ABSTRACT

WEAVER, ALLISON DEANNE. Evaluating the Relationship Between Water Use and Broiler Breeder Pullet Welfare at the Individual and Flock Level (Under the direction of Dr. Allison Pullin).

Broilers breeders are genetically selected to produce offspring with increased appetite and growth rates. As a result, breeders have similar genetic traits for fast growth, but production cycles that can be over 60 weeks longer; therefore, feeding breeders *ad libitum* can cause obesity and impair reproductive performance. Feed restriction practices are implemented to maintain health and production, but this can result in increased water use, water spillage, poor litter quality, and pododermatitis. Increased drinker use in feed restricted breeders could be the result of stereotypic pecking at the drinker (i.e., polydipsia) caused by hunger and frustration or as a result of an underlying physiological issue or demand. Water restriction practices are sometimes used to mitigate water spillage. The objective of this thesis was to evaluate the effect of feed and water restriction on drinking behavior at the flock and individual level to determine the underlying motivation of increased drinker use and to assess overall welfare.

This thesis consists of one overall trial with three different components: flock level drinker use and welfare, validation of radio frequency identification (RFID) technology to monitor individual drinking behavior, and individual drinker use and bird health. Overall, 960 Cobb 500 FF pullets were placed in a 16-floor pen curtain-sided house and randomly assigned to one of four treatments: skip-a-day feeding+*ad libitum* water (SAD+ADLIB), skip-a-day feeding+water restriction (SAD+WR), every-day feeding+*ad libitum* water (ED+ADLIB), and every-day feeding+water restriction (ED+WR). Water restriction involved turning water lines off for 3 h daily. Ten birds/pen were selected as focal birds and uniquely colored with livestock marker for identification

In the first chapter, one video camera was placed in each pen and drinking duration was coded by trained observers at 16 and 22 wks. Average bird water use was measured at 4, 10, 16, and 22 wks . Litter samples were taken at 10, 16 and 22 weeks from under the waterline and in the general pen area. Footpad scoring was performed on focal birds at 16 and 22 weeks. Increased drinker use in SAD treatments was observed at later times during the day, while increased water use occurred in this treatment at 10 wks under both WR and ADLIB. In contrast, increased drinker use was observed in ED treatments at earlier times, while increased water use occurred at later ages under ADLIB. This could be attributed to differences in portion size and sensitivities to dietary changes. Although WR birds displayed compensatory drinker use when water access resumed after restriction periods, WR still limited water use in ED treatments at 16 and 22 wks. While litter moisture patterns tended to follow water use, no differences were found for footpad scores.

The use of RFID to monitor drinking behavior in pullets, was validated by comparing different reader and tag placements, then comparing accuracy, precision, sensitivity, and specificity across ages. Results showed that placing one RFID reader on the water line and RFID tags on the wings of focal birds was the most accurate. This was used to identify high drinkers (HD) and low drinkers (LD), which were further validated using precision water measurements. Blood and feces were sampled from these HD and LD at 10, 16, and 22 wks and hematocrit, fecal moisture, and FITC-d were measured to assess hydration and intestinal permeability. There were no treatment or drinker type differences in FTIC-d and no consistent differences in hydration metrics, indicating that increased drinker use is not the result of intestinal permeability or dehydration, and instead may be another physiological cause or individual behavioral

differences. Further research into other motivations for increased drinker use in broiler breeder pullets is needed.

Evaluating the Relationship Between Water Use and Broiler Breeder Pullet Welfare at the Individual and Flock Level

by
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DEDICATION

To my family and friends who have supported me through this journey. I wholeheartedly know that I would not be where I am today without your love and encouragement, and I am so immensely grateful to have you in my life.

BIOGRAPHY

Allison Deanne Weaver was born in Asheboro, North Carolina on June 27th, 2002, and was raised in Troy, North Carolina. She is the daughter of Ron and Dedie Weaver, both of whom are teachers. She was very inquisitive from an early age and had a natural love of animals, particularly cats. Allison fostered kittens for the local humane society throughout her middle and high school years, while also developing her love for science by participating in a Field Biology Camp at the North Carolina Zoo. From there, Allison knew she wanted to pursue a career in animal science.

Allison attended high school as a part of the first graduating class of Montgomery County Early College, where she simultaneously received her high school diploma and Associate of Science. Afterwards, she attended North Carolina State University where she graduated with her Bachelor of Science in Animal Science and Bachelor of Science in Poultry Science in 2023. During her time as an undergraduate student at NCSU, Allison was an undergraduate research assistant within Dr. Allison Pullin's Poultry Welfare Lab where she studied fearfulness in turkey hens in response to varying photoperiods. She was also a member of the NCSU Animal Welfare Judging Team. These experiences instilled her with a passion for animal behavior and welfare and inspired her to pursue a master's degree and continue working in the Pullin Lab as a graduate research assistant. During her master's program, she studied the effect of feed restriction strategies on drinking behavior and water use in broiler breeder pullets. After graduation Allison plans to pursue her PhD at the University of Arkansas under the direction of Dr. Shawna Weimer, continuing research in poultry behavior and welfare.

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CHAPTER 1

Literature Review

In 2023, over 1.2 million broiler breeder pullets were placed in the United States, producing an average of 1.18 billion eggs each month, with a resulting broiler production that has an economic impact of $450 billion annually (USDA, 2023; National Chicken Council, 2024). Increased genetic selection for rapid growth in offspring has led to standard use of feed and water restriction practices for broiler breeder management, creating a welfare paradox (Savory and Maros, 1993). While these production protocols are necessary for improving and maintaining biological health and functioning, they place a strain on species-specific behavioral needs (Riber, 2020). Methods for alleviating these behavioral concerns while also maintaining ideal body weights and uniformity have become an essential part of breeder research, with increasing focus on alternative diets to increase satiety through qualitative or quantitative modifications to feed rations (de Jong and van Emous, 2017). However, research examining the implications of these practices on broiler breeder behavior and welfare, particularly on how feeding protocols impact drinking behavior, are limited. In addition, current investigations of the topic have led to inconclusive results. In order to continuously optimize broiler breeder production and welfare, further evaluation of production practices on behavior is crucial.

Behavioral Repertoire of Broiler Breeder Pullets

Feeding Behavior: Development

Chickens are precocial in nature, meaning they are born with the ability to see and move on their own. While it is not necessary for them to be fed by their mothers, maternal guidance during feeding is observed in both junglefowl and domestic hens (Nicol, 2015). Upon hatching, chicks will peck at both edible and inedible objects on the ground; however, in a natural setting,

hens will display food calls to direct the chicks towards edible, high quality food particles (Nicol, 2015) In a study that examined the food-calling behavior of domestic hens in various situations, it was found that hens were aware of the location and behavior of their chicks, increasing the intensity and duration of their calls when the chicks were separated from them (Wauters et al, 1999). While maternal guidance is important for chick feeding behavior in a natural setting, social learning of feeding behavior is the primary tool used by chicks in commercial settings where no mother is present (Nicol, 2015). In a study examining social facilitation of feeding behavior, the visual stimulus of another chick pecking, along with the corresponding sound, increased pecking and feed consumption among chicks (Tolman, 1964). Social facilitation is also important for avoidance learning in chicks (Nicol, 2015). While social learning is significant for behavioral patterns in chicks, it is not as important for older birds as their feeding behavior predominantly relies on operant conditioning (Nicol, 2015). Development of feeding behavior is crucial, as it leads to important behavioral pathways in adulthood relating to feed consumption, hunger, and satiety.

Feeding Behavior: Behavioral Pathway

The behavioral pathway of feeding in chickens consists of two phases: appetitive and consummatory (Mench, 2002). The appetitive phase of feeding is known as foraging, which is the process of searching for feed materials (Riber, 2020). Birds use both their beak and feet to scratch at the ground to uncover feed, then use their beak to peck at the ground and ingest the material. They will then either consume the material if it is edible or shake their heads rapidly to remove the material from their beak if it is inedible (Nicol, 2015). All foraging activities occur during the day and take up a significant portion of daily activity (Nicol, 2015). The foraging behaviors seen in modern chickens are nearly identical to that of their evolutionary ancestor, the

red jungle fowl, which spends 60% of daylight hours foraging (Dawkins, 1989). Genetic and environmental factors have decreased the amount of time modern laying hens forage to 40%, although this is still a significant portion of their daily activity (Nicol, 2015).

Foraging is the progenitor for the consummatory action of feeding, which is when the bird ingests the feed. While the accessibility and quantity of feed impacts the degree of foraging, this behavior is not eliminated when feed is freely available (i.e., *ad libitum*). This is a phenomenon called contrafreeloading, where birds prefer to forage even when food is provided *ad libitum* (Hemsworth and Edwards, 2020). This preferential status of foraging is indicative of a highly motivated behavior. While all animal behaviors are regulated by both internal and external factors, highly motivated behaviors are greatly influenced by internal cues, meaning that the inability to perform these behaviors and receive the desired stimulus can result in frustration (Nicol, 2015). Birds will forage with or without the presence of feed; however, preventing a bird from feeding will increase its motivation to forage above the normal level (Nicol, 2015). Subsequently, when birds are given the opportunity to feed after this restriction, the intensity of the foraging/feeding behavior will substantially increase due to a "rebound" effect (Nicol, 2015). Excessive rebound behaviors after a period of restriction are indicative of high motivation as well (Nicol, 2015).

