ABSTRACT

BOLGER, BRITTANY NICOLE. Investigating Underlying Causes of Injurious Pecking in Turkey Toms (Under the direction of Dr. Allison Pullin).

Injurious pecking is a welfare and economic concern in commercial turkey production, given that repeated pecking at plumage or skin can lead to painful injuries, feather loss, and, in extreme cases, cannibalism and mortality. Despite its prevalence in commercial flocks, the underlying motivations of this abnormal behavior in turkeys are not well understood. Injurious pecking is hypothesized to be the result of redirected foraging behavior, but this relationship has yet to be determined in turkeys. Providing environmental enrichment may also be an opportunity to redirect pecking behavior. The first objective of this thesis was to assess how environmental enrichment affected pecking and foraging behaviors and production performance in two commercial Large White genetic strains of turkey toms. The second objective was to determine if an individual's prevalence of feather pecking predicted their preference to forage or feed, as well as physiological markers for dominance and hunger.

For the first objective, 1,056 turkey toms from two commercial Large White genetic strains were randomly assigned to 24 pens/strain. At 5 wk, hanging jute rope enrichment was added to half the pens (ROPE), while the other half was not provided with enrichment (CON; n=12 pens/strain/enrichment treatment). Production measurements (body weight, body weight gain, feed intake, and feed conversion ratio) were taken approximately every 3 wk from placement to 19 wk. At 4 and 18wk, video recordings were taken in half the pens for 20 min in the morning and 20 min in the afternoon to analyze the number of head/neck pecks (HN), back/wing/tail pecks (BWT), and foraging bouts. Pecking at the jute rope enrichment was evaluated at 5, 8, 11, 14, and 18 wk. The rope enrichment was not effective at mitigating either of the pecking behaviors or foraging. The 4 wk poults performed all behaviors more frequently

than the 18 wk toms. The heaviest, fastest growing strain performed more HN pecking and rope pecking. Both strains had similar BWT pecking and foraging frequencies. However, both BWT pecking and foraging frequencies increased in the afternoon, suggesting a temporal relationship. We aimed to further investigate the relationship between BWT pecking and foraging.

For the second objective, 176 toms from a single commercial strain were randomly assigned to 8 pens (22 birds/pen). Eight focal birds were randomly chosen and colored for identification. At 14 wk, live observations were conducted, with continuous focal animal behavioral sampling, to count BWT pecking frequencies across four days (6 h total). For each pen, the two birds with the greatest sum of BWT pecking were classified as high feather pecking birds (HFP), while the two birds with the lowest sum of BWT pecking were classified as low feather pecking birds (LFP). Also at this age, blood was collected to measure testosterone and ghrelin concentrations, and home pen behavior was video recorded. At 15 wk, the HFP and LFP birds were conditioned to a place preference test, to determine if the birds preferred to contrafreeload (forage) or have feed freely available. The HFP birds fed and foraged more frequently in the home pen than the LFP birds. During conditioning, both HFP and LFP birds foraged more on the contrafreeloading side, but very few birds fed from the freely available feed. Neither the HFP nor LFP birds displayed a preference greater than chance for either side of the test. There were also no differences in plasma testosterone or ghrelin concentrations between the two groups.

Collectively, injurious pecking is a multifactorial behavior that may be influenced by genetics, environmental conditions, and individual differences. A hanging jute rope was not effective in mitigating pecking, but strain, age, and temporal patterns for pecking emerged.

Additionally, understanding individual variation in pecking behavior can improve welfare by identifying birds at risk of developing pecking behaviors or becoming a victim of pecking.

Investigating Underlying Causes of Injurious Pecking in Turkey Toms

by
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Dr. Allison Pullin Committee Chair	Dr. Jesse Grimes
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DEDICATION

To my friends, family, and colleagues who supported me throughout my master's journey. A master's degree is not something earned alone- it is built on countless moments of support, understanding, and sacrifice from the people who stand beside you. I am beyond thankful for all those who encouraged me to push forward and strive for greatness.

BIOGRAPHY

Brittany Nicole Bolger was born in Livingston, New Jersey, on June 27th, 2000, and was raised in Roxbury, New Jersey. She is the daughter of Bob Bolger, a procurement and supply executive, and Teresa Bolger, a former Special Education teacher. Brittany had a passion for animals from a young age and was always engaged in science classes growing up. Even as a child, she knew caring for animals had always been her calling.

Brittany first attended Roxbury High School before moving to Virginia, where she graduated from Blacksburg High School in 2018. She was awarded the ACCE scholarship to attend New River Community College before transferring to Virginia Tech. Brittany graduated from Virginia Tech, Cum Laude, in 2022. That same year, she earned admission to Lincoln Memorial University College of Veterinary Medicine; however, she decided to pursue another route involving animal science. In 2023, she interned at Tiger World Endangered Wildlife Preserve, where she worked with exotic carnivores and fell in love with animal behavior and promoting animal welfare. Inspired to pursue a master's degree studying such subjects, she began her degree at NC State University as a graduate research assistant under Dr. Allison Pullin. During her program, Brittany studied the underlying motivations of injurious pecking in commercial turkey toms in an attempt to expand the studies of an under-researched species. She presented her research at conferences, including the Poultry Science Association and the 58th Congress of the International Society of Applied Ethology, and earned her certification as PAACO Poultry Welfare Auditor. After graduation, Brittany plans to pursue a career promoting animal welfare across several species.

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CHAPTER 1

Literature Review

Introduction

Injurious pecking is repeated pecking at another bird's head or feathers, resulting in plumage or skin damage (Duggan et al., 2014). Cannibalism occurs when the bird performing the pecking ingests skin and tissue from another bird (Dalton et al., 2013). In the United States, cannibalism was ranked fourth in non-infectious health issues facing the turkey industry (Clark and Chiaia, 2023). Injuries on the head, neck, back, and tail regions were reported in 59.7% and 71.0% of Canadian turkey flocks, respectively (Leishman et al., 2022). Correspondingly, 38.3% of Canadian turkey farmers identified pecking injuries as a reason for culling and 41.6% of farmers listed cannibalism as a reason for mortality (van Staaveren et al., 2020). Over half (i.e., 58.8%) of culled or dead turkeys displayed severe pecking injuries (Duggan et al., 2014). This abnormal behavior stands as a welfare concern as feather pecking or plucking can be painful and result in skin tearing, bruising, and inflammation. The loss of feathers may pose challenges to thermoregulation in the recipient bird (Mench, 2009). Economic concerns with pecking injuries are a reason for condemnations at the processing plant (van Staaveren et al., 2020).

Previous research has attributed redirected foraging and feeding behavior to injurious pecking, particularly in laying hens (Brunberg et al., 2016). The objective of this literature review is to review possible underlying motivations for injurious pecking in turkeys – foraging, feeding, and social behaviors, genetics, and hormones. Environmental enrichment strategies to mitigate injurious pecking will be discussed. Ultimately, previous work will provide insights to investigate the preferences underlying injurious pecking in turkeys and enrichment opportunities to mitigate the behavior.

Foraging & Feeding Behavior

Turkeys forage by scratching and pecking at the ground to obtain insects and plant matter for feed. Foraging is a prevalent natural behavior of wild turkeys, where they can spend 89% of daytime activity engaged in foraging (Chamberlain et al., 2020). Although wild turkeys will forage over the course of a day, feeding behavior is most prevalent 2 to 3 hours after dawn and 2 to 3 hours before dusk (Klasing, 2005). In nature, these birds have access to a varied selection of feedstuffs as they forage in grass or brush, allowing birds to balance their nutritional demands and modify their intake based on what is available. In commercial farm settings, turkeys are housed in floor systems, where birds are housed on flooring layered with litter, and with feed and water provided ad libitum through feed troughs and drinker equipment (Mench, 2009). Domesticated turkeys still perform foraging behavior in loose litter on the ground, despite feed being readily available (Monckton et al., 2020). Similarly in chickens, domesticated birds forage less than wild-type birds, suggesting that while domestication has passively adapted to less energy-demanding foraging strategies, it has not completely removed the intrinsic drive to forage (Andersson et al., 2001).

It is unclear what underlying mechanisms promote foraging behavior in domesticated turkeys. Visual cues, such as ultraviolet (UV), have been used functionally for foraging behavior in wild turkeys, acting as a visual marker for birds to recognize and avoid harmful food (Werner et al., 2014). Foraging constitutes 24% of a farmed turkey's time budget, the largest portion of any behavior (Irfan et al., 2016). The amount of time turkeys engage in foraging may indicate that they value contrafreeloading, when an animal prefers to search or forage for food rather than obtain it from a freely available source, like a feeder or trough (Ferreira et al., 2021).

Alternatively, or additionally, hunger may be a contributing factor. Ghrelin is commonly used as a physiological measure of hunger in mammals as it is synthesized in the gastrointestinal (GI) tract and stimulates hunger and, consequently, promotes feed intake (Bhatti et al., 2006). In birds, however, ghrelin has been shown to have the opposite effect of that in mammals, where increased levels of ghrelin are associated with inhibiting feed intake (Kaiya et al., 2007). In chickens, ghrelin is primarily synthesized in the proventriculus of the GI tract, then circulates to reach the central nervous system, specifically the pituitary gland (Song et al., 2019). An intracerebroventricular bolus injection of rat ghrelin decreased feed intake in chicks, demonstrating a relationship between feeding behavior and ghrelin in the avian central nervous system (Ahmed and Harvey, 2002; Kaiya et al., 2002). Eight-day-old male layer chicks were injected with a bolus of ghrelin intravenously, but it did not affect feed intake compared to the control group, suggesting ghrelin does not promote feed intake like in mammals (Kaiya et al., 2007). In addition, broiler chickens fed a high-energy diet had an increased gene expression of ghrelin in the hypothalamus and an increased presence of ghrelin receptors in the proventriculus, compared to birds on a low-energy diet. The presence of ghrelin mRNA and GHS-R1a mRNA (a ghrelin receptor) in the abdominal fat pad and liver of these broilers suggests ghrelin is associated with energy storage (Song et al., 2019). Ghrelin was lower in turkeys that were fasted or feed-restricted compared to turkeys fed ad libitum, and ghrelin-inhibited turkeys displayed higher feed intake compared to control birds that were not passively immunized against ghrelin (Vizcarra et al., 2012, 2018). Lower concentrations of ghrelin are associated with hunger in turkeys as well.

Social Behavior

In the wild, turkeys live in social systems comprised of all-male groups, consisting of two to four males, or all-female groups, consisting of fifty or more hens, for most of the year, then mixed-sex groups during the breeding season (Buchholz, 1997; Krakauer, 2005; Mench, 2009; Marchewka et al., 2013). When male turkeys are six to seven months old, they will separate from the brood flock to create a lifelong sibling group (Krakauer, 2005).

Young males will establish hierarchies at an individual level within the group via agonistic behaviors to determine mating success within the group, while the group's dominance as a whole is determined either by size or combat (Watts and Stokes, 1971; Buchholz, 1997). Any lone or unrelated males that attempt to join these groups are rejected through aggressive fights to ensure closed sibling groups (Buchwalder and Huber-Eicher, 2005). Combative behaviors include wrestling and pecking at the head and neck (Watts and Stokes, 1971). Small groups of familiarized toms are capable of distinguishing non-group members and often display aggressive behavior towards them (Buchwalder & Huber-Eicher, 2005). Non-group mates maintain a larger spatial distribution from each other to mitigate aggressive encounters (Buchwalder and Huber-Eicher, 2004).

Females form their own flocks after brooding and establish individual ranks amongst themselves via fighting, where older females often win and dominate over younger females (Watts and Stokes, 1971). At the start of the breeding season, females will disband their flock when they enter the leks to be courted. Male sibling coalitions remain together, with the dominant group having first access to the females and subordinate groups attempting to display and call to females around the periphery (Watts and Stokes, 1971; Buckhholz, 1997). All siblings in the group will display for a hen by vocalizing and strutting. Strutting is described as fanning the tail feathers and drooping the wings down (Schleidt, 1970; Watts and Stokes, 1971). While

all the males will display, only the dominant brother will mate – this cooperative courtship provides indirect fitness benefits via kin selection. While the dominant tom receives the main mating benefit, subordinate toms also gain indirect benefits by ensuring the propagation of their shared genes (Krakauer, 2005).

Domestic turkeys utilize similar behaviors to establish and maintain social structures. Both toms and hens display head pecking to establish dominance, but it is most commonly noted in toms (Bartels et al., 2020). Poults display head pecking as young as 3 to 4 weeks of age (WOA; Sherwin et al., 1999; Busayi et al., 2006), learning the behavior to utilize as adults to establish hierarchies (Dalton et al., 2013). Both male and female poults also exhibit precocial strutting, possibly in relation to imprinting. Imprinted poults, raised by humans or hens, will direct their struts towards the mother hen, guardian, or siblings (Schleidt, 1970).

