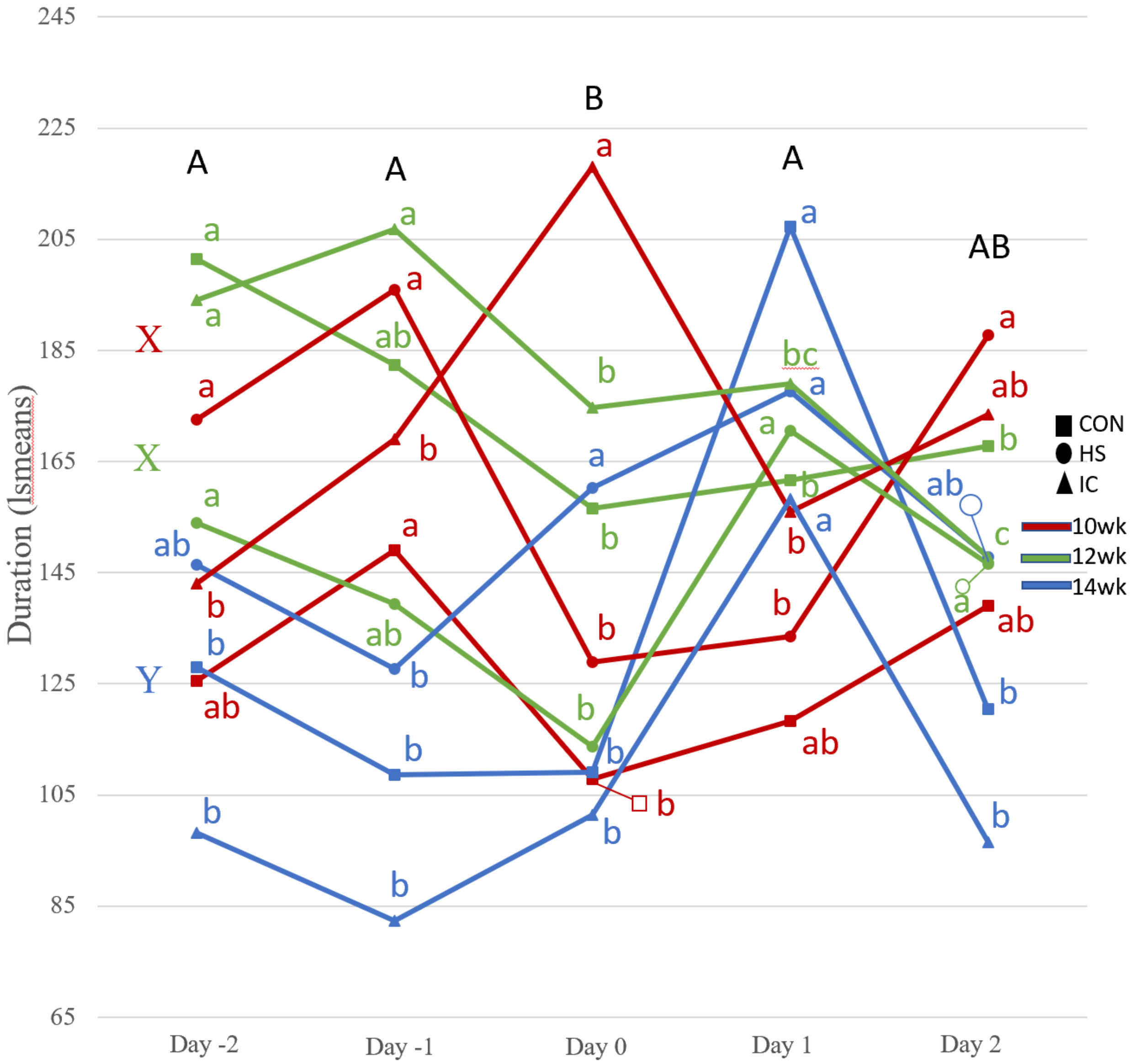

Figure 13. Duration of behaviors classified as other (lsmean ± SE) for turkeys experiencing a mild heat stress (HS), an immune challenge (IC) or no treatment (C) two days before treatment (day -2), one day before treatment (day -1), the day of treatment (day 0), one day after treatment (day +1) and two days after treatment (day +2) at 10, 12 and 14 weeks of age. Overall changes in duration at each age are denoted by X, Y, and Z. Overall changes in duration at each day in relation to the imposed treatment are denoted by A, B, C, and D. Changes in duration at each day within a treatment group and age range (i.e. CON at 10 wk) are denoted by a, b, c, and d.