Feeding Behavior: Satiety

The overall goal of feeding and foraging behaviors is hunger reduction; therefore, satiety is the internal cue that controls these behaviors. The physiological mechanisms which control feeding behavior are regulated by the peripheral and central nervous system (CNS; Denbow, 1994). Peripheral signaling molecules, such as hormones, neuropeptides, nutrients, and metabolites bind to receptors within the digestive tract, which are then interpreted by the CNS to

regulate feed intake and motivation (Richards and Proszkowiec-Weglarz, 2007; Hu et al., 2019). While there is a variety of different signaling molecules, each with their own complex interaction, common examples that alter appetite and satiety in avian species are cholecystokinin (CCK) and neuropeptide Y (NPY), which decrease and increase feed intake, respectively (Furuse et al., 2007; Richards and Proszkowiec-Weglarz, 2007). When nutrients reside in the intestines of birds, cholecystokinin (CCK) is produced, initiating a anorexigenic (i.e., appetite decreasing) pathway that promotes gut motility and the emptying of the digestive tract (Furuse et al., 2007). In contrast, during fasting NPY is co-expressed with agouti-related peptide (AgRP), which stimulates orexigenic (i.e., appetite increasing) pathways and blocks anorexigenic pathways (Song et al., 2012). The balance of these orexigenic and anorexigenic pathways have a significant impact on regulating satiety in birds (Song et al., 2012).

These pathways can be affected by a variety of different things, including energy content in feed, with high energy diets inhibiting orexigenic mechanisms, resulting in decreased appetite (Hu et al., 2019). In comparison, feed deprivation increases appetite, with 48 h fasting periods in broilers chicks resulting in the upregulation of orexigenic mechanisms, such as NPY and AgRP (Song et al., 2012). Therefore, even though satiety is primarily regulated via internal mechanisms, it is clear that external factors, such as feed quality and quantity have a significant impact on these systems. It is important to consider these factors not only when examining feeding behavior, but also when looking at development of closely related behavioral pathways, such as drinking.

Drinking Behavior: Development

In addition to feeding, drinking is another important behavior learned by birds at a young age. In general, birds learn to drink by inserting their beak into an open source of water (e.g.,

trough), performing a scooping motion with their beak, then elevating and tilting their heads backwards to transfer the water into their esophagus (Nicol, 2015). Chickens must elevate their head to swallow because they do not have an epiglottis to prevent water from entering their respiratory tract (Heidweiller, 1992). Instead, the larynx closes as they lift their head to protect the trachea while drinking (Heidweiller, 1992). The drinking behavioral sequence is typically learned within the first few days post-hatch (Nicol, 2015). However, the most commonly used drinker type on commercial farms is a nipple drinker, which requires birds to peck at an elevated metal pin to release water. Compared to troughs, nipple drinkers mitigate bacterial growth in the water source and reduce water spillage, which consequently aids in maintaining lower litter moisture and reducing the risks of pododermatitis and high ammonia concentrations (Nicol, 2015). Birds prefer to use open trough drinkers over nipple drinkers when given the choice, but their drinking behavior is flexible and they will learn to effectively utilize nipple drinkers through social facilitation if provided (Nicol, 2015).

Drinking Behavior: Behavioral Pathway and Hydration

Contrary to feeding, there is no required appetitive phase or foraging-like behavior associated with drinking (Nicol, 2015). Once a bird's physiological need for water is met, there is no further performance of drinking-related behaviors (Nicol, 2015). Therefore, the behavioral pathway of drinking behavior in chickens mainly consists of the consummatory behavior and the ultimate goal of hydration. Water plays a vital role in maintaining homeostasis in birds, as it is important for thermoregulation, digestion, nutrient absorption, and waste removal (Manning et al., 2007a). As a result, significant decreases or increases in water use can signal physiological health abnormalities in a bird and/or flock (Manning et al., 2007a).

Hydration in birds is significantly affected by intracellular and extracellular fluid volume, which are under endocrine regulation (McKinley and Johnson, 2004). One of the main hormones responsible for controlling hydration in birds is arginine vasotocin (AVT), an antidiuretic hormone produced in the posterior pituitary gland (i.e., neurohypophysis) in response to increased plasma osmolarity (i.e., an indicator of cellular dehydration; McKinley and Johnson, 2004). AVT reduces the glomerular flow rate in the kidney of chickens, subsequently reducing urine flow and plasma osmolarity (McKinley and Johnson, 2004). While AVT influences physiological mechanisms of hydration, its effects on drinking behavior remain unclear (Wu et al., 2019). However, the renin-angiotensin-aldosterone system (RAAS), another important component in regulating hydration, does have a discernable effect on avian drinking behavior (Snapir et al., 1976; Takei et al., 1988).

The RAAS, a complex communication network involving multiple organs, is responsible for the production of angiotensin II (Ang II), which not only increases aldosterone production, sodium reabsorption, and vasoconstriction, but also stimulates the brain to increase thirst and salt intake (Fournier et al., 2012). These thirst inducing actions have been noted in chickens, with the administration of Ang II resulting in increased water intake in White Leghorn males (Snapir et al., 1976). The effects of Ang II have also been reported in other avian species, where plasma Ang II concentration increased in water deprived quails, with a corresponding increase in plasma osmolarity and decrease in blood volume, indicating both intracellular and extracellular dehydration (Takei et al., 1988). Once water restriction was terminated, birds began to immediately drink water, causing Ang II, plasma osmolarity, and blood volume to return to normal levels (Takei et al., 1988). These results highlight the importance of the hormonal

regulation of hydration in birds and suggests a potential effect of external factors on these internal mechanisms.

There are many factors that affect the performance of drinking behavior and water use, a catch-all phrase for water consumption and water intake. Health status, age, sex, genetics, water quality, feed intake, feed content, drinker systems, and external temperature can all affect water use by modifying physiological indicators of hydration and/or behavior (Deeb and Cahaner, 2002). Over the past several decades, water use in broilers has increased simultaneously with genetic selection for faster growth rates (Williams et al., 2013). In a study comparing birds selected for fast growth to a group of non-selected birds, it was found that the selected line of fast-growing birds had higher water use compared to the control group (Marks, 1980). This increase in water use associated with increased growth rate is caused by the differences in weight, fat content, and metabolism between fast-growing and slow-growing birds (Marks, 1980). As the industry continuously selects for rapid growth, water use is expected to increase.

There is a positive correlation between feed intake and water intake, such that water intake increases as feed intake increases (Manning et al., 2007b). An exception to this is when ambient temperatures increase, feed consumption decreases and water intake increases (Manning et al., 2007a). The relationship between water and feed consumption can be monitored by the water consumption-to-feed consumption ratio (W:F), which examines water consumption per unit of feed consumption (Deeb and Cahaner, 2002). Research suggests that the average W:F for broilers is approximately 1.9, which remains consistent regardless of bird health status and only increases slightly as birds age (Pesti et al., 1985; Deeb and Cahaner, 2002; Williams et al., 2013). While water consumption increases with growth rate, W:F remains the same because feed consumption also increases with higher growth rates (Deeb and Cahaner, 2002). Different types

of drinkers are associated with different W:F in broilers, with bell drinkers, nipples with cups, and nipples without cups having W:Fs of 1.8, 1.7, and 1.6, respectively (Manning et al., 2007a). This can be attributed to behavioral drinking patterns with different styles of drinkers. As a result, the type of drinking system is also important to consider when examining water consumption in chickens.

Water consumption is not only impacted by feed intake and quantity, but by the quality and content of the feed as well. Altering feed content, like decreasing crude protein (CP), has been found to decrease water intake in broilers (Alleman and Leclercq, 1997). Higher protein intake increases nitrogen excretion, which is primarily done via uric acid excretion in the kidneys (Alfonso-Avila et al., 2022). Increased uric acid excretion requires more water intake, thus higher CP content in the feed results in more water consumption (Alfonso-Avila et al., 2022). Birds fed lower levels of feed additives containing CP, such as soybean meal, have reduced water intake and lower levels of litter moisture (Alfonso-Avila et al., 2022). Along with CP, fiber also increases water consumption in birds, possibly due to increased water-holding capacity of the digesta (Jiménez-Moreno et al., 2016). Electrolytes are another feed additive that can alter water consumption, as increasing the electrolyte content in feed can have an effect on the balance of sodium, potassium, and chloride within the intracellular and extracellular fluid, resulting in increased water intake (Mushtaq et al., 2013)

In addition to feed quality, environmental factors such as temperature and photoperiod also influence water use. Birds selected for faster growth rates are more sensitive to higher ambient temperatures, leading to greater water use than non-selected birds in the same environment (Deeb and Cahaner, 2002). In broilers, water use increases by 6% for every 1ºC increase in ambient temperature, which can lead to a significant increase in water intake for birds

reared in warm environments (Manning et al., 2007a). Water use in broilers is also affected by photoperiods, as water consumption decreases significantly when lights are turned off (Xin et al., 1993). Because of the noted effects various external factors have on water use, such as genetics, age, drinker types, feed quantity and quality, temperature, and lighting, it is important to consider the varying management practices implemented in breeder rearing when examining drinking beahvior.