Testosterone is a sex steroid in domestic fowl that influences behavior and physical traits. The hormone is released in a pulsatile manner following a diurnal pattern (Bacon et al., 1991). Testicular weights, the volume of semen produced, and the total amount of spermatozoa have been correlated with levels of testosterone in plasma (Cecil and Bakst, 1988). Seasonal fluctuations in circulating testosterone are responsible for changes in anatomical secondary sex characteristics and behavior. This can include increased aggression, courtship displays, and variations of facial color in wattles and caruncles in turkeys. In wild turkeys, testosterone spikes during the breeding season from late February to June, with subordinate males displaying lower testosterone levels throughout the breeding season (Lisano and Kennamer, 1977). Smith and Smyth (1963) found that toms castrated at 7 WOA had reduced snood development and displayed more feminine heads at 30 WOA, indicating a relationship between physical traits and sex steroids. This study also found that strutting steadily increased in the intact control toms, but

reduced strutting was observed in the castrated toms, with scattered instances of strutting noted at 68, 150, and 210 days of age. When two toms were treated with implants of deslorelin acetate, a gonadotropin-releasing hormone (GnRH) agonist, on three occasions, aggressive behaviors towards human caretakers and conspecifics decreased after each treatment. Birds were treated with an implant each time aggressive behaviors returned, with testosterone concentrations decreasing after each treatment, compared to nontreated birds (Molter et al., 2015).

Injurious Pecking Behavior

Injurious pecking is defined as repeated pecking directed towards another bird's head or feathers that results in the removal of feathers and/or skin damage. Ingestion of blood and tissue by the pecking bird is considered cannibalism, and recipient birds with severe injuries may die or need to be culled (Duggan et al., 2014). Injurious pecking is an animal welfare concern as the recipient turkeys experience painful injuries as indicated by inflammation, bruising, swelling, and/or bleeding (Mench, 2009). The prevalence of injurious pecking injuries varies by flock and body region being pecked, as up to 40% of birds within a flock can show head/neck pecking injuries and up to 97% of birds within a flock experiencing pecking injuries on their back/tail (Leishman et al., 2022). Feather removal can impact a bird's ability to thermoregulate, prompting additional feed intake, and skin injuries can result in downgrades at processing plants.

Economically, culling and mortality associated with injurious pecking create product losses, particularly towards the end of the flock cycle. These concerns could challenge production efficiency and profits if injurious pecking becomes prominent in a flock, and the majority of commercial turkey flocks (i.e., 58%) previously studied displayed injuries from pecking behavior (Duggan et al., 2014; Leishman et al., 2022).

Injurious pecking has been predominantly associated with environmental factors, such as stocking density, light, and enrichment. Commercial turkeys live in single-sex flocks in group sizes larger than their wild counterparts, with up to 20,000 birds in an enclosed barn with artificial or natural lighting and ventilation. Turkeys are unable to develop a stable social hierarchy in farmed group sizes (Marchewka et al., 2013). High stocking densities, such as 40 birds in a 6m × 13m pen, reduce spatial distribution between birds, so subordinate birds have minimal escape opportunities to mitigate aggressive interactions (Buchwalder and Huber-Eicher, 2004). Turkeys synchronize their behavior through social facilitation, copying their flock mates to learn new behaviors or exploit new resources (Mench, 2009). As a result, injurious pecking is difficult to mitigate once it begins to be learned through a flock (Dalton et al., 2013). Removing birds that feather peck from the flock may not be an effective strategy in mitigating this behavior as individual birds are often both feather peckers and recipients (Rodenburg et al., 2013). In laying hens, a bird considered the highest feather pecking individual in the flock at 21 WOA may not remain in that position when evaluated 3 weeks later (Daigle et al., 2015). Forty-one percent of birds that performed pecking transitioned to victims of feather pecking over the course of the 37 week trial (Daigle et al., 2015).

Light intensity and photoperiod are carefully controlled in commercial settings, as turkeys are a diurnal species and their circadian rhythms are influenced by how much light their photoreceptors are exposed to; drastic changes in either factor can increase cannibalistic behaviors (Marchewka et al., 2013; Dalton et al, 2013). Turkeys are often raised in dim lighting (21.52 to 26.9 lux) to reduce activity and increase growth rate efficiency (Mench, 2009; Hybrid, 2025). This dim lighting can also reduce natural exploratory behaviors like walking, foraging, and socializing, as turkeys have an affinity for bright environments. When provided with four

chambers of varying light intensities (1, 6, 20, and 200 lux), turkeys exhibited resting behaviors in environments with 1 lux, but feeding, pecking, and locomotion were observed in environments with 20 or 200 lux (Barber et al., 2004). However, intermittent exposure to intense white light (86.1 lux) can cause hyperactivity and flightiness in birds, suggesting that lighting schedules and the type of lighting used in housing can have varying impacts on behavior (Gill and Leighton, 1984).

Another contributing factor is that turkeys are capable of seeing with UV-A spectral vision. Birds raised under fluorescent light had injuries as early as 5 days of age, while birds raised under incandescent light had pecking injuries at 6 days of age. Fluorescent light with supplemental UV light minimized pecking injuries compared to the incandescent light, and pecking behavior decreased with lower light intensity with fluorescent light (Moinard et al., 2001). UV markings can be found on the wings and tail of turkey poults, with wing markings emerging at 8 days of age and tail markings emerging between 14 to 20 days of age (Sherwin and Devereux, 1999). These correlate with when pecking injuries were reported in these areas, as wing injuries were reported between 5 and 10 days, and tail injuries were observed between 14 to 20 days (Sherwin and Kelland, 1998). Additionally, poor ventilation within a commercial turkey house can lead to unsuitable humidity levels, fluctuating temperatures, and an influx of flies; all of which can increase stress within a flock and promote injurious pecking (Dalton et al., 2013).

Although environmental factors have been linked to injurious pecking in turkeys, there is a lack of understanding about the ontogeny of the behavior. Previous research in chickens may provide insight. In chickens, pecking directed towards the head has been associated with social interactions and establishing dominance, while feather pecking directed towards the wings, back,

and tail/vent has been associated with redirected foraging (Mench, 2009). Laying hens will perform feather pecking when a suitable foraging substrate is absent, as suggested by Huber-Eicher and Wechsler (1997). When laying hen chicks were provided with sand as a dustbathing substrate and straw as a foraging substrate, chicks raised with sand developed injurious pecking behaviors, while straw promoted foraging behaviors and mitigated pecking behaviors when behaviors were measured at 7 WOA (Huber-Eicher and Wechsler, 1997). Early exposure to the correct foraging substrate can influence the severity of pecking behavior. When wood shavings or sand were used for litter, it stimulated floor pecking in laying hens raised on it for their whole lives, while birds raised on wire mesh for the first 21 days, then given wood shavings as litter, had more feather damage when all the birds were scored at 40 WOA (de Jong et al., 2013). Young laying hens (3 to 15 WOA) that spent more time foraging as compared to resting and dustbathing were more likely to display injurious pecking behaviors as adults (17 to 37 WOA) after the onset of egg laying (Newberry et al., 2007).

Also, in chickens, there are two distinguishable forms of feather pecking: gentle and severe. Gentle feather pecking (GFP) is performed without force and is directed towards debris caught in another bird's plumage or tips of the feathers. It is considered a social and exploratory behavior and typically does not cause harm to the recipient. Severe feather pecking (SFP) involves more forceful pecking or pulling of feathers or skin of another bird, often ingesting them in bouts of cannibalism (Rodenburg et al., 2013; Dalton et al., 2018). Continuous SFP creates bald spots on recipient hens from feather damage or loss, which makes hens more susceptible to tissue damage (Rodenburg et al., 2013). Recipients of SFP react by either moving away or confronting the pecking bird, but recipients of continuous SFP may stop responding.

The ontogeny of GFP and SFP differs in chickens. To investigate the frustrated foraging motivation in laying hens, Dixon et al. (2008) investigated the fixed action pattern morphology of pecking and foraging behaviors. By observing the duration of the head fixation, duration of head fixation to contact, and the duration of the whole peck, they were able to determine that SFP and foraging peck were similar in morphology, as they both take longer to perform and have longer head fixation durations compared to GFP. This suggests that GFP and SFP are derived from different motivational systems, with SFP derived from frustrated foraging and GFP derived from preening (Dixon et al., 2008).

Genetic Influence

In domesticated turkeys, genetic selection pressures have primarily emphasized fast growth, high body weights, and improved feed conversion ratio for higher meat yield (Clark et al., 2019). However, focusing on these traits could have inadvertently selected for other undesirable traits (Flores and Grimes, 2022). Physical impairments, such as lameness, leg health issues, muscle abnormalities, and reduced immune function, are commonly associated with fast-growing breeds (Flores and Grimes, 2022). Genetic lines differ among breeding companies, with poultry producers purchasing lines based on their respective production goals.

While selecting for these growth traits has prompted physical challenges, additional behavioral abnormalities may have also arisen. Injurious pecking, feather pulling, and cannibalism are observed more in a genetic line selected for growth rate and meat yield than in a traditional line (Busayi et al., 2006). A traditional line of turkeys pecked and pulled at feathers more strongly than selected lines, but they also had better feather coverage, which provided more protection from skin damage. Across males and females, 47% of turkeys from the selected line required treatment for pecking-related injuries, while none of the birds from the traditional line

required treatment. Males of both strains pecked more frequently than females, with a majority of the severe pecking focused on the wings and backs of flockmates, and the intensity of the feather pecking increased with age in males. These results suggest that selection for production-related traits is associated with injurious feather pecking (Busayi et al., 2006).

Behavioral variations amongst the commercially selected lines have also been noted (Haug et al., 2023). A slow-growing, lightweight breed (Auburn) displayed a lower prevalence of skin injuries (31.8%) compared to a fast-growing, heavy breed (B.U.T.6; 44.1%). Auburn birds also had less plumage damage in the tail region compared to the B.U.T.6, which was attributed to the heavier body weight preventing the B.U.T.6 birds from reaching the tail area to preen. The B.U.T.6 strain showed a peak prevalence of pecking injuries from 12 to 16 WOA while the Auburn breed's injuries peaked from 16 to 20 WOA. This finding may be due to fast-growing birds maturing at a faster rate, therefore showing more injurious behavior earlier (Haug et al., 2023).

Age-related differences in growth and maturity are also observed between fast-growing commercial strains, such as the Nicholas Select and Hybrid Converters (Barbour and Lilburn, 1996). Nicholas Select toms reportedly had heavier body weights from 31 to 72 days of age, in addition to having heavier drumstick and thigh meat, compared to Hybrid Converters at. However, Hybrid Converter toms were notably heavier than Nicholas Select toms at the end of the study at 82 days of age. Thigh, drumstick, and the pectoralis major (breast meat) of Hybrid Converters all surpassed Nicholas Select muscle weights at 82 days of age. These findings suggest that Nicholas Select toms have rapid early growth while Hybrid Converters have later stages of growth (Barbour and Lilburn, 1996). While injurious pecking rates between these

strains have not been published, there may be differences in behavior alongside the different maturity timelines.

In laying hens, feather pecking is heritable. One study bred females and males to create a low feather pecking line (LFP) and a high feather pecking line (HFP). Total pecking behavior was more prevalent in the second (7.04 pecks per hour) and third generations (13.9 pecks per hour) of the HFP than in the LFP (3.85 and 2.51 pecks per hour, respectively). Plumage conditions also improved across the generations of the LFP. There was no effect of selection on aggressive pecking behavior, but body weight was positively correlated with pecking behavior. Selection had an impactful effect in influencing pecking behavior in these lines as the behavior changed accordingly throughout three generations (Kjaer et al., 2001).

Following the test conditions of the previously mentioned study, another study was conducted to determine a dominant allele associated with pecking behavior for eight generations of laying hens (Labouriau et al., 2009). The proportion of high feather pecking birds (birds that pecked over 15 times) increased throughout eight generations, except in the fifth generation due to sires acting as the control line. The data suggests the inheritance pattern of high feather pecking appears consistent with a complete dominance model, with the phenotypic frequency estimated at 0.5%. High feather pecking birds had 3.24% of the 14,077 investigated genes that were found to be differentially transcribed from the normal peckers (birds that pecked $0 \leq 15$ times). It was suggested that the dominant allele responsible for feather pecking can be efficiently removed from a population in one to two rounds of selection (Labouriau et al., 2009). Taken together, research in laying hens and turkeys suggests that injurious pecking differs between genetic strains.

Mitigating the Problem

Several practices have been implemented to mitigate injurious pecking. Immediately after poults are hatched, infrared lasers are used in hatcheries to trim the upper and lower mandible tips of beaks (Dalton et al., 2013; van Staaveren et al., 2021). However, skin injuries persist in flocks with treated beaks, possibly due to birds being less able to grasp feathers and repeatedly pecking as a result (Leighton et al., 1985). Various lighting practices have also been utilized to reduce pecking behaviors. Lower light intensities, intermittent lighting schedules, and light source (i.e., fluorescent instead of incandescent) have been used to mitigate the behavior by modifying behavioral rhythms to reduce feather pecking (Moinard et al., 2001), but lower light intensities in particular can lead to eye and musculoskeletal disorders (reviewed by Dalton et al., 2013).

