SKELETAL TRAITS AND GAIT CHARACTERISTICS OF COMMERCIAL TURKEY TOMS AS INFLUENCED BY AGE AND ACCESS TO ENVIRONMENTAL ENRICHMENT

by

STEPHANIE KULBACKI

(Under the Direction of Prafulla Regmi)

ABSTRACT

The objective of this study was to evaluate the effects of different environmental enrichments on the morphological and biomechanical properties of bones in commercial turkey toms and compare objective gait parameters with the assigned subjective gait scores. This objective was achieved by rearing Nicholas Select turkeys with select environmental enrichments. Following the rearing period, a subjective gait assessment was performed on select birds, followed by an objective test with a pressure sensitive walkway. Load and non-load bearing bones and plasma were collected for bone analysis. The inclusion of the environmental enrichments had minimal impact on the selected, body weight, bone parameters and gait parameters. However, the analysis of the effects of body weight on bone parameters demonstrated stronger correlations at younger ages, as well as confirmed more skeletal growth during the earlier stages. Additionally, a subjective gait scoring system was supported using an objective scoring method.

INDEX WORDS: Environmental enrichment, Bone health, Gait, Turkeys

SKELETAL TRAITS AND GAIT CHARACTERISTICS OF COMMERCIAL TURKEY TOMS AS INFLUENCED BY AGE AND ACCESS TO ENVIRONMENTAL ENRICHMENT

by

STEPHANIE KULBACKI

BSA, UNIVERSITY OF GEORGIA, 2023

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

MASTER OF SCIENCE

ATHENS, GEORGIA

2025

SKELETAL TRAITS AND GAIT CHARACTERISTICS OF COMMERCIAL TURKEY TOMS AS INFLUENCED BY AGE AND ACCESS TO ENVIRONMENTAL ENRICHMENT

by

STEPHANIE KULBACKI

Major Professor:	Prafulla Regmi
Committee:	Chongxiao Chen Marisa Erasmus

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
December 2025

DEDICATION

I dedicate this thesis to the following: To my wonderful father, Bob, my caring mother, Diana, my amazing sister, Nicole, and my loving fiancé, Lane.

.

ACKNOWLEDGEMENTS

To Dr. Prafulla Regmi, thank you for giving me the opportunity to discover a new passion in poultry. I am beyond grateful for your dedication, guidance and patience throughout these past two years. I thank you immensely for taking a chance on me and expanding my knowledge and skills as a scientist.

To Dr. Chongxiao Chen, thank you for serving on my committee and standing by through this journey. Thank you for sharing your expertise in turkeys and DEXA analysis.

To Dr. Marisa Erasmus, thank you for serving on my committee and your guidance during this time. Thank you for organizing and executing the experiments necessary for this research to be done.

To the current and past lab members of the Regmi lab, thank you for your constant understanding, assistance and friendship these past two years. Thank you to the many undergrads who helped me with my bone samples over the years.

To the members of the Erasmus and Brito labs at Purdue University who ensured the birds were well cared for and conducted countless samplings.

To Dr. Laura Ellestad, thank you for your guidance and assistance. Thank you to the Ellestad lab members for your teaching, help and many laughs.

To Kerry, thank you for always being willing to listen when needed and helping me navigate the logistics of being a grad student.

To my friends, who have always been there, ready with pep talks and encouraging words. Thank you for listening to endless poultry tidbits and being there for me when I needed you.

To my family and future in-laws, thank you for listening to my work and being available if I needed you. I appreciate your constant support.

To my parents, thank you for helping me be the person I am today. I would not be where I am or who I am without your love and guidance.

To Lane, who has given so much support and care to make sure I achieve my goals. Thank you for your constant flexibility and for always lending an ear when needed. You picked me up when I needed you most and I'm so grateful to have you by my side on this journey.

Finally, I thank God for the opportunity and ability to expand my knowledge and career as a scientist.

TABLE OF CONTENTS

CHAPTER

APPENDICES

LIST OF TABLES

Page

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Since 1970, the turkey industry has nearly doubled in size, heavily driven by increases in consumers' awareness of the meat's nutritional value (National Turkey Federation, 2008). The U.S. is the primary producer of turkey meat worldwide, processing 218 million birds and producing 4.7 billion pounds of meat in 2024 (Grossen, 2025). This expansion has paralleled a significant increase in the average body size of commercial turkeys. Birds typically reach market weight—averaging 37.4 pounds—between 16 and 19 weeks of age (Clark et al., 2019). However, rapid increases in weight, particularly in the breast muscles, have contributed to a rise in skeletal disorders. Leg-related issues, including tibial and femoral fractures and lameness, represent some of the industry's most serious welfare and productivity challenges. In 2024, leg problems were ranked #10 by turkey professionals in the industry (Clark and Chiaia, 2024). Such impairments hinder birds' ability to carry out essential behaviors, including feeding, drinking, and avoiding aggression from conspecifics (Corr et al., 1998). Despite ongoing efforts, cost-effective solutions to mitigate these consequences of accelerated growth remain elusive (Lendon, 2012).

The provision of environmental enrichments in the poultry industry has gained increasing attention in recent years. Common enrichments include adding straw bales, pecking blocks and/or perches to the pens. These interventions have been associated with improved animal welfare and increased economic returns by mitigating undesirable behaviors such as fearfulness and feather pecking (Jones, 2018). Studies in broiler chickens have demonstrated that increased physical activity prompted by enrichments positively influences the morphological, biophysical, and mechanical properties of the tibia (Guz, 2021). Physiological adaptations in the legs include increases in bone weight, length, diameter, cortical thickness, and tibiotarsal strength (Castellini et al., 2002). By quantifying skeletal properties based on environmental enrichment provision, a regiment suited for a commercial setting may be able to be produced to aid in the turkey's skeletal health during production.

The objective of this study is to evaluate load and non-load bearing bone health in turkey toms exposed to select environmental enrichments through the analysis of morphological traits, biomechanical properties, and mineral metabolism in the bone at 8, 12, 17 and 18 weeks of age.

REFERENCES

Castellini, C., C. Mugnai, and A. Dal Bosco. 2002. Effect of organic production system on broiler carcass and meat quality. Meat Science 60:219-225.

Clark, D. L., K. E. Nestor, and S. G. Velleman. 2019. Continual Selection for Increased 16 wk Body Weight on Turkey Growth and Meat Quality: 50 Generation Update. Poultry Science Association. doi http://dx.doi.org/10.3382/japr/pfz017

Clark, S., and L. Chiaia. 2024. 2024 Turkey Industry Annual Report - Current Health Issues Facing the US Turkey Industry, United States Animal Health Association

Corr, S. A., C. C. McCorquodale, and M. J. Gentle. 1998. Gait analysis of poultry. Research in Veterinary Science 65:233-238. doi https://doi.org/10.1016/S0034-5288(98)90149-7

Federation, N. T. 2008. Raising America's Turkeys. https://www.eatturkey.org/raising-turkeys/.

Grossen, G. 2025. Turkey Sector, Background & Statistics, USDA Economic Research Service.

Guz, B. C. 2021. Effects of pen enrichment on leg health of fast and slower-growing broiler chickens. PLoS One. doi 10.1371/journal.pone.0254462

Jones, R. B. 2018. Environmental enrichment for poultry welfare.

Lendon, J. 2012. Tackling Turkey Leg Problems. https://www.thepoultrysite.com/news/2012/11/tackling-turkey-leg-problems.

CHAPTER 2

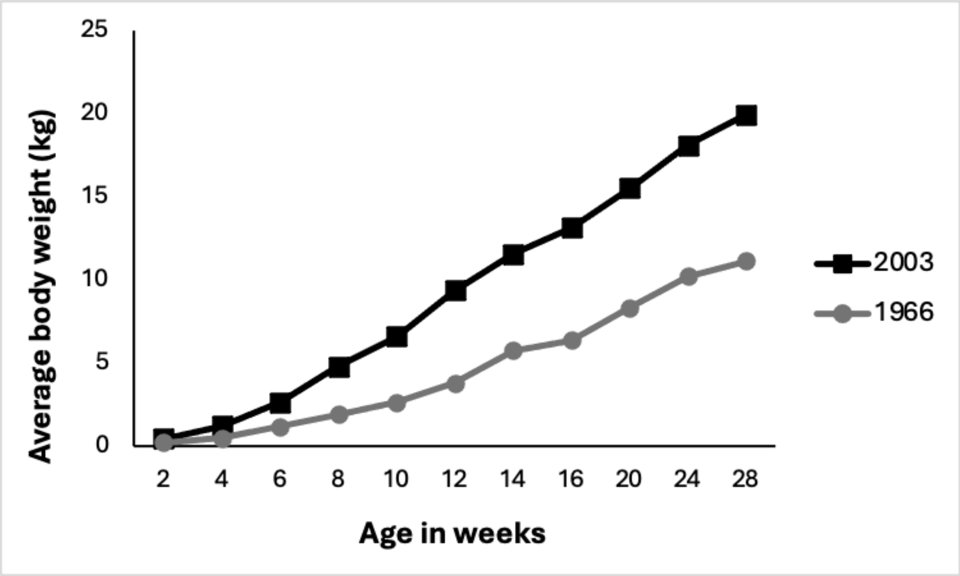
LITERATURE REVIEW

History of Turkey Production

The wild turkey found in North America, *Meleagris gallopavo*, is a key part of the poultry industry as ancestors of modern turkey breeds. Wild male turkeys usually weigh between 5 and 11 kilograms, and females weigh 2 to 5 kilograms. These weights are significantly less than the commercial turkeys we raise for the market, with toms now weighing about 14 kilograms and hens weighing nearly 9 kilograms on average. Most wild turkey breeds are not used in a commercial setting due to a slower growth rate and less white-meat production, leaving the Broad-Breasted White to be the most common commercial breed (Hulet et al., 2021). With domestication and artificial selection, the turkeys that are used commercially have very little in common genetically with their wild counterparts (Aslam et al., 2012).

The turkey industry relies on artificial insemination for efficient breeding and to maintain the physical welfare of the hens, since the large sizes of the males pose a risk of injury or pain towards females (Weber, 2012). Aside from increased body weight, commercial birds have a lower feed conversion ratio, meaning they yield more muscle while consuming less feed. Figure 2.1 was redrawn based on data collected by Havenstein et al. (2006), which compares turkeys in 2003 to turkeys from 1966 in terms of body weight from two weeks of age to 28 weeks of age. Havenstein credits these transitions to genetic selection and nutritional improvements. These vast changes in body weight and growth efficiency warrant a need to understand how production traits are associated with other systems in the body throughout the production period.

Figure 2.1. Average body weight (kg) from 2 to 28 weeks of age in commercial turkey toms in 2003 and 1966 (Havenstein et al., 2006).



Genetic Selection

Genetic selection has improved traits such as disease tolerance, which is crucial in large-scale farms with increased risk of pathogens spreading (Hu et al., 2020). Selection has allowed for white feathered birds to be bred so that dark pigmentation spots do not form on the birds when their feathers are plucked (National Turkey Federation, 2008). The top goal in terms of selection, however, is to develop birds that grow larger in a short time. While the selection has been effective in producing an economically and environmentally efficient turkey industry, it has also resulted in fast-growing birds prone to physical and welfare issues (Hunton, 1990). Major challenges in commercial turkeys include "skeletal problems, cardiac morbidity, reduced immune response to some pathogens, and some instances of meat quality issues, among others" (Barclay, 2014). In the recent years, researchers and primary breeders are slowly moving towards incorporating fitness and specific health traits in the genetic selection program (Quinton et al., 2011). Morever, rearing turkeys at higher stocking densities have led to reduced performance and increased instances of aggression and feather pecking (Buchwalder and Huber-Eicher, 2003; Ligaraba et al., 2016).

Comparison of genetic and phenotypic traits have revealed the effect that body weight can have on skeletal quality in meat birds. Prior studies have reported low phenotypic correlations between the body weight and the hip and leg structure, with correlations of 0.05 to 0.08 and 0.04 to 0.13, respectively (Kapell et al., 2016; Quinton et al., 2011). A genetic correlation of 0.34 was observed between the body weight and footpad dermatitis (Kapell et al., 2016), suggesting genetics could moderately contribute to incidences of footpad dermatitis in modern toms. Turkeys typically go to production at 16-20 weeks of age, but studies have found that traits such as bone weight, length, width and pyridinium levels aren't maximized until 25 weeks of age. Additionally, mineral content, bone density and breaking strength are not fully met until 35 weeks of age (Weber, 2012). The indication that the skeletal system continues to mature during the latter stages of the production period, and possibly beyond, calls for a deeper analysis of the age-related effects of body weight on both load and non-load bearing bones in turkeys.

Animal Welfare Principles

As animal welfare expands as a concept, places have adopted different mindsets and methods for better treatment. The concept of animal welfare generally includes three major pillars: the animals' normally function biological systems, proper emotional states, and the ability for them to express certain behaviors deemed normal (Fraser et al., 1997). Certain programs have followed the "Five Freedoms of Animal Welfare." These include freedom from hunger and thirst, freedom from discomfort, freedom from pain, injury or disease, freedom from fear and distress and freedom to express normal behavior (Farm Animal Welfare Council, 1979). However, in recent years, animal welfare has moved more towards following five specific domains instead of freedoms: nutrition, physical environment, health, behavioral interactions and mental state (Mellor et al., 2020). The behavioral interaction portion of these domains include the animal's interaction with the environment, interaction with other animals and interaction with humans. The focus on the Five Freedoms evolving into the Five Domains is derived from the notion that the Five Freedoms can result in the idea that the absence of certain negative states equates to welfare being ensured (Mellor, 2016). These welfare pillars and freedoms of welfare emphasize the need for analysis of turkeys' behavior with their environment, especially in terms of their skeletal health and gait.

A combination of information on animal physiology, ethology, pathology, and psychology can be used to determine the welfare of a farm animal (Weber, 2012). A variety of factors can prevent one or more of these criteria from being met in industry or research settings. Growing knowledge and awareness of animal welfare among consumers has driven further research into the welfare of animals, including those raised for consumption. Both producers and consumers recognize that improved animal welfare could lead to increased productivity, enhanced animal products, economic benefits, and social justification (Fragoso et al., 2023). The turkey industry faces animal welfare challenges primarily related to diseases, abnormal behaviors, and leg problems (Clark and Chiaia, 2024). These issues are multifactorial and require a multidimensional approach for mitigation. Given the current challenges associated with genetic selection, disease, and leg health, combined with the overall benefits of improved welfare, investigating the effects of age and environmental enrichment on skeletal quality and gait will provide valuable insights. The first steps are to examine bone development and growth in turkeys, identify critical periods of growth, and determine how these periods compare with body weight gain and muscle accretion.

Bone Biology

The bone is made up of two major and different components: an organic component and an inorganic component. The organic component is a majority type I collagen, making up the osteoid of the bone, with ~5% coming from non-collagenous proteins, and is responsible for the flexibility of the bone (Boskey, 2013). The inorganic component in the bone is made primarily of hydroxyapatites and calcium phosphates, which provide the strength and durability characteristics of the bone (Schlesinger et al., 2020).

The macro structure, or the morphology of bones, is made up of diaphysis, epiphysis, articular cartilage, epiphyseal plate, medullary cavity, periosteum, and endosteum. The epiphysis makes up the end points of the bones, with the metaphysis being the next section towards the middle area of the bone on each end, and the main shaft area of the bone is the diaphysis, where bone lengthening occurs (Setiawati and Rahardjo, 2018). Articular cartilage is a connective tissue covering the joints that are tasked with allowing bones to provide optimal movement (Grujjcic, 2023). The remaining bone structure is covered with another thin layer of connective tissue, or the periosteum, which has both an outer fibrous layer and an inner cellular layer (Ocran, 2023). The medullary cavity is found within the diaphysis of the bone and houses the bone marrow and has a thin layer of connective tissues surrounding it, known as the endosteum (Tosovic, 2023). The microstructure of the bone is composed of a structural unit called an osteon, which is made of collagen and calcified matrix, also known as lamella. Each osteon has a central canal, or the Haversian channel, that contains blood vessels that branch off to form a perforating canal that extends to the periosteum and endosteum (Biga et al., 2019).

Bones are primarily made of two different bone types, the trabecular (spongy) bone and cortical (compact) bone. Spongy bone contains trabeculae that provides strength and helps to redirect loads from the joint and to the cortical layer of bone (Currey, 2002). Trabeculae is a lattice-shaped matrix composed of osteocytes within lacunae (Biga et al., 2019). Osteons can be found within both layers of the bone. Whereas, cortical bone has a periosteum, which is crucial for appositional growth and repairing fractures, as well as an endosteum that expands with marrow development (Clarke, 2008).

The basic cells of bones are osteoblasts, osteoclasts, osteocytes, and chondrocytes. These cells are crucial to proper bone development by handling the processes of formation and resorption of the bone and cartilage throughout the bone. Osteoblasts are crucial to the formation of bones and they release proteins to the bone matrix like type I collagen, osteocalcin and alkaline phosphatase for bone formation (Breeland et al., 2023). Osteoblasts release RANK ligand, which will then bind to RANK receptors to regulate osteoclasts; this is important for regulation within the bone. These cells can secrete osteoprotegrin to help prevent RANK and RANK ligand interaction by binding to RANK ligand itself. This is another method of osteoclast regulation (Breeland et al., 2023). Osteoblast interaction and balance within RANK ligand and osteoprotegrin is the determining factor in osteoclast activity within the bone (Xiong et al., 2011).

Osteoclasts are the cells responsible for bone resorption and are the starting point for bone remodeling. These cells break down microscopic portions of preexisting bone matrix, allowing osteoblasts to enter and reform the matrix (Schlesinger et al., 2020). They are derived from hematopoietic cells, which are found in bone marrow (Gothlin et al., 1976). Individual osteoclasts have many processes that expand into the matrix and release hydrogen ions, and other proteolytic enzymes like cathepsin K, resulting in the acidification and breakdown of the bone and collagen (Karsenty et al., 2009). The process of bone remodeling begins when osteoclast progenitors are activated and sent to damaged bone surface, mature osteoclasts resorb the bone and die, followed by osteoblast progenitors entering, where they will produce more osteoid to mineralize the matrix (Hattner et al., 1965; Langdahl et al., 2016). Osteoclasts activity is closely and heavily regulated by various cellular functions since excessive osteoclastic activity can lead to osteoporosis, and too little osteoclastic activity will result in osteopetrosis (Khan and Bordoni, 2023).

Osteocytes are the most common cell found within the bone. Osteocytes come from osteoblasts that have been entrapped in the osteoid (Bonewald, 2011). Their primary role is vital to the transduction of mechanical stimulus to neural signals in the bone. Specifically, osteocytes will bind with one another and their surrounding environment through cytoplasmic process, allowing them to detect stress and deformation within the bone, allowing them to monitor and control the remodeling of the bone (Tresguerres et al., 2020).

Osteocalcin is a protein produced by osteoblasts, and one of the most abundant proteins found in the bone, making it an excellent biomarker for bone formation and osteoblast activity (Zoch et al., 2016). It works similarly to a hormone and impacts the pancreas, liver, muscle, fat, and other organs in the body to regulate different physiological processes (Tu et al., 2023). Osteocalcin and osteopontin are important proteins for fracture resistance, but their abundancy in bone begins to deteriorate over time (Sroga and Vashishth, 2012). Osteocalcin cells that have reached maturity will enter the bone micro-environment and complete a conformational change that places its calcium-binding γ-carboxyglutamic acid protein residues with the calcium ions in hydroxyapatite (Price et al., 1976).

Pyridinoline (PYD), a proxy bone turnover marker, can be helpful in providing bone health information. PYD is formed through the extracellular maturation of fibrillar collagens, or collagens that are trifunctional crosslinks that bridge multiple collagen peptides together (Siebel, 2005). When measured, the resulting levels are direct reflections of this matured collagen breaking down and are not influenced by collagens that have been recently synthesized; these levels are often measured via uranalysis (Siebel, 2005). PYD can be found in multiple parts of the body, including cartilage, ligaments, vessels, and bone, but bone has the highest resorption rate, so most of PYD detected in the urine comes from the skeletal system (Siebel, 2005). PYD levels are increased in diseases that cause higher rates of bone resorption, such as rickets (Fraser and Stevenson, 2013).

Bone Formation

Intramembranous ossification is when bone develops by compact and spongy bone forming directly from sheets of mesenchymal connective tissues (Biga et al., 2019). This ossification process is responsible for the formation of flat bones, like the cranial and clavicle bones (Biga et al., 2019). Long bones undergo endochondral ossification, which is when cartilage, specifically hyaline cartilage, is replaced by new bone. For this to occur, the mesenchymal cells will transform to chondroblasts, which produce hyaline cartilage, an important precursor to bone formation (Biga et al., 2019).

The epiphyseal plate is responsible for growth following the initial formation and has five different growth zones responsible for doing so: the reserve zone, the proliferative zone, the zone of hypertrophy, the zone of calcification and the zone of ossification. The reserve zone is closest to the epiphyseal line, and has the purpose of holding chondrocytes, which are necessary for securing osseous tissue in epiphysis (Sromova, 2023). The proliferative zone is below this and is made of layers of chondrocytes, which are continuously produced through the process of mitosis (Sromova, 2023). The zone of hypertrophy has the most mature chondrocytes and uses lipids, glycogen, and alkaline phosphatase to make the cartilage in the matrix calcify, resulting in the lengthening of the bones (Sromova, 2023). The zone of the calcification is the final and closest to the diaphysis of the bone; the chondrocytes in this zone have been killed through the calcifying of the matrix, and osteoblasts are able to release bone tissue to ensure the epiphyseal plate gets connected to the diaphysis (Sromova, 2023). The zone of ossification is when the osteoclasts will break down the old, calcified cartilage lining the matrix and make way for osteoblasts to form new bones, thus remodeling the bone (Sromova, 2023). Bones grow in diameter, or width, through a process called appositional growth, which occurs along the endosteum or the periosteum, where osteoclasts resorb, and osteoblasts restore the medullary cavity (Biga et al., 2019). This process will increase the diameter of both the diaphysis and the medullary cavity (Biga et al., 2019).

Properties of Bone

The most common morphological traits of bones that are measured are: bone weight, length, anterior-posterior diameter, medial-lateral diameter, and cortical thickness. Many factors can impact the morphological traits of bones, such as living conditions, age, diet, and genetics. Research has been published presenting body weight has an impact on the morphological traits of bone, but a connection between morphological changes and increased load on the bone are still being made (Zhong et al., 2012). Studies have indicated that these morphological, or geometric, properties of bone can efficiently predict the mechanical quality of bones (Soboyejo and Nestor, 2000). Key biomechanical properties measured in bones are toughness and strength. Toughness is the measurement of resistance for the bone to fracture, and strength is measurement of the bone's ability to resist total and permanent deformation, often tested and read by the maximum load applied (Ritchie et al., 2008). The toughness of bone comes from the combination of collagen and hydroxyapatite crystals present in it (Launey et al., 2010). Whereas bone strength is not only affected by the biological make-up of the bone, but the mass, geometric features, and the microstructure of the bone (Niu et al., 2023). Like morphological traits, factors such as age, genetics, diet, and environment can impact the biomechanical traits of bones. Morphological and biomechanical bone traits can indicate skeletal health and allow for the determination of how age, diet, environment and genetics influence bones. From there, these traits can be quantified and analyzed across ages and provided environmental enrichments to identify specific effects on skeletal quality.

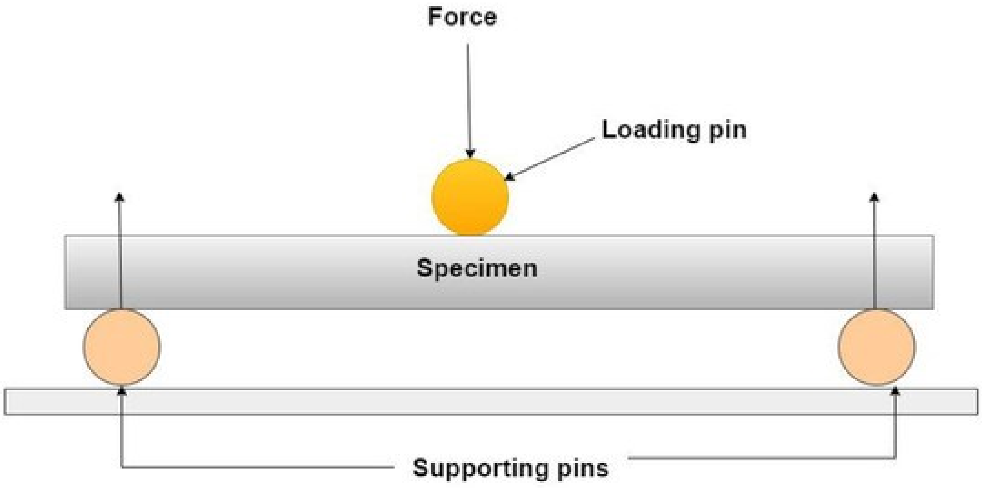
Bone Biology in Turkeys

Studies have long investigated the proportion of body mass and leg bones in the domestic turkey. Age has had effects on the morphological properties of bone since the bone will grow and remodel during the peak stages of growth in a turkey's life. Zhong et al. (2012) observed that the bone matrix is time and age dependent. Commercial birds' skeletons are not fully developed until roughly 25 weeks of age (Weber, 2012), meaning the skeletal system is likely not growing at the same rate as the body weight. It has been reported that in femurs, the bone length did not increase at the same rate as the body mass, supported by CT scans of the epiphysis (Stover et al., 2018). Crespo et al. (1999a) reported that many fractures reported in a flock of turkey toms had an insufficient amount of time for their bone matrix to mature fully, thus the femurs of the toms were exposed to loads that exceeded the yield strength of the bones. Generally, the selection is focused on an increase in breast muscles, so leg muscles are often forgotten when genetic selection is being performed. These less prominent leg muscles have been known to affect the load distribution on leg bones in turkeys, which induces bone remodeling, directly changing the structure of the bone (Jones, 2018). The concept that there is a direct correlation between body weight and the mechanical properties of bones is being evaluated (Zhong et al., 2012). This is aiding the poultry industry due to the goal of larger breast muscles and the need for the skeletal system to keep pace in terms of growth. The misalignments between skeletal development and body mass accumulation, as well as the evidence of late skeletal maturity require the quantification of how age and body weight impact the skeletal system and walking ability, including the effects of rearing conditions.