Production Practices Influencing the Behavioral Repertoire of Broiler Breeder Pullets

Restricted Feeding

Broiler breeders are genetically selected to produce offspring with high meat-yields, which results in them having similar traits for high-growth rates (Mench, 2002). This intense selection for appetite and growth can lead to issues relating to health and production (Buckley et al., 2011). If breeders are fed *ad libitum* for the entire production cycle (i.e., pullets: 0-22 wks, breeders: 23-60 wks), which is significantly longer than that of a broiler (i.e., 6-8 wks), breeders would experience increased risks for obesity, tibial dyschondroplasia, ascites, and other health related issues causing reduced welfare (Mench, 2002). In addition, higher body weights at the onset of sexual maturity lead to decreased egg production and fertility due to multiple ovulation (Hocking, 1993). Therefore, it is crucial to limit the growth of broiler breeders, especially during the rearing phase, to promote optimal physiological health status.

In order to combat genetic selection for increased appetite and growth, feed restriction is a common practice utilized within the broiler breeder industry, where birds are often limited to 20-80% of their normal *ad libitum* feed intake (Riber, 2020). Feed restriction can be classified into two types: qualitative and quantitative (D'Eath et al., 2009). Quantitative restriction occurs

when the overall portion of feed given is reduced, while qualitative restriction is when the quality and or energy content of the feed is reduced (D'Eath et al., 2009).

The conventional method of restriction used in the industry is quantitative, which can be further categorized into skip-a-day (SAD) and every-day (ED) feeding. SAD fed birds receive a large quantity of food on alternate days, where the most common schedules are either 4 on-feed days with 3 off-feed days in a week (4:3), or 5 on-feed days with 2 off-feed days in a week (5:2). ED birds are fed daily but receive a smaller portion of feed at a time compared to birds in SAD feeding programs (Riber, 2020). Quantitative feed restriction is associated with multiple welfare concerns including chronic hunger and frustration (Girard et al., 2017). Birds experiencing chronic hunger often display abnormal repetitive behaviors (ARB), including stereotypic behaviors, such as redirected oral behaviors that take the form of feather and object pecking (Mellor et al., 2018). The presence of ARB in the behavioral repertoire of an animal, particularly one in captivity, is indicative of a negative affective state (Mellor et al., 2018).There are many causes for ARB, including, but not limited to, nutritional deficiencies, individual coping style, inadequate environment, dopaminergic and serotonergic pathways, and CNS dysfunction (Mellor et al., 2018). In addition to ARB, birds undergoing qualitative feed restriction also perform increased anticipatory, aggressive, and feed-seeking (i.e., foraging) behaviors, as well as increased overall activity, which is indicative of hunger (D'Eath et al., 2009; Morrissey et al., 2014).

Since birds on ED feeding programs receive more consistent allocations of food, this regimen is perceived as more favorable for animal welfare compared to SAD feeding programs (Riber, 2020). Because birds do not have daily access to feed, SAD feeding programs are banned in many countries for their negative implications on welfare, despite the improved flock

uniformity associated with SAD feeding (Morrissey et al., 2014). However, research on the effects of SAD versus ED diets on bird behavior and welfare is inconclusive (Riber, 2020). Activity levels in breeders suggest that SAD feeding could potentially improve welfare as birds on this diet showed decreased restlessness, increased sitting, and increased performance of comfort behaviors such as preening and dust bathing compared to ED birds (Morrissey et al., 2014). ED birds have been shown to have increased activity levels before and after feeding when compared to SAD birds fed on a 5:2 schedule, which is indicative of increased anticipation and frustration likely caused by higher feed competition and/or insufficient satiation from the lower quantity of feed provided (Lindholm et al, 2018). However, research also shows that increasing feeding frequency decreases feather and object pecking in ED birds, which could potentially alleviate redirected behaviors related to frustration (Morrissey et al., 2014; van Emous et al., 2024). Novel object testing has also shown increased exploratory behavior in SAD birds compared to ED birds, which could align with a theory that birds experiencing hunger display increased risk-taking behaviors in an effort to find food (Lindholm et al., 2018). Although this behavior could also be interpreted as decreased fearfulness in SAD birds in response to novel objects since the novel object test is traditionally used to evaluate fearfulness (Forkman et al., 2007). While ED feeding may have some potential benefits for redirected behaviors, it is evident that birds still show signs of chronic hunger, anticipation, and possibly fearfulness. Overall, results regarding the effects of SAD and ED diets on bird behavior are ambiguous and more research is needed to evaluate the implications of feeding frequency on breeder welfare.

Another approach to quantitative feed restriction is precision feeding, which involves the allocation of small portions of food throughout the day on an individual basis if birds are below target weight (Zuidhof et al., 2017). This allows birds to better satisfy their foraging behavior by

eating multiple times instead of once daily (Riber, 2020). Compared to SAD feeding, precision-fed birds have been shown to have reduced activity levels, feather pecking, and foraging behaviors, possibly indicating that the precision feeding system better fulfills their need to forage (Girard et al., 2017). However, precision fed birds also had increased incidences of other redirected oral behaviors such as drinker pecking (Girard et al., 2017). Precision feeding may provide a potential option for improving welfare of feed restricted breeders; however, inconsistent results of current studies and its limited use on large-scale, commercial farms suggest a need for further research of the effects of precision feeding on behavior and welfare outcomes (Riber, 2020). Although quantitative restriction is the most common method used to control body weight in the broiler breeder industry, the welfare concerns associated with this type of feeding program has led to research into alternative forms of feed restriction.

Qualitative feed restriction, also known as an alternative diet, is another method of controlling the body weight of birds while also trying to reduce the negative welfare implications of traditional quantitative restriction. This type of feed restriction typically involves the addition of non-nutritive ingredients to increase the amount of feed while decreasing the energy content, thus improving gut fill and satiety (Buckley et al., 2011). Fibrous materials, such as oat hulls, wheat bran, and sugar beet pulp, are common low-nutritive inclusions (D'Eath et al, 2009). Another method of qualitative restriction is the addition of appetite suppressants, such as calcium propionate, to reduce the quantity of feed intake (Nielsen et al., 2011). Because qualitative feed restriction allows birds to have increased feed quantities, it takes longer for birds to eat and provides more opportunity for the performance of foraging behaviors, potentially reducing stereotypic behavior and improving welfare (D'Eath et al., 2009).

Compared to diets with insoluble fiber (ISF) or soluble fiber (SF), birds fed a standard commercial diet wihtout fiber had increased feeding rates and compensatory feeding after restriction, in addition to increased incidences of feather and object pecking (Nielsen et al., 2011). Diets with increased fiber content show indicators of increased welfare with the high ISF treatments having higher performance of comfort behaviors (e.g., dustbathing, preening, scratching, and wing stretching) and decreased compensatory feeding, while the high SF treatments display decreased intake of novel feed, decreased levels of feather pecking, and lower activity levels (Nielsen et al., 2011). However, benefits of high fiber diets have been found to be short-lived (Nielsen et al., 2011). Behavioral signs of distress and discomfort have also been seen in birds fed diets with high SF, including postural changes and redirected oral behavior (Nielsen et al., 2011). In contrast, Riber et al. (2021) found little difference in behavior between birds fed high fiber or standard diets, but this research primarily focused on *ad libitum* quantity diets (Nielsen et al., 2011; Riber et al., 2021). Qualitative feed restriction alone is not enough to completely control body weight and is often combined with quantitative restriction, known as rationed alternative diets (D'Eath et al., 2009).

When examining the use of rationed alternative diets by comparing a commercial diet and two alternative diets with both added fiber and appetite suppressants under SAD and ED conditions, results indicate that birds on the alternative diets had decreased restlessness, aggression, feather pecking, and object pecking, potentially indicating increased satiety and less frustration (Morrissey et al., 2014). A similar reduction of frustration has been found with birds fed rationed high-fiber pellet diets, as there was increased performance of comfort behaviors compared to the control treatment fed a standard diet (Hocking, 2006). However, alternative diets do not completely eliminate redirected oral behaviors, suggesting there is still a level of

chronic hunger (Morrissey et al., 2014). It should also be noted that the decreased redirected and stereotypic behaviors associated with alternative diets could be caused by increased time spent feeding, however this does not necessarily indicate improved welfare (Buckley et al., 2011). While the reduction of feeding rates could indicate satiety, they could also suggest that birds find the diet aversive (Buckley et al., 2011). Preference testing found that birds preferred the standard commercial high-energy diets in both *ad libitum* and restricted portions compared to alternative diets, particularly those with appetite suppressants (Buckley et al., 2011). While further research is still needed on broiler breeder feeding preferences, it is important to consider this when evaluating the potential welfare benefits of alternative diets.

Effects of Restricted Feeding on Water Intake

One of the most common redirected oral behaviors observed in feed restricted broiler breeders is drinker pecking or excessive drinker use, also known as polydipsia (Riber, 2020). In order to discuss excessive drinker pecking and use in breeders, it is important to discern the difference between water consumption and water use. Drinker pecking does not necessarily imply that the birds are consuming water. Instead, birds may just be pecking or "playing" in the water as a form of stereotypic behavior as a result of feed restriction (Mench, 2002). This can lead to water wastage, increased litter moisture, and increased footpad dermatitis, and ultimately decreased welfare (Riber, 2020). However, birds may also be actively consuming the water as they are pecking, which could indicate a greater physiological need for water as a result of the feeding regimen (Bennett and Leeson, 1989). Therefore, it is crucial to differentiate the cause and goal of excessive drinker use.