Adding environmental enrichment has been found to mitigate injurious pecking in laying hens. Enrichment includes items that can be manipulated or substrates that can be ingested that can be added to the environment (van Staaveren and Harlander, 2020). Adding enrichment provides motor and sensory stimuli, facilitating the natural expression of species-specific behaviors (van Staaveren et al., 2021). Poultry may have an inherent drive to reach a minimal requirement of pecking activity, with this motivation being redirected towards flock mates in barren environments (van Staaveren and Harlander, 2020). Compared to novel object and dustbathing substrate enrichment, chickens provided with foraging substrates had the lowest feather pecking frequencies compared to other enrichments. Consumable substrates, like straw or grain, are effective at reducing feather pecking than non-destructible objects (Dixon et al., 2010). A meta-analysis found that enrichment that encouraged foraging was more effective in reducing feather pecking in laying hens, and also found a stronger effect from using enrichment in barren cage systems (van Staaveren et al., 2021).

In turkeys, adding novel objects to the environment has reduced feather pecking in some cases. When provided with hanging iron sheets and straw bales on the ground as environmental enrichment, turkeys pecked at both objects (Martrenchar et al., 2001). Males had fewer head injuries and better feather coverage, and both sexes had fewer wing injuries, compared to birds without enrichment (Martrenchar et al., 2001). When provided with a variety of enrichment items (straw bale, platforms, pecking block, and tunnel), each item had an impact on feather condition. Straw bales and tunnels prompted improvement in wing feather condition as these items provided an opportunity to forage, in addition to protection from other birds. Platforms and straw bales improved neck feather condition as they offered opportunities to escape aggressive birds (Dong et al., 2023).

However, not all enrichment items mitigate pecking behavior. When offered elevated platforms, pecking stones, and alfalfa silage, two different strains of turkeys did not reduce pecking behavior, and the least injured birds were reared without enrichment (Haug et al., 2023). The researchers attributed their findings to the layout of the floor reducing the amount of time spent lying and resting, but the study also had different stocking densities and group sizes between treatments that may have confounded results (Haug et al., 2023). Providing colorful, plastic balls to turkey flocks also did not mitigate feather pecking in commercial turkeys, which was attributed to the balls losing novelty over time and/or not being friable to stimulate redirected foraging behavior (Duggan et al., 2014). Most studies have found enrichment to be beneficial to improving flock health and welfare in laying hens, although not all enrichment has been successful in reducing this injurious behavior in turkeys. Research investigating which enrichment to use based on bird preference should be conducted to determine what best deters pecking behavior (Dalton et al., 2013). Consideration should be given to factors such as the

material and the placement (hanging, free-standing) when determining which enrichment to utilize.

Understanding the Underlying Motivation

Understanding the underlying motivations that drive behavior is critical in improving animal welfare. Motivation is the affective state where a negative outcome is being avoided or a reward is being pursued (Mason and Bateson, 2009). Positive motivations can include access to resources to satisfy species-specific behaviors (e.g., food, social interactions), while negative motivations include aversive or defensive behaviors, like evading painful stimuli (Berridge, 2004). An animal's preference for a resource provides insight into which resource an animal is motivated to avoid or engage with (Kirkden and Pajor, 2006).

Preference experiments offer choices to an animal and rank how much time an animal spends between the choices to infer what the animal values (Fraser and Matthews, 1997). It is critical that preference tests provide a sufficient length of time to observe the animal's preference during fluctuations in the environment or the animal's physical condition (e.g., diurnal changes in activity or hunger; Fraser and Nicol, 2011). An animal's preference may be dependent on previous experiences with what is offered; familiarity can have diverse effects on a preference test's results, as animals can either be drawn to or avoid unfamiliar options, and preferences could change as animals acclimate to different options (Duncan, 1992); Fraser and Nicol, 2011). Conditioned place preference tests establish whether the animal has a learned preference for the location where the stimulus was presented during conditioning training, where the animal was exposed to both stimuli (Paul et al., 2018).

Preference tests have not been conducted with turkeys to our knowledge, but a preference test was used in broiler chickens to evaluate how broilers' value foraging opportunities via

contrafreeloading. A study classified free-range broilers as low-frequency rangers in the outdoor access environment or high-frequency rangers and performed a conditioned preference test for contrafreeloading (Ferreira et al., 2021). For the test, the birds were conditioned to one chamber with mealworms freely available on the floor, and the adjacent chamber with foraging substrate and mealworms, then had access to both empty chambers during testing. The low rangers foraged significantly less on the contrafreeloading side than high rangers, suggesting individual differences in foraging preferences (Ferreira et al., 2021).

Individual differences in behavior can be due to a number of factors, including strain, social and physical environments that can impact their adaptability (Väisänen and Jensen, 2003). Considering the link between foraging behavior and feather pecking behavior (Huber-Eicher and Wechsler, 1997), a similar test may provide insight into whether a high feather pecking individual may also display a preference for foraging via contrafreeloading.

Conclusion

Injurious pecking in turkeys is a multifaceted issue influenced by a complex array of behavioral, environmental, physiological, and genetic factors. While foraging and social behaviors are natural in turkeys, commercial production systems alter their expression. Social structures shaped by early life experiences and hormones, like testosterone, contribute to the development of pecking hierarchies, while overcrowded and barren environments exaggerate aggression and stress. Genetic selection for fast growth and high meat yield may have inadvertently selected birds prone to injurious pecking. Previous work in turkeys has linked injurious pecking with age and environmental conditions, but there has been limited work investigating the relationship between foraging, feeding, and injurious pecking, as well as hormones associated with social and feeding behaviors. Mitigation strategies, such as

environmental enrichment, have had mixed success. Testing additional enrichment items in turkeys is warranted. Preference testing, while underutilized in turkeys, offers a potential avenue for understanding behavioral needs and creating welfare interventions accordingly.

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CHAPTER 2

Evaluating the Prevalence of Injurious Pecking and Foraging Behaviors in Two Genetic Strains of Turkeys with or without Environmental Enrichment

ABSTRACT

Injurious pecking is an animal welfare and economic concern in commercial turkey production. Environmental enrichment has had mixed success in mitigating and redirecting pecking behavior. We aimed to assess how a hanging jute rope affected the prevalence of injurious pecking and foraging behaviors and production performance in two commercial Large White genetic strains. Turkey toms (N=1,056) from two commercial genetic strains (A and B) were randomly assigned to pens on day of hatch (22 birds/pen, 24 pens/strain). At 5 wk, a hanging jute rope enrichment was added to half of the pens (n=12 pens/strain/enrichment treatment, CON or ROPE). Body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were collected at 4, 7, 10, 13, 16, and 19 wk. Video was recorded at 4 and 18 wk in half of the pens (n=6 pens/strain/enrichment treatment) for 20 min in the morning and afternoon to analyze the number of pecks directed at head/neck (HN) and back/wings/tail (BWT) and bouts of foraging. Rope pecking was evaluated at 5, 8, 11, 14, and 18 wk. Data were analyzed in R software with linear models or generalized linear mixed effect models. The ROPE minimally affected production metrics and did not affect the prevalence of any of the behaviors ($p>0.05$). Strain A turkeys had heavier BW, faster BWG, and higher FI than strain B turkeys throughout most of the trial ($p\leq0.05$). Strain A turkeys also performed more HN pecking ($p<0.001$) and rope pecking ($p=0.002$) than strain B turkeys. Both strains performed similar frequencies of BWT pecks and foraging bouts ($p>0.05$). The 4 wk poults performed more HN pecking ($p<0.001$), BWT pecking ($p=0.014$), and foraging bouts than 18 wk toms ($p<0.001$).

Turkeys performed more BWT pecking ($p<0.001$) and more foraging ($p=0.045$) in the afternoon, while HN pecking was similar across the day ($p=0.81$). The ROPE was not an effective enrichment possibly due to loss of novelty and/or lack of complexity.

INTRODUCTION

Injurious pecking in farmed turkeys occurs when a bird repeatedly pecks at another bird's plumage and/or skin, resulting in feather removal, skin lesions, and cannibalism (Dalton et al., 2013; Duggan et al., 2014). The injuries are an animal welfare concern as feather plucking is painful and can lead to tissue pecking, which results in hemorrhaging and is associated with culling and mortality (Savory, 1995; Mench, 2009; Duggan et al., 2014). More than one-third of turkey farmers reported cannibalism as a main cause of mortality and culling within a flock (van Staaveren et al., 2020), and the prevalence of skin injuries affected up to 40% and 97% of turkeys within a flock on the head/neck and back/tail regions, respectively (Leishman et al., 2022). Consequently, this behavior also prompts economic concerns with the loss of birds before they reach processing age, and carcass downgrades at the processing plant from skin defects (Leishman et al., 2022). Over one-third of turkey farmers ranked skin condition as a leading reason for carcass condemnations, with 33.8% related to skin and 64.7% related to subcutaneous conditions (van Staaveren et al., 2020).

The cause of injurious pecking has primarily been investigated in laying hens and linked to redirected foraging behavior (van Staaveren and Harlander, 2020). Wild chickens and turkeys spend the majority of their day foraging, which involves exploratory pecking, scratching, and consuming feedstuffs (Dawkins, 1989; van Staaveren and Harlander, 2020). Feed is formulated to effectively meet the nutritional needs of farmed poultry (Bryden et al., 2021), so the time spent consuming feedstuffs is reduced compared to their wild counterparts. Exploratory pecking

from foraging is typically redirected to the back, wings, and tails of flock mates (Savory, 1995; Dalton et al., 2013). On the other hand, head, snood, and neck pecking is commonly displayed by turkeys during bouts of aggression to establish or maintain dominance (Watts and Stokes, 1971; Savory, 1995; Buchwalder and Huber-Eicher, 2003). Therefore, pecking in different body regions is associated with different underlying motivations.

Genetics may have an influence on predisposing certain turkey strains to peck more than others. A strain selected for meat production and a traditional, non-selected strain both performed feather pecking, but skin damage only occurred in the strain selected for meat production (Busayi et al., 2006). Amongst strains selected for meat production, there are differences in the prevalence of injurious pecking that appear to be associated with growth rate. A slow-growing, light-weight breed displayed less damage from pecking on the head, neck, and snood regions compared to a heavier, fast-growing breed (Haug et al., 2023). A slow growing, light-weight breed also experienced fewer body and snood injuries than moderate-weight and heavy-weight, fast growing breeds (Grün et al., 2021).

Environmental enrichment is a strategy to mitigate injurious pecking in turkeys by redirecting pecking behavior towards objects rather than flock mates. Effective environmental enrichment improves the biological functioning of animals (Newberry, 1995). Male poults reared with visual barriers, chains and plastic strips attached to walls, and straw in the litter experienced fewer pecking injuries by 35 days of age than poults reared without enrichment (Sherwin et al., 1999). However, the study did not determine which enrichments were most effective and the treatments also differed in the light sources provided. Hanging metal sheets and placing straw bales on the litter decreased aggressive pecking behavior and injuries on the wings, tail, and head for male turkeys at 10 wk compared to turkeys reared without enrichment (Martrenchar et al.,

2001). The authors noted that straw bales were quickly deconstructed and scattered by birds, starting at 12 wk. However, colored plastic balls placed on the floor in commercial turkey houses had minimal effects on pecking behavior, possibly because the balls were not destructible or manipulable to emulate foraging material (Duggan et al., 2014).

The objective of this study was to assess how a hanging, destructible jute rope environmental enrichment affected the prevalence of injurious pecking and foraging behaviors and production performance in two commercial Large White genetic strains. We hypothesized that enrichment would impact the performance of injurious pecking and foraging behavior. We predicted that birds housed with rope enrichment would redirect pecking to the rope and thus perform less injurious pecking and foraging behavior than birds housed without enrichment. We predicted that enrichment would not impair production performance. Lastly, since both genetic strains are selected for fast growth, we predicted that they would perform similar frequencies of injurious pecking and foraging, and that injurious pecking would increase with age in treatment groups and strains.

MATERIALS AND METHODS

All experimental procedures were reviewed and approved by the North Carolina State University Institutional Animal Care and Use Committee (Protocol #21-393-A). Animal care protocols were based on the 4th edition of the Guide for the Care and Use of Agricultural Animals in Research and Teaching.

Animals and housing

At 1 d of age, 1,056 turkey tom poults (*Meleagris gallopavo*) from two commercial large white turkey strains (strain A and strain B) were randomly assigned to one of 48 pens (2.44 × 3.51 m; 22 birds/pen, 24 pens/strain). Pens were organized into four blocks, with 12 pens/block,

in a curtain-sided house with concrete floors at the North Carolina State University Talley Turkey Education Unit (Raleigh, NC). The house was equipped with fans for negative air pressure power ventilation. Each pen contained one bell drinker (Plasson Turkey Bell Drinker, Plasson Livestock, Maagan Michael, Israel), one tube feeder (Manola T, Giordano Global, Springfield, MO), and fresh pine wood shavings for bedding that were top dressed as needed. During the first week, poults were reared in a brooder ring with a heat lamp and additional supplemental feeders and drinkers. Heat lamps were fitted with thermostats and placed above each brooder ring. The temperature under the lamps was set to 35°C at placement, then 32°C on day 2, 31°C on day 7, and 29°C on day 10. On day 14, the house temperature was decreased 2°C each week until ambient temperature was reached. Brooder rings and supplemental feeders and drinkers were removed at 1 wk of age, and heat lamps were removed at 4 wk of age.