Analysis of Skeletal Quality

The quality of long bones can be assessed through their structure and composition. Morphological parameters such as length, cortical thickness, and cross-sectional area influence biomechanical traits like bending resistance and ultimate fracture force. On the other hand, compositional traits such as mineral content, quality of the collagen cross-links, and density contribute to bone stiffness and toughness. In this dissertation, three-point bending tests were used to measure biomechanical properties of the bone (Figure 2.3). Bending strength is defined as "a material's ability to resist deformation under load" (Pal et al., 2022). This test applies a specific load to break the bone at its mid-point while it is supported at both ends with beams. At the concave surface, the stress reaches its maximum compressive value, whereas at the convex face, stress reaches its maximum tensile value (Martel, 2016). Compressive stress refers to the amount of pressure a material can withstand before bending at a molecular level (Mishra, 2015), while tensile strength is the maximum force a material can endure before failure (Pal et al., 2022). Both compressive and tensile stresses are often induced by consistent loading on cortical bone tissue (Havaldar et al., 2014). Due to the complex structure of bones and the presence of small defects, stress tends to concentrate locally, causing weaknesses and reducing flexural strength values (Hart, 2017). Using a 3-point bending test to quantify biomechanical properties will enable analysis of the effects of age, environmental enrichment, and body weight on the skeletal system of commercial turkey toms during production.

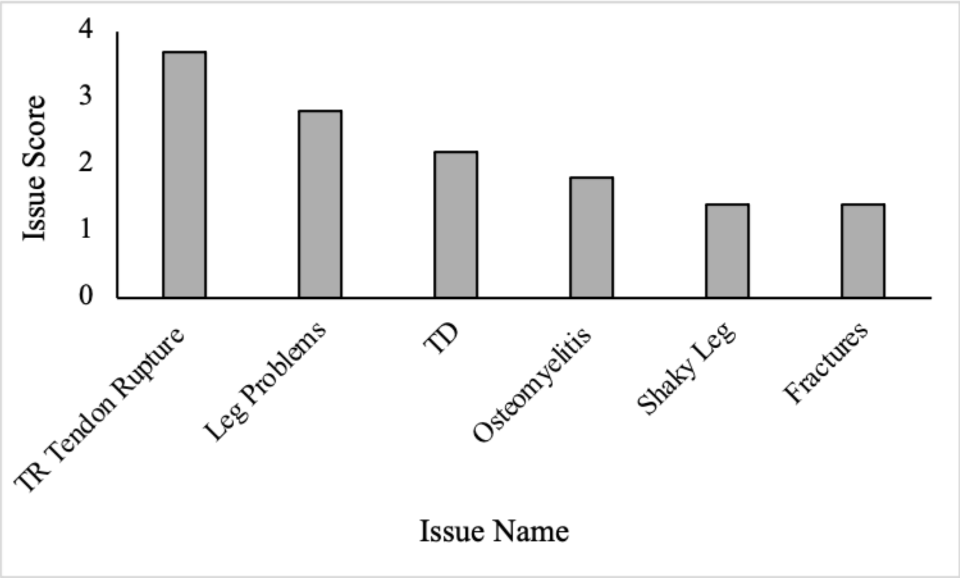
Figure 2.3. Example of a 3-point bend test set-up adapted from Thirupathy and Vadivel (2024).



Lameness: Causes and Risk Factors

Lameness in turkeys can be defined as any issue that causes turkeys to have impaired mobility, and ultimately, pain (Dibner et al., 2007). When a turkey becomes severely lame, they often will become stationary or reluctant to move, resulting in a detrimental lack of access to feed and water, and the increased risk of cannibalism and trampling. Lameness is often a multifactorial issue, with environment, genetics, nutrition, and health status playing a measure role in causation. Bone issues can become a chronic issue with a tendency to cause reduction of feed intake as well as a higher incidences of downgrades and poor carcass quality at processing (Dibner et al., 2007). Lameness is a problem that is generally irreversible once it sets in, making a push for selection programs that develop breeds with improved leg health and prevent some of the major leg disorders (Kapell et al., 2016; Granquist et al., 2019). Some leg problems are associated with incidences of footpad dermatitis (FPD) as the FPD lesions create a potential breeding area for bacteria, leading to infection and, ultimately, lameness (Clark et al., 2002). Gross abnormalities like varus, valgus, and tibial dyschondroplasia cause increased rates of lameness and culling (reviewed in Erasmus, 2018). Lameness can be caused by direct viral and bacterial infections, too. An overview of the highest-ranked issues related to lameness, based on a health survey conducted among industry professionals using a 1 to 5 scale, was adapted for Figure 2.2 (Clark and Chiaia, 2024). Figure 2.2 reinforces the multifactorial nature of lameness, indicating that various pathologies of bone and adjacent connective tissues can result in lameness of varying severity.

Figure 2.2. The 2024 turkey health survey ranking current issues where 1 = no issues and 5 = severe problem



Developmental Abnormalities

Valgus and Varus

Varus abnormalities cause the tibia to slant toward the midline, resulting in a "bow-legged" phenotype. Valgus is the opposite with the bone slanting away from the midline, resulting in a "knocked knee" phenotype. For turkeys, the rotation of the tibia is more common, but this can be accompanied with valgus or varus (Wettere, 2020). A study conducted by Kapell et al. (2016) included information on the genetic impacts of prevalence of varus and valgus and found that there is some association with purebred turkey lines, but most cases are environmental. Cases of valgus and varus provide the insight that large breasted turkeys are inadequately adapting to the excess loading caused by the breast muscles (Crespo et al., 1999a). These conditions cause the gastrocnemius tendon to flatten and then begin to cover either the lateral condyle or medial condyle, which can also make the hock appear thicker (Julian, 1984). With an additional increased risk of a leg fracture or slipped tendon (Crespo and Shivaprasad, 2011), these abnormalities prove to be an economic and welfare risk.

Tibial Dyschondroplasia

Tibial dyschondroplasia is an abnormality, commonly found in the growth plate, where the chondrocytes that make up the growth plate do not complete the ossification process to become bone (Knopov et al., 1995). It is described as a white area of unmineralized cartilage, typically in the proximal metaphysis of the tibia, but it is sometimes found in the tarsometatarsus (Pines and Reshef, 2015). Studies have found that the prevalence of tibial dyschondroplasia increases as the birds age with up to 71% of birds displaying signs by 13 weeks of age (Hocking et al., 2002). Fast growth rates and genetic selection are often the cause of this disorder, resulting in breeders working to withdraw affected birds. One breeder reported that using x-rays to find cases of dyschondroplasia early on has allowed for a reduction in future incidences by roughly 25% (Swalander et al., 2020). Increases in the calcium to phosphorus ratio has indicated to be a potential cause of dyschondroplasia. However, various studies involving feed restriction have contributed to the notion that genetic selection for fast-growth rates are the main contributor to cases of the disorder (Pines and Reshef, 2015). Successful treatments for tibial dyschondroplasia often include using 1,25-$(OH)_2$-D_3 or 1α-OH-D_3 treatments, weight management, anti-inflammatories or environmental changes to allow easy food and water access (Combs and McClung, 2017; Huang et al., 2017; Nabi et al., 2016; Nelson et al., 1992).

Tibial Rotation and Twisted Legs

Tibial rotation is the tibia rotating via the long axis of the bone, often resulting in a lateral extension; this rotation can reach up to 90 degrees in some cases (Martin, 2021). It can appear to be a slipped tibial tendon, but in the case of rotation, the tendon remains intact. This condition causes pain and discomfort and can be responsible for up to 5% of morbidity in turkeys (Boulianne et al., 2013). Similarly, perosis occurs when chicks under six weeks of age lack enough minerals, like zinc and manganese, and vitamins like choline, folic acid, niacin, pyridoxine and biotin in their systems to properly form the cartilage in their bodies (Beyer, 2008). Perosis can cause the shortening of long bones, resulting in growth issues in birds (Beyer, 2002). These symptoms can be treated by ensuring the chicks are receiving a mineral rich diet (Beyer, 2008).

Femoral Fractures

Crespo et al. (1999b) found a direct correlation between heavy body weight and the presence of lower femoral strength and femoral fractures. When different analyses were performed on turkeys with and without femoral fractures, it was found that the femoral fractures were only present in the heavy body weight birds (Crespo et al., 1999b). This study unveiled higher amounts of endosteal and periosteal cells, which indicated there was more callus formation present and thus more bone repair occurring in these birds. This contradicts previous studies, which have found that as bones undergo the repair process, they become more prone to fracture because of the inherent phase of osteoporosis within the cortical bone that takes place during this bone resorption (Nordin and Frankel, 2001). As more damage to the bone occurs and increases the frequency of remodeling, bone can have reductions in overall strength and stiffness (Kaplan et al., 1994). Femoral fractures pose a lot of issues in turkeys. They can cause severe pain, which can impact the ability to eat and drink, similarly to other forms of lameness previously observed. However, femoral fractures also pose a risk of damaging the femoral artery, leading to potential death (Van Wyhe et al., 2014).

Other Abnormalities

Other abnormalities such as crooked toes or shaky legs are often identified in commercial settings (Oviedo-Rondon et al., 2018). Crooked toes are when one or more toe(s) have a lateral or medial deviation, which some have reported could be environmentally induced by types of flooring or riboflavin deficiencies (Hicks Jr and Lerner, 1949; Nestor, 1971). A study performed by Oviedo-Rondon et al. (2009) reported that broilers who's embryos and hatchlings were exposed to an eggshell temperature of 39°C for incubations days 18 thru 21 had an increased number of crooked toe occurrences, indicating the risk begins even before hatch. Shaky legs primarily develop during 8 to 18 weeks of life, and are identified when the affected birds squat and flex their hocks, leaving their breasts resting on the litter, or they sit on their metatarsals with their body upright (Julian and Bhatnagar, 1984). The birds exhibit clear signs of pain and lack the will to stand and walk; if they do, it is a few steps at a time, described as "hobbling" and their legs quiver before they can begin walking normally (Julian and Bhatnagar, 1984). A specific cause of shaky leg is not known, but it is believed to be more environmentally caused than genetically. Studies in North America have revealed that birds displaying shaky-leg tend to have tibial dyschondroplasia or a selenium deficiency, but selenium additives in Canadian flocks did not impact the number of cases recorded (Julian and Bhatnagar, 1984). Affected birds tend to have stiff legs that are able to be straightened, but the birds express signs of discomfort with hock movement (Julian and Bhatnagar, 1984).

Footpad dermatitis can cause lameness. Footpad dermatitis is when the skin on a bird's footpad becomes damaged and inflamed, and usually demonstrates thickened scales, black lesions or ulcers in the area. Researchers have analyzed footpads at a cellular level and found issues with hyperkeratosis, as well as epithelial hyperplasia (Mayne et al., 2006). These findings contribute to the idea that subclinical forms of footpad dermatitis cause intense inflammation (Moe et al., 2018). In the industry, footpad dermatitis is typically evaluated on 1 to 5 scale, where a 1 indicates footpads with no lesions and a 5 indicates footpads with severe lesions and ulcers (Furo, 2024). Moisture levels in litter are a large contributor to the prevalence of footpad dermatitis, so it is typical to keep litter moisture below 30%. This can be accomplished through temperature and ventilation regulation to keep litter dry, repairing drinker leaks and using bedding that has a better ability to trap in moisture (Furo, 2024). Lack of biotin, methionine or zinc have been known to decrease the durability of footpad skin, contributing to potential cases of footpad dermatitis (Clopton, 2025). Footpad dermatitis poses a great risk in the industry but is manageable with proper attention and care.

Pathogenic Abnormalities

Turkey Reovirus

Avian reoviruses can cause cases of hepatitis, myocarditis, enteritis, respiratory and neurological diseases and arthritis or tenosynovitis in avian species (Porter, 2018). Turkey arthritis reovirus is a viral infection that typically causes issues for turkey toms when they near the production age, with infected birds 12 to 16 weeks of age displaying the most cases

(Sharafeldin et al., 2015). Birds with this reovirus had forms of tenosynovitis that advanced to fibroplasia and fibrosis, and their high body weights led to intense lameness and in some cases, tendon ruptures as they got older (Sharafeldin et al., 2015). Specifically, decreases in tensile strength and elasticity of the tendons in the legs can contribute to lameness (Sharafeldin et al., 2016). The infection often causes swelling in the hocks, or other issues like the erosion of intertarsal cartilage, making it painful for the birds to walk (Kumar et al., 2022a; Porter, 2018). Turkey Reovirus can cause severe weight reduction and spread into other internal organs, causing issues such as splenic necrosis (Kumar et al., 2022b).

Porter (2018) and Sharafeldin et al. (2015) remarked that strands of turkey reovirus replicated within the intestinal tract, which allowed the virus to be excreted via feces and passed on to other birds. It was observed that the reovirus can live on the litter in houses for over a week and can thrive in non-sterile drinking water for over two weeks. This led to the finding that certain disinfectants can kill the virus, such as diluted Virocid (quaternary ammonium + aldehyde mixture) or diluted Tek Trol (phenol) (Porter, 2018). In 2011, researchers introduced the use of killed vaccines, which worked to slow the number of cases until 2014, when autogenous vaccines were introduced. Producers are still utilizing these autogenous killed vaccines, but their results appear to be lackluster (Porter, 2018). In 2024, six live attenuated vaccines were developed using three different strands of the virus and tested on 10-day old poults that were challenged post vaccination. While deemed safe for the birds, the vaccines proved to have low efficacy rates (Goyal, 2024), leading reoviruses to continue posing a great risk to the industry.

Marek's Disease

Marek's disease is caused by a strain of a herpesvirus, *Mardivirus gallidalpha 2*, specifically. It causes four different phases of infection in the birds: an early cytolytic infection, a latent infection, a second phase of the cytolytic infection (associated with permanent immunosuppression) and a proliferative phase; a latent infection of the T cells can result in the virus being present in a long-term carrier state and present in lymphocytes (Nair, 2024). Marek's disease has been known to cause paralysis, edema, general inflammation and tumors in organs such as the heart, lungs and ovaries (Song, 2022). This virus is highly pathogenic and can spread quickly through turkey and chicken flocks, especially due to its unique ability to mature into an enveloped form in the epithelium of feather follicles, resulting in easy spreading via litter or dust in houses (Nair, 2024).

It is recommended to first identify and diagnose tumor presence or enlarged nerves and then perform molecular testing to officially identify the disease since Marek's disease has such a high morbidity rate (Nair, 2024). While it only recently has become more prevalent in the turkey industry, lameness has revealed to be a distinct symptom when the birds are older, between 12 and 30 weeks of age (Zlabravec et al., 2024). Like other diseases, treatment primarily revolves around vaccinations, breeding for resistance and general biosecurity protocols. For turkeys, the turkey herpesvirus vaccine (made up of the avirulent *Meleagrid alphaherpesvirus 1*) has been used to protect against Marek's disease (Nair, 2024).

Turkey Osteomyelitis Complex

Osteomyelitis is an infection in the bone. Turkey osteomyelitis complex is generally identified when an adherently normal turkey carcass is presented with the signs of green liver, arthritis, and infections in the leg bones (Huff et al., 2000). Turkey osteomyelitis complex is typically recorded in young male turkeys with lower levels of cell-mediated immunity, leading to the idea that this disease may be more of an issue with immune systems reacting to pathogens, rather than specific pathogens themselves (Huff et al., 2000). These infections can occur when rapid increases in body weight cause intense strain on vulnerable epiphyseal plates and cartilage, ultimately creating osteochondrotic clefts within the chondrocytes of the growth plates, resulting in a breeding ground for bacteria (Wideman, 2016). Lytic substances are released at lesion areas where a variety of bacteria are colonizing (i.e. *Staphylococcus aureaus, Enterococcus coreum,* etc) and induce necrosis in the calcifying area of metaphysis in the bone, destroying the structure of trabecular bone in the area, harming the growth plates' structural support. Bacterial chondronecrosis with osteomyelitis can form, causing the destruction of the proximal femoral head and tibiotarsus, resulting in severe lameness (McNamee et al., 2000). However, the bacteria harboring lesions can also be found in the distal femur and tibia, spine, ulna or radius (Wettere, 2020). The osteomyelitis and arthritis caused in these cases can be identified through examination of infected joints, which tend to swell and form a fibrinous exudate; lesions that are more subtle require histopathology for identification (Wettere, 2020). Studies have reported that taking measures such as providing probiotics via feed, adding 25-hydroxy vitamin D_3 or adding antibiotics (i.e. enrofloxacin) to the birds' drinking water can help to combat infection (Wideman, 2016).

Other Welfare Issues

Rickets is a vitamin-deficiency and cause lameness. Alongside lameness, cases of rickets often cause softer bones and beaks, as well as drops in egg production in hens (Jacob, 2024). Studies have disclosed that one of the most effective ways to treat and prevent rickets is to ensure that turkey feed contains adequate amounts of vitamin D and calcium throughout their different stages in life. Similarly, Crespo et al. (1999a) identified that minor nutritional deficiencies in things like calcium and vitamin D could have caused slight weakness in the bone shafts, even if the imbalance did not cause readily apparent clinical signs, further supporting how crucial the diet is in commercial birds. Overall, lameness can have a vast range of causes so any identifiable and practical measures should be taken to avoid welfare discrepancies and economic pitfalls.

Gait Assessments

Gait assessments, or scoring, are a common way of measuring and determining lameness in poultry (Yang et al., 2023). A six-point subjective scoring method, commonly known as the Bristol scale, is the most vastly used system in which a 0 indicates a “normal” bird and a 5 indicates a “severely lame” bird (Kestin et al., 1992). This scale is most often used in small-scale or research settings, while scales such as the 3-point gait scoring system are more often used in large scale settings (Webster et al., 2008). Subjective gait scoring systems require trained observers, but the risk of human error and bias is still present (Wurtz and Riber, 2023). To help alleviate these risks, objective gait scoring methods are being evaluated for reliability and practicality in the industry. Some of these systems include video and image analysis, accelerometers or pressure sensitive walkways (Li et al., 2023; Oviedo-Rondon et al, 2018; Pearce et al., 2023). Human error and bias in gait assessments pose a large risk, so the comparison of a subjective scoring method against quantified objective gait parameters can help to ensure the system’s reliability when used in a commercial setting.

Potential Solutions

Various approaches to help prevent and alleviate lameness in birds have been reviewed. Some breeders have created lines of slow-growing birds, but the lack of demand for them on the market has made it to where these lines aren’t often utilized. Methods like continuous gait scoring and assessing leg strength throughout the breeding process has helped to reduce leg issues in select pedigrees (Duggan et al., 2006). Another way to check for these issues is through the use of the Lixiscope, which assesses dense bone structure has been used by breeders to help with selecting for early cases of tibial dyschondroplasia (Roberson, 2009). Footpad dermatitis is a trait that companies regularly evaluate their birds for instances of and select against it to help minimize cases (Roberson, 2009). While the reduction of overall body weight and a decrease in growth weight are the primary methods in avoiding cases of lameness, neither are practical based on the demand of industry, but research is continuously conducted to find other solutions (Karcher, 2012). One of the current methods to combat these strains on the bones, specifically leg bones, is environmental enrichment. Environmental enrichments have been frequently studied in broilers, but less so in turkeys. Nonetheless, the studies conducted have reported increasing locomotive activity, positively impacting the physical and physiological welfare of farm animals (Newberry, 1995). Environmental enrichments can encourage locomotion in birds, directly impacting the skeletal system. This allows for the evaluation of how age and enrichments influence the skeletal system and walking abilities of toms during a production period.

Enrichments

As birds age and grow, they become more sedentary, so investigations on the use of enrichments and how giving animals the ability to partake in motivated behaviors may reduce boredom and increase their expression of physical activities (Vasdal, 2018). Trials have confirmed in broilers that the introduction of environmental enrichments increases their activity levels, even if only temporarily (Jacobs et al., 2022). There are findings supporting the notion that increases in activity could improve skeletal and muscular development, thus improving leg health (Reiter and Bessei, 2009).

Perches are often used to allow turkeys to perform roosting, as well as help lower the amount of bird crowding on the floor, which can impact turkeys' behaviors and sleeping habits (Martrenchar et al., 1999). However, Martrenchar et al. (2001) found that the perches were utilized in the early weeks of rearing, rather than the latter, which refuted the idea that the perches would allow for more floor space when the birds were larger in size. Elevated platforms have positively impacted the welfare of broilers by providing extra means of walking and providing more opportunities to express perching behaviors (Malchow and Schrader, 2021). Turkeys often begin to utilize elevated surfaces to rest, and studies conducted by Letzgub and Bessei (2009) and Cottin (2004) found that this resulted in better walking abilities, plumage quality and improvements to the tibia bones (Bessei, 2021). Alongside this, the idea of birds jumping on and off the perches directly aids in positive effects on the development of leg bones and muscles (Jacobs et al., 2022). When considering platforms, one of the biggest challenges is ramps are needed for the birds to access them, which can have build-up of dirt and debris that needs routinely cleaned, making it a cost-and-benefit type of enrichment, as well as requiring more space.

Litter material or roughage can be altered to provide enrichment to birds, but litter often becomes wet from things like the drinker, and is exposed to much of the birds' waste, making it the main contributor to cases of footpad dermatitis so it requires additional monitoring (Mayne et al., 2006). A range of litters can be used when rearing poultry, including wood shavings, peat, oat hulls and straw pellets (Bessei, 2021). Additionally, extra types of feed, or insects like meal worms, can be added to the litter to encourage foraging and dust bathing behaviors (Bessei, 2006; Simsek et al., 2009). These increased levels of foraging result in enhanced litter quality, which can decrease cases of footpad dermatitis and indirectly help to improve walking ability (Jacobs et al., 2022).

Straw bales are relatively easy to implement and cost effective. They can provide extra area for perching, and act as an extra medium for birds to peck, which will eventually become a part of the litter, contributing to the litter quality and foraging behaviors (Bessei, 2021). Bales may help birds in vulnerable states to hide from situations they find threatening, thus helping to reduce levels of stress (Newberry and Shackleton, 1997). Kells et al. (2001) found significant increases in activity levels of broilers who were provided with a straw bale compared to those who were not provided with one. Like litter, the introduction of straw bales has been met with caution due to studies revealing increases in the presence of lesions on the footpad, as well as bacterial infections (Thofner et al., 2019). Therefore, they are an enrichment that will need to be carefully monitored.

Some materials and devices can increase the expression of positive pecking in flocks. The more common pecking devices instilled are strings, CDs, plastic bottles, and baskets filled with hay or mineral-made pecking blocks (Bessei, 2021). Reviewed by Bessei (2021), it was reported that broilers and turkeys have little desire to utilize these objects after a short time of interaction, recommending that these enrichments be provided to the birds on a temporary basis and be switched out with different types regularly.

Select enrichment studies have observed parameters such as behavior and bone quality. A study conducted by Weber (2012) found that the addition of enrichments led to turkeys displaying more perching or climbing, which represents a possible improvement to their well-being. Since turkeys roost, forage and hide in the natural world, the provision of enrichments could help to prompt these behaviors and naturally induce positive behaviors and possibly musculoskeletal improvements. One of the most viewed positive effects of enrichment inclusion has been a decrease in injurious feather pecking. Crowe and Forbes (1999) found there was a direct correlation between the amount of time turkeys spent using enrichment and the amount of feather pecking displayed i.e. when their birds began to utilize the perches less, the number of birds pecking one another increased. Several type of enrichments, such as certain perches and tunnels, allow birds to escape aggressive or fearful situations, prompting better “moods” in the birds (Lindenwald et al., 2021). Higher stock densities, especially when turkeys are in the late stages of rearing, can prevent birds from being able to walk freely or avoid being in proximity to other birds, which can cause tension among birds. The lack of space can lead to injury in the birds attempting to crouch or rest if other birds need to get around or over them (Martrenchar et al., 1999). Because of instances like this, studies have reviewed lower stock density as a type of enrichment. These birds provided with increased space display more “play” behaviors, such as frolicking or running, as well as a greater ability to get better rest (Martrenchar et al., 1999).

Enrichments have had positive impacts on poultry welfare. There are cases in which birds may not utilize the enrichments as much as intended, making the implementation of them less desirable to producers. As birds age, they develop less of an ability to do things, such as jump on perches, leading to less use in the later weeks of production (Rayner et al., 2020). Things like CDs, strings and plastic bottles are sometimes used with chickens, but while frequently used in the beginning, the birds become less interested in the objects. Positive results, such as less fear, were observed when the enrichments were changed every few days, but it is unrealistic for producers to maintain the enrichments enough to keep them effective in a commercial setting (Bizeray et al., 2002). Finally, if the enrichment provided is not placed evenly throughout the barn, or if few are provided, there is less of a chance of most birds being able to utilize the enrichments (Baxter et al., 2020). These are some of the many factors producers must take into consideration when deciding how and what environmental enrichments to use throughout their farms. Various restraints on the design and execution of environmental enrichment designs require the need for testing enrichments that are realistic and practical for a commercial setting, while also having a positive impact on skeletal welfare and gait.

Objectives

This study is being conducted to examine the effects of age, body weight and environmental enrichments on the gait and skeletal quality of commercial turkey toms during a production period. Developing a clear idea of how the critical phases of bone development are affected by other systems, as well as how skeletal development impacts said systems. Additionally, the inclusion of objectivity in gait scoring systems will help to reduce issues such as human error and bias in pre-existing subjective gait scoring systems. The objectives of this study were:

- Determine the effects of age and environmental enrichments on the load-bearing and non-load-bearing bones in male turkeys, as well as determine the influence body weight has on specific bone parameters throughout the rearing period.
- To quantify and support the use of a two-point subjective gait score system on production age male turkeys using a pressure sensitive walkway.

REFERENCES

Aslam, M. L., J. W. Bastiaansen, M. G. Elferin, H.-J. Megens, R. P. Crooijmans, L. A. Blomberg, R. C. Fleischer, C. P. V. Tassell, T. S. Sonstegard, S. G. Schroeder, M. Groenen, and J. A. Long. 2012. Whole genome SNP discovery and analysis of genetic diversity in Turkey (*Meleagris gallopavo)*. BMC Genomics 13:391.

Barclay, E. 2014. Can Breeders Cure What Ails Our Breast-Heavy Turkeys? https://www.npr.org/sections/thesalt/2014/11/27/366850401/could-turkey-breeders-cure-the-ailments-of-our-big-breasted-birds. Accessed 04/09 2024.