Feed intake and water intake are closely related, so modifying feeding protocols results in a subsequent modification of drinking behavior (Bennett and Leeson, 1989). In general, SAD

birds drink up to 150% more on feed days than off-feed days compared to ED treatments (Bennett and Leeson, 1989). While SAD birds drink more on days when they are fed, off-feed drinking behavior is still significant as it can be influenced by hunger and frustration. When examining the effects of drinker use across various rationed alternative diets, Morrissey et al. (2014) found that SAD-fed birds displayed increased overall drinker use compared to ED-fed birds across all feed types, including a standard commercial diet and two diets diluted with fiber and appetite suppressants. This increased drinker use from quantitative restriction potentially indicates higher levels of frustration. However, Girard et al. (2017) suggests the opposite effect, as precision-fed birds performed drinker pecking significantly more than SAD-fed birds.

Qualitative feeding has also been shown to have impacts on drinker use, as birds fed diets with higher SF used over 100 g more water and had a higher W:F ratio than birds fed a conventional or high ISF diet (SF: 3.3, Conventional: 2.3, ISF 1.3), with the majority of water usage occurring around feeding time (Nielsen et al., 2011). Simultaneously, litter moisture in birds fed SF diets was significantly higher than the other treatments at both sampling ages, suggesting that much of the water used was not actually consumed or that the diet resulted in higher fecal moisture content (Nielsen et al., 2011). Similar results were found when birds fed a diet including both SF and ISF had increased water usage and litter moisture (Riber et al., 2021). While birds fed a conventional diet used less water than the SF treatment, they performed more drinker pecking than what was indicated by their water usage (Nielsen et al., 2011). Birds fed high ISF diets actually had a lower W:F ratio than the recommended 1.8, possibly indicating a reduced need for water for birds on this regimen (Nielsen et al., 2011). Decreased water use and litter moisture in birds fed a high ISF diet has also been observed in other studies (Hocking, 2006). While current research shows some consistent patterns in the effect of feed restriction on

water intake, there are still some variable results. Nevertheless, implementation of water restriction has become a common practice within the breeder industry in an attempt to prevent polydipsia and improve litter quality, particularly in broiler breeder pullets who are most impacted by feed restriction (de Jong and van Emous, 2017).

Water Restriction

In order to reduce excessive drinker pecking and litter moisture, water restriction is often applied to feed restricted birds (Mench, 2002). Typically, water is provided to birds during and immediately after feeding, but then water is restricted for the majority of the day and overnight (de Jong and van Emous, 2017). There is some research that indicates water restriction does not decrease welfare of birds. For example, in an experiment comparing three types of water provision (i.e., SAD birds with daily water restriction, SAD birds water restricted on feed days only, and ED birds with *ad libitum* water access), there were no significant differences in total water usage, suggesting that the birds were able to fulfill their water use needs regardless of restriction (Bennett and Leeson, 1989). However, there are still animal welfare concerns with the practice of limiting water access as it contradicts one of the "*Five Freedoms of Animal Welfare*" that requires animals be free from thirst (Hocking et al., 1993; FAWC, 1999; de Jong and van Emous, 2017). Another experiment found that water-restricted SAD birds and ED birds with *ad libitum* water access drank more than SAD-fed birds with *ad libitum* water access on an off-feed day (Bennett and Leeson, 1989). However, on feed days, water-restricted SAD birds and ED birds with *ad libitum* water drank less than SAD-fed birds with *ad libitum* access to water (Bennett and Leeson, 1989). Despite the differences in water consumption, water spillage remained similar across all groups based on daily measurements from a water-catching reservoir around the drinker (Bennett and Leeson, 1989). Taken together, these results indicate that

increased water use in SAD fed birds may not be a stereotypic behavior, but rather may be related to the physiological need for water after eating larger rations. Water-restricted SAD birds are likely unable to consume all of the water needed on feed days, so they are displaying an increased compensatory intake on off-feed days (Bennett and Leeson, 1989). A similar pattern can be observed when examining thirst in broilers, as birds that were deprived of water for either 6h or 12h drank significantly more in the post-deprivation period than control birds (Vanderhasselt et al., 2014).

In the same way that feed restriction has a significant effect on water intake, water restriction also has a significant impact on feed intake. Limiting water intake to 3 h daily has been shown to decrease *ad libitum* feed intake by 15% (Hocking, 1993). The relationship between water and feed intake has led to research evaluating the use of water restriction as not just a way to decrease litter moisture, but also as a form of body weight control in breeders (Ross et al., 1981). *Ad libitum*-fed birds undergoing extreme levels of water restriction, 23 h restriction daily, had similar feed intake and body weights when compared to SAD-fed birds with *ad libtum* access to water (Ross et al., 1981). However, activity levels were greater in both SAD and 23 h water restricted birds compared to those with *ad libitum* access to feed and water, suggesting there is increased frustration and stress in these treatments (Ross et al., 1981) Though overall activity levels were similar between SAD birds and birds under 23 h water restriction, SAD birds had a greater increase in activity at the age the SAD program was started, potentially indicating a greater level of distress (Ross et al., 1981). Birds who were water restricted for only 3 h with *ad libitum* access to feed had similar activity levels and feed intake compared to birds with *ad libitum* access to both feed and water, indicating that in order to get birds to voluntarily reduce their feed intake, water restriction must be extreme enough to produce discomfort (Ross et al.,

1981). This level of restriction (e.g., 23 h) is not feasible, especially with increasing ambient temperatures, and therefore is not a common practice within the industry. While some studies suggest there are no welfare drawbacks associated with water restriction, there is limited research to support this claim and further evaluation of the effects of limiting water use on the behavior and welfare of breeders is needed. In addition, the goal of drinking behavior in feed restricted birds should be further investigated to determine whether polydipsia is the result of ARB or a physiological need for water.

Physiological Implications of Production Practices in Broiler Breeder Pullets

Implications of feed and water restriction cannot only be seen in behavior, but also in physiological metrics such as blood biomarkers, gut health, and indicators of hydration such as fecal moisture (Ogunji et al., 1983; Jones et al., 2004; Sweeney et al., 2022). Quantitative feed restriction is associated with higher inflammatory markers of stress, while both quantitative and qualitative restriction have impacts on gut fill, water-holding capacity, gut permeability, and ultimately nutrient absorption (de Jong and van Emous., 2017; Nascimento et al., 2021). These factors, along with altered drinking behavior associated with feed restriction, can impact breeders' physiological need for water, resulting in differences in hydration parameters such as fecal moisture (Ogunji et al., 1983). The role of production practices on the physiology of broiler breeders is complex and should be thoroughly investigated to optimize the welfare of breeders, particularly during rearing when feed and water restriction are most limited and birds are most susceptible to the long-term effects of management procedures.

Blood Parameters and Biomarkers

One of the most common blood parameters used to assess welfare in poultry is plasma corticosterone, which can be associated with chronic stress, inflammation, and metabolic

adaptations (Aranibar et al., 2020). Research suggests that quantitative feed restriction results in higher levels of plasma corticosterone levels, while most qualitative restriction typically has no significant effect on this metric (de Jong et al., 2003; Hocking et al., 2004; Hocking, 2006; Nielsen et al., 2011). One exception is qualitative diets containing sugar beet pulp, which have been shown to increase plasma corticosterone levels potentially due to its high water-holding capacity (WHC; Hocking et al., 2004). Increase of plasma corticosterone can also result in increased heterophil-lymphocyte ratios (H:L), which is another indicator of increased stress and has been shown in birds undergoing both quantitative and qualitative feed restriction (Hocking et al., 2004; de Jong et al., 2005). While these physiological parameters indicate that feed restriction, particularly quantitative, increases stress in birds, another interpretation could be that it is the result of metabolic adjustments made to cope with decreased caloric intake (Aranibar et al., 2020).

Glucose, non-esterified fatty acids (NEFA), phospholipids, and β-hydroxybutyrate (BHB) are other biomarkers found in plasma that are indicative of metabolic rate (de Jong et al., 2003; Nielsen et al., 2011). Birds fed standard commercial diets *ad lbitum* have lower levels of BHB when compared to birds fed diluted diets, indicating less metabolic hunger when feed is not qualitatively restricted (Nielsen et al., 2011). However, the *ad libitum*-fed birds also had decreased levels of phospholipids 4 to 5 h after feeding, which signals that the high energy content of commercial feed may be short-lived (Nielsen et al., 2011). Glucose/NEFA ratios were found to increase with increasing levels of quantitative restriction (i.e. decreasing feed level as a percent of *ad libtum* intake) in breeders (de Jong et al., 2003). These increases in glucose/NEFA ratios were the result of glucose levels remaining similar while NEFA values decreased, suggesting that reducing the quantity of feed also affects metabolic rate by increasing energy

storage (de Jong et al., 2003). Taken together, qualitative and quantitative feed restriction have metabolic effects on birds.

Other blood biomarkers important in evaluating the effects of restrictive practices on bird health include fluorescein isothiocyanate-dextran (FITC-d), lipocalin, calprotectin, α-1-acid glycoprotein (AGP), and total bile acid, which are indicative of intestinal health (Ducatelle et al., 2023). These metrics allow for the measurement of gut permeability and inflammation, which is significantly altered by production practices and are crucial components of breeder health and welfare and will be reviewed in the next section (Baxter et al., 2019; Ducatelle et al., 2023).