All feed was manufactured at the North Carolina State University Feed Mill Education Unit (Raleigh, NC). Feed and water were provided *ad libitum*. Feed was formulated following the appropriate breeder guidelines and provided in six diet phases throughout the trial (Table 1.1). Diet phase changes occurred approximately every 3 wk: starter 1 (0 to 4 wk), starter 2 (4 to 7 wk), grower 1 (7 to 10 wk), grower 2 (10 to 13 wk), finisher 1 (13 to 16 wk), and finisher 2 (16 to 19 wk). The weight of feed added during each phase was recorded, and feed and feeders were weighed at the end of each phase. The lighting schedule was maintained with natural and artificial light, with 24 hours of light for the first 2 days, then 18 hours of light for the remainder of the trial.

Experimental design

A 2x2 complete randomized block design was utilized with two commercial Large White genetic strains – strain A and strain B – and two enrichment treatments – control with no

enrichment (CON) or a hanging jute rope provided at 5 wk of age (ROPE). At 5 wk, a twisted jute rope (Yuzenet, Amazon.com; 3.8 cm diameter, 30.5 cm L, Figure 1.1) was hung from the ceiling at turkey eye level in half of the pens for each genetic strain. The rope was continuously adjusted as birds grew to maintain it at bird eye level, and the rope was replaced as needed when only a few rope strands remained. Treatments were randomly and evenly distributed within each block of the house (n=12 pens/strain/enrichment treatment). One pen of strain B was removed due to high mortality (50%) from leg health issues, resulting in 11 pens of CON for strain B.

Production performance

Turkeys were group-weighed at placement and then individually weighed at 4, 7, 10, 13, 16, and 19 wk of age to correspond with feed phase changes. Body weights and feed weights from each pen were utilized to determine average bird body weight (BW), average bird feed intake (FI), average bird body weight gain (BWG), and feed conversion ratio (FCR). The FCR was corrected for mortality by dividing total feed consumed by the sum of the final body weights of surviving birds and the body weights of the mortalities. All mortalities and birds identified for humane culling were promptly removed from pens, and their BW and feeder weights were recorded.

Behavior

Color video cameras (4K Smart IP Fixed Bullet Camera, Lorex Corporation, Irvine, CA) were placed in the top left corner of 6 pens/strain/enrichment treatment to continuously capture footage. One pen of strain B CON was removed from the behavior analysis due to the aforementioned high mortality, so 5 pens were recorded for behavior for this treatment combination. Each camera was wired to a network video recorder (4K 16-channel Wired NVR System, Lorex Corporation, Irvine, CA) to review and save video files.

At 4 and 18 wk, head/neck (HN) pecking, back/wing/tail (BWT) pecking, and foraging were recorded and analyzed (Table 1.2). At 5, 8, 11, 14, and 18 wk, rope pecking was recorded and analyzed for ROPE pens. At all ages, the frequency of behaviors was coded continuously for 20-min segments at 10 AM and 4 PM. These times were selected to capture diurnal changes during the photoperiod when there were no routine husbandry disruptions. Behaviors were coded from video files using Behavioral Observation Research Interactive Software (BORIS, version 8.19.3, http://www.boris.unito.it/, Friard and Gamba, 2016) by 9 trained observers for behaviors at 4 and 18 wks. Inter-rater agreement was calculated by comparing behavioral point event counts between the trainer and trainee for the three behaviors using Google Sheets (version 91.0, Googleplex, Mountain View, CA) to achieve 90% or higher inter-rater agreement across all observers. Rope pecking behavior was coded by one observer with the same BORIS software.

Statistical analysis

Data were analyzed with R statistical software (version 4.4.2; R Core Team, 2021) using RStudio (version 2024.12.0+467) for mac OS Sequoia 15.6. Production performance data (BW, BWG, FI, and FCR) were analyzed with separate linear models for each age (lm function in base R). Genetic strain (factor with 2 levels: A and B), enrichment treatment (factor with 2 levels: CON and ROPE), their two-way interaction, and block (factor with 4 levels: 1, 2, 3, and 4) were included as fixed effects. Linear model fits were assessed for normality and homogeneity of variance using quantile-quantile plots and plots of residuals versus fitted values (plot function in base R). A type III ANOVA (car package, version 3.1.3, Fox and Weisberg, 2019) was used to obtain P-values for main effects and interactions.

Behavior data (BWT pecking, HN pecking, foraging, and rope pecking) were analyzed with generalized linear mixed effects models with a negative binomial distribution (glmmTMB

package, version 1.1.10, Brooks et al., 2017). Genetic strain (factor with 2 levels: A and B), enrichment treatment (factor with 2 levels: CON and ROPE), age (factor with 2 levels: 4 and 18 wk), time of day (factor with 2 levels: 10 AM and 4 PM), and their two-way, three-way, and four-way interactions were included as fixed effects for BWT pecking, HN pecking and foraging. Rope pecking included genetic strain (factor with 2 levels: A and B), age (factor with 5 levels: 5, 8, 11, 14, and 18 wk), time of day (factor with 2 levels: AM and PM), and their two-way and three-way interactions as fixed effects. Pen and an offset of the number of birds in each pen were included as random effects in all models. Generalized linear mixed effects model fits were assessed for deviations from their expected distribution, overdispersion, outliers, and homogeneity of variance via plot and test functions in the DHARMa package (version 0.4.7; Hartig, 2024). The final generalized linear mixed effect models were obtained by a stepwise backward reduction using ANOVA for model comparison with a P value of >0.05 as the criterion of exclusion. All model estimates and 95% confidence intervals were obtained with the emmeans package (version 1.10.5, Lenth, 2024)

RESULTS

Production performance

Body weight. Strain A had heavier BW than strain B at placement ($F=44.75$, $p<0.001$), 10 wk ($F=5.19$, $p=0.028$), 13 wk ($F=12.68$, $p<0.001$), 16 wk ($F=15.77$, $p<0.001$), and 19 wk ($F=42.04$, $p<0.001$; Table 1.3). The ROPE toms were heavier than CON toms at 16 wk ($F=4.04$, $p=0.051$). Enrichment did not affect BW at any other age, and there were no significant interactions between strain and enrichment at any age.

Body weight gain. Strain A displayed higher BWG than strain B from 7 to 10 wk ($F=14.54$, $p<0.001$), 10 to 13 wk ($F=11.76$, $p=0.001$), 13 to 16 wk ($F=5.82$, $p=0.020$), and 16 to

19 wk (F=24.23, p<0.001). Enrichment did not affect BWG during any period, and there were no significant interactions between strain and enrichment during any period.

Feed intake. Strain A toms consumed more feed than strain B toms from 10 to 13 wk (F=4.77, p=0.035), 13 to 16 wk (F=13.79, p<0.001), and 16 to 19 wk (F=13.77, p<0.001). The ROPE toms consumed more feed than CON toms from 13 to 16 wk (F=4.11, p=0.049). Enrichment did not affect FI during any other period, and there were no significant interactions between strain and enrichment during any period.

Feed conversion ratio. There were no effects of strain, enrichment, or their interaction on FCR.

Behavior

Back/Wing/Tail pecking. Both genetic strains performed similar frequencies of BWT pecking (χ^2=2.45, p=0.12; Figure 1.2). Enrichment did not mitigate the incidence of BWT pecking (χ^2=0.58, p=0.45). The 4 wk poults performed more BWT pecking than the 18 wk toms (χ^2=6.02, p=0.014). More BWT pecking was observed in the afternoon than in the morning (χ^2=15.10, p<0.001). There were no significant two-way, three-way, or four-way interactions between genetic strain, enrichment, age, or time of day.

Head/Neck pecking. Strain A performed more HN pecking than strain B (χ^2=15.76, p<0.001; Figure 1.3). Enrichment did not affect the frequency of HN pecking (χ^2=0.07, p=0.79). The 4 wk poults displayed more HN pecking than the 18 wk toms (χ^2=42.56, p<0.001). Similar frequencies of HN pecking were observed in the morning and afternoon (χ^2=0.05, p=0.81). There were no significant two-way, three-way, or four-way interactions between genetic strain, enrichment, age, or time of day.

Foraging. Both genetic strains displayed similar frequencies of foraging behavior (χ^2=0.52, p=0.47; Figure 1.4). Enrichment did not affect the number of foraging bouts observed (χ^2=2.65, p=0.10). Foraging was observed more frequently in the 4 wk poults than the 18 wk toms (χ^2=178.43, p<0.001). Birds performed more foraging bouts in the afternoon than in the morning (χ^2=4.00, p=0.045). There were no significant two-way, three-way, or four-way interactions between genetic strain, enrichment, age, or time of day (p>0.05 for all).

Rope pecking. Strain A pecked at the rope more than strain B throughout the trial (χ^2=9.54, p=0.002; Figure 1.5). At 5 wk, birds pecked the rope more than at 8 wk (χ^2=10.33, p=0.035), but no other pairwise differences between ages were detected. Rope pecking was similar between the morning and afternoon (χ^2=3.52, p=0.061). There were no significant two-way or three-way interactions between genetic strain, age, or time of day.

DISCUSSION

Environmental enrichment

Turkeys pecked at the hanging rope enrichment throughout rearing, but the presence of the rope did not mitigate BWT pecking, HN pecking, or foraging and had minimal influence on production performance metrics. This aligns with the findings from Busatta et al. (2024), where hanging plastic bottles filled with blue water as enrichment for turkey hens also did not affect gentle pecking or injurious pecking. Similarly, previous research reported that dispersing colored plastic balls throughout a commercial house did not reduce severe pecking behavior in turkey toms (i.e., pecking that removed/damaged feathers or skin; Duggan et al., 2014). Both studies hypothesized that these enrichment items were ineffective because the objects did not address the underlying causes of injurious pecking. The objects did not serve as foraging material as they were not destructible or manipulable and may have lost their novelty to birds over time. We

hypothesized that the destructible hanging rope in the present experiment would serve these functions. However, it is possible that the rope did not provide enough novelty for birds to redirect pecking from flock mates. Previous work found that rotating novel hanging objects weekly was more effective in reducing injurious pecking than scattering straw or grain two to three times per week, respectively (Crowe and Forbes, 1999).

Providing a variety of enrichments may be more effective in redirecting turkeys than a single enrichment, as they can serve multiple functions. Compared to turkeys housed without enrichment, the prevalence of head, wing, and tail injuries decreased when hanging metal sheets and straw bales on the litter were both available as enrichment (Martrenchar et al., 2001). Turkeys pecked at the straw bales as often or even more than the hanging metal sheets (Martrenchar et al., 2001). Dong et al. (2023) evaluated a variety of enrichment items for turkey toms and found different effects on feather condition. Neck feather condition improved when toms had access to platforms or platforms and straw bales simultaneously, as they provided a space for turkeys to escape aggressive encounters with other birds. Wing feather condition improved when toms had access to straw bales or tunnels, which was attributed to fulfilling foraging motivations and providing a hiding place from other birds, respectively (Dong et al., 2023). Turkey hens provided with a "turkey tree" structure consisting of three elevated tiers displayed less feather pecking and mostly improved feather and injury scores compared to the control group, as these birds had an opportunity to evade aggressive encounters (Lindenwald et al., 2021). Therefore, offering a variety of enrichments, including opportunities for birds to escape from other birds, may be most effective. One study found that a combination of pecking blocks, an elevated platform, and silage feeding resulted in more severe head injuries than birds housed without enrichment (Haug et al., 2023). However, the control and enriched treatments

were confounded with different stocking densities and group sizes, which also affect pecking behavior (Erasmus, 2017; Haug et al., 2023).

Genetic strain

Strain A displayed more HN pecking and rope pecking than strain B. Pecking directed at HN is associated with aggression (Dalton et al., 2018). With the rope placed at turkey eye level, it was a more attractive enrichment item for the strain predisposed to HN pecking. Previous work associated a faster growth rate with a higher prevalence of injuries on the head, neck, and snood regions specifically (Grün et al., 2021; Haug et al., 2023). Correspondingly, strain A had higher body weights, faster body weight gain, and higher feed intake than strain B throughout most of the current trial. In behavioral ecology, positive correlations have been reported between growth rate and aggressive territorial behavior as a means of ensuring food resources in brown trout (Lahti et al., 2001). In farmed laying hens and pigs, feather pecking and tail biting have also been correlated with production traits (reviewed by Brunberg et al., 2016). The underlying mechanism linking growth rate, HN pecking, and injuries remains unclear in turkeys.

In the present study, both genetic strains performed similar rates of BWT pecking and foraging. Another study also found no differences between fast- and slow-growing turkey strains for back and wing injuries (Haug et al., 2023). However, a different study reported more body injuries in faster-growing strains compared to a slow-growing strain (Grün et al., 2021). We did not measure injuries, but since strains with different growth rates showed no behavioral differences in these body regions, BWT pecking and foraging likely lack a genetic-related mechanism in commercial strains.

Age

Contrary to our hypothesis, the younger 4 wk-old poults performed BWT pecking, HN pecking, and foraging more frequently than the older 18 wk-old toms. Previous research reported the prevalence and severity of pecking-related injuries to increase as turkeys age (Leishman et al., 2022; Haug et al., 2023). Similarly, pecking-related skin lesions identified at the processing plant indicates pecking injuries made at an older age (Allain et al., 2013; van Staaveren et al., 2020). This is likely due to turkeys becoming stronger and thus more damaging as they get older, even if they are performing lower frequencies of the behavior (Busayi et al., 2006).