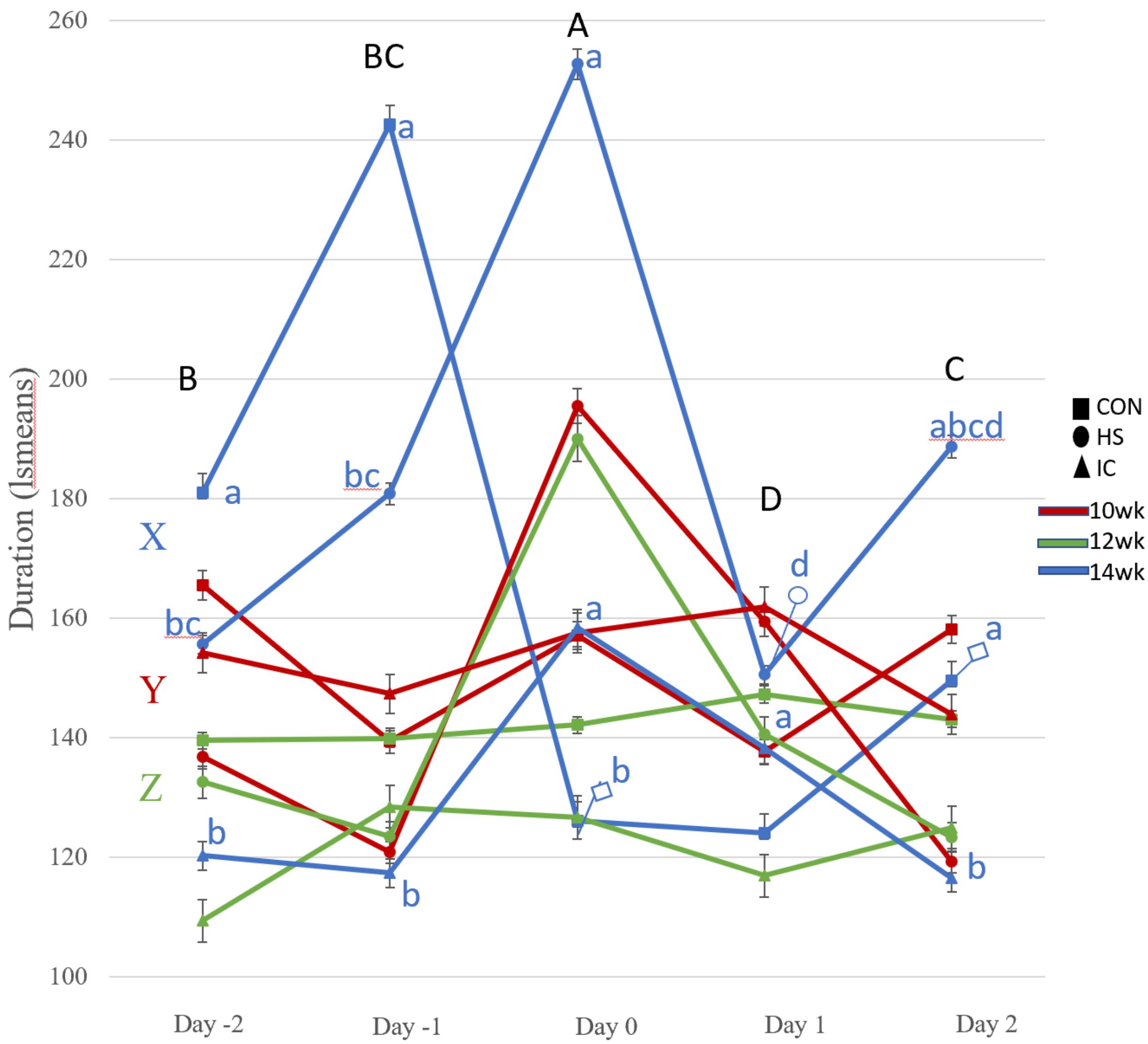

Figure 14. Duration of feather pecking and aggressive interaction behaviors (lsmean ± SE) for turkeys experiencing a mild heat stress (HS), an immune challenge (IC) or no treatment (C) two days before treatment (day -2), one day before treatment (day -1), the day of treatment (day 0), one day after treatment (day +1) and two days after treatment (day +2) at 10, 12 and 14 weeks of age. Overall changes in duration at each age are denoted by X, Y, and Z. Overall changes in duration at each day in relation to the imposed treatment are denoted by A, B, C, and D. Changes in duration at each day within a treatment group and age range (i.e. CON at 10 wk) are denoted by a, b, c, and d.

3.4 Discussion

In general, turkey welfare was unaffected by treatment; however, tail and wing feather condition and footpad condition worsened with age. These results are similar to those of other studies that reported an increase in injurious pecking as turkeys age (Busayi et al., 2006; Duggan et al., 2014; Dalton et al., 2016b). Although treatment did not affect welfare scores, video behavior analysis revealed a higher display of aggressive interactions and feather pecking for turkeys subjected to heat stress on the day of the imposed treatment (day 0). Turkey welfare was assessed two days before treatments were imposed; therefore, it is possible that welfare scores differed among turkeys post-treatment, but this was not assessed.

Peak temperature in each of the heat stress rooms was selected by measuring the humidity in each room and then using the heat index of Xin and Harmon (1998). It could be argued that because rooms were not heated to the same temperature, that each room did not impose a similar heat stress; however, each room was set to the same heat index level. Cloacal temperatures indicated that during heat stress, body temperatures increased by an average of 0.7 ± 0.18°C. The increase in body temperature observed here is comparable to that reported by Brown-Brandl et al. (1997), who reported an increase of 0.5 to 1°C in turkeys following heat stress. The increase in body temperature corresponded with heat stress-related behavior, such as