Baxter, M., A. Richmond, U. Lavery, and N. O'Connell. 2020. Investigating optimal levels of platform perch provision for windowed broiler housing. Applied Animal Behaviour Science 225. doi https://doi.org/10.1016/j.applanim.2020.104967

Bessei, W. 2006. Welfare of broilers: a review. World's Poultry Science Journal 62:455-466. doi http://dx.doi.org/10.1017/S0043933906001085

Bessei, W. 2021. Enrichment for broilers and turkeys – from theoretical consideration to practical application, Lohmann Breeders.

Beyer, R. S. 2002. Leg Problems in Broilers and Turkeys in EP-113Kansas State University Agricultural Experiment Station and Cooperative Extension Service.

Beyer, R. S. 2008. Leg Problems in Broilers and TurkeysEngormix.com, Metabolic and nutritional diseases in poultry.

Biga, L., S. Bronson, S. Dawson, A. Harwell, R. Hopkins, J. Kaufmann, M. LeMaster, P. Mater, K. Morrison-Graham, K. Oja, D. Quick, and J. Runyeon. 2019. Bone Formation and Development in Anatomy & PhysiologyPressbooks, Oregon State University.

Bizeray, D., I. Estevez, C. Leterrier, and J. M. Faure. 2002. Influence of Increased Environmental Complexity on Leg Condition, Performance, and Level of Fearfulness in Broilers. Poultry Science Association:6. doi https://doi.org/10.1093/ps/81.6.767

Bonewald, L. F. 2011. The amazing osteocyte. Journal of Bone and Mineral Research 26:229-238. doi 10.1002/jbmr.320

Boskey, A. L. 2013. Bone composition: relationship to bone fragility and antiosteoporotic drug effects. Bonekey Reports 2. doi 10.1038/bonekey.2013.181

Boulianne, M., M. L. Brash, B. R. Charlton, S. H. Fitz-Coy, R. M. Fulton, R. J. Julian, M. W. Jackwood, D. Ojkic, L. J. Newman, J. E. Sander, H. L. Shivaprasad, E. Wallner-Pendleton, and P. R. Woolcock. 2013. Avian Disease Manual: Seventh Edition. M. Boulianne ed. American Association of Avian Pathologists.

Breeland, G., M. Sinkler, and R. Menezes. 2023. Embryology, Bone Ossification in StatPearls, National Library of Medicine.

Buchwalder, T., and B. Huber-Eicher. 2003. A brief report on aggressive interactions within and between groups of domestic turkeys. Applied Animal Behaviour Science 84.

Clark, S., and L. Chiaia. 2024. 2024 Turkey Industry Annual Report - Current Health Issues Facing the US Turkey Industry, United States Animal Health Association.

Clark, S., G. Hansen, P. McLean, P. Bond Jr., W. Wakeman, R. Meadows, and S. Buda. 2002. Pododermatitis in Turkeys. Pages 1038-1044 in Avian Diseases.

Clarke, B. 2008. Normal bone anatomy and physiology. Clin J Am Soc Nephrol 3 Suppl 3:S131-139. doi 10.2215/CJN.04151206

Clopton, J. 2025. Preventing Footpad Dermatitis (FPD) in Poultry: Practical Tips for Success, Global.

Combs, G., and J. McClung. 2017. Tibial Dyschondroplasia in The Vitamins, ScienceDirect.

Cottin, E. 2004. Influence of enriched husbandry environment and origin on performance, behaviour, plumage condition, leg position, running ability and tibial dyschondroplasia in male fattening turkeys. University of Veterinary Medicine Hanover.

Crespo, R., S. M. Stover, K. T. Taylor, R. P. Chin, and H. L. Shivaprasad. 1999. Morphometric and Mechanical Properties of Femora in Young Adult Male Turkeys with and without Femoral Fractures.

Crespo, R., S. M. Stover, R. Droual, R. P. Chin, and H. L. Shivaprasad. 1999. Femoral fractures in a young male turkey breeder flock. Avian Diseases 43.

Crespo, R., and H. L. Shivaprasad. 2011. Rupture of Gastrocnemius Tendon in Broiler Breeder Hens. Avian Diseases 55:495-498. doi 10.1637/9669-012711-case.1

Crowe, R., and J. M. Forbes. 1999. Effects of four different environmental enrichment treatments on pecking behaviour in turkeys. Br Poult Sci 40 Suppl:S11-12. doi 10.1080/00071669986558

Currey, J. D. 2002. Bones: structure and mechanics. Princeton university press.

Dibner, J. J., J. D. Richards, M. L. Kitchell, and M. A. Quiroz. 2007. Metabolic Challenges and Early Bone Development. Poultry Science Association.

Duggan, B., J. Ralph, S. Avendano, A.-M. Neeteson, T. Burnside, and A. Koerhuis. 2006. Methods like continuous gait scoring and assessing leg strength throughout the breeding process.

Erasmus, M. 2018. Welfare issues in turkey production. Food Science, Technology and Nutrition:28.

Federation, N. T. 2008. Raising America's Turkeys. https://www.eatturkey.org/raising-turkeys/.

Fragoso, A. A. H., K. Capilé, C. A. Taconeli, G. C. De Almeida, P. P. De Freitas, and C. F. M. Molento. 2023. Animal Welfare Science: Why and for Whom? Animals 13:1833. doi 10.3390/ani13111833

Fraser, D., D. M. Weary, E. A. Pajor, and B. N. Milligan. 1997. A Scientific Conception of Animal Welfare that Reflects Ethical Concerns. Wellbeing International 6:187-205.

Fraser, W., and J. Stevenson. 2013. Bone and Calcium Metabolism in the Immunoassay Handbook, Science Direct.

Furo, G. 2024. Managing footpad dermatitis in turkeys, University of Minnesota Extension.

Gothlin, G., and J. L. Ericsson. 1976. The osteoclast: review of ultrastructure, origin, and structure-function relationship. Clin Orthop Relat Res:201-231.

Goyal, S. M. 2024. Development of Live Attenuated Vaccine for Reoviruses Causing Arthritis and Hepatitis in TurkeysU.S. Poultry & Egg Association and the USPOULTRY Foundation, University of Minnesota.

Granquist, E. G., G. Vasdal, I. C. de Jong, and R. O. Moe. 2019. Lameness and its relationship with health and production measures in broiler chickens. The Animal Consortium 13:7. doi doi:10.1017/S1751731119000466

Grujjcic, R. 2023. Articular cartilage. https://www.kenhub.com/en/library/anatomy/articular-cartilage2024.

Hart, N. H., S. Nimphius, T. Rantalainen, A. Ireland, A. Siafarikas, and R. U. Newton. 2017. Mechanical basis of bone strength: influence of bone material, bone structure and muscle action. J Musculoskelet Neuronal Interact 17:114-139.

Hattner, R., B. N. Epker, and H. M. Frost. 1965. Suggested sequential mode of control of changes in cell behaviour in adult bone remodelling. Nature 206:489-490. doi 10.1038/206489a0

Havaldar, R., S. C. Pilli, and B. B. Putti. 2014. Insights into the effects of tensile and compressive loadings on human femur bone. Adv Biomed Res 3:101. doi 10.4103/2277-9175.129375

Havenstein, G. B., P. R. Ferket, J. L. Grimes, M. A. Qureshi, and K. E. Nestor. 2006. Comparison of the Performance of 1966- Versus 2003-Type Turkeys When Fed Representative 1966 and 2003 Turkey Diets: Growth Rate, Livability, and Feed Conversion. Poultry Science.

Hicks Jr, A. F., and M. Lerner. 1949. Hereditary Crooked Toes in Chickens. Poultry Science 28:625-626. doi https://doi.org/10.3382/ps.0280625

Hocking, P. M., S. Wilson, L. Dick, L. N. Dunn, G. W. Robertson, and C. Nixey. 2002. Role of dietary calcium and available phosphorus in the aetiology of tibial dyschondroplasia in growing turkeys. British Poultry Science 43:432-441. doi 10.1080/00071660120103729

Hu, G., D. N. Do, J. Gray, and Y. Miar. 2020. Selection for Favorable Health Traits: A Potential Approach to Cope with Diseases in Farm Animals. Animals 10:1717. doi 10.3390/ani10091717

Huang, S.-C., M. U. Rehman, Y.-F. Lan, G. Qiu, H. Zhang, M. K. Iqbal, H.-Q. Luo, K. Mehmood, L.-H. Zhang, and J.-K. Li. 2017. Tibial dyschondroplasia is highly associated with suppression of tibial angiogenesis through regulating the HIF-1α/VEGF/VEGFR signaling pathway in chickens. Scientific Reports 7. doi 10.1038/s41598-017-09664-6

Huff, G. r., W. E. Huff, N. C. Rath, and J. M. Balog. 2000. Turkey Osteomyelitis Complex. Poultry Science 79:1050-1056.

Hulet, M., P. Clauer, J. Boney, J. Harper, and L. Kime 2021. Small-Flock Turkey Production. https://extension.psu.edu/small-flock-turkey-production.

Hunton, P. 1990. Industrial breeding and selection [in poultry]. Developments in Animal and Veterinary Sciences (Netherlands).

Jacobs, L., R. A. Blatchford, I. C. de Jong, M. Erasmus, M. A. Levengood, R. C. Newberry, P. Regmi, and S. L. Weimer. 2022. Enhancing their quality of life: environmental enrichment for poultry. 102. doi 10.1016/j.psj.2022.102233

Jones, R. B. 2018. Environmental enrichment for poultry welfare.

Julian, R. J., and M. K. Bhatnagar. 1984. Cartilage Lesions Associated with Shaky-Leg Lameness. Avian Diseases 29:14.

Julian, R. J. 1984. Valgus-Varus Deformity of the Intertarsal Joint in Broiler Chickens. The Canadian Veterinary Journal 25:254-258.

Kapell, D. N. R. G., P. M. Hocking, P. K. Glover, V. D. Kremer, and S. Avendano. 2016. Genetic basis of leg health and its relationship with body weight in purebred turkey lines. Oxford University Press.

Kaplan, F. S., C. M. Strear, and M. A. Zasloff. 1994. Radiographic and scintigraphic features of modeling and remodeling in the heterotopic skeleton of patients who have fibrodysplasia ossificans progressiva. Clin Orthop Relat Res:238-247.

Karsenty, G., H. M. Kronenberg, and C. Settembre. 2009. Genetic control of bone formation. Annu Rev Cell Dev Biol 25:629-648. doi 10.1146/annurev.cellbio.042308.113308

Kells, A., M. S. Dawkins, and M. Cortina-Borja. 2001. The Effect of a 'Freedom Food' Enrichment on the Behaviour of Broilers on Commercial Farms. Animal Welfare 10:347-356. doi http://dx.doi.org/10.1017/S0962728600032620

Kestin, S. C., T. G. Knowles, A. E. Tinch, and N. G. Gregory. 1992. Prevalence of leg weakness in broiler chickens and its relationship with genotype. Vet Rec 131:190-194. doi 10.1136/vr.131.9.190

Khan, I. A., and B. Bordoni. 2025. Histology, Osteoclasts in StatPearls, Treasure Island (FL).

Knopov, V., R. M. Leach, T. Barak-Shalom, S. Hurwitz, and M. Pines. 1995. Osteopontin gene expression and alkaline phosphatase activity in avian tibial dyschondroplasia. Bone 16:S329-S334.

Kumar, R., T. A. Sharafeldin, S. M. Goyal, S. K. Mor, and R. E. Porter. 2022. Infection and transmission dynamics of Turkey arthritis reovirus in different age Turkeys. Microbial Pathogenesis 173.

Kumar, R., T. A. Sharafeldin, N. M. Sobhy, S. M. Goyal, R. E. Porter, and S. K. Mor. 2022. Comparative pathogenesis of turkey reoviruses. Avian Pathology 51:435-444. doi 10.1080/03079457.2022.2079474

Langdahl, B., S. Ferrari, and D. W. Dempster. 2016. Bone modeling and remodeling: potential as therapeutic targets for the treatment of osteoporosis. Ther Adv Musculoskelet Dis 8:225-235. doi 10.1177/1759720X16670154

Launey, M. E., M. J. Buehler, and R. O. Ritchie. 2010. On the mechanistic origins of toughness in bone. Annual review of materials research 40:25-53.

Letzgub, H., and W. Bessei. Year. Effects of environmental enrichment on the locomotor activity of turkeys. Proc. Poultry Welfare Symposium, Cervia, Italy.

Li, G., R. S. Gates, M. M. Meyer, and E. A. Bobeck. 2023. Tracking and Characterizing Spatiotemporal and Three-Dimensional Locomotive Behaviors of Individual Broilers in the Three-Point Gait-Scoring System. Animals 13:717. doi 10.3390/ani13040717

Ligaraba, T., K. Benyi, and J. Baloyi. 2016. Response of broiler chickens from three genetic groups to different stocking densities. Springer 48:1227-1234.

Lindenwald, R., H. J. Schuberth, B. Spindler, and S. Rautenschlein. 2021. Influence of environmental enrichment on circulating white blood cell counts and behavior of female turkeys. Poult Sci 100:101360. doi 10.1016/j.psj.2021.101360

Malchow, J., and L. Schrader. 2021. Effects of an Elevated Platform on Welfare Aspects in Male Conventional Broilers and Dual-Purpose Chickens. Frontiers in Veterinary Science 8. doi 10.3389/fvets.2021.660602

Martel, S. 2016. Effects of small-amplitude periodic topography on combined stresses due to gravity and tectonics. International Journal of Rock Mechanics & Mining Sciences 89:1-13. doi http://dx.doi.org/10.1016/j.ijrmms.2016.07.026

Martin, E. 2021. Lameness disorder in poultry. Pages 22 in AHL NewsletterUniversity of Guelph, Animal Health Laboratory.

Martrenchar, A., D. Huonnic, J. P. Cotte, E. Boilletot, and J. P. Morisse. 1999. Influence of stocking density on behavioural, health and productivity traits of turkeys in large flocks. British Poultry Science 40:323-331. doi 10.1080/00071669987403

Mayne, R. K., P. M. Hocking, and R. W. Else. 2006. Foot pad dermatitis develops at an early age in commercial turkeys. British Poultry Science 47:36-42. doi DOI: 10.1080/00071660500475392

McNamee, P. T., J. A. Smyth, and J. A. Smyth. 2000. Bacterial chondronecrosis with osteomyelitis ('femoral head necrosis') of broiler chickens: A review. Avian Pathology 29:253-270. doi 10.1080/03079450050118386

Mellor, D. 2016. Updating Animal Welfare Thinking: Moving beyond the “Five Freedoms” towards “A Life Worth Living”. Animals 6:21. doi 10.3390/ani6030021

Mellor, D. J., N. J. Beausoleil, K. E. Littlewood, A. N. McLean, P. D. McGreevy, B. Jones, and C. Wilkins. 2020. The 2020 Five Domains Model: Including Human–Animal Interactions in Assessments of Animal Welfare. Animals 10:1870. doi 10.3390/ani10101870

Mishra, A. n.d. What is Compressive Strength? Definition, Uses, and Formula, Testronix.

Moe, R. O., J. Bohlin, A. Flo, G. Vasdal, H. Erlandsen, E. Guneriussen, E. C. Sjokvist, and S. M. Stubsjoen. 2018. Effects of subclinical footpad dermatitis and emotional arousal on surface foot temperature recorded with infrared thermography in turkey toms (Meleagris gallopavo). Poultry Science 97:2249-2257.

Nabi, F., M. Shahzad, J. Liu, K. Li, Z. Han, D. Zhang, M. K. Iqbal, and J. Li. 2016. Hsp90 inhibitor celastrol reinstates growth plate angiogenesis in thiram-induced tibial dyschondroplasia. Avian Pathology 45:187-193. doi 10.1080/03079457.2016.1141170

Nair, V. 2024. Marek's Disease in Poultry, Merck Manual Veterinary Manual.

Nelson, T. S., L. K. Kirby, J. W. Purdy, and Z. B. Johnson. 1992. Effect of Diet on Growth and the Incidence of Tibial Dyschondroplasia in Broilers. The Professional Animal Scientist 8:8-11. doi https://doi.org/10.15232/S1080-7446(15)32116-1

Nestor, K. E. 1971. Crooked Toes in Turkeys. O. A. R. a. D. Center ed.

Newberry, R. C. 1995. Environmental enrichment: Increasing the biological relevance of captive environments. Applied Animal Behaviour Science 44:229-243. doi 10.1016/0168-1591(95)00616-Z

Newberry, R. C., and D. M. Shackleton. 1997. Use of visual cover by domestic fowl: A Venetian blind effect? Animal Behaviour 54:387-395. doi http://dx.doi.org/10.1006/anbe.1996.0421

Niu, Y., T. Du, and Y. Liu. 2023. Biomechanical Characteristics and Analysis Approaches of Bone and Bone Substitute Materials. J Funct Biomater 14. doi 10.3390/jfb14040212

Nordin, M., and V. H. Frankel. 2001. Basic Biomechanics of the Musculoskeletal System. Lippincott Williams & Wilkins.

Ocran, E. 2023. Periosteum. https://www.kenhub.com/en/library/anatomy/periosteum2024.

Oviedo-Rondon, E. O., M. J. Wineland, J. Small, H. Cutchin, A. McElroy, A. Barri, and S. Martin. 2009. Effect of incubation temperatures and chick transportation conditions on bone development and leg health. The Journal of Applied Poultry Research 18:671-678. doi http://dx.doi.org/10.3382/japr.2008-00135

Oviedo-Rondon, E. O., P. L. Mente, C. Arellano, B. D. X. Lascelles, and A. Mitchell. 2018. Influence of gait on bone strength in turkeys with leg defects. Pages 16. N. C. S. University ed., Poultry Science Association.

Pal, D., R. Janardhana, R. Samuel Rajesh Babu, and S. Vijayakumar. 2022. An examination of the tensile strength, hardness and SEM analysis of Al 5456 alloy by addition of different percentage of SiC/flyash. Materials Today Proceedings 62. doi http://dx.doi.org/10.1016/j.matpr.2022.02.288

Pearce, J., Y.-M. Chang, and S. Abeyesinghe. 2023. Individual Monitoring of Activity and Lameness in Conventional and Slower-Growing Breeds of Broiler Chickens Using Accelerometers. Animals 13:1432. doi 10.3390/ani13091432

Pines, M., and R. Reshef. 2015. Tibial Dyschondroplasia in Sturkie's Avian Physiology, ScienceDirect.

Porter, R. 2018. Turkey Reoviral Arthritis Update Turkey Health Workshop. U. o. Minnesota ed., Midwest Poultry Federation.

Price, P. A., A. A. Otsuka, J. W. Poser, J. Kristaponis, and N. Raman. 1976. Characterization of a gamma-carboxyglutamic acid-containing protein from bone. Proceedings of the National Academy of Sciences 73:1447-1451. doi 10.1073/pnas.73.5.1447

Program, A. H. F. 2020. Animal Welfare Standards for Turkeys. Pages 46.

Quinton, C. D., B. J. Wood, and S. P. Miller. 2011. Genetic analysis of survival and fitness in turkeys with multiple-trait animal models. Poultry Science Association.

Rayner, A. C., R. C. Newberry, J. Vas, and S. Mullan. 2020. Slow-growing broilers are healthier and express more behavioural indicators of positive welfare. Scientific Reports 10. doi 10.1038/s41598-020-72198-x

Reiter, K., and W. Bessei. 2009. Effect of locomotor activity on leg disorder in fattening chicken. Berliner und munchener tierarztliche wochenschrift 122:264-270.

Ritchie, R. O., K. J. Koester, S. Ionova, W. Yao, N. E. Lane, and J. W. Ager III. 2008. Measurement of the toughness of bone: A tutorial with special reference to small animal studies. Bone 43:798-812.

Roberson, K. D. 2009. Growth performance and spontaneous bone fracture incidence of turkey toms fed various levels of calcium and nonphytate phosphorus to heavy market weight. Journal of Applied Poultry Research 18:158-164. doi https://doi.org/10.3382/japr.2008-00083

Schlesinger, P. H., H. C. Blair, D. Beer Stolz, V. Riazanski, E. C. Ray, I. L. Tourkova, and D. J. Nelson. 2020. Cellular and extracellular matrix of bone, with principles of synthesis and dependency of mineral deposition on cell membrane transport. American Journal of Physiology-Cell Physiology 318:C111-C124. doi 10.1152/ajpcell.00120.2019

Setiawati, R., and P. Rahardjo. 2018. Osteogenesis and bone regeneration in Bone Development and Growth, Research Gate.

Sharafeldin, T. A., S. K. Mor, A. Z. Bekele, H. Verma, S. L. Noll, S. M. Goyal, and R. E. Porter. 2015. Experimentally induced lameness in turkeys inoculated with a newly emergent turkey reovirus. Veterinary Research.

Sharafeldin, T. A., Q. Chen, S. K. Mor, S. M. Goyal, and R. E. Porter. 2016. Altered Biomechanical Properties of Gastrocnemius Tendons of Turkeys Infected with Turkey Arthritis Reovirus, Veterinary Medicine International.

Siebel, M. 2005. Biochemical Markers of Bone Turnover Part 1: Biochemistry and Variability. Clin Biochem 26.

Simsek, U. G., I. H. Cerci, B. Dalkilie, O. Yilmaz, and M. Ciftci. 2009. Impact of stocking density and feeding regimen on broilers: Chicken meat consumption, fatty acids and serum cholesterol levels. Poultry Science Association 18:514-520. doi 10.3382/japr.2008-00141

Soboyejo, A. B. O., and K. E. Nestor. 2000. A NEW STATISTICAL BIOMECHANICS APPROACH TO MODELING BONE STRENGTH IN TURKEYS AND BROILER CHICKENS. Transactions of the ASABE 43:1997-2006.

Song, B., J. Zeb, S. Hussain, M. U. Aziz, E. Circella, G. Casalino, A. Camarda, G. Yang, N. Buchon, and O. Sparagano. 2022. A Review on the Marek's Disease Outbreak and Its Virulence-Related meq Genovariation in Asia between 2011 and 2021. Animals (Basel) 12. doi 10.3390/ani12050540

Šromová, V., D. Sobola, and P. Kaspar. 2023. A Brief Review of Bone Cell Function and Importance. Cells 12:2576. doi 10.3390/cells12212576

Sroga, G. E., and D. Vashishth. 2012. Effects of Bone Matrix Proteins on Fracture and Fragility in Osteoporosis. Current Osteoporosis Reports 10:141-150. doi 10.1007/s11914-012-0103-6

Stover, K. K., D. M. Weinreich, T. J. Roberts, and E. L. Brainerd. 2018. Patterns of musculoskeletal growth and dimensional changes associated with selection and developmental plasticity in domestic and wild strain turkeys. Ecology and Evolution 8:3229-3239. doi 10.1002/ece3.3881

Swalander, L. M., T. A. Burnside, and P. K. Glover. 2020. Leg Health in Commercial Turkeys, Aviagen Turkeys.

Thirupathy, M., and M. Vadivel. 2024. Effects of Tribology and Mechanical Properties on Silicon Carbide and Glass Fiber-Reinforced Hybrid Nanocomposites in The International Conference on Processing and Performance of Materials (ICPPM 2023).

Thofner, I. C. N., L. L. Poulsen, M. Bisgaard, H. Christensen, R. H. Olsen, and J. P. Christensen. 2019. Correlation between footpad lesions and systemic bacterial infections in broiler breeders. Vet Res 50:38. doi 10.1186/s13567-019-0657-8

Tosovic, D. 2023. Medullary cavity. https://www.kenhub.com/en/library/anatomy/medullary-cavity2024.

Tresguerres, F. G. F., J. Torres, J. Lopez-Quiles, G. Hernandez, J. A. Vega, and I. F. Tresguerres. 2020. The osteocyte: A multifunctional cell within the bone. Annals of Anatomy 227. doi https://doi.org/10.1016/j.aanat.2019.151422

Tu, W., Y. Zhang, K. Jiang, and S. Jiang. 2023. Osteocalcin and Its Potential Functions for Preventing Fatty Liver Hemorrhagic Syndrome in Poultry. Animals 13:1380. doi 10.3390/ani13081380

Van Wyhe, R. C., P. Regmi, B. J. Powell, R. C. Haut, M. W. Orth, and D. M. Karcher. 2014. Bone characteristics and femoral strength in commercial toms: The effect of protein and energy restriction. Poultry Science 93:943-952. doi https://doi.org/10.3382/ps.2013-03604

Vasdal, G. 2018. Effects of environmental enrichment on activity and lameness in commercial broiler production. Journal of Applied Animal Welfare Science 22:8.

Weber, P. A. 2012. The Effects of Social and Environmental Enrichments on Leg Strength and Welfare of Tom Turkeys. Master of Science in Animal Science. University of Nebraska.

Webster, A. B., B. D. Fairchild, T. S. Cummings, and P. A. Stayer. 2008. Validation of a Three-Point Gait-Scoring System for Field Assessment of Walking Ability of Commercial Broilers. Poultry Science 17:529-539. doi 10.3382/japr.2008-00013

Wettere, A. J. V. 2020. Infectious Skeletal Disorders in Poultry. https://www.merckvetmanual.com/poultry/disorders-of-the-skeletal-system-in-poultry/infectious-skeletal-disorders-in-poultry.

Wideman, R. 2016. Bacterial chondronecrosis with osteomyelitis and lameness in broilers: a review. Poultry Science 95:325-344.

Wurtz, K. E., and A. B. Riber. 2024. Overview of the various methods used to assess walking ability in broiler chickens. Veterinary Record 195. doi 10.1002/vetr.4398

Xiong, J., M. Onal, R. L. Jilka, R. S. Weinstein, S. C. Manolagas, and C. A. O'Brien. 2011. Matrix-embedded cells control osteoclast formation. Nature Medicine 17:1235-1241. doi 10.1038/nm.2448

Yang, X., Y. Zhao, S. Hawkins, L. Eckelkamp, M. Prado, R. Burns, J. Purswell, and T. Tabler. 2023. Modeling gait score of broiler chicken via production and behavioral data. The international journal of animal biosciences. doi 10.1016/j.animal.2022.100692

Zhong, Z., M. Muckley, S. Agcaoglu, M. E. Grisham, H. Zhao, M. Orth, M. S. Lilburn, O. Akkus, and D. M. Karcher. 2012. The morphological, material-level, and ash properties of turkey femurs from 3 different genetic strains during production, Poultry Science Association.