Injurious pecking reportedly begins as early as 1 to 2 wk of age in turkeys (Moinard et al., 2001). Young poults tend to be more active by engaging in more foraging, feeding, and exploratory pecking, followed by reducing activity and sitting more as they age (Marchewka et al., 2013). Young laying hen pullets (13 to 15 wk) also performed more gentle feather pecking than adult laying hens (17 to 37 wk; Newberry et al., 2007). Additionally, the frequency of performing severe and stereotyped gentle feather pecks as an adult laying hen was positively correlated with foraging behavior as a pullet (Newberry et al., 2007). There may be a relationship between BWT pecking and foraging in turkeys that warrants further investigation, considering that both are more frequent in younger poults.

To investigate this relationship, it will be critical to distinguish between gentle feather pecking and severe feather pecking in future work. In the present study, we counted each individual peck directed at the HN and BWT regions, but did not differentiate between gentle and severe pecking. Gentle feather pecking is defined as a turkey opening and closing its beak on the back, wing, or tail feathers of a flock mate without pulling on the feathers, while severe feather pecking is defined as visibly pulling on the feathers or skin of a flock mate (Dalton et al., 2018). The prevalence of gentle and severe feather pecks in turkeys varies among age, sex, and

genetic strain (Busayi et al., 2006). When comparing turkeys that performed more severe feather pecking to turkeys that performed more HN pecking, the severe feather pecking turkeys tended to perform more gentle feather pecking (Dalton et al., 2018). However, individual variation between turkeys and small sample sizes in previous work made it difficult to understand the relationships between the different types of pecking and feeding and foraging behavior, so disentangling these connections would be a valuable topic for future work (Dalton et al., 2018).

Time of day

While HN pecking frequencies remained similar across the morning and afternoon, BWT pecking and foraging bouts both increased in the afternoon. Historically, BWT pecking has been associated with redirected foraging in laying hens (reviewed by Brunberg et al., 2016), but this relationship has not been established in turkeys. Laying hens and turkeys are both diurnal, with their behaviors being influenced by light intensity and duration (Mench, 2009). Laying hens housed in cage-free aviaries were more commonly observed in the open litter area that is designated for foraging in the afternoon (Campbell et al., 2016). We observed a similar timing for turkeys' foraging behavior in the present study, which, to the best of our knowledge, has not been previously reported.

Our turkeys were reared in a curtain-sided house, and turkeys are more active in curtain-sided barns with natural sunlight compared to environmentally controlled houses (Duggan et al., 2014). We did not measure light intensity, but it is possible that light was brighter in the afternoon, stimulated activity, and made litter and feathers more visible for foraging and BWT pecking, respectively (Duggan et al., 2014). Turkeys reared in low-intensity light are generally less active and display fewer BWT injuries (Sherwin et al., 1999; Moinard et al., 2001; Marchewka et al., 2013). Elucidating the relationship between circadian rhythm, light intensity,

and pecking and foraging behaviors would offer additional management tools for mitigating pecking behavior.

CONCLUSION

Hanging jute rope enrichment was not effective in mitigating injurious pecking or redirecting foraging behavior in two commercial Large White turkey strains, regardless of consistent interaction with the rope. However, this study found insights into HN and BWT pecking and foraging amongst genetic strains, ages, and times of day. Strain A had a heavier body weight, faster body weight gain, higher feed intake, and more HN and rope pecking than strain B. Foraging and BWT pecking followed similar temporal patterns, where both behaviors were more frequent in younger poults and in the afternoon. These insights may offer an association between BWT pecking and foraging in turkeys and highlight temporal predictors as to when these behaviors occur. Environmental enrichment programs that address underlying causes of pecking behavior related to genetics, age, and time of day may be more effective.

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Table 1.1 Diet ingredients and nutrient composition of all diet phases fed to turkey toms from 0 to 19 wk.

Ingredient, % "as fed"	**Starter 1**	**Starter 2**	**Grower 1**	**Grower 2**	**Finisher 1**	**Finisher 2**
Age (wk)	0-4	4-7	7-10	10-13	13-16	16-19
Form	Crumble	Small Pellet	Pellet	Pellet	Pellet	Pellet
Corn	18.4	23.6	29.5	37.2	40.6	44.9
Wheat	20	20	20	20	20	20
Soybean meal	37	33	28	20	17.5	13.8
Poultry meal	10	10	10	10	10	10
Poultry Fat	8	7.005	7.4	7.975	8	7.315
Limestone	1.875	1.725	1.475	1.35	1.05	1.1
Monocalcium Phosphate	2.35	2.65	1.8	1.7	1.3	1.35
Salt	0.2	0.2	0.2	0.2	0.2	0.2
Mineral Mix	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin Mix	0.2	0.2	0.2	0.2	0.2	0.2
Selenium Mix	0.05	0.05	0.05	0.05	0.05	0.05
Choline Chloride	0.2	0.2	0.2	0.2	0.2	0.2
Lysine	0.65	0.4	0.425	0.425	0.31	0.325
Methionine	0.475	0.4	0.35	0.3	0.23	0.21
Sodium Bicarbonate	0.2	0.21	0.1	0.1	0.1	0.1
Threonine	0.2	0.16	0.1	0.1	0.06	0.05
Total %	100	100	100	100	100	100

Table 1.1 continued

Calculated analysis, % (unless otherwise noted)						
Crude Protein	29.7	28	25.5	22	20.8	19.2
Crude Fat	10.9	10.2	10.6	11.3	10.9	10.9
Digestible Lysine	2.02	1.76	1.62	1.41	11.4	1.18
Digestible Methionine	0.87	0.79	0.72	0.63	0.55	0.52
Calcium	1.5	1.45	1.25	1.18	1	1.02
Available Phosphate	0.75	0.81	0.62	0.59	0.5	0.51
Sodium	0.2	0.2	0.17	0.17	0.17	0.17
Chloride	0.32	0.29	0.29	0.29	0.28	0.28
Metabolizable energy kcal/kg	3020.33	3060.02	3154.81	3289.3	3351.03	3364.25

Table 1.2 Definitions of behaviors observed in two genetic strains of Large White turkey toms. All behaviors were assessed as frequencies.

Behavior	Definition
Head/Neck pecking	Bird's beak comes into contact with another bird's head or neck.
Back/Wings/Tail pecking	Bird's beak comes into contact with another bird's body on their back, wings, and/or tail.
Foraging	Bird's beak comes into contact with the ground, sometimes coupled with their feet pawing at the ground. Behavior can be performed while the bird is sitting or standing. The foraging bout begins when the bird's beak comes into contact with the ground. If the beak is not in contact with the ground for 3 sec, or if the bird performs a different behavior before their beak contacts the ground again, the foraging bout ends.
Rope pecking	Bird's beak comes into contact with the rope enrichment.

Table 1.3 Production performance data for two genetic strains of Large White turkey toms (A and B) that were reared without (CON) or with (ROPE) a hanging rope enrichment. Model estimated means (95% Confidence Intervals) are presented for body weight (BW), body weight gain (BWG), feed intake (FI), and mortality-corrected feed conversion ratio (FCR). Superscripts differing within a row indicate statistically significant differences ($p<0.05$).

	Treatments				***P-Values***		
	A+CON	**A+ROPE**	**B+CON**	**B+ROPE**	**Strain**	**Enrichment**	**Strain × Enrichment**
Placement							
BW (kg)	0.07^{a} (0.06, 0.07)	0.07^{a} (0.06, 0.07)	0.05^{b} (0.05, 0.06)	0.07^{b} (0.05, 0.06)	<0.001	0.946	0.950
4 wk							
BW (kg)	1.30 (1.25, 1.36)	1.30 (1.25, 1.35)	1.25 (1.19, 1.30)	1.30 (1.25, 1.35)	0.152	0.178	0.301
BWG (kg)	1.27 (1.22, 1.33)	1.27 (1.21, 1.32)	1.22 (1.17, 1.28)	1.27 (1.22, 1.33)	0.258	0.194	0.312
FI (kg)	1.58 (1.51, 1.66)	1.57 (1.50, 1.65)	1.48 (1.41, 1.56)	1.56 (1.48, 1.63)	0.068	0.174	0.277
FCR	1.22 (1.18, 1.26)	1.22 (1.19, 1.26)	1.20 (1.16, 1.24)	1.21 (1.17, 1.24)	0.464	0.766	0.912
7 wk							
BW (kg)	3.93 (3.80, 4.06)	3.96 (3.82, 4.09)	3.86 (3.72, 4.00)	3.97 (3.83, 4.01)	0.467	0.275	0.565

BWG (kg)	2.63 (2.54, 2.72)	2.66 (2.57, 2.75)	2.61 (2.52, 2.71)	2.67 (2.57, 2.76)	0.821	0.412	0.828

Table 1.3 (continued).

FI (kg)	3.70 (3.59, 3.80)	3.73 (3.62, 3.83)	3.68 (3.57, 3.79)	3.64 (3.54, 3.75)	0.781	0.656	0.569
FCR	1.35 (1.32, 1.37)	1.34 (1.32, 1.37)	1.33 (1.31, 1.36)	1.31 (1.28, 1.33)	0.433	0.126	0.338
10 wk							
BW kg)	8.03[a] (7.83, 8.22)	8.08[a] (7.88, 8.27)	7.70[b] (7.50, 7.91)	7.95[b] (7.45, 8.14)	0.028	0.096	0.342
BWG (kg)	4.10[a] (4.01, 4.19)	4.11[a] (4.03, 4.21)	3.85[b] (3.75, 3.94)	3.98[b] (3.88, 4.07)	<0.001	0.055	0.243
FI (kg)	6.81 (6.58, 7.05)	7.00 (6.77, 7.23)	6.49 (6.25, 6.74)	6.79 (6.55, 7.02)	0.062	0.087	0.664
FCR	1.52 (1.49, 1.55)	1.55 (1.52, 1.57)	1.53 (1.51, 1.56)	1.52 (1.49, 1.55)	0.504	0.504	0.122
13 wk							
BW (kg)	12.82[a] (12.56, 13.09)	12.80[a] (12.53, 13.06)	12.16[b] (11.88, 12.43)	12.45[b] (12.18, 12.71)	<0.001	0.130	0.237
BWG (kg)	4.80[a] (4.66, 4.94)	4.72[a] (4.58, 4.86)	4.45[b] (4.30, 4.60)	4.50[b] (4.36, 4.64)	0.001	0.634	0.382

FI (kg)	10.24[a] (9.91,10.58)	10.02[a] (9.68, 10.36)	9.72[b] (9.37, 10.07)	9.86[b] (9.52, 10.19)	0.035	0.565	0.293

Table 1.3 (continued).

FCR	1.71 (1.67, 1.76)	1.75 (1.70, 1.80)	1.72 (1.67, 1.77)	1.72 (1.67, 1.76)	0.832	0.909	0.395
16 wk							
BW (kg)	17.60[ad] (17.25, 17.95)	17.81[ac] (17.46, 18.16)	16.61[bd] (16.25, 16.97)	17.11[bc] (16.76, 17.46)	<0.001	0.051	0.409
BWG (kg)	4.78[a] (4.59, 4.96)	5.01[a] (4.82, 5.20)	4.45[b] (4.26, 4.65)	4.66[b] (4.78, 4.85)	0.020	0.121	0.903
FI (kg)	11.89[a] (11.55, 12.22)	11.85[a] (11.52, 12.19)	11.00[b] (10.65, 11.35)	11.48[b] (11.15, 11.82)	<0.001	0.049	0.134
FCR	1.92 (1.88, 1.96)	1.93 (1.89, 1.98)	1.91 (1.87, 1.95)	1.92 (1.88, 1.96)	0.821	0.756	0.850
19 wk							
BW (kg)	22.07[a] (21.72, 22.42)	22.21[a] (21.86, 22.55)	20.45[b] (20.09, 20.82)	20.90[b] (20.56, 21.25)	<0.001	0.079	0.386
BWG (kg)	4.47[a] (4.29, 4.64)	4.40[a] (4.22, 4.58)	3.85[b] (3.66, 4.03)	3.79[b] (3.62, 3.97)	<0.001	0.665	0.948

FI (kg)	13.23[a] (12.74, 13.72)	13.00[a] (12.51, 13.49)	11.93[b] (11.42, 12.44)	12.05[b] (11.56, 12.54)	<0.001	0.735	0.485
FCR	2.10 (2.05, 2.15)	2.14 (2.10, 2.19)	2.13 (2.08, 2.18)	2.13 (2.08, 2.18)	0.421	0.932	0.419

Figure 1.1 A twisted jute rope was hung from the ceiling at turkey eye level as an enrichment item for turkey toms. Left image: Rope condition of a newly placed rope. Right image: Rope condition after turkey interaction.

Figure 1.2 The frequency of pecking on the back/wings/tail (BWT) region of flock mates by turkey toms in a 20-min continuous observation period. An asterisk (*) indicates significant differences between factors. Raw data are displayed in box plots, where boxes represent the interquartile range, the black line represents the median, the top of the box represents the 75th quartile, and the bottom of the box represents the 25th quartile. Whiskers represent the minimum and maximum values, while gray dots indicate outliers. The back X overlaying the box plots is the estimated mean value.