panting and wing spreading, which were consistently observed in turkeys in HS treatment groups. Birds in non-HS groups also occasionally performed these behaviors, despite not being subjected to increased room temperatures. Sometimes birds would naturally sit with wings away from their body (being defined as wing splay in the ethogram) when not exposed to HS. The temperatures in the rooms were slightly higher than what is recommended by industry (Aviagen, 2015), which may have resulted in some birds displaying these behaviors, despite not being subjected to HS per se. Turkeys in HS groups also performed more aggressive behaviors during the period of heating.

Many differences in accelerometer walking activity were found in this study, including age, day, treatment, age-day interactions, and day-treatment interactions. Turkeys' activity levels decreased with age: the number of steps/hr decreased and amount of time spent sitting increased with age. Age related changes in turkeys' gait resulting from poorer leg health and increased body weight have been reported in several studies (Martrenchar et al., 1999; Krautwald-Junghanns et al., 2011; Dalton et al., 2016b).

Ours is the first study to report age-related changes in step counts, and daily variations in step counts for turkeys. Steps/hr varied among all days, except for day -1 and day 2. It is possible that each bird does not have a consistent total steps/hr each day, as day -2 and day -1 should have no differences in total number of steps since no treatments were imposed. However, a limitation of this study, and a possible explanation for the difference in steps/hr between days -2 and -1 and days -2 and 4 is that we were unable to record step counts for the first 3 hr on day-2. Turkeys are likely to be most active in the morning around feeding time shortly after the lights come on, which could have contributed to the differences observed in steps/hr. Other aspects of turkey behavior were greatly affected by age. Higher activity related behaviors, such as walking, decreased with age. The frequency of each of the active behaviors followed a similar pattern, with the frequencies of these behavior declining as turkeys aged.