Gastrointestinal Health

Maintaining gastrointestinal (GI) health is important for efficient production, nutrient absorption, and welfare in poultry and factors that can impact GI health, and subsequently nutrient availability, include genetics, nutrition, temperature, age, etc. (Yegani and Korver, 2008). In broiler breeders, where uniformity and growth are considerable topics of interest, optimal nutrient absorption is critical. Altering feeding and growth rate, which is commonly done in breeder management, can impact gut fill, intestinal permeability, inflammation, microbiota, as well as the surface-area of the small intestine (Baxter et al., 2019; Nascimento et al., 2021; Sweeney et al, 2022; Shterzer et al., 2023). The relationship between qualitative restriction and satiety is primarily related to increased gut fill from higher WHC of feed ingredients, which is the ability of feed inclusions to hold water (Nascimento et al., 2021). Increasing WHC of diets through the inclusion of fibers like cellulose, rice husk, and sugar beet pulp decreases both feed intake and scaled feed intake (i.e., feed intake adjusted based on body weight), indicating an increase in satiety; however, dietary changes can also negatively impact gut health (Hocking et al., 2004; Nascimento et al., 2021).

The intestinal barrier is composed of epithelial cells with tight junctions in between that regulate paracellular transport (Vicuña et al., 2015). When this barrier is compromised, the intestinal wall becomes more susceptible to toxins and subsequently increase inflammation in the gut (Gilani et al., 2018). This increased enteric permeability can impact both nutrient and water absorption, resulting in greater water loss, also referred to as leaky gut (Collett, 2012). FITC-d is a marker for intestinal permeability in poultry, as it is typically unable to cross the intestinal barrier except in the case of birds with increased permeability (Vicuña et al., 2015). Therefore, increased FITC-d levels in blood serum after inoculation are indicative of enteric permeability and inflammation (Kuttappan et al., 2015). Broilers given rye-based diets, which can be used to stimulate intestinal inflammation and decreased nutrient absorption, have been found to have increased levels of serum FITC-d concentration compared to a corn-based diet (Baxter et al., 2019). However, when comparing the effect of rye diets between a modern broiler strain, a 1995 broiler strain, and an unselected jungle fowl strain, increased serum FITC-d concentrations were only observed in the modern strain and the 1995 strain (Baxter et al., 2019). In addition, the modern strain had the highest FITC-d concentrations compared to the other strains, despite dietary treatment, suggesting a link between genetic selection for fast-growth and intestinal permeability (Baxter et al., 2019). Quantitative restriction also has implications on gut permeability, as feed-restricted birds have significantly higher serum FITC-d concentrations, indicating increased incidence of gut leakage and greater susceptibility to pathogens (Kuttappan et al., 2015). These findings suggest that feed restriction practices have a significant effect on markers of enteric health, which not only impacts the physiological health status of feed-restricted breeders but can also affect the behavioral response to these protocols.

The gut-brain axis explains the relationship between bird behavior and gastrointestinal tract (GIT) status (Fu and Cheng, 2024). The hypothalamic-pituitary-adrenal (HPA) axis, which is responsible for communication between the brain and other organs via hormonal activity, is not only responsible for coping and behavior in animals, but also has effects on intestinal permeability and the gut microbiome (Brunberg et al., 2016). These effects can be seen in laying hens where high feather pecking, an ARB that can be reduced by increasing dietary fiber, is associated with differences in gut microbiota (Meyer et al., 2013; Brunberg et al., 2016). This evidence suggests that qualitative restriction in broiler breeders may potentially mediate ARB, such as excessive drinker use, by altering the intestinal microbiome. Differences in microbiota have been found in modern broiler breeders compared to older strains, such as an increase in *Akkermansia*, a mucin-degrading bacteria Genus (Shterzer et al., 2023). Interestingly, these differences were not found in modern broiler strains, indicating that current breeder management practices may be the cause of this phenomenon, as opposed to genetic selection (Shterzer et al., 2023).

Another important aspect of enteric health in breeders relates to the surface area and absorption of the small intestine. Structures called villi are small projections lining the small intestine that are responsible for nutrient absorption and are made from cells produced by structures called crypts (Yamauchi, 2002). Three main cell types that makeup intestinal villi include absorptive enterocytes, goblet cells, and enteroendocrine cells, which are responsible for nutrient uptake, mucin secretion, and neurotransmitter secretion, respectively (Zhang et al., 2019). Increased villi height and the ratio of villi height to crypt depth (V:C) is associated with increased nutrient absorption, energy utilization, and cellular turnover (Zhang et al., 2019; Sweeney et al., 2022). Rationed alternative diets have been shown to impact intestinal surface

area, as birds fed an ED high-fiber diet had increased villi height in the jejunum at 16 wks of age compared to birds fed the same diet on a SAD regimen (Sweeney et al., 2022). While this increase in villi height could indicate increased nutrient utilization during crucial developmental time periods in ED-fed birds, it should also be noted that SAD birds had increased V:C at 8, 16 and 20 wks compared to ED-fed birds, which is another positive indicator of nutrient absorption (Sweeney et al., 2022). Feeding protocols clearly affect gut health, but the associations are complex and further research is needed to understand the full implications of both quantitative and qualitative restriction on intestinal morphology.

Fecal Moisture as a Marker of Hydration

The multifaceted implication of feeding protocols on behavior and intestinal health, in addition to the subsequent effects of water restriction, places an emphasis on the physiological utilization of water in broiler breeders. Excessive water use in response to feeding protocols could potentially be indicative of less efficient physiological water utilization, particularly if the modifications to enteric health result in an increased physiological requirement for water. However, excessive water use could also be the result of redirected behavioral responses causing birds to "play" with the drinkers in order to redirect and satisfy their foraging needs (Mench, 2002). Both of these theories result in increased litter moisture either through the result of wet droppings or water wastage (Ogunji et al., 1983; Nielsen et al., 2011). This can further impact bird welfare as wet litter is associated with higher incidences of pododermatitis (Kaukonen et al., 2016).

Fecal moisture is a potential method for measuring the physiological need for water (Ogunji et al., 1983). There is evidence that qualitative feed restriction has an effect on fecal moisture, with soluble fibers increasing fecal moisture due to increased digesta viscosity, while

insoluble fiber decreases fecal moisture due to its high WHC (van der Hoeven-Hangoor et al., 2014). Genetics may also play a role in fecal moisture as two different strains of broiler breeders showed significant differences in fecal moisture, most likely caused by a substantial increase in water intake by one strain which was evident across all dietary treatments (Ogunji et al., 1983). This increased water intake and fecal moisture content in a particular strain of breeders could indicate a difference in physiological water utilization due to genetics, suggesting that genetics may have an even greater effect than some dietary alterations (Ogunji et al., 1983). The confounding role of genetics is further supported by the limited effects of dietary protein on both fecal and litter moisture in broiler breeders (van Emous et al., 2019). There is also some evidence that dilution of diets with roughage may not have substantial impacts on fecal or litter moisture either (Kittelsen et al., 2023). However, further research is needed to thoroughly understand the impacts of feed inclusions and practices on indicators of hydration, such as fecal moisture.

In conclusion, the quantity and quality of feed and water provided to chickens influences their behavioral repertoire. Therefore, production practices within the United States aimed at maintaining optimal body weight and uniformity in broiler breeder pullets results in changes to the natural feeding and drinking behaviors, as well as increasing physiological indicators of stress and impaired intestinal health. This ultimately leads to a reduction of breeder welfare. While methods for resolving these behavioral and physiological issues have been evaluated, the effectiveness of alternative feed and water protocols is still not completely understood. Further research to evaluate the impacts of feed and water restriction on breeder behavior and physiology, especially related to water use, can establish practices that improve welfare of pullets during rearing.

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CHAPTER 2

The Effect of Feed and Water Provision Strategies on Broiler Breeder Pullet Performance and Welfare

ABSTRACT

Feed restriction practices are implemented in the broiler breeder industry to maintain optimal health and production. However, these practices are often associated with increased drinker use by the birds that can cause water spillage, higher litter moisture, and increased incidence of footpad lesions. As a result, some parts of the industry have implemented water restriction protocols to mitigate water spillage. The underlying cause of increased drinker use by the birds is not entirely clear. The objective of this study was to assess the effect of skip-a-day (SAD) feeding versus every-day (ED) feeding and water restriction (WR) versus *ad libitum* access to water (ADLIB) on flock performance and welfare metrics, such as drinking behavior, water use, litter moisture, footpad condition, body weight, and uniformity. A total of 960 Cobb 500 FF pullets (*Gallus gallus domesticus*) were randomly allocated to one of four treatments: SAD+ADLIB, ED+ADLIB, SAD+WR, and ED+WR. Ten focal birds per pen were uniquely colored with livestock marker for individual identification. Drinking behavior was recorded at 16 and 22 wks using one video camera per pen. Trained observes continuously coded three 20-min segments of video for each focal bird at an hour after feeding (HAF), when water was turned off for the WR treatments (12:00), and when water access resumed for 1 h for the WR treatments (14:30). During the on-feed day, ED-fed pullets displayed more drinker use at HAF at both ages ($P=0.0135$), while SAD-fed pullets had increased drinker use at 12:00 ($P<0.0001$) and 14:30 ($P=0.0028$) at 22 wks. Also during the on-feed day, pullets from the WR treatments displayed higher drinker use than pullets from the ADLIB treatments during HAF at 22 wks ($P<0.0001$)