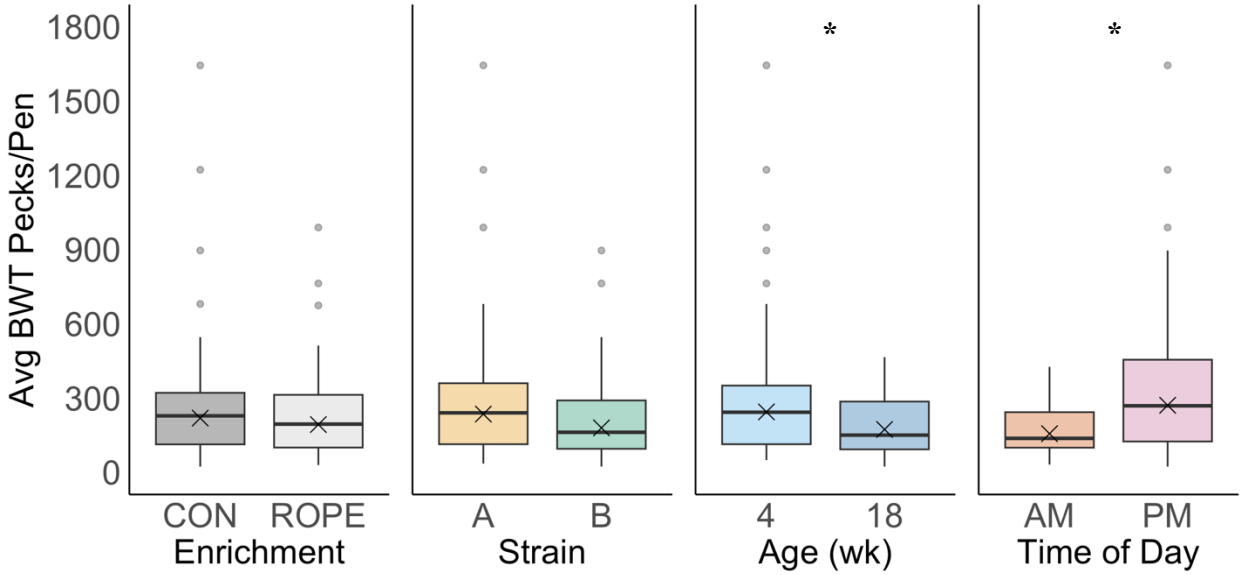


Figure 1.3 The frequency of pecking on the head/neck (HN) region of flock mates by turkey toms in a 20-min continuous observation period. An asterisk (*) indicates significant differences between factors. Raw data are displayed in box plots, where boxes represent the interquartile range, the black line represents the median, the top of the box represents the 75th quartile, and the bottom of the box represents the 25th quartile. Whiskers represent the minimum and maximum values, while gray dots indicate outliers. The back X overlaying the box plots is the estimated mean value.

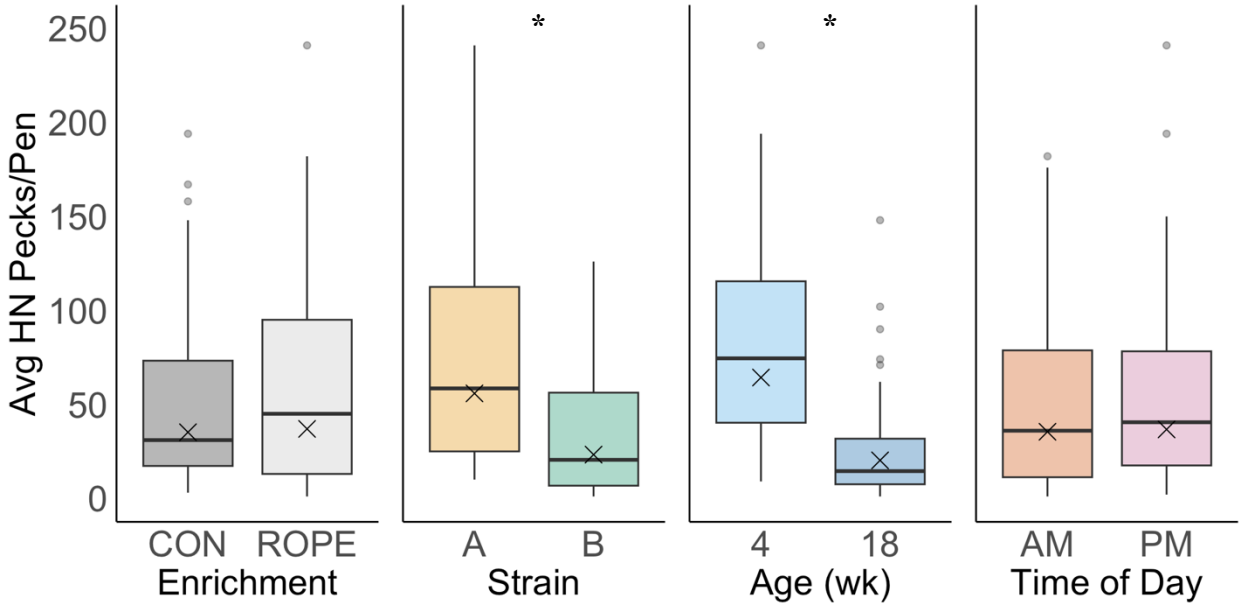


Figure 1.4 The frequency of foraging bouts by turkey toms in a 20-min continuous observation period. An asterisk (*) indicates significant differences between factors. Raw data are displayed in box plots, where boxes represent the interquartile range, the black line represents the median, the top of the box represents the 75th quartile, and the bottom of the box represents the 25th quartile. Whiskers represent the minimum and maximum values, while gray dots indicate outliers. The back X overlaying the box plots is the estimated mean value.

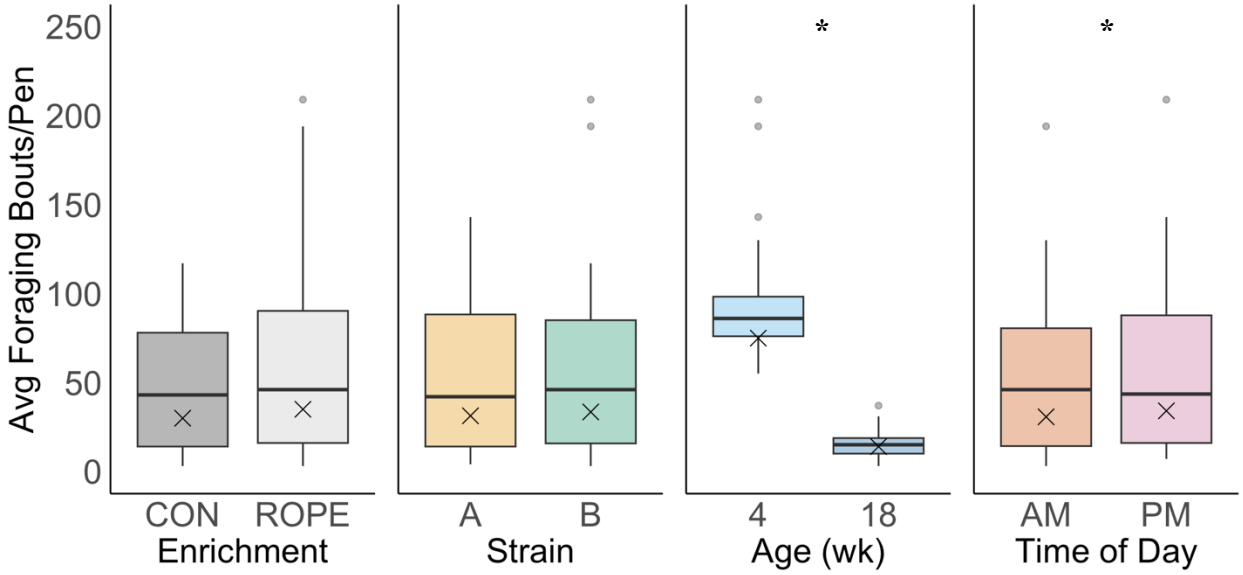
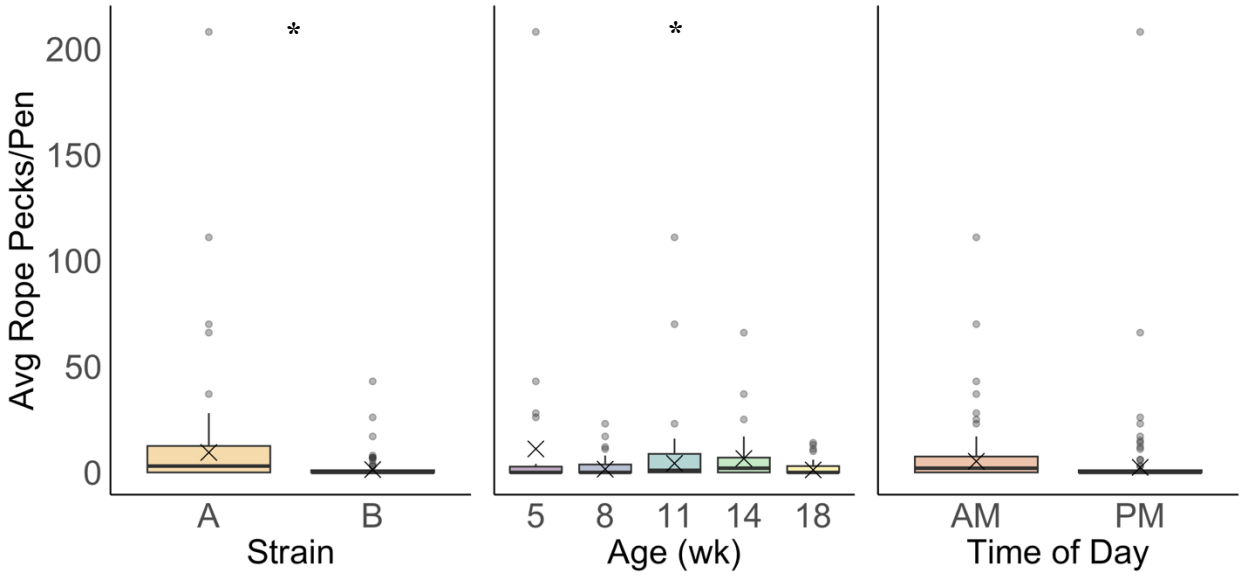


Figure 1.5 The frequency of pecking on a hanging rope enrichment by turkey toms in a 20-min continuous observation period. An asterisk (*) indicates significant differences between factors. Raw data are displayed in box plots, where boxes represent the interquartile range, the black line represents the median, the top of the box represents the 75th quartile, and the bottom of the box represents the 25th quartile. Whiskers represent the minimum and maximum values, while gray dots indicate outliers. The back X overlaying the box plots is the estimated mean value.



CHAPTER 3

How the Prevalence of Feather Pecking Relates to the Preference to Perform Foraging and Physiological Markers for Dominance and Hunger

ABSTRACT

Injurious pecking in turkeys is hypothesized to be caused, in part, by redirected foraging and consummatory behavior. Foraging behavior in farmed poultry is an example of contrafreeloading (CF), where an animal prefers to work for food rather than obtain it from a readily available source. We aimed to determine whether an individual turkey's prevalence of feather pecking was associated with foraging and feeding preferences (i.e., home pen behavior, preference test, and ghrelin concentrations) and social dominance (i.e., testosterone). A total of 176 turkey toms from one commercial strain were randomly assigned to 8 pens (22 birds/pen). Eight focal birds/pen were colored for identification. At 14 wk, the frequencies of body/wing/tail (BWT) pecking/6 h for each focal bird were determined via live observations. Two birds/pen with the highest BWT pecks were deemed High Feather Peckers (HFP; n=16), while two focal birds/pen with the lowest were classified as Low Feather Peckers (LFP; n=16). Also at 14 wk, home pen behavior was video recorded and blood was collected for testosterone and ghrelin concentrations. At 15 wk, a conditioned place preference test was conducted to associate wall patterns paired with either the presence of dried mealworms and feed mixed into bedding (CF side) or a pan feeder offering the same feed mix (FF side). Birds were conditioned for 3 d, then tested on the fourth day. All data were analyzed with R software. The HFP performed more foraging ($p=0.048$) and feeding bouts ($p=0.044$) than LFP in the home pen, but did not differ in their ghrelin concentrations ($p=0.60$). During conditioning, both HFP and LFP spent more time foraging on the CF side than the FF side ($p=0.027$). Neither HFP nor LFP turkeys displayed a

preference greater than chance for the CF or FF side ($p>0.05$). To our knowledge, this is the first CF preference test conducted in turkeys. Turkeys also did not differ in plasma testosterone concentrations ($p=0.71$). The HFP turkeys perform more exploratory and consummatory behaviors, which provides support for the redirected foraging hypothesis. However, individual turkeys vary widely in their feather pecking behavior, so further investigation of the underlying causes regarding individual temperament and group dynamics is warranted.