The imposed mild heat stress and immune challenge significantly affected the number of steps/hr and turkey behavior. Both HS and IC treatments could be expected to cause a decrease in walking activity if birds are feeling ill (IC) or if birds are trying to cool down (HS). Activity levels have been reported to decrease when poultry are heat stressed or sick (Mahmoud et al., 2015; Li et al., 2015; Quinteiro-Filho et al., 2010; Hart, 1988). During the day of the imposed treatments and the day afterwards, steps/hr for both HS and IC were lower compared to CON groups. This was to be expected as it was thought that turkeys may adjust their behavior up to several days after an induced stressor. HS and IC turkeys performed more sitting behaviors and less standing and walking behaviors compared to CON groups in terms of both duration of sitting and the frequency of sitting. Similar to the decrease in steps/hr detected by accelerometers, turkeys under HS and IC stressors may have chosen to sit longer and more frequently if they felt unwell or too hot (Mahmoud et al., 2015; Li et al., 2015). Behavioral differences between days were found on day 0 and day 1 compared to all other days. Birds under a mild heat stress performed more aggressive interactions on the day of the imposed treatment than on all other days and when compared to turkeys not experiencing a mild heat stress. Although no studies have documented an increase in aggression among turkeys under heat stress, chickens have been reported to display signs of frustration and aggression under high ambient temperature compared to cooler housing temperatures (Muir and Craig, 1998).

Chapter 2 examined changes in turkey welfare and gait due to the presence of an accelerometer attached to the leg, and it was determined that a one wk habituation period did not adversely affect turkey gait. However, no behavioral data had been collected in Chapter 2, and in this chapter, results demonstrated that the presence of an accelerometer did not change turkeys' behavior. Therefore, a one week habituation period to AXY-3 accelerometers was sufficient enough to get turkeys accustomed to wearing an accelerometer on their leg.

Based on the results of this study, turkeys experiencing an immune challenge will take less steps, walk and stand less, and spent more time sitting up to one day after the imposed stressor. similarly, turkeys under a mild heat stress will sit and pant more and have more aggressive interactions during the heat stress period. Up to one day after the heat stress, turkeys will still sit more frequently and for longer durations compared to turkeys at lower temperatures.

3.5 Conclusion

Heat stress and disease are major welfare concerns for the turkey industry. Our study is the first to report changes in turkey's behavior in response to mild heat stress and an immune challenge with a live-virus vaccine. In addition, we report that accelerometers can be used to detect changes in turkeys' walking activity in response to heat stress and an immune challenge. Under an imposed heat stress and immune challenge, turkeys were less active; took fewer steps/hr and walked less and for longer durations, while sitting more frequently and for longer durations. Turkeys exposed to HS also performed behaviors such as wing splay, panting, and aggressive interactions more frequently than turkeys not experiencing HS. Future studies should look into how time of day effects both accelerometer and behavior data to indicate whether accelerometers have the potential to detect welfare issues earlier than visual observations.

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CHAPTER 4: SUMMARY AND FUTURE DIRECTIONS

Accelerometers were shown to provide valuable information about turkeys' gait, activity, and behavior at different ages. Although many aspects of a turkey's gait appear unaffected by the presence of an accelerometer, habituation was necessary to achieve higher accuracy and specificity of accelerometer data. No welfare implications were found for wearing an accelerometer, but lower walking frequencies were observed for turkeys experiencing HS or an IC that were wearing and accelerometer vs. birds not wearing anything. Turkeys experiencing a heat stress or immune challenge caused a decrease in steps/hr up to 24 hours after the imposed treatment began. Age related changes in turkey gait and behavior provide insight into changes a turkey experiences over time. Activity levels decreased with age, as shown from accelerometer output and behavioral analysis, and gait dynamics shifted depending on the age of a turkey.