and 14:30 at both ages (P<0.0001). However, at 12:00, pullets from the ADLIB treatments displayed more drinker use at both ages than WR pullets (P=0.008). Pen water use was measured for 48 h at 4, 10, 16 and 22 wks using a gravity water system for each pen. At 10 wks, pullets from both SAD treatments had the highest water use compared to pullets from both ED treatments (P=0.0419). The ED+ADLIB pullets used more water at 16 and 22 wks than SAD+ADLIB pullets (P=0.0419). Water use was similar between ED+WR and SAD+WR treatments at 16 and 22 wks (P>0.05). Litter samples were taken from under the waterline (WL) and 1 meter away from the waterline in the general pen area (GEN) at 10, 16, and 22 wks. Samples were weighed at ~70g, placed in an aluminum pan, dried at 100°C for 48 h, then reweighed to calculate litter moisture content. Litter moisture for WL samples coincided with water use at 16 wks, where the ED+ADLIB treatments displayed the highest WL litter moisture of all treatments when they simultaneously displayed the highest water use (P=0.0011). However, the highest WL litter moisture did not correspond to the highest water use treatment at 10 or 22 wks (P=0.011). For GEN litter samples, both SAD treatments had increased moisture at 10 wks compared to both ED treatments, while ADLIB pens had increased moisture compared to WR pens at all ages (P=0.0036). Footpad condition scoring was performed on 10 focal birds from each pen at 16 and 22 wks of age by two trained observers; however, no significant differences were found (P>0.05). Body weight and uniformity was also recorded weekly, but did not appear to influence drinking behavior or water use. The results of this study suggest that ED-fed pullets displayed more drinker use at earlier times in the day and increased overall water use at later ages, while SAD-fed pullets displayed more drinker use at later times in the day and leveled off water use as they aged. In addition, compensatory drinking behavior suggests a potential welfare concern for WR treatments.

INTRODUCTION

Broiler breeders are genetically selected for traits that result in offspring with high carcass yields, such as a strong appetite and fast growth rate (Mench, 2002). However, because broiler breeders have a longer life cycle than their offspring (e.g., 60 to 70 wks versus 6 to 8 wks), these traits must be carefully managed in breeders to mitigate the risk of developing obesity-related physiological issues, such as tibial dyschondroplasia, ascites, heart disease, and impaired reproductive function (Mench 2002; D'Eath et al., 2009). Growth management strategies are particularly critical during the pullet phase, where birds are more heavily feed restricted in order to follow a recommended growth curve provided by the breeding company to optimize development (de Jong and van Emous, 2017). Feed restriction is implemented throughout the industry to limit nutrient intake either quantitatively or qualitatively (Buckley et al., 2011).

Quantitative feed restriction provides a limited ration of feed through one of two possible regimens: skip-a-day (SAD) or every-day (ED) feeding. SAD feeding is a program that allows for large portions of feed to be fed on alternate days. Most commonly, SAD birds are fed on either a 4:3 (4 days on feed and 3 days off feed each week) or a 5:2 (5 days on feed and 2 days off feed each week) schedule (de Jong and van Emous, 2017). Alternatively, an ED feeding program provides birds with feed daily, but the portion is smaller than what would be provided to SAD birds on a feeding day (Riber, 2020). Though feed restriction is necessary to maintain the physiological health status of breeder pullets, the severe reduction of up to 80% of *ad libitum* feed intake during rearing can result in chronic hunger and increased indicators of poor welfare, such as abnormal repetitive behaviors, stereotypic object pecking, and restlessness (D'Eath et al.,

2009; Morrissey et al., 2014; Mellor et al., 2018; Riber, 2020). ED feeding regimens have been shown to improve both behavioral and physiological metrics through the decrease of feather and object pecking, as well as increased villi height in the small intestine indicating increased nutrient absorption (Morrissey et al., 2014; Sweeney et al, 2022; van Emous et al., 2024). However, ED feeding is also associated with increased anticipatory activity prior to feeding compared to SAD feeding, suggesting possible insufficient satiation due to the smaller portion (Lindholm et al, 2018).

Qualitative feed restriction is another feeding method that limits the caloric intake of birds by introducing non-nutritive ingredients to the diet that promote gut fill and satiety (Buckley et al., 2011). For example, insoluble fiber is a feed ingredient that increases the water-holding capacity of digesta within the gut, causing birds to feel satiated for longer (Hocking et al., 2004). The addition of fiber in breeder diets has also been noted to decrease feather and object pecking and increase the performance of comfort behaviors, such as preening, dustbathing, scratching, and stretching (Nielsen et al., 2011). However, qualitative-restricted diets cannot be fed *ad libitum* as they are not effective at maintaining ideal body weight and uniformity in flocks on their own. Qualitative-restricted diets are often used in combination with quantitative restriction methods (D'Eath et al., 2009).

While there is some evidence that qualitative feed restriction reduces the incidence of abnormal behaviors associated with quantitative restriction, signs of chronic hunger are still apparent (Morrissey et al., 2014). For example, excessive drinker use (i.e., polydipsia) is often associated with feed restriction in breeder pullets (Riber, 2020). This may be the result of stereotypic drinker pecking – an abnormal repetitive behavior associated with chronic hunger, where birds redirect pecking from empty feeders onto drinkers and waterlines (D'Eath et al.,

2009). Prolonged pecking on drinkers can increase the risks for water spillage, high litter moisture, and high incidence of pododermatitis or footpad lesions (de Jong and van Emous, 2017). However, high drinker use could also be the result of an increased physiological need for water caused by feed restriction practices (Bennett and Leeson, 1989). Nevertheless, water restriction is a method applied to reduce excessive drinker use and subsequently improve litter quality and footpad condition (Mench, 2002). Waterlines are often turned off overnight but may also be turned off intermittently throughout the day (e.g., from 09:00 to 13:00h, 4 h total; Bennett and Leeson, 1989). Though restricting water access may benefit some welfare metrics by maintaining dryer litter, the practice's effect on animal welfare remains understudied and may result in similar drinker pecking behavior observed with feed restriction (de Jong and van Emous, 2017).

The objective of this study was to evaluate the effect of feed and water restriction strategies (i.e., SAD versus ED feeding and *ad libitum* versus restricted access to water) on broiler breeder pullet welfare. We hypothesized that the most restricted feed and water provision practices (i.e., SAD feeding and water restriction) would impair broiler breeder pullet welfare. Specifically, we predicted that SAD-fed and water-restricted pullets would display more drinking behavior and higher water use that may indicate compensatory drinking and/or stereotypic behavior associated with restriction. With higher water use, we also predicted that SAD-fed and water-restricted pullets would have higher litter moisture and consequently a higher prevalence of footpad lesions than ED-fed pullets and pullets with *ad libitum* water access. For performance, we predicted the SAD-fed and water-restricted pullets would maintain lower body weights and a lower coefficient of variation, indicating better uniformity.

MATERIALS AND METHODS

The North Carolina State University Institutional Animal Care and Use Committee reviewed and approved the experimental procedures for this study (Protocol #23-417). The animals were cared for in accordance with the 4th edition of the Guide for the Care and Use of Agricultural Animals in Research and Teaching.

Animals and Housing

A total of 960 female Cobb500 FF (Cobb-Vantress Genetics, Siloam Springs, AR) one-day-old chicks (*Gallus gallus domesticus*) were randomly assigned to one of 16 floor pens (3.81 x 2.44 m, L x W; 60 birds/pen) in a curtain-sided poultry house at the North Carolina State University Chicken Education Unit in Raleigh, North Carolina (60 chicks/pen, 1,549.4 cm^2/bird). For the first two wks of the experiment, mortalities and culls were replaced with extra birds from the same chick flock that were housed in the same location to maintain similar bird numbers across pens.

A 2x2 factorial design was utilized with two feeding protocol treatments: skip-a-day (SAD); or every-day (ED), and two water protocol treatments: *ad libitum* (ADLIB) water during lights on, and water restriction intermittently during lights on (WR). The house was divided into quadrants with four pens/quadrant. Due to animal welfare and infrastructure considerations, treatments were unable to be randomly distributed throughout the house. All SAD pens were located on the west half of the house in two quadrants, while all ED pens were located on the east half of the house in two quadrants (8 pens/feeding protocol). The two halves were separated by a 3.6 m storage area, and curtains were hung over the openings to each side of the house. From an animal welfare standpoint, maintaining SAD and ED pens on separate sides of the house allowed us to reduce visual and auditory stimuli of feeding that could result in

anticipation, frustration, and anxiety, particularly for SAD birds during off-feed days. For infrastructure, water clocks were added to water lines to ensure consistency in the timing of when water was turned on and off across treatment pens. However, water clocks were only able to be installed on a waterline for each quadrant, so an entire quadrant of four pens was assigned to the same water treatment. As a result, one quadrant on each side was assigned to ADLIB and the other was assigned to WR (8 pens/water treatment). The feeding and water protocol treatment combinations included SAD+WR, SAD+ADLIB, ED+WR, and ED+ADLIB (n=4 pens/treatment).

At placement, birds received 23 h of light, which was decreased by 1 to 2 h per day until the photoperiod became 8 h (07:00-15:00) by day 14 and remained through 22 wks of age. Pens were bedded with pine wood shavings (Suncoast Pine Shavings, Fitzgerald, GA). Each pen contained four circular pan feeders (36.83 cm diameter; 2.45 cm/bird total) and one water line with 5 nipple drinkers (12 birds/nipple; Ziggity Systems Inc.©, Middlebury, IN). Supplemental feeders and waterers were also provided at chick placement and removed after the first week. At 15 wks of age, two additional trough feeders (50.80 cm each) were added to increase feeder space (4.15 cm/bird total).