Zlabravec, Z., B. Slavec, E. Rozmanec, S. Koprivec, A. Doc, and O. Rojs. 2024. First Report of Marek’s Disease Virus in Commercial Turkeys in Slovenia. Animals (Basel) 14. doi 10.3390/ani14020250

Zoch, M. L., T. L. Clemens, and R. C. Riddle. 2016. New insights into the biology of osteocalcin. Bone 82:42-49. doi 10.1016/j.bone.2015.05.046

CHAPTER 3

THE EFFECTS OF ENVIRONMENTAL ENRICHMENTS ON THE BODY WEIGHT, MORPHOLOGICAL AND BIOMECHANICAL BONE TRAITS OF COMMERCIAL TURKEY TOMS

Kulbacki, Stephanie. To be Submitted to Poultry Science

ABSTRACT

In recent years, increased body weight and breast size in commercial turkeys have coincided with a rise in leg abnormalities and lameness, indicating that the skeletal structure may not have been proportionally favored by genetic selection for performance traits. Inclusion of environmental enrichments (EE) might alleviate these issues by promoting active behavior and, subsequently, bone strength. This study evaluated the relationship between body weight (BW) and skeletal parameters at different ages in male turkeys and assessed the effects of different EE treatments - Pecking Block (PB), Straw Bale (SB), Tunnel (TU), and ground Robot (RO) compared to a control group (CO) in male commercial turkeys. Across two experiments, tibia, femur, and humerus samples were collected at various production ages. Body weight, morphological (bone weight, length, and diameter) and biomechanical (peak breaking force and energy required to fracture) bone traits were measured. Data were analyzed with a mixed model ANOVA with treatment and age as main effects, pen as a random effect, and BW as a linear covariate. Tukey's HSD was used for pairwise comparisons. As expected, age had a significant impact on femur and tibia properties, with bone growth slowing notably between 12 and 18 woa (weeks of age) compared to the earlier 8 to 12 woa. The humerus was only analyzed at two age points, presenting substantial growth in most parameters, but biomechanical traits only increased slightly. Minimal changes were observed in bone properties between the different EE treatments. Select diameters in the femur and humerus were enhanced with the provision of the TU at 8 woa. Few biomechanical properties, such as the PF of tibias at 18 woa, were improved with the inclusion of a PB. Correlation analysis revealed that at 8 woa, BW was strongly correlated with femur and tibia weight, and moderately with the peak force to fracture (PF). At 18 woa, the correlation was quite weak, particularly for tibia PF, which presented no correlation with BW.

Overall, long bone properties in apparently healthy toms were primarily influenced by age and BW, rather than EE. Interestingly, market BW exhibited weak or no correlation with bone traits of turkey toms, suggesting that heavier toms may not always have stronger bones. These findings support the potential for genetic selection targeting skeletal traits without compromising performance.

INTRODUCTION

As the commercial turkey industry grows in terms of overall production, targets for body weight and feed conversion ratio have evolved accordingly. Current performance goals for hybrid male turkeys include reaching a body weight of 14.3 kg and achieving a cumulative feed conversion ratio of 1.1 kg/kg by 18 weeks of age (Aviagen, 2022.). This represents approximately an 8.33% increase in the body weight target for male turkeys compared to standards from 50 years ago (Clark et al., 2019). Variation in trait heritability has enabled the substantial increase in body weight to be driven primarily by growth in breast muscles mass, rather than by increases in wing and leg muscles or the skeletal system overall (Stover et al., 2018).

Various genetic, metabolic and structural issues in modern turkeys have been associated with body weight-driven selection (Stover et al., 2018). The skeletal system has not developed at the same rate as body mass. Traits like increased shank width, which is directly related to improved walking ability, have been negatively impacted by genetic selection for increased body weight (Nestor et al., 1985). Skeletal development in turkeys occurs at different rates throughout the production cycle and longitudinal growth slows considerably as the birds age. Biomechanical properties such as yield stress and toughness of turkey femurs increased by 19% and 35%, respectively, from 8 to 12 weeks, but displayed modest increases of 9% and 8% from 16 to 18 weeks (Zhong et al. (2012a). Overall, the disproportionate growth between the muscular and the skeletal system may have partially contributed to the higher incidences of lameness observed in commercial turkeys. Turkey industry reports indicate that up to 20% of turkeys experience some form of lameness; lameness and other skeletal issues have ranked consistently among the top ten concerns in the industry (Clark, 2020; Lilburn, 1994).

Lameness can be caused by a variety of factors, ranging from developmental disorders to pathogens (Bradshaw et al., 2002). Conditions like tibial dyschondroplasia, valgus-varus disorders, and viral and bacterial infections that can cause drastic damage to the bone are often prompted by abnormal bone development (Dibner et al., 2007). Pre-existing bone lesions can lead to stress fractures, resulting in overall weaker bones and increased probability of complete fractures (Crespo et al., 1999a; Crespo et al., 1999b). Skeletal issues become more prevalent and severe with age, resulting in less mobility and impacting access to feeders and drinkers (Beaulac and Schwean-Lardner, 2018). Additionally, a greater frequency of damaging behaviors, such as feather pecking, has been observed in flocks with a higher prevalence of skeletal issues (Reviewed in Erasmus, 2018). Damage to the load-bearing bones impedes ambulatory activity in turkeys, which can indirectly affect non-load-bearing bones such as the wings, as turkeys with gait issues rely heavily on their wings for support. Unfortunately, skeletal issues recorded in fast-growing lines can contribute to up to 5% of flock mortality (Ferket and Sell, 1989; Julian, 2004). Some cases are severe and directly related to leg fractures, like when a femoral artery is punctured by a fractured femur, whereas others are indirect, like lame birds being killed due to aggression from dominant conspecifics (Crespo et al., 1999a).

Lameness can further compromise turkey welfare by reducing their ability to perform natural and comfort behaviors such as preening, foraging, walking, and dustbathing (Weeks et al., 2000). The provision of environmental enrichments, defined as a method to modify an animal's environment to improve their behaviors and biological functions, are being studied throughout the poultry industry (Newberry, 1995). Turkeys have been encouraged to perch, climb, and display signs of "play" with the addition of environmental enrichments, suggesting improved welfare (Weber, 2012). These additions have also reduced aggression and handling stress, resulting in both welfare and economic benefits (Day et al., 2008). In caged layers provided perches, improved bone mineral content and bone area at 12 weeks of age, and wider shanks at 71 weeks of age were observed, indicating improved skeletal quality (Enneking et al., 2012; Yan et al., 2014). Other enrichments like straw bales and platforms enhanced walking ability and reduced footpad dermatitis in broilers (Jacobs et al., 2022). Broilers reared with access to platforms had improved gait, as well as lower prevalence of tibial dyschondroplasia (Kaukonen et al., 2017). While bone health studies in turkeys exposed to environmental enrichments are few, gait changes under environmental provision have been observed. Briefly, turkey gait worsened regardless of the inclusion of enrichments, but impaired gait was not reported until roughly 19 weeks of age (close to market age), in those subjects provided enrichments; whereas those without enrichment presented signs of gait impairment beginning at 16 weeks of age (Dong, 2023).

This study aims to evaluate how age and environmental enrichments affect long bone characteristics of commercial turkey toms at different stages during the production period. It also examines the correlation between performance and skeletal traits.

MATERIALS AND METHODS

Animals and Housing

This experiment was approved by the Institutional Animal Care and Use Committee at Purdue University.

Two experiments were conducted (E1 and E2), each with 400 beak-trimmed and vaccinated Nicholas Select male turkey poults from Aviagen® Turkeys/experiment. The turkey poults were collected from a commercial hatchery and taken to Purdue University's Animal Sciences Research and Teaching Center in West Lafayette, Indiana. The poults were randomly divided among 24 pens containing wood-shavings based litter. These 24 pens were evenly divided into two rooms, each pen measuring 10 feet in length by 8 feet in width. The turkeys were housed with a stocking density of 9 to 11 lbs/ft^2. Each pen held two bell drinkers and one feeder, providing *ad libitum* access to water and feed. Diets were determined based on Aviagen's recommendations and the NRC nutrition guidelines. Twenty-four hours of continuous photo period was provided for the first day of placement. After that, a schedule of 22 hours of light and 2 hours of dark was implemented until the poults were 9 weeks of age (woa). The lighting schedule then changed to 20 hours of light and 4 hours of dark until the end of the study. The lighting schedule was determined per Aviagen's recommendations for the Nicholas Select performance line. Housing temperatures were set based on Aviagen's recommendations, with the brooding phase being controlled to 40 °C.

Treatments

The following environmental enrichments were described by Dong et al (2023). Briefly, the 24 pens were randomly assigned to six groups: five treatment groups and a Control (CO), with four pens per group. The treatment groups consisted of four environmental enrichment types: Platform (PL), Pecking Block (PB), Straw Bale (SB), and Tunnel (TU). In addition, a fifth treatment involved using a ground robot (RO; Supplemental Figures S3.1 – S3.5) to induce physical activity among turkeys. The control group had no added enrichments resembling a standard commercial production environment. The PL treatment provided a square wooden platform (42" x 42") placed at a height of 40" from the floor, accessible via two ramps placed at a 45° angle. The PB treatment offered access to a suspended pecking-block from (Peck Stone, York Ag Products Inc., Manheim, PA), while the SB treatment consisted of a single straw bale (28" x 40" x 12") placed on litter. The TU treatment featured a tunnel designed to provide shelter and protection from aggressive behaviors of conspecifics. The RO treatment consisted of a manually maneuvered ground robot (GPK-32 Tracked Inspection Robot, SuperDroid Robotics, IL, USA) for 20 minutes every other day. Access to enrichment began when toms reached 5 woa. For RO pens, the robot was moved along random paths from 5 to 9 woa and then along a set path until the end of the study. Additionally, turkeys were individually marked with non-toxic livestock markers starting at 2 woa for focal behavioral assessments.

Sampling

Two experiments were conducted for this study. Experiment 1 (E1) included two toms with no apparent gait issues being chosen at 8 (n = 48 birds) and 12 (n = 63 birds) woa from each pen, followed by all remaining birds being sacrificed and sampled at 18 (n = 155 birds) woa. Their right femur and tibiotarsus were collected and any major skin and muscle removed and stored in bags for freezing. For Experiment 2 (E2), at 8 (n = 42 birds) woa, two toms with no apparent gait issues were selected per pen and at 17 (n = 227 birds) woa all remaining birds were sacrificed and sampled. The right humerus and right tibiotarsus were sampled from each bird, with major skin and tissue being removed before storage. E2 included plasma analysis for bone biomarkers. 387 birds at 8 woa and 178 birds at 17 woa had blood collected from their brachial veins. The blood collected was spun at 4000 g for five minutes and the plasma extracted into pre-labeled tubes. The extracted plasma was then stored in -80 °C until further analysis.

Bone Mechanical Properties

All bone samples were stored in freezers at -20 °C until further analysis. Bone samples were frozen regardless of whether soft tissues were present. If necessary, samples were thawed to remove surrounding soft tissues, then allowed to dry at room temperature prior to biomechanical testing. Drying times varied based on the size of the samples and the age of the bird, typically ranging from 2 to 4 hours. If the samples were not tested on the same day, bones were wrapped in a paper towel moistened with phosphate-buffered saline (PBS) and stored in a refrigerator at 4 °C.

Prior to analysis, each bone was weighed and the length, mid-shaft anteroposterior and mediolateral diameter were recorded using a vernier caliper (INSIZE Co., LTD, China). The samples were then subjected to a 3-point bend test (TA.HDPlus, Texture Technologies Corp., MA, USA). The machine was calibrated to a force of 25 kg with a height of 15 mm. The test was performed with either a 250 kg (for 8 and 12 woa) or 750 kg (for 17 and 18 woa) load cell, depending on the age of the bones, and at a test speed of 1 mm/s. The resulting force-distance curve was used to calculate the peak force and energy to fracture using custom software (Exponent Connect, Texture Technologies Corp., MA, USA). The standard set-up settings for testing are found in Supplemental Table S3.1.

Plasma Pyridinoline Concentrations

Blood samples were collected from the toms in E2 only at 8 (n = 387 birds) and 17 (n = 178 birds) woa. The samples were used to measure circulating pyridinoline (PYD) concentrations as an indicator of bone resorption. PYD is a collagen byproduct which is released into the bloodstream, and eventually excreted through urine when the skeletal system undergoes remodeling. Pyridinoline levels were analyzed using PYD Urine ELISA kits (QuidelOrtho, CA, USA). A trial plate was analyzed using plasma samples from 28-day old turkey poults and a 16-fold dilution factor was determined to be appropriate for the assay. All plates were processed according to the manufacturer's instructions and read at 405 nm, using a 4-PL curve with the equation: $y = (A-D) / (1+(x/C^{B}) + D$ to quantify PYD concentrations for each sample. This equation contained the minimal absorbance obtained (A), the maximum absorbance obtained

(D), the inflection points between A and D (C), and Hill's slope of the curve relating to the steepness of the curve (B). The average intra-assay CV% for all plates was 7.2% and the inter-assay CV% was 14.2%.

Statistical Analysis

For body weight comparisons a mixed model was performed measuring the effects of age, treatment and the interaction. Tukey HSD was used for mean-wise comparisons. Data from both E1 and E2 are presented.

Statistical analysis for age and treatment effects on bone traits was completed. A mixed model was performed with age, treatment and the interaction as main effects. Data from E1 and E2 were combined and a model for 8-, 12-, 17- and 18-week-old tibias was used. The data from E2 was used for 8- and 17- weeks of age analysis for the humerus. The analysis performed for the tibia parameters included experiment as a random effect. Body weight was used as a covariate for age comparisons, but not treatment comparisons at the different age points.

The correlations between body weight, bone weight, peak force and average diameter were determined. A multivariate method test was analyzed for each bone type at each age point, which yielded the Pearson correlation coefficient (r), as well as the corresponding p-values. Data from E1 and E2 are presented.

Pyridinoline concentration differences between treatments and ages were analyzed. For treatment effects, a Kruskal-Wallis test with PYD concentration (nmol/L) as the response variable, and treatment as the factor variable were performed at each age point. For age comparisons, a Wilcoxon Two-Sample test with PYD concentration as the response variable and age as the factor variable was performed. Both are non-parametric tests since the assumptions of normality and heterogeneity could not be met for a one-way ANOVA test. Data presented is only from E2.

All analyses were conducted in JMP Pro 18 (JMP Statistical Discovery LLC., NC, USA) and significant differences were identified at $P \leq 0.05$ and significant trends were identified at $P \leq 0.1$

RESULTS

For the following results an interaction effect was not observed, so the data presented is from analyses performed at each of the sampled age points.

Body Weight

The average body weights of turkeys from E1 and E2 at different ages are presented in Figure 3.1 and Table 3.1.

Average body weights of 3.09 ± 0.21 kg at 8 weeks, 8.38 ± 0.35 kg at 12 weeks, 14.15 ± 0.32 kg at 17 weeks and 17.28 ± 0.32 kg at 18 weeks were recorded (Figure 3.1). This reflects a 174.34% increase between 8 weeks and 12 weeks, a 66.86% increase between 12 and 17 weeks, and an increase of 22.54% from 17 to 18 weeks. Overall, there was an increase of 461.17% between 8 and 18 woa, consistent with typical growth observed in male commercial turkeys.

In general, treatments used in this study had minimal impact on body weight. The only difference was observed at 17 woa between toms from the Platform (PL) group and the Pecking Block (PB) group (Table 3.1). Birds from PL were 1.3 kg lighter on average compared to the PB birds ($P < 0.05$).

Bone Morphological and Biomechanical Properties

Tables 3.2 through 3.7 present the morphological and biomechanical properties of turkey bones collected from toms in E1 and E2 at various ages during production.

Femur

At 8 woa, toms from the Tunnel (TU) treatment group had wider antero-posterior (AP) and medio-lateral (ML) diameters compared to those in the Platform (PL) group ($P < 0.05$; Table 3.2). A similar trend was observed for average total diameter, with TU toms exhibiting wider femurs than PL toms (11.38 ± 0.27 mm vs. 10.14 ± 0.19 mm; $P = 0.002$). A trend in femur weight was also noted between treatments ($P = 0.072$), with femurs from the TU group weighing 24% more than those from the PL group (Table 3.3). Treatment effects for femur parameters were not observed at other ages in this study.

Tibia

Several differences in tibia parameters were observed between the treatment groups across the production period (Table 3.4). At 8 woa, turkeys in the PB group had tibias with ML diameters 0.88 mm wider than those in the PL group ($P = 0.03$). Tibias from the PB group also required higher peak force to fracture (PF) compared to the Control (CO) and Straw Bale (SB) groups (49.61 ± 3.50 kg vs. 38.92 ± 3.45 kg and 40.01 ± 3.40 kg, respectively; $P = 0.02$; Table 3.5). At 12 woa, tibiae from the Robot (RO) group were approximately 20 g heavier than those from the PL group. Tibiae from RO toms also tended to have wider average diameter than PL toms ($P = 0.09$). Some differences were observed between treatment for tibia parameters at 18 woa. Tibia was longer in SB compared to RO toms ($P = 0.02$). A significant difference was also found in the energy required to fracture the tibia ($P < 0.001$). Tibiae from SB toms absorbed more energy before fracture (350.64 ± 19.23 kg) than those from PL and PB toms (261.41 ± 14.23 kg and 250.60 ± 24.04 kg, respectively).

Humerus

At 8 woa, a difference in ML diameter was observed, with TU toms having humeri that were 1 mm wider on average compared to those in the PL group (Table 3.6). A trend in which the average humerus diameter was 0.85 mm wider in the TU compared to the PL group (***P*** = *0.084*). At 17 woa, humeri from toms of the PL, PB, and TU group had wider ML diameters than those from the SB (***P*** = *0.012*). The PB group had greater average humerus diameter (17.08 ± 0.09 mm) compared to the SB group (16.55 ± 0.09 mm; *P* = *0.019*). Additionally, a treatment effect was observed for humerus weight (*P* = *0.03*), with birds reared with tunnel enrichment (TU) having heavier humeri (69.06 ± 1.52 g) than those reared with straw bales (61.53 ± 1.40 g; Table 3.7).

Age Effects Observed on Load-bearing and Non-Load-Bearing Bones

Tables 3.8 through 3.10 present the differences in morphological and biomechanical properties from toms in E1 and E2 at select ages.

Femur

Significant differences were observed across all femur parameters when comparing birds sampled at 8, 12, and 18 woa (*P* < *0.001*; Table 3.8). Femur length increased by 39.17% from 8 to 12 weeks, followed by a slower growth rate of 7.57% between 12 and 18 weeks. From 8 to 18 weeks, anterior-posterior (AP) diameters, medial-lateral (ML) diameters, and average diameters nearly doubled, reflecting substantial morphological development over time (*P* < *0.001*). Femur weight provided a clear indication of bone development, increasing by 229.43% from 8 to 12 woa. This growth slowed substantially between 12 and 18 weeks, with only a 17.81% increase.

In terms of peak force to fracture (PF), measurements nearly doubled from 8 to 18 weeks, while the energy required to fracture (AUC) increased nearly fourfold over the same period.

Tibia

All tibia measurements increased with age in male turkeys (Table 3.9). Tibia length increased by 39.49% from 8 to 12 weeks but only increased by 7.83% from 12 to 18 weeks. Similarly, the AP, ML, and average diameters also increased more substantially during the earlier growth phase. From 8 to 12 weeks, diameters increased by 45–57%, whereas from 12 to 18 woa the increases only ranged from 7-15%. These results highlight the rapid skeletal development occurring in the first half of the growth period. The findings were further supported by age differences in bone weight, peak force to fracture and the amount of energy to fracture. Tibia weight and PF were substantially different in the earlier stages of production with tibia weight being twice as much at 12 woa compared to 8 woa and nearly 20 kg more of force required to fracture the bone. As the birds transitioned into 12 to 18 woa, the tibia weight increased by less than 15 g and the bones only required 13.74% more force to fracture at 18 woa compared to 12 woa.

Humerus

The humerus was evaluated for morphological traits at both 8 and 17 woa (Table 3.10). The average length of the humerus increased by 34% from 8 to 17 woa. Alongside this, the AP diameter, ML diameter and average diameter increased by approximately 2.5 mm during the 9 weeks of growth and development. The average bone weight increased by 18.34 g from 8 to 17 woa. Aging did not influence the PF or AUC, and the AUC appeared to decrease from 8 to 17 woa. This is likely due to error during the 3-point bend test.

Correlations Between BW and Bone Parameters

Tables 3.11 through 3.13 present the Pearson's correlations in body weight (BW), bone weight (WoB), peak force to fracture (PF) and average diameter for load and non-load-bearing bones at various ages across the production period.

Femur

Femurs collected at 8 woa were strongly correlated to other phenotypic traits. A 72% variation in femur weights could be explained by BW ($P < 0.01$; Table 3.11). The correlations of BW with PF and average diameter were moderate (r = 0.41 and r = 0.63, respectively). However, the highest correlation observed at 8 woa was an 83% correlation between WoB and average diameter. As the birds aged, the correlations decreased. At 12 woa, the most variation explained by the BW was in the diameter (r = 0.33). The effects of BW on PF and WoB had lessened greatly (r = 0.03 and r = 0.21, respectively). Some moderate interactions were observed from the WoB influencing PF and average diameter (r = 0.60 and r = 0.81, respectively). By 18 woa, most correlation observed between BW and other parameters was low. BW could only explain 10% of WoB, 29% of PF and 5% of the average diameter. The correlation between WoB and PF remained lowly moderate (r = 0.22).

Tibia

At 8 woa, BW had moderate influences on WoB, PF and average diameter (r = 0.59, r = 0.48 and r = 0.56, respectively; Table 3.12). Similarly to BW, 48% of the PF could be explained by the WoB. A higher correlation was determined between the WoB and the average diameter measured (r = 0.66). The effects observed decreased as the birds aged, similarly to the femurs, with BW only explaining 22 to 36% variation in the parameters measured. A similar trend to 8 woa was identified in which the average diameter and WoB explained 86% of one another. This is likely because wider diameters are acquired through more appositional growth in the bone, which can directly contribute to the overall weight. Once the birds reached 18 woa, low correlation was observed when considering BW. BW explained 5% of the WoB and the average diameter. Interestingly, negative correlation between BW and PF was observed (r = -0.02). WoB and average diameter were 63% related, but PF and average diameter had a correlation of -19%. These values at the latter end of the rearing period give insight into that lack of effect BW has on skeletal development.

Humerus

Pearson's correlation coefficient was determined for the same traits in the humerus collected at 8 and 17 woa in E2. At 8 woa, BW had moderate correlations with WoB, PF and average diameter (r = 0.43, r = 0.47, r = 0.51, respectively). Higher correlations were observed between the WoB and PF, with a 67% correlation, and the average diameter and PF with a 65% correlation. Most correlations decreased slightly at 17 woa but remained positive and lowly moderate. The largest decrease in correlation was observed between the PF and average diameter, which were only 17% correlated at the older age.

Treatment Effects at Various Ages on Pyridinoline Concentrations

Pyridinoline (PYD) was measured as an indicator of bone resorption at 8 and 17 woa in commercial turkey toms. Figures 3.8 through 3.10 present circulating PYD concentrations of toms at 8 and 17 woa. At 8 woa, there was no difference in PYD between the experimental groups (χ^2 = 5.74; *P = 0.33*). The mean rank scores for PYD concentration ranged from 182.69 nmol/L in PL toms to 218.75 nmol/L in SB toms but lacked statistical significance. At 17 woa, difference was observed between the PL toms and the CO toms (χ^2 = 17.19; *P = 0.0041*). The PL toms had a mean rank score of 115.80 nmol/L whereas the CO toms had a mean rank score of 69.12 nmol/L.

The PYD concentrations declined drastically between 8 and 17 woa (Z = -10.85; Wilcoxon Two-Sample test; $P < 0.0001$) suggesting a lower overall bone remodeling among older toms.

DISCUSSION

This study examined the effects of environmental enrichment, age and body weight on load-bearing and non-load-bearing bones in commercial turkey toms. Overall, environmental enrichments had limited impact on body weight and bone parameters. However, specific enrichments influenced certain bone traits, particularly cross-sectional diameters. These effects were primarily observed in the femur, tibia, and humerus at 8 weeks. Enrichment treatments such as provision of tunnels and pecking blocks contributed to increased cross-sectional geometry and biomechanical traits. Enrichment effects on bone properties further diminished with age. At 12 weeks, differences were mainly observed in tibia diameter and weight. By market age, humerus traits varied by treatment, favoring the inclusion of tunnel and pecking block in rearing environment whereas minor differences in tibia length and fracture energy remained. Further, age-related changes revealed that majority of the bone growth occurred in the earlier weeks of the toms' development, and the rate of skeletal growth decreased substantially as they got older. A similar trend was observed in the correlations between body weight and other key bone parameters. Body weight had little influence on the bone weight, peak force to fracture and diameter, particularly as the birds grew to market age.

Bone remodeling is a continuous physiological process crucial to maintaining and improving bone health within the skeletal system (Rowe et al., 2025). This process can be stimulated by increased physical activity. For example, a study conducted in post-menopausal women demonstrated forms of exercise significantly improved bone mineral density (Kemmler et al., 2020). These findings support the hypothesis that increased locomotor activities may enhance the quality of load-bearing bones in fast growing poultry, prompting further investigation into the use of environmental enrichments during rearing to promote skeletal development.

The birds provided the platform enrichment had femur diameters measuring less than those provided a tunnel. The platform enrichment involved ascending or descending a ramp, which may have instilled less strain on the femur than the repeated flexing required for the tunnel. The tunnel enrichment, often used as a space of safety or for birds to hide away, resulted in antero-posterior, medio-lateral and average diameters that were wider in femurs as well as increased femur weight at 8 woa. Use of the tunnel may have generated sufficient mechanical tension on the bone to induce microfractures, thereby stimulating bone deposition and resulting in morphometric changes (Nazareno et al., 2024). A direct cause-and-effect relationship between specific locomotor actions and bone properties could have been clarified through behavioral analysis of individual birds exposed to each enrichment treatment. At 8 woa, the provision of a pecking block resulted in wider tibia medio-lateral diameters compared to the platform enrichment group. The pecking block also resulted in a higher peak force to fracture compared to the birds in the control group and those provided a straw bale. Increased tibia fracture force in turkeys supplemented with higher levels of non-phytate phosphorus have been reported (Roberson et al., 2009). Therefore, additional levels of phosphorus provided by the pecking block may have contributed to improved bone strength in this group. By 12 woa, trends in tibia weight and average diameter emerged, with birds exposed to the robot enrichment exhibiting heavier and wider tibias than those in the platform group. The robot, classified as a form of dynamic enrichment, forced movement by prompting birds to stand and walk when activated. Previous research supports this through the recording of increased mechanical loading, such as walking while supporting large breast muscles, which enhances bone deposition. Additionally, at 18 woa, birds provided straw bales had longer tibias that required more energy to fracture compared to those given pecking blocks or platforms. Straw bales often encourage pecking and perching behavior, which may increase jumping activity. A study conducted in adolescent human males found that jumping can improve bone mass and stiffness (Vlachopoulos et al., 2018), suggesting that similar activity in poultry may have contributed to the development of longer, stiffer tibiae in the straw bale group.