Further research is needed to explore the best step threshold for specific turkey ages in order to get the most accurate data in future analysis of walking activity. I found that at each age, a different stepping threshold was best to get the most accurate number of step counts for each bird. Unfortunately, due to individual variation among turkeys, a false discovery rate cannot (at this point) be eliminated from acceleration output. Stepping thresholds should be analyzed at each week of age as a reference for accelerometer analysis in order to be as accurate as possible. As of now, only 10, 12, 14, and 16 wk age old turkeys have a unique stepping threshold. Research should be conducted on how step thresholds change for younger turkeys. Furthermore, sex differences were not a factor in these studies as only male turkeys were used which may differ from females in terms of gait and behavior under heat stress and an immune challenge. Further exploration of turkey gait may be beneficial as there were many discrepancies in the literature and this study concerning age related differences in gait (duty factor, gait velocity, step time, and cadence).

Future research should focus on the early detection potential accelerometers can provide to the turkey industry for welfare concerns. As shown by our results, walking activity decreases under both a mild heat stress and immune challenge, so future studies should now determine if this decrease in activity level is detectable before overt visual behavioral signs. If accelerometers can detect signs of stress more objectively, accurately, and quicker than visual inspection, then both farmers and researchers could benefit from utilizing these devices to improve animal welfare in the future.

APPENDIX

Table 1. Differences in the proportion of turkeys performing behaviors at 10, 12, and 14 wk of age within each treatment group (CON, HS, and IC) as median (25^{th}, 75^{th} quartile). Significance ($P < 0.05$) denoted with superscripts: $^{a\ b\ c\ d}$

Behavior	CON			HS			IC		
	10 wk	12 wk	14 wk	10 wk	12 wk	14 wk	10 wk	12 wk	14 wk
Sitting	0.17 (0, 0.33)^{a}	0.17 (0, 0.33)^{a}	0.17 (0, 0.5)^{b}	0.17 (0, 0.5)^{b}	0.2 (0.14, 0.5)^{c}	0.2 (0.14, 0.5)^{c}	0.17 (0, 0.5)^{b}	0.2 (0.14, 0.5)^{c}	0.2 (0.14, 0.5)^{c}
Standing	0 (0, 0.2)^{a}	0 (0, 0.2)^{a}	0 (0, 0.14)^{b}	0 (0, 0.2)^{a}	0 (0, 0.17)^{c}	0 (0, 0.14)^{b}	0 (0, 0.2)^{a}	0 (0, 0.17)^{c}	0 (0, 0.14)^{b}
Walking	0 (0, 0.17)^{a}	0 (0, 0.17)^{a}	0 (0, 0.14)^{b}	0 (0, 0.14)^{b}	0 (0, 0.14)^{a}	0 (0, 0.06)^{c}	0 (0, 0.14)^{b}	0 (0, 0.14)^{a}	0 (0, 0.06)^{c}
Eating	0.14 (0, 0.33)^{a}	0.14 (0, 0.29)^{b}	0.14 (0, 0.33)^{a}	0.14 (0, 0.29)^{b}	0.14 (0, 0.33)^{a}	0.14 (0, 0.33)^{a}	0.14 (0, 0.29)^{b}	0.14 (0, 0.33)^{a}	0.14 (0, 0.33)^{a}
Drinking	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
Preening	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
Aggressive interaction	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0.2)^{b}	0 (0, 0.2)^{b}	0 (0, 0.2)^{b}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}
Feather Pecking	0 (0, 0.29)	0 (0, 0.29)	0 (0, 0.29)	0 (0, 0.29)	0 (0, 0.29)	0 (0, 0.29)	0 (0, 0.29)	0 (0, 0.29)	0 (0, 0.29)
Panting while sitting	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0.29)^{c}	0 (0, 0.2)^{b}	0 (0, 0.2)^{b}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}
Panting while standing	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{b}	0 (0, 0.29)^{d}	0 (0, 0.2)^{c}	0 (0, 0.2)^{c}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{b}
Wing splay	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0.29)^{c}	0 (0, 0.2)^{b}	0 (0, 0.2)^{b}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}
Missing/ other	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)