Feeding and Water Treatments

All birds were fed the same standard starter diet (2,850 kcal/kg, 19% Crude Protein, CP) until 3 wks of age. At 3 wks, birds were redistributed to maintain similar bird numbers/pen prior to switching to grower diets and starting their respective feed and water treatments. Both ED and SAD grower diets had similar nutritional content (ED: 2,799kcal/kg, 13.5% CP, 1.09% Ca; SAD: 2,799kcal/kg, 15% CP, 1.10% Ca), but the ED diet had additional fiber to promote gut fill (Table 1.1, Table 1.2). Birds were switched to a developer diet at 16 wks of age, again with similar

overall nutritional content (ED: 2,623 kcal/kg, 13.40% CP, 1.10% Ca; SAD: 2,799 kcal/kg, 14.80% CP, 1.18% Ca), but increased fiber in the ED diet (Table 1.1, Table 1.2). At the onset of the feeding regimens, SAD birds initially followed a 4:3 feeding program, where they were fed four out of seven days of the week and had three off-feed days. At 14 wks of age, they were switched to a 5:2 feeding program. Weekly feed quantities were determined for both treatments by following Cobb Genetics (2020) recommendations pending age and average bird weight for each pen.

All birds had *ad libitum* access to water until 6 wks of age. At this time, nocturnal water restriction (15:00 to 07:00; total of 16 h) was applied to all pens. After a one-week acclimation to nocturnal water restriction, daytime water restriction was applied to the SAD+WR and ED+WR pens at 7 wks of age. Water restricted birds had access to water for the first 4 h of the photoperiod, which included during and after feeding (07:00-11:00), and also for 1 h before the photoperiod ended (14:00-15:00). Water lines were verified to be empty during the daytime restriction period (11:00-14:00).

Drinking Behavior

Ten birds were randomly selected at placement to be focal behavior pullets. They were marked with unique colors for identification, using food dye on down feathers at placement and livestock markers after primary feather development (All-Weather Twist-Stik, LA-CO Markal, Elk Grove Village, IL). Markings were touched up as needed to ensure visibility in video recordings. In each pen, one color video camera (4K Smart IP Fixed Bullet Camera, Lorex Corporation, Irvine, CA) was directed at the water line to continuously record video footage for 1 wk at 16 and 22 wks of age. All cameras were linked to a network video recorder (4K 16-channel Wired NVR System, Lorex Corporation, Irvine, CA) to save video files.

At each age, behavior was coded for two consecutive days for SAD pens, including one on-feed and one off-feed day, while behavior was only coded for one day for ED pens, which was the on-feed day for SAD pens. On the selected days, 20-min segments of video were coded at an hour after feeding (HAF), 12:00 hrs (12:00), and 14:30 hrs (14:30), for a total of 60 min/day. Due to video hard-drive malfunctions, footage from the SAD off-feed day at 22 wks was not coded. Inter-rater agreement was calculated by comparing drinking durations (sec) between the trainer and trainee for 10 pullets using Microsoft Excel 2016 (version 16.0.5465.1000, Microsoft Corporation, Redmond, WA) to achieve ≥90% inter-rater agreement across all observers.

During video observations, drinking behavior was defined as when the bird's neck is extended towards the nipple and the beak is in contact with the nipple. The drinking bout ends when beak contact with nipple pauses for >3 seconds, and/or when the bird turns and its head reaches 1 bird length away from the drinker (3.8 cm on the computer screen) or reaches the end of the drinker line. Drinking behavior of the 10 focal birds/pen was coded from video files by six trained observers using Behavioral Observation Research Interactive Software (BORIS, version 8.19.3, http://www.boris.unito.it/, Friard and Gamba, 2016).

Average Bird Water Use

Water usage for a 48-h period was measured for each pen at 4, 10, 16, and 22 wks of age using a gravity water system. The two 24-h periods represented an on-feed day (Day 1) and an off-feed day for SAD treatments (Day 2). At each timepoint, 16.5-gallon carboys were filled, weighed, and placed on top of a ladder in front of each pen (n=4 pens/treatmetn). Waterlines in each pen were disconnected from the barn's main water supply and reconnected to their respective carboys. For ADLIB pens, carboys were weighed at the end of the photoperiod each

day (8 h), prior to manually turning off water flow for nocturnal restriction. For WR pens, carboys were manually turned on and off to maintain the daytime and nocturnal restriction schedule, and they were weighed each time they were turned off. Carboys were refilled and weighed as needed to ensure birds had access to water for the entirety of the treatment's water access period. Pen water use (g/pen) was calculated by subtracting carboy weights from the beginning of the water access period from the end of the access period. The average pullet water use (g/pullet) was determined by dividing the pen water use by the number of birds per pen.

Litter Moisture

At 10, 16, and 22 wks of age, litter samples were collected from both the general pen area (GEN) and under the waterline (WL). For the GEN sample, four handfuls of litter (one from each quadrant of the pen) were collected 1 m from the waterline and mixed in a plastic bag for each pen. For the WL sample, one handful of litter was collected directly under each nipple on the waterline (five handfuls total) and mixed in a plastic bag for each pen. Approximately 70 g of each sample was weighed then dried in an oven at 100°C for 48-hrs. Dried samples were reweighed. Litter moisture was calculated using the following equation: (wet weight-dry weight)/wet weight.

Footpad Condition Scoring

Footpad scoring (Table 1.3) was performed on the 10 focal birds from each pen at 16 and 22 wks of age. Two trained scorers achieved an inter-rater agreement ≥90%.

Body Weight and Uniformity

Body weights of a subsample of 10 birds per pen were measured weekly from 0 to 22 wks. Coefficient of variation (CV) was calculated from 3 to 22 wks by dividing the standard deviation of weights by the mean.

Statistical Analysis

All data were analyzed using R statistical software (version 4.4.2; R Core Team, 2021) using RStudio (version 2022.12.0+353) for macOS Sonoma 14.7.1. Both generalized linear mixed effects models (glmmTMB package, version 1.1.10, Brooks et al., 2017) and linear mixed effects models (nlme package, version 3.1.166, Pinheiro et al., 2024) were used. Generalized linear mixed effects model fits were tested using DHARMa package (version 0.4.7; Hartig, 2022). Linear mixed effects model fits were evaluated for normality and homogeneity of variance with visual inspection of quantile-quantile plots and plots of residuals versus fitted values (*qqnorm*, *plot*, and *hist* functions in base R). A stepwise backward reduction using ANOVA for model comparison was used for all analyses to obtain the final models, where $p>0.05$ was the criterion of exclusion. Where there were significant main effects or interactions, differences between specific fixed effects were determined with Tukey's pairwise comparisons (emmeans package, version 1.10.5, Lenth, 2024).

Drinking behavior durations were summed by focal bird for each time period (i.e., HAF, 12:00, and 14:30) in each pen at two ages. Separate linear mixed effects models were run for each time period for on-feed days to improve model fit. Feed treatment (factor with two levels: SAD and ED), water treatment (factor with two levels: ADLIB and WR), age (factor with two levels: 16 and 22 wks), and their 2-way and 3-way interactions were included as fixed effects, and focal bird nested within pen was included as a random effect. Data were square-root transformed to improve model fit. Due to off-feed day data loss at 22 wks, the off-feed day model was assessed only for 16 wks. Feed treatment (factor with two levels: SAD and ED), water treatment (factor with two levels: ADLIB and WR), time period (factor with three levels:

HAF, 12:00, and 14:30), and their 2-way and 3-way interactions were included as fixed effects, and focal bird nested within pen was included as a random effect.

Pen water usage data were summarized by the water usage/pen/day (g) divided by the number of birds in the pen to obtain an average bird water usage/pen/day (g). Separate linear mixed effects models were run for on-feed and off-feed days to improve model fit. Feed treatment (factor with two levels: SAD and ED), water treatment (factor with two levels: ADLIB and WR), age (factor with four levels: 4, 10, 16 and 22 wks), and their 2-way and 3-way interactions were included as fixed effects. Pen was included as a random effect.

Litter moisture was calculated for WL and GEN areas and analyzed using generalized linear mixed effects models with a negative binomial distribution. Separate models were run for WL and GEN areas, where feed treatment (factor with two levels: SAD and ED), water treatment (factor with two levels: ADLIB and WR), age (factor with three levels: 10, 16 and 22 wks), and their 2-way and 3-way interactions were included as fixed effects. Pen was included as a random effect.

Footpad condition was summarized as the proportion of footpad scores of 0 and 1 in each pen and analyzed using generalized linear mixed effects models with a negative binomial distribution. Separate models were run for scores of 0 and scores of 1. Fixed effects included feed treatment (factor with two levels: SAD and ED), water treatment (factor with two levels: ADLIB and WR), age (factor with two levels: 16 and 22 wks), and their 2-way and 3-way interactions, with pen as a random effect. Footpad scores of 2 were not statistically analyzed due to their low prevalence (only one bird out of 160 birds scored as 2 at 22 wks of age).

Body weight and CV data were analyzed using linear models to assess the effect of feed treatment and water treatment. Separate models were run for each week and significance was determined at $p \leq 0.05$.