INTRODUCTION

Turkeys perform injurious pecking when they forcefully peck and pluck the feathers and skin of another bird, resulting in feather loss, skin damage, and cannibalism (Dalton et al., 2013; Rodenburg et al., 2013). Severe pecking injuries have constituted the majority of culls and mortality in commercial turkey production (Duggan et al., 2014). Management practices, such as lighting programs, high stocking density, lack of enrichment, and husbandry disturbances, are linked with higher prevalences of this behavior (reviewed by Dalton et al., 2013; Leishman et al., 2022). Injurious pecking has been extensively studied in laying hens, where characteristics of individual birds also contribute to the development of the behavior (Brunberg et al., 2016). This has yet to be determined in turkeys.

Injurious pecking is often associated with redirected pecking behavior due to insufficient foraging and/or feeding opportunities (van Staaveren et al., 2021). Indeed, foraging behavior in young laying hens (3 to 15 weeks of age) was correlated with severe feather pecking in the same adult laying hens (17 to 37 weeks of age; Newberry et al., 2007). The duration and head fixations of severe pecking were similar to those of foraging pecks in laying hens, further suggesting that this abnormal behavior is associated with foraging (Dixon et al., 2008). Redirected foraging pecks are directed at the back, wings, and tail regions, as opposed to the head and neck regions

that are associated with aggressive social hierarchy pecks (Savory, 1995; Dalton et al., 2013). To our knowledge, the relationship between foraging behavior and pecking behavior has not been evaluated in individual turkeys.

Deficiencies in feeding behavior, gut health, and nutrition are also associated with injurious pecking in laying hens and turkeys (Dalton et al., 2013; Rodenburg et al., 2013). Laying hen genetic lines bred to be "high feather pecking" and "low feather pecking" differed in their gut microbiota, and "high feather pecking" birds ingested more feathers despite being fed *ad libitum* (Kjaer et al., 2001; Harlander-Matauschek et al., 2007; Meyer et al., 2013). Turkeys are also fed *ad libitum*, but individual differences in pecking behavior may have a relationship with feed intake. Ghrelin is a peptide hormone that regulates appetite, where lower concentrations are associated with hunger and higher concentrations indicate satiety in turkeys (Vizcarra et al., 2012, 2018). To our knowledge, the relationship between ghrelin, feeding, and pecking behavior has not been assessed in individual turkeys.

Sex steroids have been linked with the onset of injurious pecking. In laying hens, increasing levels of progesterone and estrogen correlated with higher prevalences of pecking behavior around the onset of sexual maturity (Hughes, 1973). In turkeys, toms are more likely to have back and tail injuries than hens, and injuries increase for both sexes as they age (Leishman et al., 2022). Testosterone production fluctuates throughout the seasons in wild turkeys, with levels peaking during the breeding season. Additionally, dominant males have higher plasma testosterone than subordinate males (Lisano and Kennamer, 1977). Turkey toms treated with multiple doses of a gonadotropin-releasing hormone (GnRH) agonist decreased aggression for 3.5 to 7 months and lowered plasma testosterone (Molter et al., 2015). However, both of the

latter studies were assessed in wild turkeys, and the relationship between testosterone and pecking behavior has not been evaluated in a domesticated commercial turkey strain.

The objective of this study was to evaluate possible underlying causes of injurious pecking in turkeys. Specifically, we aimed to determine if an individual turkey tom's prevalence of feather pecking would predict their foraging behavior in their home pen and their preference to forage in a conditioned place preference test. We also aimed to assess whether an individual turkey tom's prevalence of feather pecking was related to their feeding behavior in their home pen, their preference to feed in a conditioned place preference test, and their plasma ghrelin concentrations as an indicator of hunger. Lastly, we aimed to compare individual tom's prevalence of feather pecking with their plasma testosterone concentrations as an indicator of social dominance. We hypothesized that High Feather Pecking (HFP) toms would differ from Low Feather Pecking (LFP) toms in foraging and feeding markers. Specifically, we predicted that HFP toms would forage more in their home pens and spend more time on the contrafreeloading side of the preference test, while LFP toms would forage less in their home pens and display no preference between sides. We also predicted that HFP toms would feed more in their home pens and have lower levels of ghrelin than LFP toms, indicating more hunger; however, the two types of birds would not differ in plasma testosterone.

MATERIALS AND METHODS

All experimental procedures were reviewed and approved by the North Carolina State University Institutional Animal Care and Use Committee (Protocol #24-202). Animal care protocols were based on the 4th edition of the Guide for the Care and Use of Agricultural Animals in Research and Teaching.

Animals and Housing

At 1 d of age, 704 turkey tom poults (*Meleagris gallopavo*) from four genetic strain sources were randomly assigned to one of 32 pens (2.44 × 3.51 m; 22 birds/pen, 8 pens/strain) in a curtain-sided house at the North Carolina State University Talley Turkey Education Unit (Raleigh, NC) as part of a larger study. Pens were divided into 4 blocks, with 8 pens/block, and strains were randomized across blocks. Each pen contained one tube feeder (Manola T, Giordano Global, Springfield, MO), one bell drinker (Plasson Turkey Bell Drinker, Plasson Livestock, Maagan Michael, Israel), and fresh pine wood shavings for bedding, which were top-dressed as needed. The house utilized fans negative air pressure power ventilation. Poults were reared in a brooder ring with a heat lamp and additional supplemental feeders and drinkers during the first week. Heat lamps were fitted with thermostats and placed above each brooder ring. The temperature under the lamps was set to 35°C at placement, then 32°C on day 2, 31°C on day 7, and 29°C on day 10. Beginning in week 2, the house temperature was decreased by 3 to 5°C each week until the ambient temperature was reached. Brooder rings and supplemental feeders and drinkers were removed at 1 wk of age, and heat lamps were removed at 5 wk of age.

All feed was manufactured at the North Carolina State University Feed Mill Education Unit (Raleigh, NC). Both feed and water were provided *ad libitum*. The feed was formulated according to breeder guidelines (Aviagen-Turkey, 2024) and supplied in six diet phases throughout the trial. Diet phase changes occurred approximately every 3 wk: starter 1 (0 to 4 wk), starter 2 (4 to 7 wk), grower 1 (7 to 10 wk), grower 2 (10 to 13 wk), finisher 1 (13 to 16 wk), and finisher 2 (16 to 19 wk). Feed and feeders were weighed at the end of each phase, and the weight of feed added during each phase was recorded. The lighting schedule was maintained with natural and artificial light, with 24 hours of light for the first 3 days, then 20 hours of light

from day 4 to 6 wk, then 19 hours at 7 wk, 18 hours at 8 wk, 17 hours at 9 wk, and 16 hours until the end of the trial.

Experimental Design

A completely randomized block design was utilized with one of the genetic strains (8 pens). At 1 d of age, 8 focal birds were randomly chosen, then colored using food dye on the down feathers, then livestock marker after primary feather development (All-Weather Twist-Stik, LA-CO Markal, Elk Grove Village, IL). Color was remarked every 3 wk, or as needed, and birds were weighed individually at 5, 8, 11, 14, 17, and 19 wk.

Live Behavior Observations

At 14 wk, live observations were conducted in the home pen using continuous focal animal sampling to determine the high feather pecking (HFP) and low feather pecking (LFP) toms in each pen. Each pen was observed for 90 min/day at one of four time points (8 AM, 10:15 AM, 1 PM, or 3:15 PM). Pens were observed for four consecutive days to balance blocks across times of day, and pen order was randomized within a day (Table 2.1). A total of 6 hours was observed per pen.

Observers were stationed 0.3 m in front of the home pen, either standing or seated on a stool, for a 15 min acclimation period before beginning observations. There were 2 observers per pen, with each observer assigned to one-half of the pen. Back/Wing/Tail (BWT) pecking and Head/Neck (HN) pecking were scored continuously for all focal birds (Table 2.2). Inter-observer reliability was evaluated prior to data collection for a total of 13 observers. During reliability, the birds were acclimated to the observers for 10 min, then the observers counted pecking for focal birds for 20 min. This process was repeated three times for each observer. Behavior counts were

compared between the observer and the trainer and between observers to achieve an inter-rater agreement of 90%.

The HFP were the two individuals with the greatest total sum of BWT pecking frequencies in each pen, while the LFP were the two individuals with the lowest total sum of BWT pecking frequencies in each pen. The same 32 toms (16 HFP, 16 LFP) were utilized for the remaining metrics.

Blood Sampling

At 14 wk, one day after live observations were completed, blood samples were collected via venipuncture of the brachial vein in vacutainer tubes (BD Vacutainer, Franklin Lakes, NJ). After collection, the tubes were inverted, placed on ice, and centrifuged at 200 rpm for 15 min at 4° C within the hour. After centrifugation, the plasma was aliquoted into 2 mL bullet tubes and stored at -20 °C until the samples were ready to be analyzed. Concentrations of testosterone were quantified via an ELISA (Enzyme-Linked Immunosorbent Assay) kit (Cayman Chemical, Ann Arbor, MI). All samples were run in triplicate across two 96-well assays, coefficients of variation (CV) are reported (CV sample range: 0.99-12.78%). The Cusabio ELISA kit designed for chickens was used to measure ghrelin concentrations (Cusabio Biotech, Houston, TX). All samples were run in duplicates in one 96-well assay, CV are reported (CV sample range: 1.05-19.59%).

Home Pen Behavioral Coding

Color video cameras (4K Smart IP Fixed Bullet Camera, Lorex Corporation, Irvine, CA) were placed above the top corner of each home pen to continuously capture footage at 14 wk, the day after blood sampling. Each camera was wired to a network video recorder (4K 16-channel Wired NVR System, Lorex Corporation, Irvine, CA) to review and save video files. The

frequency of foraging and feeding behaviors by the HFP and LFP birds were coded for 1 h at 8 AM, 10 AM, and 1 PM (Table 2.2). These times were chosen because they aligned with times of day that feather pecking behavior was recorded for live observations and did not include husbandry disturbances. Behaviors were coded from video files using Behavioral Observation Research Interactive Software (BORIS, version 8.19.3, http://www.boris.unito.it/, Friard and Gamba, 2016) by three trained observers. Inter-rater agreement was calculated by comparing behavioral point event counts between the trainer and trainee for the two behaviors using Google Sheets (version 91.0, Googleplex, Mountain View, CA) to achieve 90% or greater inter-rater agreement across all observers.

Conditioned Place Preference Test

Conditioning Training. Our test apparatus, conditioning training, and test design were based on Ferreira et al. (2021), who designed a conditioned place preference test to compare preferences for contrafreeloading between low ranging and high ranging outdoor access broiler chickens. The test apparatus consisted of a pen with a metal frame (4.57 × 3.47 × 1.6 m; L × W × H) and concrete floors topped with wood shavings. During conditioning, the test apparatus was divided into two chambers with a removable wooden board (3.5 m × 1.6 m; L × H). The two chambers had different geometric patterns painted along the back wall for the birds to learn a visual to associate with each chamber (Figure 2.1). To mitigate social isolation stress, both sides of the test apparatus had an additional chamber separated by a chicken wire fence to allow visual contact with flock mates from each bird's original home pen.

The two chambers were associated with the contrafreeloading (CF) side or the free feed (FF) side. On the CF side, dried mealworms and familiar feed were scattered in the wood shavings bedding to promote foraging behavior. On the FF side, familiar feed and dried

mealworms were freely available in a familiar tube feeder. There was a test apparatus on both sides of the turkey house to ensure an equal distance of carrying turkeys to the test chambers. Test apparatuses were balanced to minimize side bias (left or right) and pattern bias (stripes or solid).

At 15 wk, conditioning occurred over three consecutive days. Toms were conditioned in pairs, with both of the HFP from the same pen together then both of the LFP from the same pen together. The pairs would be placed in one chamber at a time for 5 min. After conditioning in one chamber, all birds would be returned to their home pen for at least 1 h, then they would be conditioned to the opposite chamber for 5 min. Conditioning occurred at 8:00 AM and 1:00 PM each day to balance possible differences in diurnal motivations. The order of pens and whether HFP or LFP were conditioned first were balanced between the two times of day.

The same color video cameras described above were also placed above each test chamber. During conditioning, the duration of foraging and feeding behaviors (Table 2.2) were coded by the same three observers described for home pen behavior.

Testing. On the fourth day at 15 wk, birds were tested in the apparatus. The center wall was removed, and dried mealworms and familiar feed were scattered on the CF side and the filled tube feeder remained on the FF side. Four pens were tested at 7:00 AM (8 HFP, 8 LFP), while the pens were tested at 1:00 PM (8 HFP, 8 LFP) to balance possible differences in diurnal motivations, similar to conditioning. Birds were tested individually, where they were placed in the center of the arena facing the back wall with the geometric shapes. Birds remained in the arena for a total of 2 min 30 sec. Two observers, one per chamber, recorded the time the bird spent in their respective chamber of the test arena with a stopwatch. Observers started their watch when the focal bird entered their chamber and stopped when the bird left. If the bird

returned to their chamber, the observer continued to record from the last recorded time. The focal birds completed the test for a single trial before returning to their home pen. The duration of time spent on each chamber side was recorded live, and durations of foraging and feeding behavior were evaluated from video recordings by one observer with the same BORIS software.