The potential effects of environmental enrichments on non-load-bearing bones were also explored in this study. In younger birds, the humerus diameter was impacted by the tunnel enrichment. By 17 woa, the tunnel, pecking block and platform enrichments promoted increased medial-lateral diameter as compared to the straw bale enrichment. It has been reported that humerus development in geese accelerated between 3 and 6 woa, coinciding with increased mobility and wing use (Osiak-Wicha et al., 2023). Enrichments such as tunnels and platforms may require balance, prompting birds to engage their wings for stability. This wing activity, combined with the structural role of the humerus in supporting relatively large breast muscles, may stimulate bone remodeling and, as a result, improve bone quality. At 17 weeks, birds provided with tunnel enrichment also exhibited a slightly heavier humerus. Research has demonstrated that increased mechanical loading on bone can stimulate bone mass development, contributing to greater overall bone weight (Gusmao and Belangero, 2009). Like other bones examined in this study, morphological parameters of the humerus increased substantially with age. However, since only two sample points were explored, it remains unclear during which period the most rapid growth occurred.

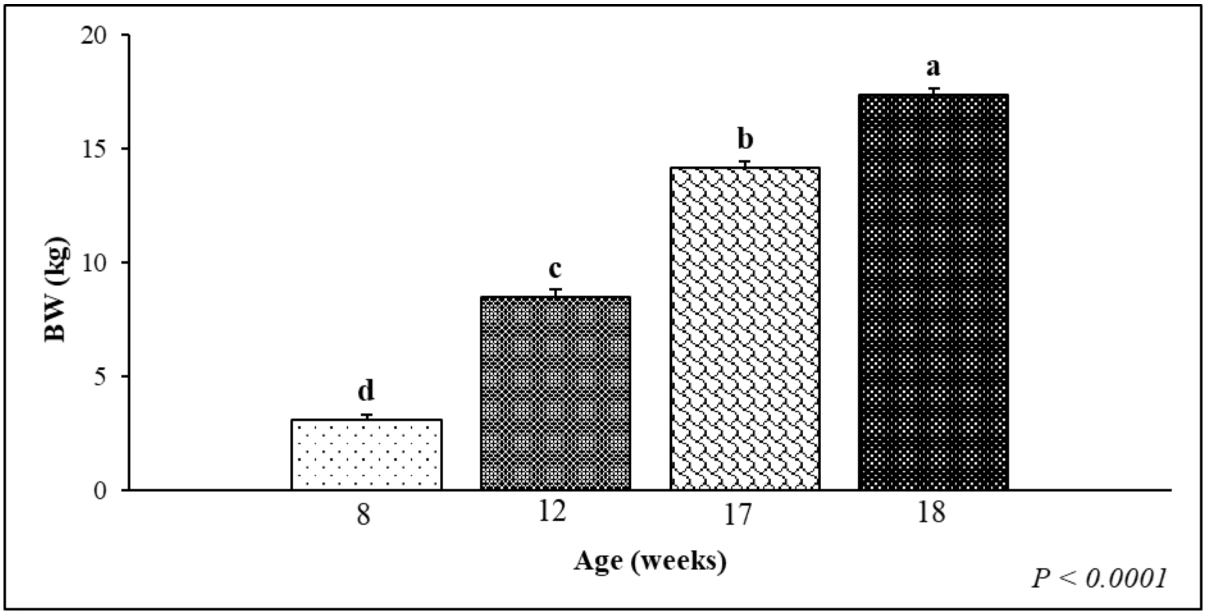
Significant age-related differences were found across all parameters of the bones measured at the different age points. The average body weight of commercial turkey toms has been recorded to increase by approximately 150% from 8 to 12 woa, and approximately 60% from 12 to 18 woa (Lilburn, 1994). These growth patterns align with the findings of this study, reinforcing the concept that the most rapid growth occurs during the earlier stages of development. Consistent with these results, significant increases in femur length and body weight have been observed as toms age (Van Wyhe et al., 2014). Although fewer studies have been conducted on commercial turkey toms, a majority of leg bone growth occurs in the first 10 weeks of life, with bone length and width only increasing 20 to 30% from 14 to 22 woa (Lilburn et al., 2006). Notably, the percentage of growth observed in bone parameters decreased drastically from 12 to 18 weeks compared to 8 to 12 woa. While the birds from E2 that were sampled at 17 woa were analyzed and considered, the data differs from those analyzed at 18 woa in E1. At the beginning of E2, cases of *E. coli, Salmonella* and Coccidiosis were diagnosed throughout the flock, which impaired the birds' growth, resulting in them being lighter than those sacrificed at 18 woa in E1. Even considering this, we can still conclude that the earlier weeks of life are critical developmental stages for skeletal growth in commercial turkeys.

Correlations among body weight, bone weight, peak force, and average diameter were evaluated in femurs, tibias and the humerus at each age point. The highest correlation observed was between the body weight and femur weight at 8 weeks of age. Other studies have discussed body weight influencing the size and remodeling of femurs (Zhong et al., 2012b), however our results indicate that this influence lessens drastically as the birds age. This pattern continues to suggest that bone development is more responsive to mechanical loading during earlier growth phases, when birds are concurrently more active and experiencing rapid weight gain. This study resulted in the bone weight having a consistent moderate to high correlation with the bone diameter, as found in previous studies (Gonz´alez-Cer´on et al., 2015). Additionally, our findings support the idea that bone width contributes significantly to overall bone weight. This correlation ranged from 36% to 86% between the femur and tibias measured, with the samples analyzed at 17 and 18 woa having lower correlations. Some studies have explored the use of morphological traits, such as diameter, as predictors of biomechanical properties like peak force (Soboyejo and Nestor, 2000). Our study demonstrated that bone weight and average diameter can be used to explain the peak force to fracture in the tibia and femur, but only at 8 and 12 woa. The correlations decrease as the birds approach 17 and 18 woa, expressed through negative correlations that were present. The humerus was examined to gather information regarding body weight effects on non-load-bearing bones, as well. While a similar trend was found with low-to-moderate correlations that decreased as the birds aged from 8 to 17 woa, the decline was not nearly as intense as the analyzed load-bearing bones. Body weight continued to somewhat explain the bone weight, peak force to fracture and the average diameter of the humerus at both age points. While this topic has not been widely researched, the lack of changing correlation is likely because, unlike the leg bones, the increased body mass does not induce bone remodeling as heavily in the wing bones. When considering all the found correlations, the low influences observed suggest that body weight does not account for many of the morphological and biomechanical traits as the birds grow through a production period. This highlights the potential for genetic selection as a strategy to improve skeletal health in commercial poultry without sacrificing fast growth and heavier body weights.

Plasma samples from E2 were analyzed to assess pyridinoline (PYD) concentrations across treatment and control groups at 8 and 17 woa. No treatment effects were observed in 8-week-old birds. However, in 17 weeks, a significant difference in PYD concentration was found between the control group and birds provided the platform enrichment. Mouse models have displayed that exercise activates the PYD pathway, increasing concentrations of PYD and other mature collagen cross-links (McNerny et al., 2015). In this study, platform enrichment required birds to climb a ramp, encouraging additional movement compared to the control group. These extra steps, particularly in heavier 17-week-old birds with substantial breast muscle mass, likely contributed to increased mechanical loading and subsequent bone remodeling. A significant age-related difference in PYD concentration was also observed, with 8-week-old birds exhibiting nearly twice the levels found in 17-week-olds. This differs from Roberson et al. (2005), who reported PYD concentrations increasing from 7 to 17 woa. It is important to note that each of these analyses were conducted two decades apart. During this time, genetic selection has greatly altered body weight and skeletal systems in poultry. Our morphological and biomechanical findings suggest most bone development occurs early in life, supporting the notion that bone remodeling, and thus bone resorption, is more active at 8 woa.

The findings from this study explain that environmental enrichments can minimally influence skeletal development in commercial turkey toms, especially in the earlier weeks of life when bone remodeling is most active. Enrichments such as pecking blocks, straw bales, platforms, tunnels and dynamic enrichments can affect bone morphology and strength in load bearing bones. These results align with previous research indicating that mechanical loading during peak growth promotes bone deposition and improved bone quality. While enrichment effects on non-load-bearing bones, such as the humerus, were more discreet, increased diameters and bone weights in older birds suggest that even moderate activity can induce remodeling in these structures. Correlation analyses revealed that bone weight and diameter were more closely related to each other than body weight, emphasizing the importance of localized mechanical stimuli over general mass gain. This analysis determined more correlation occurred at younger ages, such as 8, 12 and even 17 woa compared to the analysis performed on birds at 18 woa. Additionally, age-related declines in PYD concentrations and bone growth rates reinforce the critical window for skeletal development early in life. Although enrichment alone may not fully alleviate skeletal issues in fast growing poultry, these results highlight its potential as a management tool and underscore the need for additional strategies, such as genetic selection, to improve bone quality and welfare in commercial production systems.

Figure 3.1. Average body weight of turkey toms based on age in E1 and E2.



^{a,b,c} Means lacking a common superscript differ significantly ($P \leq 0.05$)

Table 3.1. Average body weight (Mean ± SEM) of commercial male turkeys at 8, 12, 17 and 18 woa in E1 and E2.

Treatment	Age (weeks)			
	8	12	17	18
BW (kg)				
CO	3.00 ± 0.22^{d}	8.30 ± 0.37^{c}	14.24 ± 0.25^{bxy}	17.14 ± 0.55^{a}
PL	2.98 ± 0.22^{d}	7.81 ± 0.37^{c}	13.49 ± 0.23^{by}	17.58 ± 0.53^{a}
PB	3.39 ± 0.22^{d}	7.85 ± 0.34^{c}	14.79 ± 0.23^{bx}	17.70 ± 0.60^{a}
RO	3.02 ± 0.22^{d}	9.03 ± 0.34^{c}	14.47 ± 0.24^{bxy}	17.33 ± 0.57^{a}
SB	2.94 ± 0.22^{d}	8.21 ± 0.35^{c}	13.87 ± 0.24^{bxy}	16.39 ± 0.57^{a}
TU	3.23 ± 0.22^{d}	9.06 ± 0.35^{c}	14.59 ± 0.28^{bxy}	17.47 ± 0.58^{a}

Groups: CO (Control), PL (Platform), PB (Pecking Block), RO (Robot), SB (Straw Bale) and TU (Tunnel)
abc Means within the same row lacking a common superscript differ significantly ($P \leq 0.05$)
xyz Means within the same column lacking a common superscript differ significantly ($P \leq 0.05$)

Table 3.2. Average diameter and lengths (Mean ± SEM) of commercial male turkey femurs at 8, 12 and 18 woa in E1.

Parameter	Treatment	Age (weeks)		
		8	12	18
Length (mm)				
	CO	106.63 ± 1.51^{c}	146.28 ± 1.60^{b}	159.29 ± 0.94^{a}
	PL	103.00 ± 0.80^{c}	145.67 ± 2.89^{b}	158.50 ± 0.91^{a}
	PB	107.81 ± 1.19^{c}	147.00 ± 2.88^{b}	158.85 ± 1.67^{a}
	RO	104.49 ± 1.82^{c}	151.17 ± 6.56^{b}	158.54 ± 1.11^{a}
	SB	104.75 ± 1.77^{c}	145.27 ± 2.90^{b}	157.44 ± 1.35^{a}
	TU	108.00 ± 1.34^{c}	147.18 ± 1.20^{b}	157.13 ± 1.13^{a}
Anterior-posterior (mm)				
	CO	10.13 ± 0.36^{cz}	15.70 ± 0.48^{b}	18.30 ± 0.23^{a}
	PL	9.97 ± 0.77^{cz}	15.27 ± 0.37^{b}	18.33 ± 0.17^{a}
	PB	10.95 ± 0.11^{cxy}	16.23 ± 0.47^{b}	18.15 ± 0.21^{a}
	RO	10.44 ± 0.23^{cxyz}	16.50 ± 0.36^{b}	18.16 ± 0.20^{a}
	SB	10.32 ± 0.40^{cyz}	16.02 ± 0.57^{b}	18.13 ± 0.20^{a}
	TU	11.43 ± 0.26^{cx}	16.30 ± 0.53^{b}	18.42 ± 0.22^{a}
Medial-lateral (mm)				
	CO	10.49 ± 0.45^{cxy}	16.56 ± 0.39^{b}	19.20 ± 0.19^{a}
	PL	10.31 ± 0.27^{cy}	16.27 ± 0.38^{b}	19.53 ± 0.90^{a}

	PB	11.09 ± 0.13^{cx}	17.06 ± 0.51^{b}	19.16 ± 0.24^{a}
	RO	10.65 ± 0.15^{cxy}	18.04 ± 0.44^{b}	19.02 ± 0.21^{a}
	SB	10.70 ± 0.39^{cxy}	16.54 ± 0.77^{b}	19.26 ± 0.18^{a}
	TU	11.33 ± 0.32^{cxy}	17.22 ± 0.46^{b}	19.34 ± 0.18^{a}
Avg. Diameter (mm)				
	CO	10.31 ± 0.40^{cxy}	16.13 ± 0.39^{b}	18.75 ± 0.20^{a}
	PL	10.14 ± 0.26^{cy}	15.87 ± 0.36^{b}	18.93 ± 0.15^{a}
	PB	11.02 ± 0.10^{cx}	16.65 ± 0.46^{b}	18.65 ± 0.21^{a}
	RO	10.55 ± 0.17^{cxy}	17.27 ± 0.38^{b}	18.59 ± 0.19^{a}
	SB	10.51 ± 0.39^{cxy}	16.28 ± 0.66^{b}	18.69 ± 0.17^{a}
	TU	11.38 ± 0.27^{cx}	16.76 ± 0.49^{b}	18.88 ± 0.17^{a}

Groups: CO (Control), PL (Platform), PB (Pecking Block), RO (Robot), SB (Straw Bale) and TU (Tunnel)
^{abc} Means within the same row lacking a common superscript differ significantly ($P \leq 0.05$)
^{xyz} Means within the same column lacking a common superscript differ significantly ($P \leq 0.05$)

Table 3.3. Average bone weight and biomechanical properties (Mean ± SEM) of commercial male turkey femurs at 8, 12 and 18 woa in E2.

Parameter	Treatment	Age (weeks)		
		8	12	18
Bone Wt. (g)				
	CO	19.94 ± 1.13^{c}	66.38 ± 1.55^{b}	81.34 ± 1.43^{a}
	PL	18.18 ± 0.93^{c}	62.92 ± 3.14^{b}	81.08 ± 1.46^{a}
	PB	21.59 ± 0.86^{c}	68.45 ± 4.12^{b}	79.97 ± 1.80^{a}
	RO	19.91 ± 0.91^{c}	74.47 ± 3.36^{b}	78.88 ± 1.34^{a}
	SB	20.60 ± 1.49^{c}	64.40 ± 4.69^{b}	77.11 ± 1.03^{a}
	TU	23.14 ± 0.89^{c}	67.80 ± 2.19^{b}	80.04 ± 1.40^{a}
Peak force (kg)				
	CO	45.87 ± 5.88^{c}	70.89 ± 4.87^{b}	107.98 ± 3.62^{a}
	PL	47.79 ± 3.05^{c}	54.36 ± 15.56^{b}	104.77 ± 4.45^{a}
	PB	57.62 ± 2.74^{c}	72.17 ± 29.51^{b}	97.31 ± 3.76^{a}
	RO	47.96 ± 5.02^{c}	70.11 ± 5.88^{b}	99.66 ± 3.55^{a}
	SB	49.63 ± 6.50^{c}	73.01 ± 10.28^{b}	101.07 ± 3.05^{a}
	TU	55.55 ± 4.89^{c}	65.50 ± 6.48^{b}	102.55 ± 3.80^{a}
*Energy (kg×cm)				
	CO	73.99 ± 13.27^{c}	165.22 ± 20.62^{b}	271.11 ± 30.48^{a}
	PL	76.77 ± 8.15^{c}	203.04 ± 29.18^{b}	255.05 ± 28.09^{a}

	PB	120.10 ± 26.64^{c}	207.16 ± 25.26^{b}	243.69 ± 38.34^{a}
	RO	83.08 ± 12.65^{c}	251.23 ± 39.70^{b}	220.23 ± 24.63^{a}
	SB	80.29 ± 12.11^{c}	180.63 ± 31.06^{b}	254.25 ± 25.78^{a}
	TU	97.27 ± 14.98^{c}	182.30 ± 25.83^{b}	262.51 ± 30.86^{a}

Groups: CO (Control), PL (Platform), PB (Pecking Block), RO (Robot), SB (Straw Bale) and TU (Tunnel)

^{abc} Means within the same row lacking a common superscript differ significantly ($P \leq 0.05$)

^{xyz} Means within the same column lacking a common superscript differ significantly ($P \leq 0.05$)

*Energy determined by AUC, or area under the curve

Table 3.4. Average bone diameters and lengths (Mean ± SEM) of commercial male turkey tibias at 8, 12, 17 and 18 woa in E1 and E2.

Parameter	Treatment	Age (weeks)			
		8	12	17	18
Length (mm)					
	CO	152.13 ± 1.48^{c}	212.11 ± 3.24^{b}	235.42 ± 0.92^{a}	243.66 ± 1.16^{ayz}
	PL	151.00 ± 1.60^{c}	212.11 ± 5.09^{b}	234.01 ± 0.87^{a}	245.37 ± 1.15^{axy}
	PB	156.50 ± 2.18^{c}	215.45 ± 6.47^{b}	236.69 ± 0.91^{a}	245.55 ± 1.60^{axy}
	RO	151.44 ± 2.60^{c}	221.89 ± 3.60^{b}	236.67 ± 0.91^{a}	243.58 ± 1.21^{az}
	SB	153.63 ± 2.91^{c}	219.64 ± 5.79^{b}	236.28 ± 0.94^{a}	245.80 ± 1.41^{ax}
	TU	159.88 ± 2.20^{c}	217.00 ± 3.51^{b}	238.26 ± 1.03^{a}	244.65 ± 1.58^{axy}
Anterior-posterior (mm)					
	CO	8.30 ± 0.30^{c}	12.10 ± 0.47^{b}	14.33 ± 0.19^{b}	15.60 ± 0.17^{a}
	PL	8.58 ± 0.27^{c}	12.25 ± 0.56^{b}	14.36 ± 0.18^{b}	15.68 ± 0.19^{a}
	PB	8.93 ± 0.19^{c}	13.08 ± 0.47^{b}	14.53 ± 0.18^{b}	15.72 ± 0.23^{a}
	RO	8.80 ± 0.31^{c}	13.45 ± 0.49^{b}	14.39 ± 0.18^{b}	15.71 ± 0.20^{a}
	SB	8.49 ± 0.24^{c}	12.85 ± 0.69^{b}	14.28 ± 0.19^{b}	15.75 ± 0.18^{a}
	TU	8.69 ± 0.17^{c}	12.92 ± 0.27^{b}	14.44 ± 0.20^{b}	15.66 ± 0.21^{a}
Medial-lateral (mm)					
	CO	10.17 ± 0.38^{cxy}	16.13 ± 0.40^{b}	18.21 ± 0.17^{b}	19.58 ± 0.18^{a}
	PL	10.01 ± 0.32^{cy}	15.34 ± 0.93^{b}	17.89 ± 0.16^{b}	19.56 ± 0.18^{a}

	PB	10.81 ± 0.20^{cx}	16.73 ± 0.57^{b}	18.31 ± 0.17^{b}	19.66 ± 0.24^{a}
	RO	10.44 ± 0.20^{cxy}	17.73 ± 0.51^{b}	18.13 ± 0.17^{b}	19.39 ± 0.22^{a}
	SB	10.50 ± 0.33^{cxy}	16.76 ± 0.84^{b}	17.92 ± 0.17^{b}	19.58 ± 0.19^{a}
	TU	10.80 ± 0.23^{cxy}	17.03 ± 0.45^{b}	18.43 ± 0.18^{b}	19.40 ± 0.19^{a}
Avg. Diameter (mm)					
	CO	9.24 ± 0.33^{c}	14.11 ± 0.39^{b}	16.27 ± 0.16^{b}	17.52 ± 0.18^{a}
	PL	9.29 ± 0.15^{c}	13.79 ± 2.03^{b}	16.13 ± 0.16^{b}	17.62 ± 0.17^{a}
	PB	9.87 ± 0.19^{c}	14.91 ± 0.51^{b}	16.41 ± 0.16^{b}	17.69 ± 0.22^{a}
	RO	9.62 ± 0.24^{c}	15.59 ± 0.49^{b}	16.26 ± 0.16^{b}	17.55 ± 0.19^{a}
	SB	9.49 ± 0.29^{c}	14.80 ± 0.75^{b}	16.10 ± 0.17^{b}	17.67 ± 0.17^{a}
	TU	9.75 ± 0.20^{c}	14.97 ± 0.36^{b}	16.43 ± 0.43^{b}	17.53 ± 0.18^{a}

Groups: CO (Control), PL (Platform), PB (Pecking Block), RO (Robot), SB (Straw Bale) and TU (Tunnel)

^{abc} Means within the same row lacking a common superscript differ significantly ($P \leq 0.05$)

^{xyz} Means within the same column lacking a common superscript differ significantly ($P \leq 0.05$)

Table 3.5. Average bone weight and biomechanical properties (Mean ± SEM) of commercial male turkey tibias at 8, 12, 17 and 18 woa in E1 and E2.

Parameter	Treatment	Age (weeks)			
		8	12	17	18
Bone Wt. (g)					
	CO	31.00 ± 1.55^{c}	78.74 ± 4.70^{b}	103.78 ± 1.58^{a}	111.41 ± 1.53^{a}
	PL	30.90 ± 1.17^{c}	78.46 ± 5.90^{b}	104.13 ± 1.52^{a}	111.88 ± 1.59^{a}
	PB	33.66 ± 1.21^{c}	84.62 ± 6.56^{b}	106.41 ± 1.53^{a}	112.58 ± 1.68^{a}
	RO	32.66 ± 1.82^{c}	96.05 ± 6.07^{b}	103.78 ± 1.54^{a}	111.29 ± 1.52^{a}
	SB	32.86 ± 1.77^{c}	89.23 ± 6.61^{b}	101.28 ± 1.59^{a}	111.72 ± 1.55^{a}
	TU	35.74 ± 1.87^{c}	84.62 ± 5.26^{b}	107.18 ± 1.67^{a}	110.61 ± 1.87^{a}
Peak force (kg)					
	CO	35.98 ± 3.27^{dy}	67.73 ± 7.64^{c}	84.62 ± 2.89^{b}	97.26 ± 1.84^{a}
	PL	35.45 ± 2.81^{dxy}	58.78 ± 4.81^{c}	80.24 ± 2.74^{b}	94.19 ± 3.74^{a}
	PB	48.53 ± 3.38^{dx}	66.78 ± 8.41^{c}	85.60 ± 2.78^{b}	93.68 ± 3.29^{a}
	RO	37.98 ± 1.99^{dxy}	72.34 ± 5.95^{c}	86.68 ± 2.81^{b}	93.41 ± 2.16^{a}
	SB	39.40 ± 4.92^{dy}	56.30 ± 6.07^{c}	83.01 ± 2.91^{b}	96.11 ± 2.74^{a}
	TU	43.47 ± 2.31^{dxy}	75.63 ± 5.80^{c}	87.96 ± 3.05^{b}	93.94 ± 3.16^{a}
*Energy (kg×cm)					
	CO	186.07 ± 24.09^{b}	283.36 ± 42.45^{ab}	283.73 ± 31.28^{a}	323.24 ± 28.78^{a}
	PL	180.43 ± 20.54^{b}	274.17 ± 23.07^{ab}	258.20 ± 29.94^{a}	257.61 ± 23.58^{a}

PB	214.31 ± 19.14^{b}	276.17 ± 28.06^{ab}	306.08 ± 30.40^{a}	256.71 ± 24.85^{a}
RO	176.32 ± 21.22^{b}	265.82 ± 39.38^{ab}	318.12 ± 30.79^{a}	272.77 ± 23.19^{a}
SB	195.53 ± 19.21^{b}	206.40 ± 32.30^{ab}	259.85 ± 32.26^{a}	351.05 ± 38.95^{a}
TU	208.34 ± 25.80^{b}	302.43 ± 34.76^{ab}	310.87 ± 33.51^{a}	297.25 ± 20.32^{ax}

Groups: CO (Control), PL (Platform), PB (Pecking Block), RO (Robot), SB (Straw Bale) and TU (Tunnel)

^{abc} Means within the same row lacking a common superscript differ significantly ($P \leq 0.05$)

^{xyz} Means within the same column lacking a common superscript differ significantly ($P \leq 0.05$)

*Energy determined by AUC, or area under the curve

Table 3.6. Average bone diameters and lengths (Mean ± SEM) of commercial male turkey humerus at 8 and 17 woa in E2.