Table 2. Differences in treatment (CON, HS, and IC) at each day (2 days before imposed treatment (-2), 1 day before (-1), the day of imposed treatment (0), the day after the imposed treatment (1), and 2 days after the imposed treatments (2)) as median (25th, 75th quartile). Significance denoted with superscripts: $^{a\,b\,c\,d}$ ($P < 0.05$)

	Day -2			Day -1			Day 0			Day 1			Day 2		
	CON	HS	IC	CON	HS	IC	CON	HS	IC	CON	HS	IC	CON	HS	IC
Sitting	0.185 (0, 0.33)^{a}	0.17 (0, 0.365)^{a}	0.17 (0, 0.33)^{a}	0.14 (0, 0.29)^{a}	0.17 (0, 0.33)^{a}	0.17 (0, 0.33)^{a}	0.17 (0, 0.33)^{a}	0.5 (0.2, 0.83)^{b}	0.66 (0.33, 1)^{b}	0.17 (0, 0.33)^{a}	0.47 (0, 0.73)^{b}	0.47 (0, 0.83)^{b}	0.17 (0, 0.33)^{a}	0.17 (0, 0.33)^{a}	0.17 (0, 0.29)^{a}
Standing	0 (0, 0.2)^{a}	0 (0, 0.2)^{a}	0 (0, 0.2)^{a}	0 (0, 0.2)^{a}	0 (0, 0.2)^{a}	0 (0, 0.2)^{a}	0 (0, 0.2)^{a}	0 (0, 0.17)^{b}	0 (0, 0.17)^{b}	0 (0, 0.2)^{a}	0 (0, 0.17)^{a}	0 (0, 0.17)^{a}	0 (0, 0.2)^{a}	0 (0, 0.185)^{a}	0 (0, 0.2)^{a}
Walking	0 (0, 0.14)^{a}	0 (0, 0.17)^{a}	0 (0, 0.17)^{a}	0 (0, 0.14)^{a}	0 (0, 0.14)	0 (0, 0.155)^{a}	0 (0, 0.17)^{a}	0 (0, 0)^{b}	0 (0, 0)^{a}	0 (0, 0.14)^{a}	0 (0, 0.14)^{a}	0 (0, 0.17)^{a}	0 (0, 0.14)^{a}	0 (0, 0.17)^{a}	0 (0, 0.14)^{a}
Eating	0.14 (0, 0.33)	0.14 (0, 0.31)	0.14 (0, 0.33)	0.14 (0, 0.29)	0.14 (0, 0.33)	0.14 (0, 0.33)	0.14 (0, 0.29)	0 (0, 0.17)	0 (0, 0.17)	0.14 (0, 0.33)	0.14 (0, 0.29)	0.14 (0, 0.33)	0.14 (0, 0.29)	0.14 (0, 0.29)	0.14 (0, 0.31)
Drinking	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
Preening	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
Aggressive interaction	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0.17)^{b}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}
Feather Pecking	0 (0, 0.14)^{a}	0 (0, 0.14)^{a}	0 (0, 0.14)^{a}	0 (0, 0.14)^{a}	0 (0, 0.14)^{a}	0 (0, 0.14)^{a}	0 (0, 0.14)^{a}	0 (0, 0.29)^{b}	0 (0, 0.14)^{a}	0 (0, 0.14)^{a}	0 (0, 0.14)^{a}	0 (0, 0.14)^{a}	0 (0, 0.14)^{a}	0 (0, 0.14)^{a}	0 (0, 0.14)^{a}
Panting while sitting	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{b}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}
Panting while standing	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{b}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}
Wing splay	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{b}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}
Missing/ other	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0.14)^{b}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}	0 (0, 0)^{a}