Statistical analysis

Identifying the HFP and LFP birds in each pen was conducted using Google Sheets (version 91.0, Googleplex, Mountain View, CA). All data was analyzed using R statistical software (version 4.4.2; R Core Team, 2021) with RStudio (version 2024.12.0+467) for mac OS Sequoia 15.6. Conditioning data were analyzed with generalized linear mixed effects models. Conditioned side (factor with 2 levels: CF and FF), pecking category (factor with 2 levels: HFP and LFP), and their two-way interaction were included as fixed effects for foraging and feeding behaviors. Individual bird ID were included as a random effect.

Foraging and feeding durations during testing were analyzed with a t-test to compare the means of pecking category (factor with 2 levels: HFP and LFP). Side preferences were analyzed via a Wilcoxon Signed Rank test to determine if the focal birds spent more than 50% of the tested time on one side of the arena, with pecking category (factor with 2 levels: HFP and LFP) and a μ value of 0.5.

Assay data were analyzed in R (version 4.5.1). Linear mixed-effects models and generalized linear mixed-effects models were fitted using lme4 and glmmTMB. Feather pecking category was used as the fixed effect, while pen was used as the random factor. Model residuals were checked for uniformity and other assumptions using simulated residuals from the DHARMa package. Estimated marginal means for fixed effects were obtained using the emmeans package, with 95% confidence intervals. Outlying observations were identified and

removed in sensitivity analyses when biologically implausible (one data point for ghrelin concentration because of a high CV). Significance was set at $\alpha = 0.05$. Data are presented as means [95% confidence interval] unless otherwise stated.

RESULTS

HFP/LFP Identification. The two focal birds per pen with the highest sum of BWT were categorized as HFP (343.1 ± 248.8 pecks/6 h, mean ± SD). The two focal birds per pen with the lowest sum of BWT were categorized as LFP (59.2 ± 31.7/6 h).

Blood assays. There was no difference between HFP (0.23 pg/ml [0.21, 0.26]) and LFP (0.23 pg/ml [0.21, 0.25]; mean [95% CI]) plasma testosterone concentrations (χ^2=0.28, p=0.60). There was also no difference between HFP (0.98 pg/ml [0.94, 1.02]) and LFP (0.97 pg/ml [0.93, 1.01]) plasma ghrelin concentrations (χ^2=0.14, p=0.71).

Home pen behavior. The HFP turkeys displayed more foraging bouts than the LFP turkeys (χ^2=3.92, p=0.048; HFP: 20.8 bouts/3 h [16.2, 26.7], LFP: 14.4 bouts/3 h [11.1, 18.7]). The HFP turkeys also displayed more feeding bouts than the LFP turkeys (χ^2=4.07, p=0.044; HFP: 24.4 bouts/3 h [15.8, 37.8], LFP: 12.8 bouts/3 h [8.2, 20.0]).

Conditioning. All turkeys foraged during conditioning and spent more time foraging on the CF side than the FF side (p=0.027; CF: 32.66 sec [23.15, 43.81], FF: 23.87 sec [15.86, 33.51]). HFP and LFP birds spent a similar amount of time foraging (p=0.704; HFP: 29.67 sec [18.42, 43.61], LFP: 26.55 sec [15.96, 39.82]). Only 10 of the 32 total birds ate from the feeder on the FF side, of which 7 birds were HFP and 3 birds were LFP.

Testing. HFP and LFP spent a similar amount of time foraging during testing (p=0.951). Only 5 of the 32 toms ate from the feeder on the FF side during testing, all of which were HFP

birds (p=0.19). Neither HFP (p=0.38) nor LFP (p=0.95) displayed a preference greater than chance for the CF or FF side (p>0.05; Figure 2.2).

DISCUSSION

HFP and LFP identification

In the current study, the HFP and LFP birds were selected within each pen based on the total sum of BWT pecking during live observations, where the HFP displayed a 5.8-fold increase in pecking on average. In laying hens, third-generation lines selected for HFP and LFP similarly displayed a 5.5-fold difference between pecking on average (HFP: 13.9 pecks/bird/h, LFP: 2.51 pecks/bird/h; Kjaer et al., 2001). However, there was overlap in pecking counts between the two categories of turkeys in the present study, as indicated by the standard deviations. The HFP turkeys in some pens had similar counts as the LFP turkeys in other pens. When selecting for divergent genetic lines of HFP and LFP laying hens, researchers created cut-off points to prevent overlap between the two categories (Labouriau et al., 2009). They reported that feather pecking behavior was variable amongst individuals, with 10.6% of laying hens identified as high peckers, 35.9% of hens not displaying the behavior at all, and the majority of hens as low peckers (Labouriau et al., 2009). Taken together with our results, categorizing feather pecking into categories may risk losing the nuance and variation across a population.

Foraging

The HFP birds performed more foraging bouts in their home pens than the LFP birds. Injurious pecking is often associated with redirected foraging behavior in poultry, with redirected foraging pecks directed at the BWT regions (Savory, 1995; Dalton et al., 2013; van Staaveren and Harlander, 2020). We categorized turkeys based on their prevalence of BWT pecking specifically, so this finding supports a relationship between BWT pecking and foraging in turkeys.

We attempted to expand on this relationship between foraging and BWT pecking with a conditioned place preference test, which to our knowledge, was the first contrafreeloading conditioned place preference test conducted in turkeys. Our conditioning training and test design was based on Ferreira et al. (2021). In the prior study, contrafreeloading (CF) and freely available feed (FF) sides were differentiated between feeding method, flooring, and wall pattern – the FF feeders were placed on concrete floors while the CF side offered feed scattered in wood shavings. We aimed to minimize the flooring confound in the current study. Anecdotally, turkeys find concrete flooring aversive and are typically housed on wood shavings; therefore, we added bedding to both sides to prevent a side bias of aversive concrete (Fraser and Nicol, 2011). This resulted in the birds also foraging on the FF side, even without feed items scattered throughout it. All turkeys foraged during conditioning, and this may be due to a preference to explore the fresh bedding that was available, regardless of side. Indeed, earlier work also found that the majority of turkeys were motivated to access fresh bedding (Monckton et al., 2020), so future contrafreeloading preference tests in turkeys should consider how the flooring type affects behavior between sides.

Feeding

The HFP birds also performed more feeding bouts in their home pens than the LFP birds. Nutritional deficiencies or differences in gut health have been reported to be a possible underlying cause of injurious pecking. In domestic fowl, birds can detect when their diet is deficient, which increases exploratory behavior in search of resources to correct the deficiency, including redirecting pecking toward conspecifics' plumage (Kjaer and Bessei, 2013). A laying hen study found HFP birds, both food-deprived and non-food-deprived, had a stronger preference to consume feathers than LFP birds (Harlander-Matauschek et al., 2007). Although

our birds were fed *ad libitum* throughout the trial, our findings suggest that variation in feather pecking within a flock could also be associated with consummatory behavior. We did not measure gut health in the current study, but the HFP and LFP turkeys did not differ in plasma ghrelin concentrations as a marker of hunger. Previous research reported lower ghrelin concentrations, indicating higher hunger, in turkeys that were fasted compared to those fed *ad libitum* (Vizcarra et al., 2018). When all birds are on the same feeding program, ghrelin is not an underlying mechanism for individual bird differences in feather pecking, so individual variation in other drivers of feed intake warrant further investigation.

In the conditioned place preference test, feeding was not valued for the turkeys. Only 10 of the 32 focal birds ate from the feeder on the FF side during conditioning (7 HFP, 3 LFP), and only 5 of the 32 focal birds ate from the feeder during testing (5 HFP, 0 LFP). It is worth noting that the HFP toms were typically the ones feeding, which aligns with their home pen behavior, but this should be interpreted with caution because the majority of HFP toms did not feed during conditioning or testing. Turkeys may not have been interested in feeding due to being fed *ad libitum* in their home pens, or they may not have learned that feed was available. The purpose of conditioning was to ensure birds engage with and learn what is available between two options in a preference test, so future work should ensure that all birds feed from the FF side in conditioning before proceeding to testing.

In comparison to Ferreira et al. (2021), we did not feed restrict the focal birds before testing, nor did we remove the feed items in the bedding or the feeder during testing. Given that not all the birds fed during conditioning, the feeder and substrates remained on their respective sides during testing to reinforce the options available. However, the removal of the substrate and feed would show which side is preferred based on what was learned (Ferreira et al., 2021). A

conditioned place preference test in free-range broiler chickens utilized feed and social companions as the options, and found that the birds gradually lost their learned preference for the conditioned chambers after 6 days of an extinction phase, where both rewards were removed (Ferreira et al., 2020). Our findings support this study's implications that memory is nuanced and the effectiveness of associating a visual cue offered in a preference test may vary due to individual differences. Furthermore, despite minimizing social isolation and aversive flooring, turkeys may have behaved differently in the test arena versus the home pen as they were still separated from the majority of their flock and handled by caretakers, which may have altered their behavior for testing compared to their home pen (Schrader, 2002).

Social dominance

Testosterone concentrations did not differ between HFP and LFP, which aligns with our original hypothesis. Our HFP and LFP categories were based on BWT pecks, while social dominance is typically associated with HN pecks (Mench, 2009; Bartels et al., 2020). Testosterone increases in turkeys drastically between 13 and 17 WOA as they reach sexual maturity (Classen et al., 1994). The early observation of spermatozoa within the seminiferous tubules at 15 wk and the production of mature spermatozoa within semen at 26 wk suggests toms reach sexual maturity between 15 to 26 wk (Cecil and Bakst, 1991). This correlates with the ages the National Research Council's (NRC) nutrient guidelines list for semen production in turkey toms (Sifri, 1995). The birds of our study may not have reached the age of maturation where increases in testosterone are prompted. Testosterone fluctuates according to the season and is responsible for anatomical secondary sex characteristics and behavior, such as aggression (Lisano and Kennamer, 1977; Moore et al., 2019). The blood we measured was from 14 wk toms, so these birds were within the range, but testosterone did not contribute to feather pecking.

Correlating testosterone with HN pecking prevalence should be explored further by measuring plasma testosterone in older toms and comparing it to the HN pecking counts or injuries at the same ages.

CONCLUSION

In their home pens, HFP turkey toms forage and feed more often than LFP turkey toms, suggesting a relationship between feather pecking and exploratory and consummatory behaviors. However, this relationship was not maintained in a conditioned place preference test with foraging and feeding opportunities. Multiple aspects of the test, including flooring material, lack of feed restriction, and novelty, influenced how turkeys behaved in the test apparatus versus the home pen. Overlap in pecking counts between the HFP and LFP categories may have also contributed to a lack of categorical differences that warrant further exploration on a continuous scale.

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Table 2.1 Randomization of blocks within a day and across days for live behavior observations to identify the two highest feather pecking and two lowest feather pecking toms in each pen. Each block contains two pens of Nicholas Select turkey toms (22 toms/pen). Behavior observations (90 min) were started after a 15 min acclimation period to live observers.

Monday	Tuesday	Wednesday	Thursday
Block A (8 - 9:45 AM)	Block C (8 - 9:45 AM)	Block B (8 - 9:45 AM)	Block D (8 - 9:45 AM)
Block B (10:15 AM - 12 PM)	Block D (10:15 AM - 12 PM)	Block A (10:15 AM - 12 PM)	Block C (10:15 AM - 12 PM)
Block C (1 - 2:45 PM)	Block A (1 - 2:45 PM)	Block D (1 - 2:45 PM)	Block B (1 - 2:45 PM)
Block D (3:15 - 5 PM)	Block B (3:15 - 5 PM)	Block C (3:15 - 5 PM)	Block A (3:15 - 5 PM)

Table 2.2 Definitions of behaviors observed in Nicholas Select turkey toms in the home pen (frequencies) and conditioned place preference test (durations). The HN pecking and BWT pecking were recorded live in the home pen, while foraging and feeding were coded from video recordings in the home pen and conditioned place preference test.

Behavior	Definition
Head/Neck pecking	Bird's beak comes into contact with another bird's head or neck. The neck area includes the feathered base of the neck. Mark each time a bird pecks at the head or neck of another bird.
Back/Wings/Tail pecking	Bird's beak comes into contact with another bird's body on their back, wings, and/or tail. Mark for each time a bird pecks at the body areas of another bird
Foraging	Foraging begins when bird's head drops below shoulder level. Behavior continues if beak comes in contact with the ground, or head hovers above the ground. Foraging behavior ends when bird's head raises above shoulder height.
Feeding	Feeding begins when bird's beak comes into contact with feed in the feeder. Feeding can be a single peck or a repetitive action. Feeding behavior ends when the bird stops feeding for 3 seconds, or performs a different behavior.

Figure 2.1 Contrafreeloading conditioned place preference test. The contrafreeloading side is identified with a striped wall, where familiar feed and dried mealworms were scattered in the bedding. The free feed side is identified with the white wall, where familiar feed was available in a familiar feeder. On either side, flock mates from the same home pen were visible through wire fencing to minimize social isolation stress during testing.

Figure 2.2 The orange graph depicts the percentage of time HFP spent on the FF and CF side during testing. The blue graph depicts the percentage of time LFP spent on the FF and CF side during testing. Neither HFP or LFP display a preference greater than chance for the CF or FF side ($p>0.05$).