Parameter	Treatment	Age (weeks)	
		8	17
Length (mm)			
	CO	121.5 ± 2.93^{b}	171.32 ± 0.63^{a}
	PL	120.63 ± 2.54^{b}	171.59 ± 0.61^{a}
	PB	126.38 ± 2.64^{b}	172.00 ± 0.62^{a}
	RO	125.41 ± 2.64^{b}	170.81 ± 0.63^{a}
	SB	122.80 ± 2.59^{b}	170.66 ± 0.65^{a}
	TU	122.63 ± 2.54^{b}	172.08 ± 0.73^{a}
Anterior-posterior (mm)			
	CO	10.02 ± 0.25^{b}	14.84 ± 0.12^{a}
	PL	9.67 ± 0.21^{b}	14.64 ± 0.12^{a}
	PB	10.32 ± 0.23^{b}	15.02 ± 0.12^{a}
	RO	10.35 ± 0.23^{b}	14.74 ± 0.12^{a}
	SB	10.19 ± 0.22^{b}	14.52 ± 0.12^{a}
	TU	10.41 ± 0.21^{b}	14.77 ± 0.13^{a}
Medial-lateral (mm)			
	CO	12.94 ± 0.24^{bxy}	18.97 ± 0.07^{axy}
	PL	12.71 ± 0.21^{by}	19.01 ± 0.07^{ax}

	PB	13.39 ± 0.22^{bxy}	19.15 ± 0.07^{ax}
	RO	13.39 ± 0.22^{bxy}	18.91 ± 0.08^{axy}
	SB	12.89 ± 0.21^{bxy}	18.60 ± 0.07^{ay}
	TU	13.71 ± 0.21^{bx}	19.08 ± 0.09^{ax}
Avg. Diameter (mm)			
	CO	11.48 ± 0.23^{b}	16.90 ± 0.09^{axy}
	PL	11.21 ± 0.20^{b}	16.86 ± 0.09^{axy}
	PB	11.86± 0.22^{b}	17.08 ± 0.09^{ax}
	RO	11.87± 0.22^{b}	16.82 ± 0.09^{axy}
	SB	11.53 ± 0.21^{b}	16.55 ± 0.09^{ay}
	TU	12.06 ± 0.20^{b}	16.91 ± 0.10^{axy}

Groups: CO (Control), PL (Platform), PB (Pecking Block), RO (Robot), SB (Straw Bale) and TU (Tunnel)

^{ab} Means within the same row lacking a common superscript differ significantly ($P \leq 0.05$)

^{xy} Means within the same column lacking a common superscript differ significantly ($P \leq 0.05$)

Table 3.7. Average bone weight and biomechanical properties (Mean ± SEM) of commercial male turkey humerus at 8 and 17 woa in E2.

Parameter	Treatment	Age (weeks)	
		8	17
Bone Wt. (g)			
	CO	26.78 ± 2.12^{b}	62.71 ± 1.37^{axy}
	PL	25.23 ± 1.83^{b}	64.79 ± 1.33^{axy}
	PB	28.49 ± 1.90^{b}	66.32 ± 1.35^{axy}
	RO	28.00 ± 1.90^{b}	65.02 ± 1.36^{axy}
	SB	26.21 ± 1.87^{b}	61.53 ± 1.40^{ay}
	TU	27.63 ± 1.83^{b}	69.06 ± 1.52^{ax}
Peak Force (kg)			
	CO	49.25 ± 5.37^{b}	105.51 ± 5.15^{a}
	PL	47.86 ± 4.65^{b}	112.10 ± 5.01^{a}
	PB	52.72 ± 4.83^{b}	110.98 ± 5.07^{a}
	RO	57.07 ± 4.83^{b}	108.81 ± 5.06^{a}
	SB	51.27 ± 4.75^{b}	106.72 ± 5.25^{a}
	TU	59.97 ± 4.65^{b}	115.05 ± 5.51^{a}
*Energy (kg·cm)			
	CO	149.64 ± 20.55^{b}	489.98 ± 72.77^{a}
	PL	122.75 ± 17.80^{b}	553.59 ± 70.86^{a}

PB	155.64 ± 18.94^{b}	585.76 ± 71.39^{a}
RO	158.15 ± 18.94^{b}	586.92 ± 71.47^{a}
SB	136.13 ± 18.29^{b}	560.47 ± 75.74^{a}
TU	174.52 ± 17.80^{b}	621.48 ± 77.13^{a}

Groups: CO (Control), PL (Platform), PB (Pecking Block), RO (Robot), SB (Straw Bale) and TU (Tunnel)

^{abc} Means within the same row lacking a common superscript differ significantly ($P \leq 0.05$)

*Energy determined by AUC, or area under the curve

Table 3.8. Femur length, diameters, weight, peak force to fracture and energy to fracture (Mean ± SEM) at 8, 12 and 18 weeks of age.

Parameter	Age (weeks)		
	8	12	18
Length (mm)	105.36 ± 2.59^{c}	147.05 ± 1.32^{b}	158.54 ± 1.3^{a}
Anterior-posterior (mm)	11.04 ± 0.49^{c}	16.25 ± 0.25^{b}	18.03 ± 0.25^{a}
Medial-lateral (mm)	11.84 ± 0.50^{c}	17.50 ± 0.25^{b}	18.73 ± 0.25^{a}
Avg. Diameter (mm)	11.45 ± 0.47^{c}	16.88 ± 0.24^{b}	18.38 ± 0.23^{a}
Weight (g)	26.51 ± 3.39^{c}	70.26 ± 1.72^{a}	76.92 ± 1.70^{a}
Peak force (kg)	71.84 ± 8.01	77.39 ± 4.01	91.65 ± 3.96
*Energy (kg·cm)	127.68 ± 51.84^{b}	219.83 ± 26.04^{a}	225.80 ± 25.24^{ab}

^{abc} Means within the same row lacking a common superscript differ significantly ($P \leq 0.05$)

*Energy determined by AUC, or area under the curve

Table 3.9. Tibia length, diameters, weight, peak force to fracture and energy to fracture (Mean ± SEM) at 8, 12, 17 and 18 weeks of age.

Parameter	Age (weeks)			
	8	12	17	18
Length (mm)	167.95 ± 4.43^{c}	224.83 ± 4.09^{b}	231.03 ± 3.92^{b}	243.16 ± 4.09^{a}
Anterior-posterior (mm)	9.75 ± 0.55^{c}	13.54 ± 0.49^{b}	13.85 ± 0.47^{b}	15.79 ± 0.49^{a}
Medial-lateral (mm)	12.50 ± 0.51^{c}	17.77 ± 0.45^{b}	17.49 ± 0.42^{b}	19.08 ± 0.45^{a}
Avg. Diameter (mm)	11.12 ± 0.52^{c}	15.66 ± 0.47^{b}	15.67 ± 0.44^{b}	17.44 ± 0.47^{a}
Weight (g)	46.88 ± 3.00^{c}	91.89 ± 2.49^{b}	101.55 ± 2.06^{ab}	105.66 ± 2.57^{a}
Peak force (kg)	60.64 ± 4.69^{b}	75.99 ± 3.79^{a}	80.47 ± 3.14^{a}	87.20 ± 3.86^{a}
*Energy (kg·cm)	209.31± 45.29^{a}	277.47 ± 46.59^{a}	279.71 ± 9.67^{a}	280.72 ± 36.18^{a}

^{abc} Means within the same row lacking a common superscript differ significantly (*P ≤ 0.05*)

*Energy determined by AUC, or area under the curve

Table 3.10. Humerus length, diameters, weight, peak force to fracture and energy to fracture (Mean ± SEM) at 8 and 17 weeks of age.

Parameter	Age	
	8	17
Length (mm)	126.07 ± 2.01^{b}	170.74 ± 0.50^{a}
Anterior-posterior (mm)	12.22 ± 0.29^{b}	14.33 ± 0.71^{a}
Medial-lateral (mm)	15.38 ± 0.31^{b}	18.48 ± 0.08^{a}
Avg. Diameter (mm)	13.82 ± 0.28^{b}	16.41 ± 0.06^{a}
Weight (g)	43.45 ± 3.48^{b}	61.79 ± 0.98^{a}
Peak force (kg)	95.76 ± 1.65	101.78 ± 2.98
*Energy (kg·cm)	545.18 ± 141.96	491.08 ± 38.53

^{ab} Means within the same row lacking a common superscript differ significantly ($P \leq 0.05$)
*Energy determined by AUC, or area under the curve

Table 3.11. Pearson's correlation coefficient among body weight, bone weight, peak force and average diameter in commercial turkey femurs at 8, 12 and 18 woa in E1.

8 weeks

	BW	Bone Wt.	Peak Force	Avg. Diameter
BW		0.72**	0.41**	0.63**
Bone Wt.	0.72**		0.43**	0.83**
Peak Force	0.41**	0.43**		0.45**
Avg. Diameter	0.63**	0.83**	0.45**	

12 weeks

	BW	Bone Wt.	Peak Force	Avg. Diameter
BW		0.21	0.03	0.33**
Bone Wt.	0.21		0.60**	0.81**
Peak Force	0.03	0.60**		0.53**
Avg. Diameter	0.33**	0.81**	0.53**	

18 weeks

	BW	Bone Wt.	Peak Force	Avg. Diameter
BW		0.10	0.29**	0.05
Bone Wt.	0.10		0.22**	0.36**
Peak Force	0.29**	0.22**		-0.04
Avg. Diameter	0.05	0.36**	-0.04	

*Represents $P \leq 0.05$
** Represents $P \leq 0.01$

Table 3.12. Pearson's correlation coefficient among body weight, bone weight, peak force and average diameter in commercial turkey tibias at 8, 12, 17 and 18 woa in E1 and E2.

8 weeks

	BW	Bone Wt.	Peak Force	Avg. Diameter
BW		0.59**	0.48**	0.56**
Bone Wt.	0.59**		0.48**	0.66**
Peak Force	0.48**	0.48**		0.62**
Avg. Diameter	0.56**	0.66**	0.62**	

12 weeks

	BW	Bone Wt.	Peak Force	Avg. Diameter
BW		0.27*	0.30*	0.22
Bone Wt.	0.27*		0.36**	0.86**
Peak Force	0.30*	0.36**		0.36**
Avg. Diameter	0.22	0.86**	0.36**	

17 weeks

	BW	Bone Wt.	Peak Force	Avg. Diameter
BW		0.39**	0.36**	0.31**
Bone Wt.	0.39**		0.23**	0.57**
Peak Force	0.36**	0.23**		0.08
Avg. Diameter	0.31**	0.57**	0.08	

18 weeks

	BW	Bone Wt.	Peak Force	Avg. Diameter
BW		0.05	-0.02	0.04
Bone Wt.	0.05		0.11	0.63**
Peak Force	-0.02	0.11		-0.19*
Avg. Diameter	0.05	0.63**	-0.19*	

*Represents $P \leq 0.05$
** Represents $P \leq 0.01$

Table 3.13. Pearson's correlation coefficient among body weight, bone weight, peak force and average diameter in commercial turkey humerus at 8 and 17 woa in E2.

8 weeks

	BW	Bone Wt.	Peak Force	Avg. Diameter
BW		0.43**	0.47**	0.51**
Bone Wt.	0.43**		0.67**	0.41**
Peak Force	0.47**	0.67**		0.65**
Avg. Diameter	0.51**	0.41**	0.65**	

17 weeks

	BW	Bone Wt.	Peak Force	Avg. Diameter
BW		0.32**	0.24**	0.47**
Bone Wt.	0.32**		0.49**	0.25**
Peak Force	0.24**	0.49**		0.17**
Avg. Diameter	0.47**	0.25**	0.17**	

*Represents $P \leq 0.05$
** Represents $P \leq 0.01$

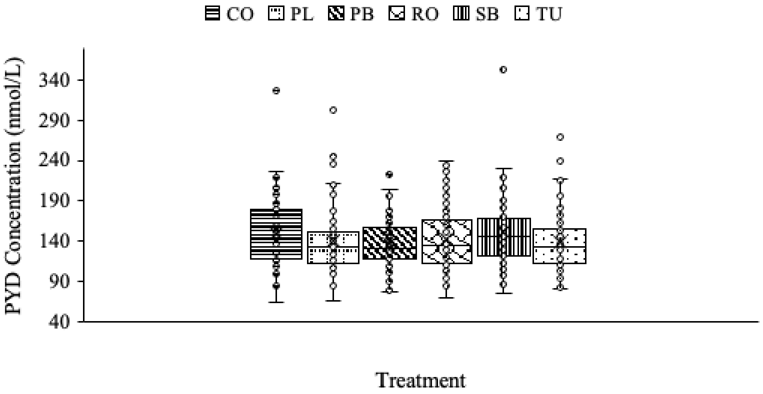


Figure 3.8. PYD concentrations compared between environmental enrichment treatments at 8 woa (n = 387).

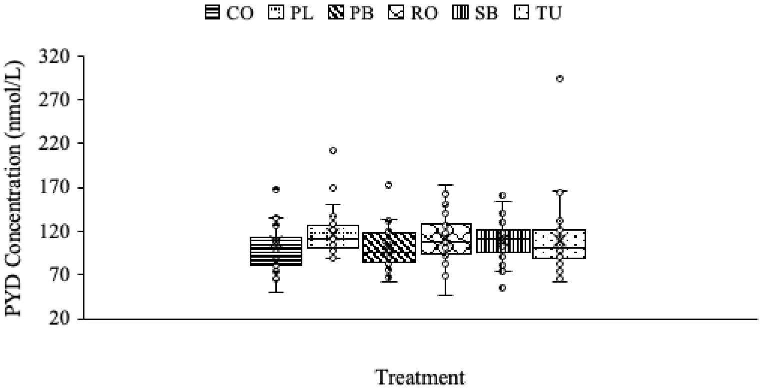


Figure 3.9. PYD concentrations compared between environmental enrichment treatments at 17 woa (n = 178).

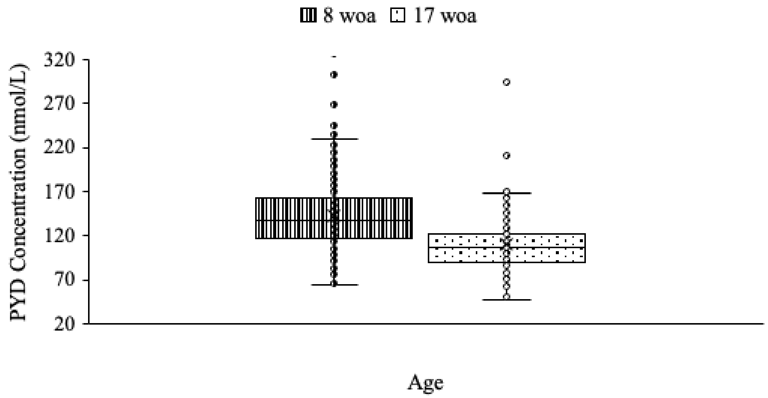


Figure 3.10. Wilcoxon Two-Sample test results for PYD concentrations measured in male turkey toms at 8 and 17 woa in E2.

SUPPLEMENTAL FIGURES AND TABLES

Supplemental Figure S3.1: Photo of the platform (PL) enrichment, placed at 5 woa.

Supplemental Figure S3.2: Photo of the tunnel enrichment (TU), placed at 5 woa.

Supplemental Figure S3.3: Photo of the straw bale enrichment (SB), placed at 5 woa.

Supplemental Figure S3.4: Photo of the pecking block enrichment (PB), placed at 5 woa.

Supplemental Figure S3.5: Photo of the dynamic robot enrichment (RO), placed at 5 woa.

Supplemental Table S3.1. Testing speeds, trigger force, load cell size and support beam placements for femur and tibia (8,12 and 18 woa) and humerus and tibia (8 and 17 woa) for 3-point bend test.

Age and Bone type	Load Cell (kg)	Test Speed (mm/sec)	Trigger Force (g)	Support Beam Distance (mm)	Distance from Midpoint on Each Side (mm)
8-week-old Femurs	250	1	100	40	20
8-week-old Tibias	250	1	100	70	35
12-week-old Femurs	250	1	300	60	30
12-week-old Tibias	250	1	300	100	50
18-week-old Femurs	750	1	400	60	30
18-week-old Tibias	750	1	400	120	60
8-week-old Humerus	250	1	200	50	25
17-week-old Humerus	750	1	400	70	35
8-week-old Tibias	250	1	200	70	35
17-week-old Tibias	750	1	400	120	60

REFERENCES

Aviagen, T. 2022 Management Guidelines for Raising Commercial Turkeys. Pages 36.

Beaulac, K., and K. Schwean-Lardner. 2018. Assessing the effects of stocking density on turkey tom health and welfare to 16 weeks of age. Frontiers in Veterinary Science 5:213.

Bradshaw, R. H., R. D. Kirkden, and D. M. Broom. 2002. A review of the aetiology and pathology of leg weakness in broilers in relation to welfare. Avian and Poultry Biology 13:45-103. doi 10.3184/147020602783698421

Clark, D. L., K. E. Nestor, and S. G. Velleman. 2019. Continual Selection for Increased 16 wk Body Weight on Turkey Growth and Meat Quality: 50 Generation Update. Poultry Science Association. doi http://dx.doi.org/10.3382/japr/pfz017

Clark, S., Dr. 2020. 2020 Turkey Industry Annual Report - Current Health and Industry Issues Facing the US Turkey Industry, Huvepharma.

Crespo, R., S. M. Stover, R. Droual, R. P. Chin, and H. L. Shivaprasad. 1999a. Femoral fractures in a young male turkey breeder flock. Avian Diseases 43.

Crespo, R., S. M. Stover, K. T. Taylor, R. P. Chin, and H. L. Shivaprasad. 1999b. Morphometric and Mechanical Properties of Femora in Young Adult Male Turkeys with and without Femoral Fractures.

Day, J., H. A. Van de Weerd, and S. A. Edwards. 2008. The effect of varying lengths of straw bedding on the behaviour of growing pigs. Applied Animal Behaviour Science 109:249-260.

Dibner, J. J., J. D. Richards, M. L. Kitchell, and M. A. Quiroz. 2007. Metabolic Challenges and Early Bone Development. Poultry Science Association.

Dong, Y. 2023. Effects of environmental enrichment on turkey behavior, welfare and walking ability. Doctor of Philosophy Dissertation. Purdue University.

Enneking, S. A., H. W. Cheng, K. Y. Jefferson-Moore, M. E. Einstein, D. A. Rubin, and P. Y. Hester. 2012. Early access to perches in caged White Leghorn pullets. Poultry Science Association 91:2114-2120. doi 10.3382/ps.2012-02328

Erasmus, M. 2018. Welfare issues in turkey production. Food Science, Technology and Nutrition:28.

Ferket, P. R., and J. L. Sell. 1989. Effect of Severity of Early Protein Restriction on Large Turkey Toms. 1. Performance Characteristics and Leg Weakness. Poultry Science 68:676-686.

Gonz´alez-Cer´on, F., R. Rekaya, and S. E. Aggrey. 2015. Genetic analysis of bone quality traits and growth in a random mating broiler population. Poultry Science 94:883-889. doi 10.3382/ps/pev056

Gusmao, C. V., and W. D. Belangero. 2009. How Do Bone Cells Sense Mechanical Loading? Rev Bras Ortop 44:299-305. doi 10.1016/S2255-4971(15)30157-9

Jacobs, L., R. A. Blatchford, I. C. de Jong, M. Erasmus, M. A. Levengood, R. C. Newberry, P. Regmi, and S. L. Weimer. 2022. Enhancing their quality of life: environmental enrichment for poultry. 102. doi 10.1016/j.psj.2022.102233

Julian, R. J. 2004. Production and growth related disorders and other metabolic diseases of poultry – A review. The Veterinary Journal 169:350-369. doi 10.1016/j.tvjl.2004.04.015

Kaukonen, E., M. Norring, and A. Valros. 2017. Perches and elevated platforms in commercial broiler farms: use and effect on walking ability, incidence of tibial dyschondroplasia and bone mineral content. The Animal Consortium 11:7. doi https://doi.org/10.1017/S1751731116002160

Kemmler, W., M. Shojaa, M. Kohl, and S. Von Stengel. 2020. Effects of Different Types of Exercise on Bone Mineral Density in Postmenopausal Women: A Systematic Review and Meta-analysis. Calcified Tissue International 107:409-439. doi 10.1007/s00223-020-00744-w

Lilburn, M., A. Mitchell, and J. Anderson. 2006. The relationship between growth of commercial toms and linear skeletal development. Poult. Sci 85:31.

Lilburn, M. S. 1994. Skeletal Growth of Commercial Poultry Species. Poultry Science 73:897-903.

McNerny, E. M. B., J. D. Gardinier, and D. H. Kohn. 2015. Exercise increases pyridinoline cross-linking and counters the mechanical effects of concurrent lathyrogenic treatment. Bone 81:327-337. doi 10.1016/j.bone.2015.07.030

Nazareno, A. C., R. M. F. Silveira, D. P. B. Fernandes, J. Chierri, L. O. Pradella, and I. J. Oliveira Da Silva. 2024. Perches used as environmental enrichment influence fast-growth broilers' biomechanics and locomotor morphometry at the age of 42 days. PLOS ONE 19:e0313214. doi 10.1371/journal.pone.0313214

Nestor, K. E., W. L. Bacon, Y. M. Saif, and P. A. Renner. 1985. The Influence of Genetic Increases in Shank Width on Body Weight, Walking Ability, and Reproduction of Turkeys. Poultry Science 64:2248-2255.

Newberry, R. C. 1995. Environmental enrichment: Increasing the biological relevance of captive environments. Applied Animal Behaviour Science 44:229-243. doi 10.1016/0168-1591(95)00616-Z

Osiak-Wicha, C., E. Tomaszewska, S. Muszynski, P. Dobrowolski, K. Andres, T. Schwarz, M.

Swietlicki, M. Mielnik-Blaszczak, and M. B. Arciszewski. 2023. Developmental changes in tibia and humerus of goose: morphometric, densitometric, and mechanical analysis. Animal 17:100960. doi 10.1016/j.animal.2023.100960

Roberson, K. D., M. W. Klunzinger, R. A. Charbeneau, and M. W. Orth. 2005. Evaluation of phytase concentration needed for growing–finishing commercial turkey toms. British Poultry Science 46:470-477. doi 10.1080/00071660500190769

Roberson, K. D. 2009. Growth performance and spontaneous bone fracture incidence of turkey toms fed various levels of calcium and nonphytate phosphorus to heavy market weight. Journal of Applied Poultry Research 18:158-164. doi 10.3382/japr.2008-00083

Rowe, P., A. Koller, and S. Sharma. 2015. Physiology, Bone Remodeling in StatPearls, Treasure Island (FL).

Soboyejo, A. B. O., and K. E. Nestor. 2000. A NEW STATISTICAL BIOMECHANICS APPROACH TO MODELING BONE STRENGTH IN TURKEYS AND BROILER CHICKENS. Transactions of the ASABE 43:1997-2006.

Stover, K. K., D. M. Weinreich, T. J. Roberts, and E. L. Brainerd. 2018. Patterns of musculoskeletal growth and dimensional changes associated with selection and developmental plasticity in domestic and wild strain turkeys. Ecology and Evolution 8:3229-3239. doi 10.1002/ece3.3881

Van Wyhe, R. C., P. Regmi, B. J. Powell, R. C. Haut, M. W. Orth, and D. M. Karcher. 2014.

Bone characteristics and femoral strength in commercial toms: The effect of protein and energy restriction. Poultry Science 93:943-952. doi https://doi.org/10.3382/ps.2013-03604

Vlachopoulos, D., A. R. Barker, E. Ubago-Guisado, C. A. Williams, and L. Gracia-Marco. 2018.

The effect of a high-impact jumping intervention on bone mass, bone stiffness and fitness parameters in adolescent athletes. Arch Osteoporos 13:128. doi 10.1007/s11657-018-0543-4

Weber, P. A. 2012. The Effects of Social and Environmental Enrichments on Leg Strength and Welfare of Tom Turkeys. Master of Science in Animal Science. University of Nebraska.

Weeks, C. A., T. D. Danbury, H. C. Davies, P. Hunt, and S. C. Kestiin. 2000. The behaviour of broiler chickens and its modification by lameness. Applied Animal Behaviour Science 67:111-125. doi https://doi.org/10.1016/S0168-1591(99)00102-1

Yan, F. F., P. Y. Hester, and H. W. Cheng. 2014. The effect of perch access during pullet rearing and egg laying on physiological measures of stress in White Leghorns at 71 weeks of age. Poultry Science 93:1318-1326. doi 10.3382/ps.2013-03572

Zhong, Z., M. Muckley, S. Agcaoglu, M. E. Grisham, H. Zhao, M. Orth, M. S. Lilburn, O. Akkus, and D. M. Karcher. 2012b. The morphological, material-level, and ash properties of turkey femurs from 3 different genetic strains during production. Poultry Science 91:2736-2746. doi 10.3382/ps.2012-02322

CHAPTER 4

BONE AND QUANTITATIVE GAIT PROPERTIES ASSOCIATED WITH SUBJECTIVE GAIT SCORES IN MALE TURKEYS REARED WITH ACCESS TO ENVIRONMENTAL ENRICHMENT

Kulbacki, Stephanie. To be submitted to Poultry Science

ABSTRACT

Heavier body weight of commercial turkey toms is associated with a higher prevalence of mobility issues. While economically beneficial, weight gain that outpaces skeletal development can lead to abnormalities such as valgus or varus deformities, crooked toes, shaky legs, and ultimately, lameness. Reliable and simple gait assessment tools are crucial for testing the effectiveness of intervention strategies to reduce lameness. In this study, we compared objective gait parameters in toms with normal or mildly impaired gait scores (GS) generated from a subjective assessment technique. At the day of hatch, 400 male Nicholas Select turkeys were randomly distributed among 24 pens (n=16 birds/pen). Each pen was assigned one of five EE treatments or a control (CO) group without enrichment, resulting in 4 pens/treatment. The EE groups included the Platform (PL), Pecking Block (PB), Straw Bale (SB), Tunnel (TU) and a ground-robot (RO) for induced locomotion. At 16 weeks of age, gait scoring was conducted on toms and 2 birds from each pen, one with a score of ‘0’ (no gait issues) and one with a score of ‘1’ (mild gait impairment), were selected for objective gait assessment using a pressure sensing walkway (Strideway, Tekscan™). After habituation, gait parameters were recorded and analyzed using custom software. Gait parameters, including cadence (steps/min), gait time (s), gait distance (cm), gait velocity (cm/s), step length (cm), peak pressure (kPa), and maximum force (g) were calculated for each bird. A One-Way ANOVA (JMP 18) was performed to analyze the data, with GS and EE as the main effects, and pen as a random effect. A p-value of $P \leq 0.05$ was considered statistically significant, while a p-value of $P \leq 0.10$ was considered a trend. The average body weight (15.10 ± 0.64 kg) was not different between EE or GS groups. Environmental enrichments did not influence any of the gait parameters observed. Cadence, gait time, and gait velocity were affected by gait scores. Toms with a GS of ‘1’ had a longer gait time

($P = 0.05$) than those with a GS of ‘0’. Opposite of this, toms with a GS of ‘0’ had greater cadence ($P = 0.06$) and gait velocity ($P = 0.09$) than those with a GS of ‘1’. Certain parameters were measured across both limbs, as well. Parameters such as maximum force and peak pressure were higher in the left limb of birds with impaired gait ($P = 0.10$ and $P = 0.09$, respectively). Differences within the limbs were measured, resulting in the right – left limb of the birds with impaired gait having a longer single support time compared to the unimpaired birds ($P = 0.10$). These results demonstrated that birds with mild gait impairment walked slower and took fewer steps than birds without impairment. Overall, these results indicate that enrichments had minimal impact on gait and confirm that some gait parameters can be distinguished clearly using subjective scoring.

INTRODUCTION

Lameness in poultry encompasses birds' having mobility issues, or gait impairment. The prevalence of lameness among fast-growing birds such as broilers or turkeys has been a concern in the poultry industry. Developmental abnormalities, such as valgus and varus, or pathogenic issues like osteomyelitis can contribute to cases of lameness (Van Wettere, 2020), but a host of other risk factors can induce gait problems as well. The secondary consequences to lameness includes compromised ability to access feed and water, skeletal deformations, and experiencing aggression from other birds (Corr et al., 1998; Weeks et al., 2000). Overall, lameness is a prominent concern that can compromise the welfare of meat birds. Currently, research efforts are geared towards finding genetic and management solutions to lameness.

Lameness in turkeys has been associated with genetic selection for improved performance traits. Selection for increased breast muscle mass and higher body weight gain leads to birds having a disproportionate growth between the muscular and the skeletal system, which often predisposes them to lameness. To identify and categorize lameness, primary turkey breeders examine pure and hybrid lines using subjective gait assessment protocols. Gait scoring is a process in which a set, descriptive scale is used to categorize individuals based on their walking ability; this allows for further selection of birds with inherently better phenotypic leg bone characteristics (Swalander et al., 2020). Gait is generally characterized on a 3-point or a 6-point scale, with each category having a description regarding what walking pattern or ability is being observed. Kestin et al. (1992) developed the ordinal 6-point scale, with a score of 0 indicating birds have no gait issues, whereas a score of 5 indicates the bird under observation is unable to take a full step. The scale also provides specific gait characteristics associated with each score. For example, a bird with a score of 2 would have a consistently flat foot and shortened stride. Since this scale requires greater attention to detail, particularly during on-farm gait assessment in commercial settings, a 3-point scale has also been used. The 3-point scale ranges from 0-2 with increasing severity of lameness or gait abnormalities and has broad definitions for each category. A score of 0 implies no impairments are present, a score of 1 presents with signs of mild struggles but the bird is still able to effectively move, and a score of 2 signifies that the bird has great difficulty in taking steps (Webster et al., 2008). Regardless of the scale used, accuracy and inter-observer reliability of the subjective gait scoring protocols depend on appropriate training and experience.

Despite being effective, especially in large commercial settings, subjective gait scoring methods are qualitative in nature and have greater probability of human error. As a result, various objective methods have been investigated primarily in research settings. Video recordings have been used to identify individual birds within large groups, not only in terms of gait scoring, but also identification of other behaviors. For example, Weeks et al. (2000) reported that 39 to 49-day old broilers spent 76-86% of their time lying down, which can serve as a proxy indicator of mobility issues within a flock. Similarly, automated computer-assisted technologies that can read gait score have begun to emerge within the industry (Dong et al., 2023; Van Hertem et al., 2018). Examples of these systems are ones that focus on combining the "activity index" collected by camera systems, paired with body weights collected by an automatic weighing system to assess gait score in a production setting (Dong et al., 2023; Van Hertem et al., 2018). Additionally, these systems can account for factors like age and stocking density. Objective and automated methods can help reduce the labor required for in-person gait assessments, as well as minimize human errors involved with assessing large number of birds in commercial settings.

Accelerometry is another objective method that can be used to help assess gait impairment in poultry. Accelerometers can measure vertical and horizontal accelerations exerted during walking, which, alongside body weight measurements, can help predict gait issues (Kavanagh and Menz, 2008; Pearce et al., 2023). However, accelerometers suffer from limitations such as short battery life, high cost, and acclimation of birds to the sensors which make them a less desirable technique for long-term monitoring systems (Wurtz and Riber, 2024). The gold standard gait analysis tool is the use of pressure sensitive walkways (PSW) which have been used to detect lameness in poultry research. Pressure sensitive walkways often collect quantifiable parameters like vertical impulse, peak force, kinetic data, step time, step length etc. from both limbs (Kremer et al., 2018). Nääs et al. (2009) created a chamber with a pressure plate to measure forces exerted by broilers at different ages when the plate was stepped on. This also allowed for the measurement of the drug (metamizole) in terms of efficacy for reducing pain from lameness in broilers.

Since gait issues in meat birds are related to issues in the skeletal system, measuring biomechanical properties of bones can give insight to different gaits. Research has revealed positive associations between tibia breaking force and gait score in broiler chickens (Kittelsen et al., 2017). These results warrant the investigation of biomarkers of bone formation and resorption as another potential tool to longitudinally track skeletal dynamics. Pyridinoline, or PYD, is a collagen turnover marker that can be measured in plasma and can be used as an indicator of bone resorption since it is released into the blood stream during collagen degradation (Kline et al., 2017). Studies in human subjects have found positive correlations between PYD and bone resorption area (r = 0.77) as well as PYD and osteoclast surfaces (r = 0.59) (Nemoto et al., 1997; Urena et al., 1995). However, analysis of bone markers in poultry species with different severity of lameness has not been explored previously.

This study aimed to quantify the use of a qualitative six-point gait scoring system (Dong, et al., 2023) with quantitative gait parameters collected by a pressure sensitive walkway (Strideway, Tekscan, Inc., MA, USA). Alongside this, humerus and tibia were analyzed for biomechanical and morphological traits based on gait score of toms at an individual level. Finally, plasma PYD levels were compared between toms differing in gait scores.

MATERIALS AND METHODS

Animals and Housing

The experimental procedures used in this study were approved by the Institutional Animal Care and Use Committee at Purdue University (PACUC: 2111002216).

The housing and other husbandry practices used in this study were described previously described by Griffith (2025). Briefly, 400 one-day-old Nicholas Select (Aviagen Turkeys Inc. Lewisburg, WV, USA) male turkey poults were procured from a commercial hatchery and placed in a turkey facility at the Purdue University's Animal Science Research and Education Center (West Lafayette, IN, USA). Poults were reared in four pens (10 feet by 8 feet) within the same room. Cardboard brood rings were provided that allowed access to heat lamps until approximately 2.5 weeks of age (woa), whereas wood shavings were provided as the litter substrate. At 3 woa, poults were weighed and assigned to 24 littered pens (12 pens/room; 16 turkeys/pen) to ensure each pen had similar average pen weights at the beginning of the study. Each pen held two bell drinkers and one feeder, providing *ad libitum* access to water and feed. Diets were determined based on Aviagen's recommendations and the NRC nutrition guidelines. Twenty-four hours of continuous photoperiod was provided for the first day of placement. After that, a schedule of 22 hours of light and 2 hours of dark was implemented until the poults were 9 woa. The lighting schedule then changed to 20 hours of light and 4 hours of dark until the end of the study. The lighting schedule was determined per Aviagen's recommendations for the Nicholas Select performance line. Housing temperatures were set based on Aviagen's recommendations, with the brooding phase being controlled to 40 °C.

Experimental Design

The following environmental enrichments were described by Dong et al (2023). Briefly, the experiment included 5 treatment groups, each with 4 replicate pens. The assigned treatments provided access to various environmental enrichments: a platform, tunnel shelter, straw bale, and pecking block. One group was exposed to a motorized robot to induce locomotor activity among the turkeys. All enrichments were introduced at 5 woa. A control group, which did not receive any additional enrichment, was also included in the study.

Measurement of Gait Parameters

At 16 woa, toms from each pen were visually assessed by trained individuals, and their gait scores were recorded using a six-point gait scale adapted by Dong et al. (2023), which was a modification of the original scale reported by Kestin et al. (1992) (Table 4.1). For quantitative gait assessment using a pressure-sensing gait analysis system (Strideway, Tekscan, Inc., MA, USA), two toms were selected from each pen: one with a score of '0' and another with a score of '1'. There were very few toms that presented gait issues greater than a score of '1', hence only score '0' and '1' were used. Prior to testing, focal toms were habituated to walking across the walkway for five consecutive days. The birds being evaluated were habituated and tested with gentle coaxing and guidance along the pressure pad (Supplemental Figure S4.1).

Table 4.1. Description of subjective gait scoring system utilized (from (Dong et al., 2023; Kestin et al., 1992). Description includes numerical score, a summary of the degree of impairment for each score, as well as what specific gait qualities observers should identify for each score.

Gait Score	Degree of Impairment	Description
0	"None"	"Straight legs, smooth fluid movement. The foot is furled while raised."
1	"Detectable, unidentifiable abnormality"	"The bird is unsteady or wobbles when it walks. However, the problem leg is unclear or cannot be identified in the first 20 seconds of observation. The bird readily runs from the observer in the pen. The foot may remain flat when raised, but the rest of the stride is fluid and unimpaired. Gait appears unstable (shaky or stomping)."
2	"Identifiable abnormality that has little impact on overall function"	"The leg producing the gait defect can be identified within 20 seconds of observation. If a problem leg is determined after 20 seconds of observed locomotor behavior, the bird is classed as having a gait score of 1. However, the defect seems to have only a minor impact on biological function. Thus, the bird will run from the observer spontaneously or if touched or nudged with the padded stick. If the bird does not run at full speed, it runs, walks, or remains standing for at least 15 seconds after the observer in the pen has ceased to move towards or nudge it. Birds in this and previous, scores are often observed to scratch their face with their feet, again indicating little impact on function. (The most common abnormality in this score is for the bird to make short, quick, unsteady steps with one leg, where the foot remains flat during the step)"
3	"Identifiable abnormality impairs function"	"Although the bird will move away from the observer when approached, touched, or nudged, it will not run and squat within 15 seconds or less of the observer in pen ceasing to approach or nudge it. If the bird squats after 15 s have elapsed, it is classified as a gait score of 2."
4	"Severe impairment of function but still capable of walking"	"The bird remains squatting when approached or nudged. This criterion is assessed by approaching the bird, and if it remains squatting, gently nudging or touching the animal for 5 s. Animals may appear to rise but still rest upon their hocks. Only rising to stand on both feet within 5 seconds of handling is counted. A bird that takes longer than 5 s to rise, or that does not rise at all, is scored as 4, while a bird that rises in 5 s or less is counted as a 3 (or lower if its gait is good). Nevertheless, the bird can walk when picked up by the observer and placed in a standing position. Still, squats immediately

		following one or 2 steps (Squatting often involves a characteristic ungainly backward fall). Bird requires wings for balance.”
5	“Complete lameness”	“The bird cannot walk and instead may shuffle along on its hocks. It may attempt to stand when approached but cannot do so, and when placed on its feet, it is unable to complete a step with one or both legs.”

As the birds walked across the pressure-sensing walkway (PSW), footprint images were generated in real time, with color variations indicating different amounts of pressure, e.g. red pixels represented high amounts of pressure detected (Supplemental Figures S4.2a and S4.2b). Images were collected for each bird during four passes on the PSW. Following collection, image samples were individually reviewed and classified as either “readable” or “unreadable.” To be classified as “readable,” at least two continuous footprints had to be detected with at least one from each limb being recorded (Supplemental Figure S4.2a). “Unreadable” images were those in which footprints overlapped, there were not enough consecutive strikes detected or there were only strikes from a single limb (Supplemental Figure S4.2b). The average number of strikes detected across the files was four, and each strike was manually labeled as being the left or right limb and provided the appropriate strike box for data collection. To ensure the accuracy of the images used for quantifying gait parameters, video recordings of the birds walking were reviewed and cross-referenced with the corresponding image data. Footprint images were analyzed using the PSW’s custom software to generate various gait parameters (Table 4.3).

Different gait parameters (Table 4.2) were collected using custom software provided by the PSW manufacturer. Gait cycle time (GCT), maximum force (MF), maximum peak pressure (PP), single support time (SST), stance time (SAT), swing time (SWT), step length (SL), step time (ST), step velocity (SV), and stride length (SRL) were measured for each limb, and the differences between the left and right limbs were also recorded. For other gait traits including cadence (CA), gait time (GT), gait distance (GD), and gait velocity (GV), the average of the right and left limbs were recorded for analysis.

Table 4.2. Select parameters and their descriptions analyzed based on data exported from PSW custom program (Tekscan, 2024).

Strikes/Steps	Total number of steps taken by the patient
Cadence (steps/min)	Number of steps taken per minute
Gait Time (s)	Time of first contact of first step to the time of first contact of last step registered on sensor
Gait Distance (cm)	Measured along the line of progression, from posterior heel of the first stance to the posterior heel of the last stance
Gait Velocity (cm/s)	Gait distance divided by gait time
Gait Cycle Time (s)	Average time from first contact of the foot to subsequent first contact of same foot
Maximum Force (g)	Maximum force value reported in common standard unit
Maximum Peak Pressure (kPa)	Maximum pressure value reported in common standard unit
Single Support Time (s)	Time the foot is in contact with the sensor, measured from the last contact of the opposing foot's preceding stance to the first contact of the opposing foot's next stance
Stance Time (s)	Average time from first contact of the foot to last contact of the same foot
Swing Time (s)	Average time that the foot is not in contact with the ground Gait cycle time – the stance time
Step Length (cm)	Length measure parallel to line of progression of the body, from the most posterior contact (heel) of previous footfall to most posterior contact (heel) of opposing footfall If there is no previous contra lateral footfall, length cannot be calculated If next contra lateral footfall is "partial" but the posterior heel strike is registered, length can be calculated
Step Time (s)	Elapsed time from first contact of the foot to the first contact of the opposing foot The heel may or may not contact first. Be careful of random sensel (single sensor) flicker When contact begins, it must be immediately followed by a larger region of contact for several frames The first contact sensel must continue to be loaded for at least 15 milliseconds If there are no sufficient footfalls detected, N/A will be displayed
Step Velocity (cm/s)	Step length of the foot divided by step time of the same foot
Stride Length (cm)	Distance measured parallel to the line of progression, between the posterior heel points of two consecutive footprints of the foot in question

Bone Mechanical Properties

After testing on the PSW, toms were euthanized and the right humerus and tibiotarsus were excised from surrounding soft tissues and stored at -20 °C until further analysis. Two days prior to the biomechanical testing, bones were placed in the refrigerator at 4 °C for 12 to 24 hours for thawing. The bones were removed from the refrigerator and any remaining soft tissues, and the periosteum were removed. After ensuring the bones were completely clean, paper towels were lightly sprayed with phosphate-buffered saline (PBS) and wrapped around the bones before they were placed back into the 4 °C refrigerator. Between 12 to 24 hours later, the bones were removed from the refrigerator and were allowed to dry for 2 to 4 hours at room temperature before the biomechanical test was performed.

Bones were weighed and their length and mid-shaft anteroposterior and mediolateral diameter were recorded using a vernier caliper (INSIZE Co., LTD, China). Bones were then subjected to a 3-point bend test (TA.HDPlus, Texture Technologies Corp., MA, USA). The equipment was calibrated to a force of 25 kg and a height of 15 mm based on user settings and a load cell of 750 kg was used, with the test speed set at 1 mm/s. The resulting force-distance curve was used to calculate peak force and energy required to fracture using custom software (Exponent Connect, Texture Technologies, MA, USA). The standard set-up settings for testing are found in Supplemental Table S4.1.

Plasma Pyridinoline Concentrations

Blood samples were collected from the toms selected for gait analysis at 8 (n = 387 birds) and 16 (n = 180 birds) woa to measure circulating pyridinoline (PYD) concentrations as an indicator of bone resorption. The birds were gait scored prior to blood sampling at each time point. The birds that underwent gait analysis using the PSW did not have plasma drawn at 16 woa when testing was conducted, but their 8 woa samples were included in the overall analysis. Pyridinoline levels were analyzed using PYD Urine ELISA kits (QuidelOrtho,CA, USA). A trial plate was analyzed using plasma samples from 28-day old turkey poults to determine the appropriate dilution factor for subsequent analyses; plasma samples were diluted by 16-folds for the assay. All plates were processed according to the manufacturer's instructions and read at 405 nm, using a 4-PL curve with the equation: $y = (A-D) / (1+(x/C^{B}) + D$ to quantify PYD concentrations for each sample. This equation contained the minimal absorbance obtained (A), the maximum absorbance obtained (D), the inflection points between A and D (C), and Hill's slope of the curve relating to the steepness of the curve (B). The average intra-assay CV% for all plates was 7.2% and the inter-assay CV% was 14.2%.

Statistical Analyses

A One-Way ANOVA was performed using JMP Pro 18 (JMP Statistical Discovery LLC., NC, USA) to compare quantitative gait traits between toms with gait scores of 0 and 1. The statistical model included gait score as the main effect, while environmental enrichment treatments and pen ID were included as random effects.

$$Y = \mu + \alpha + \beta + \gamma + \varepsilon$$

Where Y is the response variable, μ is the mean, α is the main effect of gait score, β is the random effect of treatment, γ is the random effect of pen and ε as the residual errors. All analyses were performed with statistical significance being observed at $P \leq 0.05$ and statistical trends being observed at $0.05 \leq P \leq 0.1$. Tukey's HSD was used for multiple comparison when significance was observed.

The pyridinoline concentrations measured were quantified through a Wilcoxon Two-Sample test, a nonparametric test due to the inability to assume normality and heterogeneity conditions were met for a one-way ANOVA. PYD concentration (nmol/L) was used as the response variable and gait score was used as the factor variable. Significant differences were identified at $P \leq 0.05$ and statistical trends were identified at $P \leq 0.1$

RESULTS

Quantitative Gait and Body Weight Measurements

The average body weight and quantitative gait parameters collected from focal toms with gait scores of 0 and 1 are presented in Tables 4.3 and 4.4.

Body weight was not different between toms with gait scores of 0 and 1 (Table 4.3). A statistical trend was observed when average cadence (CA) was compared between toms with gait scores 0 and 1 (*P = 0.06*). Toms with normal gait (gait score 0) had higher cadence than those with mild gait impairment (gait score 1), taking 26% more steps per minute. Like cadence, toms with mild gait impairment exhibited longer gait times (3.31± 0.45 s vs. 2.33 ± 033 s; *P = 0.05*). This result is further supported by the faster GV recorded in toms with normal gait than those with mild gait impairment (19.30 ± 2.73 cm/s vs. 13.66 ± 1.95 cm/s; *P = 0.09*). However, gait distance (GD) did not differ between the groups.

Single support time (SST), stance time (SAT), swing time (SWT) and gait cycle time (GCT) were measured at different phases of the gait cycle and are indicators of gait stability (Table 4.4). Single support time (SST) and SAT are measured during the stance phase of the gait cycle. Turkey toms with a gait score of 0 tended to have a lower right-left (R-L) support time (0.033 ± 0.06 s) compared to those with gait scores of 1 (0.067 ± 0.05 s), indicating a more asymmetrical gait in birds with mild gait impairment (*P = 0.10*). The SAT, however, was not different between the groups with gait scores of 0 and 1. Similarly, the SWT and the overall GCT were not different between the toms with gait scores of 0 and 1.

Stride length (SRL) provides insight into how far birds can effectively walk. A statistical trend was observed between the groups, with birds having a gait score of 0 exhibiting longer SRL in the right limb compared to those with gait scores of 1 ($P = 0.10$; Table 4.4). A similar trend was observed in the step length (SL) of the left limb, where toms with gait scores of 1 took approximately 3 cm longer steps than those with gait scores of 0 ($P = 0.08$). Furthermore, toms with gait scores of 1 took more time for each step with the left limb (1.08 ± 0.12 s) than toms with gait scores of 0 (0.95 ± 0.09 s; $P = 0.003$). Step velocity did not differ between the groups.

The maximum force (MF) exerted by the left limb also tended to be greater in toms with gait scores of 1 compared to those that had gait scores of 0 ($P = 0.1$; Table 4.4). A similar result was observed for the peak pressure (PP) exerted by the left limb, with PP in toms with gait scores of 1 being higher than those with gait scores of 0 ($P = 0.09$).

Bone Morphological and Biomechanical Properties

Tibia and humerus properties from toms with gait scores of 0 and 1 are presented in Table 4.5 and Table 4.6.

Humerus samples of toms with gait scores of 0 and 1 were not different in morphological and biomechanical traits examined in this study (Table 4.5). Unlike humerus, tibiae from toms with gait scores of 0 tended to be heavier (105.23 ± 1.74 g vs. 109.47 ± 1.67 g; $P = 0.09$) and shorter ($P = 0.1$; Table 4.6) compared to toms with gait scores of 1. Average diameter and peak force to fracture of tibia, however, were not different between the two groups of toms.

Gait Score and Age Dependent Pyridinoline Concentrations

Pyridinoline (PYD), a collagen cross-link that is often used as a bone resorption biomarker was measured. At 8 woa, there was no significant difference in plasma PYD concentration between toms with gait scores of 0 and 1 ($Z = -0.79$; $P = 0.43$). Similar results were observed when toms were 16 woa ($Z = 0.94$; $P = 0.34$). Although not statistically significant, PYD concentrations were numerically higher in toms with gait scores of 0 than in those with gait scores of 1 at both ages.

DISCUSSION

In this study, we aimed to determine if quantitative gait parameters differed among birds with subjective gait scores. The results indicate that birds with mild gait impairments exhibited differences in parameters such as cadence, gait time, and velocity, supporting the validity of the subjective scoring system. These measurable parameters could enhance the objectivity of current gait assessments. Additionally, impaired birds consistently relied more on their left limb for propulsion unlike birds with normal gait. Overall, the study confirms measurable differences in gait characteristics between toms with and without impairment.

Cadence generally increases with walking speed in birds (Corr et al., 1998); that trend was confirmed in this study where birds with gait impairment took fewer steps per minute at lower speeds. These findings confirm the positive relationship between gait velocity and cadence, as both were lower in impaired birds. Gait time, the duration of time it takes to maneuver a set distance, was significantly longer in birds with gait impairments, possibly due to slower movement or pauses for stability (Bazzi and Cacace, 2023). The change in cadence and gait time align with the observed change in gait velocity, or the speed at which the birds were walking. Gait velocity has been known to influence a wide range of gait parameters (Ziegler et al., 2024). However, gait distance, or how long the subjects walk for, remained consistent regardless of the gait scores in this study. However, locomotive behavior tracking in birds of various gait scores was reported to determine differences in gait distance based on the subjective gait scores (Li et al., 2023). The lack of change in this study is likely due to the birds being guided to walk a set path through multiple passes, rather than being able to walk freely. However, the difference observed in cadence, gait time, and velocity lay the foundation for the effectiveness of the used subjective gait scoring system. Previous studies have concluded that cadence in toms measured with a pressure sensitive walkway lowered when the birds reached 16 weeks of age, potentially due to either their heavy body weights making it more difficult to take steps, or due to impending leg impairments (Stevenson et al., 2019).

The gait cycle consists of two phases: stance phase (when the limb is in contact with the ground) and swing phase (when the limb is lifted off of the ground) (Winter, 1987). Across all analyzed birds, stance time exceeded swing time, mimicking human gait patterns where reduced stability leads to longer stance phases and shorter swing phases (Corr et al., 2003); this is likely to maintain balance throughout their steps. Birds with normal gait were found to have a higher stance time in the right limb, opposite that of the impaired birds. Single support time, a key component in the stance phase, can indicate how much weight a bird bears on a certain lone limb, displaying the highest point of stress and instability for the birds (Caplen et al., 2012; Tekscan, 2024). In this study, birds with impaired gaits had a higher single support time in their right limb compared to the birds with normal gaits. Contrastingly, both groups swung their left limb for longer compared to the right limb. Birds with gait impairments required more time to complete a full gait cycle, consistent with reports of broilers with leg disorders taking 2 s per cycle compared to 1.2 s in birds with normal gait (Reiter and Bessei, 1997).

Stride length is calculated using steps from both limbs, whereas step length, time, and velocity are calculated using only the motion from the specific limb in observation (Frothingham, 2018; Winter, 1987). This study observed birds with normal gait exhibiting longer right-limb stride lengths compared to birds with impaired gait, but both groups generally tend to have longer strides on their right side. When the birds have a longer stride length, they are generally covering more distance with each step and showing to have an efficient gait (Oviedo-Rondon et al., 2017). Step velocity, comprised of the step length and step time, had no apparent differences between the two groups. Birds with normal gait had slightly higher right-limb velocity, while impaired birds moved more quickly with the left limb. Step time was statistically longer in the left limb of impaired birds compared to the unimpaired birds, though both groups had longer step times on the right compared to the left. The left limb of unimpaired birds had a shorter step length than the left limb of the impaired birds. The stride length was longer in the right limb for unimpaired birds, but the step length and step time being higher in the left limb of impaired birds. This could indicate the impaired birds compensating for stiffness and discomfort by being more cautious with their steps. Additionally, within each group, the overall longer step occurred in the right limb for unimpaired birds and the left limb for impaired birds. This difference in step length may be linked to limb length. The right tibia of birds with mild gait impairment were longer than those with normal gait, and longer limbs have been associated with longer steps in some species, including turkeys (Reiter, 2002; Stevenson et al., 2019). Although left tibiae were not measured, assuming bilateral symmetry suggests the left limbs may also have been longer.

Maximum force reflects the total load applied to the foot during each step, while peak pressure indicates how that load is distributed; both occur during the stance phase (Kim et al., 2020; Pirani and Azizi, 2020). In this study, impaired birds exerted a higher peak pressure and maximum force on their left limb compared to unimpaired birds, suggesting a reliance on the left foot during locomotion. A limb preference was consistent across both parameters with impaired birds favoring the left limb, while unimpaired birds favored the right. Paxton et al. (2013) found that commercial broilers exerted more force on their right limb compared to the left, indicating a preference for balance and support. Although these birds were not gait scored, the findings support the idea that birds prone to potential gait abnormalities may compensate by favoring one limb over the other. In terms of force and pressure in general, both were higher in the impaired birds compared to the unimpaired birds. This is similar to Oviedo-Rondon et al., 2018, who reported turkeys with shaky legs and crooked toes having higher peak vertical forces than normal turkeys throughout the latter part of a production period. These findings could be due to the birds making contact with the ground more quickly and forcibly, but typically a decrease in forces is observed in subjects with lameness (Oviedo-Rondon et al., 2017; Corr et al., 2007).

Comparisons within each gait score group resulted in unimpaired birds utilizing their right limb, whereas the left limb is utilized more by birds with mild gait issues. This aligns with previous research in which pure line birds with gait issues tended to laterally displace one limb and use the opposite more as the swing leg (Paxton et al., 2013). Concurrently, dominant limbs have exerted more vertical forces (Polk et al., 2016). In this study, the left limb of impaired birds had longer swing times, greater step lengths, and generated more pressure and force with each step.

Some parameters that seemingly contradicted this conclusion were the apparent higher single support and step times, as well as longer stride lengths observed in the right limb. However, longer strides can indicate a more energy-efficient gait (Oviedo-Rondon et al., 2017), a pattern that may be present due to compensation for issues in the right limb. The preferred limb is one that typically bears more load, leaving the non-preferred limb to be the supporting and stabilizing limb (Sadeghi et al., 2000). Since the analysis was performed on a flat surface, the preferred limb was not influenced by uneven surfaces or steps, reinforcing that birds with impaired gait relied more on their left limb. The increased step time and single support time in the right limb can be a continued indication of its role as the stabilizing limb.

Minimal differences were observed in the right tibia and humerus across gait score groups. As mentioned, the trends in tibia weight and length only indirectly correspond with the differences observed in gait parameters such as maximum force, peak pressure, and step length; this suggests birds with gait impairment relied more on the left leg. This is supported by Resch-Magras et al. (1993), cited by Oviedo-Rondon (2007), in which different leg conditions in turkeys potentially lead to uneven walking forces and potential lameness. However, without data from the left limbs of the sampled birds, we cannot confidently refute bone characteristics contributing to gait asymmetry between limbs. The lack of bilateral bone analysis limits our ability to draw comprehensive conclusions about the relationship between bone morphology and functional gait differences.

Although this study did not find significant differences in pyridinoline (PYD) concentrations based on walking ability, PYD is vastly recognized as a biomarker of bone resorption, particularly in human studies involving osteoarthritis. Elevated PYD levels in symptomatic osteoarthritis patients have been reported (Ok et al., 2017), indicating more bone resorption occurs when the subject is experiencing issues within the bone. This current study lacked samples from birds with severe gait impairments, as these birds are typically euthanized for welfare purposes. This limitation may have reduced the ability to detect PYD changes in birds with more progressive or severe gait abnormalities. In the future, it will be helpful to measure additional biomarkers, such as osteocalcin, to better assess the ratio of bone formation to bone resorption across different gait scores at various ages.

In conclusion, the subjective gait scoring system used in this study was supported by multiple quantitative parameters, establishing its effectiveness in assessing walking ability in commercial turkey toms. Parameters like cadence and gait time can be measured through cameras and visual tracking, and aligned with subjective scores from this study, suggesting potential cost-effective options to be used in a farm setting. Birds with apparent gait impairments tended to utilize the left limb for propulsion, as indicated by parameters like maximum force or peak pressure. Additionally, body weight appeared to have minimal effect on gait score and the objective parameters measured. Both morphological and biomechanical traits in load-bearing (e.g. tibia) and non-load-bearing (e.g. humerus) bones were not affected by gait score, nor were pyridinoloine concentrations. Overall, these findings give insight into biological and kinetic changes associated with age and gait score in turkey toms during a typical commercial growth period.

Table 4.3. Body weight and quantitative gait parameters (Mean ± SEM) collected from 16-week-old male turkeys gait scored either 0 = no impairment (n = 23) or 1 = mild impairment (n = 24) using a pressure sensitive walkway.

Parameter	**Gait Score 0**	**Gait Score 1**	**P-value**
Body weight (kg)	14.39 ± 0.29	14.89 ± 0.28	0.23
Cadence (CA; steps/min)	73.85 ± 6.33	58.57 ± 4.78	0.06
Gait time (GT; s)	2.33 ± 0.33	3.31 ± 0.45	0.05
Gait velocity (GV; cm/s)	19.30 ± 2.73	13.66 ± 1.95	0.09
Gait distance (GD; cm)	32.90 ± 3.04	33.52 ± 3.1	0.80

Statistical differences observed at $P \leq 0.05$

Statistical trends observed at $P \leq 0.1$

Table 4.4. Quantitative gait parameters (Mean ± SEM) collected from 16-week-old male turkeys gait scored either 0 = no impairment (n = 23) or 1 = mild impairment (n = 24) using a pressure sensitive walkway.

Parameter	Gait Score 0	Gait Score 1	P-value
Single support time right (SST; s)	0.31 ± 0.02	0.31 ± 0.02	0.98
Single support time left (SST; s)	0.33 ± 0.03	0.28 ± 0.03	0.58
Single support time right – left (SST; s)	0.03 ± 0.06	0.07 ± 0.05	0.10
Stance time right (SAT; s)	1.84 ± 0.23	1.89 ± 0.18	0.99
Stance time left (SAT; s)	1.66 ± 0.15	1.98 ± 0.20	0.23
Stance time right - left (SAT; s)	0.18 ± 0.19	-0.08 ± 0.08	0.58
Swing time right (SWT; s)	0.33 ± 0.14	0.24 ± 0.19	0.21
Swing time left (SWT; s)	0.68 ± 0.2	0.64 ± 0.2	0.82
Swing time right - left (SWT; s)	-0.35 ± 0.27	-0.40 ± 0.4	0.19
Gait cycle time right (GCT; s)	2.30 ± 0.29	2.67 ± 0.38	0.47
Gait cycle time left (GCT; s)	2.40 ± 0.32	2.51 ± 0.29	0.74
Gait cycle time right - left (GCT; s)	0.17 ± 0.56	0.42 ± 0.4	0.77
Stride length right (SRL; cm)	35.45 ± 3.3	27.64 ± 3.67	0.10
Stride length left (SRL; cm)	27.50 ± 3.97	25.77 ± 3.35	0.62
Stride length right - left (SRL; cm)	7.05 ± 8.46	1.97 ± 3.9	0.31
Step length right (SL; cm)	15.13 ± 5.68	15.76 ± 1.32	0.66
Step length left (SL; cm)	14 ± 4.71	16.67 ± 1.01	0.08
Step length right - left (SL; cm)	0.94 ± 1.57	-0.49 ± 1.78	0.50
Step time right (ST; s)	1.11 ± 0.13	1.45 ± 0.2	0.15
Step time left (ST; s)	0.95 ± 0.09	1.08 ± 0.12	0.0026
Step time right – left (ST; s)	0.16 ± 0.11	0.36 ± 0.21	0.47
Step velocity right (SV; cm/s)	19.14 ± 2.53	15.96 ± 2.53	0.46
Step velocity left (SV; cm/s)	18.06 ± 2.16	19.23 ± 2.04	0.24
Step velocity right – left (SV; cm/s)	0.10 ± 2.18	-2.37 ± 2.72	0.58
Max force right (MF; kg)	28.90 ± 1.1	30.17 ± 0.86	0.41
Max force left (MF; kg)	28.03 ± 1.15	31.02 ± 0.99	0.10
Max force right - left (MF; kg)	0.59 ± 0.68	-0.85 ± 0.78	0.14
Peak pressure right (PP; pKa)	680.58 ± 23.13	717.27 ± 26.13	0.31
Peak pressure left (PP; pKa)	666.69 ± 28.44	738.23 ± 20.21	0.09

Peak pressure right – left (PP; pKa)	14.04 ± 31.02	-20.88 ± 25.67	0.40

Statistical differences observed at $P \leq 0.05$
Statistical trends observed at $P \leq 0.1$

Table 4.5. Morphological and biomechanical properties (Mean ± SEM) measured in the humerus of 16-week-old male turkeys gait scored either 0 = no impairment (n = 23) or 1 = mild impairment (n = 24).

Parameter	**Gait Score 0**	**Gait Score 1**	**P-value**
Weight (g)	69.92 ± 1.93	74.03 ± 1.88	0.13
Length (mm)	171.71 ± 0.82	171.48 ± 0.81	0.83
Average Diameter (mm)	17.03 ± 0.13	16.95 ± 0.13	0.69
Peak Force (kg)	128.08 ± 5.46	119.66 ± 5.30	0.27

Statistical differences observed at $P \leq 0.05$

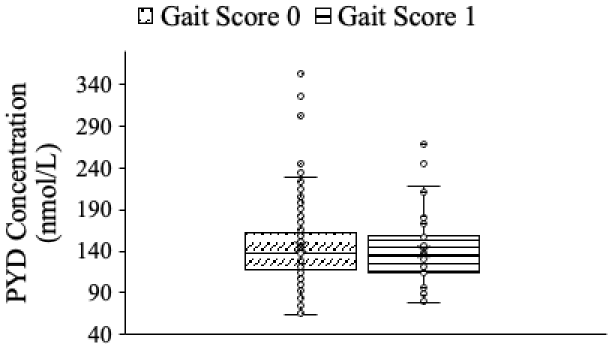
Statistical trends observed at $P \leq 0.1$

Table 4.6. Morphological and biomechanical properties (Mean ± SEM) measured in the tibia of 16-week-old male turkeys gait scored either 0 = no impairment (n = 23) or 1 = mild impairment (n = 24).

Parameter	**Gait Score 0**	**Gait Score 1**	**P-value**
Weight (g)	105.23 ± 1.74	109.27 ± 1.67	0.09
Length (mm)	234.08 ± 1.47	237.38 ± 1.43	0.10
Average Diameter (mm)	16.48 ± 0.19	16.53 ± 0.19	0.86
Peak Force (kg)	86.99 ± 3.16	90.89 ± 3.11	0.35

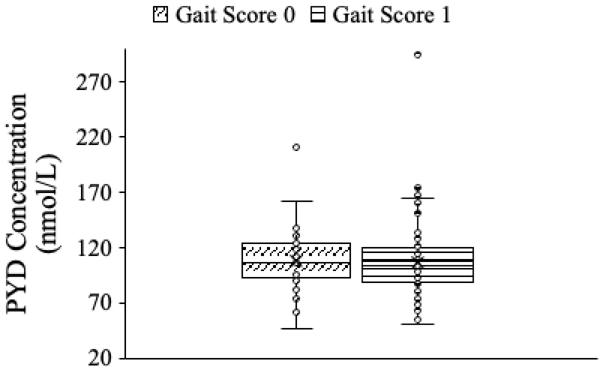
Statistical differences observed at $P \leq 0.05$

Statistical trends observed at $P \leq 0.1$



P = 0.42

Figure 4.1. PYD concentrations compared between gait scores 0 (n = 334) and 1 (n = 53) at 8 woa.

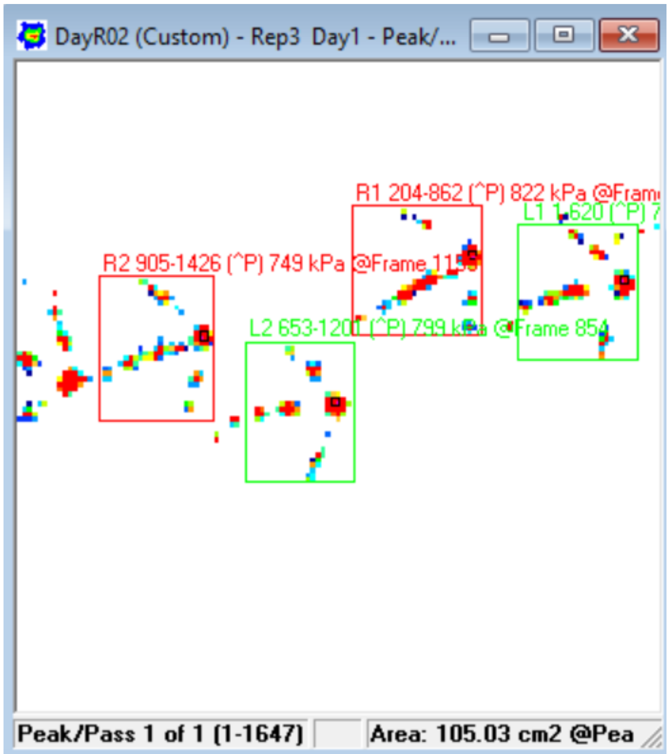


P = 0.34

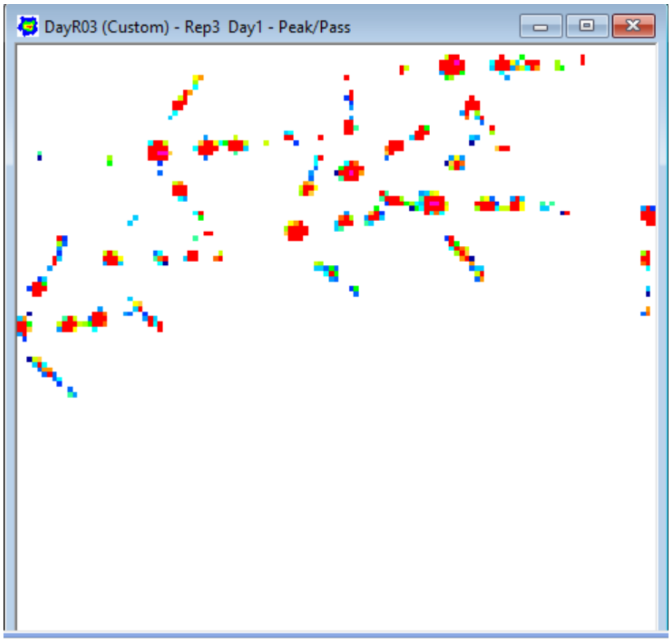
Figure 4.2. PYD concentrations compared between gait scores 0 (n = 59) and 1 (n = 121) at 16 woa.

SUPPLEMENTAL FIGURES AND TABLES

Supplemental Figure S4.1. A sample photo of a 16-week-old turkey tom being gently coaxed to cross the PSW to collect quantitative gait parameters following a subjective gait scoring.



Supplemental Figure S4.2a. An imaged deemed "readable" based on the presence of at least one strike per limb and the ability to provide strike boxes.



Supplemental Figure S4.2b. An imaged deemed "unreadable" due to the inability to provide strike box and differentiate footprints from one another.

Age and Bone type	Load Cell Weight (kg)	Test Speed (mm/sec)	Trigger Force (g)	Support Beam Distance (mm)	Distance from Midpoint on Each Side (mm)
16-week-old Humerus	750	1	400	70	35
16-week-old Tibias	750	1	400	120	60

Supplemental Table S4.1. Each of the parameters used when performing a 3-point bend test used for 16-week-old humerus and tibias, including: the load cell weight (kg), test speed (mm/sec), trigger force (g), support beam distance (mm) and the distance on each side of the bone from the midpoint (mm).

REFERENCES

Bazzi, H., and A. T. Cacace. 2023. Altered gait parameters in distracted walking: a bio-evolutionary and prognostic health perspective on passive listening and active responding during cell phone use. Frontiers in Integrative Neuroscience 17. doi 10.3389/fnint.2023.1135495

Caplen, G., B. Hothersall, J. C. Murrell, C. J. Nicol, A. E. Waterman-Pearson, C. A. Weeks, and G. R. Colborne. 2012. Kinematic Analysis Quantifies Gait Abnormalities Associated with Lameness in Broiler Chickens and Identifies Evolutionary Gait Differences. PLoS ONE 7:e40800. doi 10.1371/journal.pone.0040800

Corr, S. A., C. C. McCorquodale, and M. J. Gentle. 1998. Gait analysis of poultry. Research in Veterinary Science 65:233-238. doi https://doi.org/10.1016/S0034-5288(98)90149-7

Corr, S. A., C. C. McCorquodale, R. E. McGovern, M. J. Gentle, and D. Bennett. 2003. Evaluation of ground reaction forces produced by chickens walking on a force plate. American Journal of Veterinary Research 64:76-82. doi 10.2460/ajvr.2003.64.76

Corr, S. A., C. C. McCorquodale, J. McDonald, M. J. Gentle, and R. McGovern. 2007. A force plate study of avian gait. Journal of Biomechanics 40:2037-2043.

Dong, Y., G. Fraley, J. Siegford, F. Zhu, and M. Erasmus. 2023. Environmental and genetic strategies for improving leg health and walking ability of commercial turkeys. PLoS One:20.

Frothingham, S. 2018. How to Calculate Stride Length and Step LengthHealthline.

Griffith, N. 2025. ENVIRONMENTAL MODIFICATION STRATEGIES FOR IMPROVING LEG HEALTH AND WALKING ABILITY OF COMMERCIAL TURKEY TOMS. Master of Science. Purdue University.

Kavanagh, J., and H. Menz. 2008. Accelerometry: A technique for quantifying movement patterns during walking. Gait & Posture 28:1-15. doi 10.1016/j.gaitpost.2007.10.010

Kestin, S. C., T. G. Knowles, A. E. Tinch, and N. G. Gregory. 1992. Prevalence of leg weakness in broiler chickens and its relationship with genotype. Vet Rec 131:190-194. doi 10.1136/vr.131.9.190

Kim, I., M. Gallagher, and R. A. Speckman 2020. Biomechanics of Normal Gait. https://now.aapmr.org/biomechanics-normal-gait/.

Kittelsen, K. E., B. David, R. O. Moe, H. D. Poulsen, J. F. Young, and E. G. Granquist. 2017. Associations among gait score, production data, abattoir registrations, and postmortem tibia measurements in broiler chickens. Poultry Science 96:1033-1040. doi https://doi.org/10.3382/ps/pew433

Kline, G., D. Orton, and H. Sadrzadeh. 2017. Chapter 4 - Bone Metabolism. Endocrine Biomarkers:157-180. doi https://doi.org/10.1016/B978-0-12-803412-5.00004-5

Kremer, J. A., C. I. Robison, and D. M. Karcher. 2018. Growth dependent changes in pressure sensing walkway data for turkeys. Frontiers in Veterinary Science 9.

Li, G., R. S. Gates, M. M. Meyer, and E. A. Bobeck. 2023. Tracking and Characterizing Spatiotemporal and Three-Dimensional Locomotive Behaviors of Individual Broilers in the Three-Point Gait-Scoring System. Animals 13:717. doi 10.3390/ani13040717

Nääs, I. A., I. C. L. A. Paz, M. S. Baracho, A. G. Menezes, G. F. Bueno, I. C. L. Almeida, and D. J. Moura. 2009. Impact of lameness on broiler well-being. Poultry Science Association 18:432-439. doi 10.3382/japr.2008-00061

Nemoto, R., I. Nakamura, Y. Nishijima, K. Shiobara, M. Shimizu, T. Takehara, T. Ohta, and M.

Kiyoki. 1997. Serum pyridinoline crosslinks as markers of tumour-induced bone resorption. British Journal of Urology 80:274-280. doi 10.1046/j.1464-410x.1997.00237.x

Ok, S.-M., S.-M. Lee, H. R. Park, S.-H. Jeong, C.-C. Ko, and Y.-I. Kim. 2017. Concentrations of CTX I, CTX II, DPD, and PYD in the urine as a biomarker for the diagnosis of temporomandibular joint osteoarthritis: A preliminary study. CRANIO®:1-7. doi 10.1080/08869634.2017.1361624

Oviedo-Rondon, E. O. Year. Predisposing Factors that Affect Walking Ability in Turkeys and Broilers. Proc. Carolina Poultry Nutrition Conference.

Oviedo-Rondon, E. O. Year. Predisposing Factors that Affect Walking Ability in Turkeys and Broilers. Proc. Carolina Poultry Nutrition Conference.

Oviedo-Rondon, E. O., B. D. X. Lascelles, C. Arellano, P. L. Mente, P. Eusebio-Balcazar, J. L. Grimes, and A. Mitchell. 2017. Gait parameters in four strains of turkeys and correlations with bone strength. Poultry Science 96:1989-2005. doi http://dx.doi.org/10.3382/ps/pew502

Paxton, H., M. Daley, S. Corr, and J. Hutchinson. 2013. The gait dynamics of the modern broiler chicken: A cautionary tale of selective breeding. Journal of Experimental Biology 216:3237-3248. doi 10.1242/jeb.080309

Pearce, J., Y.-M. Chang, and S. Abeyesinghe. 2023. Individual Monitoring of Activity and Lameness in Conventional and Slower-Growing Breeds of Broiler Chickens Using Accelerometers. Animals 13:1432. doi 10.3390/ani13091432

Pirani, H., and M. Azizi. 2020. Comparison of Peak Pressure, Maximum Force, Contact Area, and Contact Time Between the Right and Left Foot in Elite Weightlifters. Journal of kermanshah University of Medical Sciences. doi 10.5812/jkums.96967.

Polk, J., R. Stumpf, and K. Rosengren. 2016. Limb dominance, foot orientation and functional asymmetry during walking gait. Gait & Posture 52:140-146. doi http://dx.doi.org/10.1016/j.gaitpost.2016.11.028

Reiter, K. 2002. Analyse of locomotion in laying hen and broiler. Arch. Geflu¨gelk 66:133-140.

Reiter, K., and W. Bessei. 1997. Gait analysis in laying hens and broilers with and without leg disorders. Equine Veterinary Journal 29:110-112. doi 10.1111/j.2042-3306.1997.tb05067.x

Roberson, K. D., M. W. Klunzinger, R. A. Charbeneau, and M. W. Orth. 2005. Evaluation of phytase concentration needed for growing–finishing commercial turkey toms. British Poultry Science 46:470-477. doi 10.1080/00071660500190769

Sadeghi, H., P. Allard, F. Prince, and H. Labelle. 2000. Symmetry and limb dominance in able-bodied gait: a review. Gait Posture 12:34-45. doi 10.1016/s0966-6362(00)00070-9

Soyalp, S., E. Hartono, O. W. Willems, B. J. Wood, S. E. Aggrey, and R. Rekaya. 2023. Research Note: Analysis of body weight and walking ability in turkeys and the prediction of categorical responses across systematic effect classes using a linear threshold model. Poult Sci 102:102993. doi 10.1016/j.psj.2023.102993

Stevenson, R., H. A. Dalton, and M. Erasmus. 2019. Validity of Micro-Data Loggers to Determine Walking Activity of Turkeys and Effects on Turkey Gait. Frontiers in Veterinary Science 5. doi 10.3389/fvets.2018.00319

Swalander, L. M., T. A. Burnside, and P. K. Glover. 2020. Leg Health in Commercial Turkeys.

Tekscan The Gait Cycle: Phases, Parameters to Evaluate & Technology. https://www.tekscan.com/blog/medical/gait-cycle-phases-parameters-evaluate-technology.

Urena, P., A. Ferreira, V. T. Kung, C. Morieux, P. Simon, K. S. Ang, J. C. Souberbielle, G. V. Segre, T. B. Drueke, and M. C. De Vernejoul. 1995. Serum pyridinoline as a specific marker of collagen breakdown and bone metabolism in hemodialysis patients. J Bone Miner Res 10:932-939. doi 10.1002/jbmr.5650100614

Van Hertem, T., T. Norton, D. Berckmans, and E. Vranken. 2018. Predicting broiler gait scores from activity monitoring and flock data. Biosystems Engineering 173:93-102. doi 10.1016/j.biosystemseng.2018.07.002

Van Wettere, A. 2020. Noninfectuous Skeletal Disorders in Poultry Broilers. https://www.merckvetmanual.com/poultry/disorders-of-the-skeletal-system-in-poultry/noninfectious-skeletal-disorders-in-poultry-broilers#:~:text=Varus%2Fvalgus%20deformation%20is%20less,in%20turkeys%20than%20in%20chickens. Accessed 04/10 2024.

Webster, A. B., B. D. Fairchild, T. S. Cummings, and P. A. Stayer. 2008. Validation of a Three-Point Gait-Scoring System for Field Assessment of Walking Ability of Commercial Broilers. Poultry Science 17:529-539. doi 10.3382/japr.2008-00013

Weeks, C. A., T. D. Danbury, H. C. Davies, P. Hunt, and S. C. Kestiin. 2000. The behaviour of broiler chickens and its modification by lameness. Applied Animal Behaviour Science 67:111-125. doi https://doi.org/10.1016/S0168-1591(99)00102-1

Winter, D. 1987. The Biomechanics and Motor Control of Human Gait. university of Waterloo Press.

Wurtz, K. E., and A. B. Riber. 2024. Overview of the various methods used to assess walking ability in broiler chickens. Veterinary Record 195. doi 10.1002/vetr.4398

Ziegler, J., H. Gattringer, and A. Muller. 2024. On the relation between gait speed and gait cycle duration for walking on even ground. Journal of Biomechanics 164. doi https://doi.org/10.1016/j.jbiomech.2024.111976

CHAPTER 5

CONCLUSIONS

Genetic selection in domestic turkeys has prioritized increased body masses and rapid growth, but often at the expense of skeletal development and resulting in issues such as lameness. This study evaluated the effects of environmental enrichment, both stationary and dynamic, on the body weight and skeletal quality of a line of commercial turkey toms at various ages throughout a standard production period. While minimal effects were observed from the enrichments selected, some transient differences were observed when the birds were at a younger age. Many of these findings diminished with age, indicating a limitation in the long-term benefits of enrichments for skeletal health. Subsequent analysis revealed a vast amount of skeletal development in load-bearing bones, specifically through parameters such as bone weight, bone diameter and peak force to fracture, happens in the early ages (8 to 12 weeks of age). As the birds aged, the phenotypic relationship between these bone properties and body weight also declined. This trend was observed in both load-bearing (tibia and femur) and non-load-bearing (humerus) bones. However, the changes in the humerus were less pronounced, indicating the relationship with body weight remains stable over time. These findings ultimately indicate a possibility for skeletal selection in commercial breeds, without the need to sacrifice increased body masses. Further phenotypic and genotypic analyses are needed to determine if this concept is valid and if the selection is feasible in breeding programs.

Gait is a trait heavily influenced by the selection for increased body mass in commercial turkey toms. Gait score is typically performed throughout the rearing period using subjective scoring systems, which require trained observed but are prone to the risk of human bias and error. Therefore, this study evaluated a simplified subjective two-point gait score system and assessed its efficacy and validity through the collection of objective gait parameters. Birds classified as either having no gait impairments or having mild gait impairments were analyzed using a pressure sensitive walkway to measure parameters such as cadence, step time and maximum force. Significant differences in these parameters quantified and supported the subjective scoring system and its ability to detect lameness in birds. Alongside this, the potential for commercially feasible objective measurements like cadence, gait time and gait velocity were determined. These are parameters that can be recorded using cameras or basic timing tools (e.g. a stopwatch). The integration of a subjective scoring system with select objective gait measures can work well to ensure correct and adequate gait score analysis. This supports genetic selection strategies and welfare improvements in aging toms throughout commercial production periods.