RESULTS

Drinking Behavior

During on-feed days at HAF, there was a main effect of feed treatment with ED birds spending more time at the drinker than SAD birds at both ages (LLR=6.10, p=0.0135; Figure 1.1). There was also a significant interaction between age and water treatment, with WR pullets drinking more than ADLIB pullets at 22 wks (LLR=14.61, p<0.0001; Figure 1.2). There was no feed x water interaction at HAF (LLR=1.77, p=0.1836). At 12:00 during on-feed days, when water was turned off for WR birds, there was an age x feed treatment interaction, with SAD birds spending more time at the drinker than ED birds at 22 wks (LLR=16.30, p<0.0001; Figure 1.1). There was also an age x water treatment interaction at 12:00 where ADLIB spent more time at the drinker than WR birds at both ages, but WR pullets showed a larger increase in drinking time between ages than ADLIB pullets (LLR=7.03, p=0.008; Figure 1.2). There was no feed x water interaction during the on-feed day at 12:00 (LLR=0.0073, p=0.93). At 14:30, when water access resumed for 1 h for the WR treatments, there was an interaction between age and feed treatment, with SAD pullets drinking longer than ED pullets at 22 wks (LLR=8.94, p=0.0028; Figure 1.1). A main effect of water treatment was also observed at this time of day, where WR pullets drank more than ADLIB pullets at both ages (LLR= 33.99, P<0.001; Figure 1.2). There was no feed x water interaction at 14:30 on the on-feed day (LLR=0.60, 0.4377).

On the off-feed day for SAD pullets only, there was an interaction between time period and water treatment with ADLIB birds drinking more than WR birds at 12:00 (when water was turned off for WR birds), but WR birds drank more than ADLIB birds at 14:30 (LLR=46.16, p<0.0001; Figure 1.3). There was no significant difference between the water treatments at HAF.

Average Bird Water Use

During the on-feed day, there was a three-way interaction between age, feed treatment, and water treatment (LLR=8.21, p=0.0419; Figure 1.4). At 4 wks, water use was similar across all treatments. At 10 wks, SAD + ADLIB and SAD + WR pullets used more water than ED + ADLIB and ED + WR pullets. With ADLIB water access, ED pullets used more water at 16 and 22 wks than SAD pullets. However, the WR access resulted in similar water use between SAD and ED pullets at 16 and 22 wks.

There was another three-way interaction between age, feed treatment, and water treatment on the off-feed day (LLR=10.35, p=0.0158; Figure 1.5). At 4 wks, ED + ADLIB and ED + WR pullets used more water than SAD + ADLIB and SAD + WR pullets. The ED + ADLIB pullets continued using more water than SAD + ADLIB pullets at 16 and 22 wks, but they used similar amounts of water during week 10. The ED + WR pullets used more water at 10 wks than SAD + WR pullets; however, there were no differences in water use between the treatments at 16 and 22 wks.

Litter Moisture

There was a three-way interaction between feed treatment, water treatment, and age for the WL samples (χ^2=13.67, df=2, P=0.0011; Table 1.4). At 10 wks, ED + ADLIB pens had the highest WL litter moisture, followed by the other three treatments with a similar moisture level. At 16 wks, ED + ADLIB had the highest WL litter moisture again, with SAD + ADLIB and ED

+ WR in the middle and SAD + WR with the lowest litter moisture. However, by 22 wks the SAD + ADLIB pens had the highest litter moisture, followed by the ED + ADLIB and SAD + WR, with the ED + WR having the lowest WL litter moisture. Data were analyzed as proportions but are presented as percentages for readability.

For GEN litter samples, there was an age x feed interaction, where SAD-fed birds had increased litter moisture compared to ED birds at 10 wks only, and litter moisture content was similar between these treatments at 16 and 22 wks (χ^2=11.25, df=2, P=0.0036; Table 1.5). There was also a main effect of water, with ADLIB pens having greater GEN moisture compared to WR pens at all ages (χ^2=7.88, df=1, P=0.005; ADLIB: 22.90 [21.10, 24.80], WR: 18.90 [17.30, 20.60] %, mean [95% CI]). Data were analyzed as proportions but are presented as percentages for readability.

Footpad Condition Scoring

There were no significant interactions or main effects of feed treatment, water treatment, or age on footpad condition scores of 0 (P>0.05; Table 1.6). Similarly, for footpad condition scores of 1, there were also no significant interactions or main effects of feed treatment, water treatment, or age (P>0.05; Table 1.7). Data were analyzed as proportions but are presented as percentages for readability.

Body Weight and Uniformity

For body weights, both ED treatments were significantly higher than both SAD treatments despite water provision at 8, 12, 13, 17, 19, and 20 wks. In contrast, both SAD treatments had higher body weights than both ED treatments at 2 and 22 wks (Table 1.8). There was an interaction between feed and water treatments at 18 wks, with the ED+ADLIB treatment having the highest body weights, followed by ED+WR, SAD+WR, and SAD+ADLIB (Table

1.8). There was another interaction between feed and water at 21 wks as SAD+ADLIB had greater body weights than ED+ADLIB (Table 1.8). There were no significant effects or interactions at any of the other ages (Table 1.8).

For uniformity there was a main effect of water treatment at 9 wks, with both ADLIB treatments having a higher CV than both WR treatments ($p=0.0292$; Table 1.9). There was an interaction between feed and water treatments at 19 wks, where SAD+ALIB had a higher CV than SAD+WR and ED+ADLIB ($p=0.014$; Table 1.9). There were no significant effects or interaction at any other age ($p>0.05$; Table 1.9)

DISCUSSION

The objective of this study was to assess the effect of feed (i.e., SAD versus ED) and water protocols (i.e., WR versus ADLIB) on broiler breeder pullet water use, drinking behavior, and key welfare indicators of litter moisture and footpad condition scores. We hypothesized that SAD feeding would increase drinking behavior and water use when compared to ED feeding as a result of redirected consummatory behaviors caused by longer temporal gaps in feed provision. We further hypothesized that more drinking behavior would result in water spillage that increased litter moisture and worsened footpad condition, which would be exacerbated by ADLIB access to water. While we expected WR to decrease litter moisture and improve footpad condition, we also hypothesized that this water protocol would result in compensatory drinking behavior.

Drinking Behavior

We anticipated that SAD and ED groups would show differences in drinking behavior at different timepoints throughout the on-feed day due to differences in quantity of feed and fasting

periods, which was observed. The ED-fed birds drank more at HAF at both ages, while SAD-fed birds drank more at 12:00 and 14:30 at 22 wks. This is most likely because ED-fed pullets have a smaller quantity of feed to consume than SAD-fed pullets, so they finished eating and began drinking earlier. In contrast, SAD-fed birds have a larger quantity of feed to consume on feed days, particularly as they age. Larger feed quantities leads to more overall water use, which has been noted in SAD-fed birds when compared to ED-fed birds (Bennett and Leeson, 1989). However, it is important to consider the timing of this increased drinking behavior when examining the implications of water restriction practices used alongside SAD or ED feeding programs. Because of the larger feed rations, SAD-fed birds might not be finished consuming their feed at HAF, thus drinking more at later times in the day. Our findings suggest that, for SAD-fed pullets, restricting access to water even several hours after feeding could prevent them from fulfilling physiological needs and risking adequate hydration (El Sabry et al., 2023).

We also expected differences in drinking behavior between ADLIB and WR treatments, with ADLIB treatments drinking more than WR during the restricted time period (i.e., 12:00) and WR treatments drinking more than ADLIB when access to water resumed (i.e., 14:30). These results were observed, as ADLIB birds drank more at 12:00 at both ages and WR birds drank more at 14:30 at both ages. Water restriction practices are often implemented simultaneously with feed restriction to prevent water spillage from birds "playing" with the drinkers, as anecdotally described in the industry. We hypothesized that "playing" with drinkers was stereotypic pecking behavior directed towards the drinker line (Riber, 2020). The results from our study indicate that the WR protocols were successful in minimizing drinking behavior when water was turned off, as pullets learned the WR schedule and mostly did not "play" with, or peck at, the drinker when the water was off. Water output appears to be a critical component

of satisfying the pecking behavior at the drinker line. However, despite 12 weeks of training on the WR schedule, the WR pullets increased their drinking behavior attempts at 12:00 at 22 wks, possibly reflecting a greater need for water at this age.

While the WR protocol was effective in minimizing drinking behavior when water was off, the WR pullets displayed an increase in drinking behavior at 14:30, resulting in compensatory drinking when the water is turned back on. WR treatments also displayed more drinking behavior at HAF at week 22, potentially indicating an anticipatory reaction to losing water access. Increased water consumption after a period of deprivation has been previously observed in both broilers and broiler breeders (Bennett and Leeson, 1989; Vanderhasselt et al., 2014; El Sabry et al., 2023). Bennett and Leeson (1989) noted that SAD birds with ad libitum access to water actually drank less on an off-feed day than SAD-fed birds with restricted access to water, similarly suggesting that water restriction elicits a compensatory consumption at the end of the restriction period. Compensatory behavior could aim to fulfill behavioral and/or physiological needs, such that water restriction may contribute to dehydration or discomfort (Vanderhasselt et al.,2014). Our observations contradict findings by Hocking (1993), where water-restricted birds did not display signs of thirst after a period of water deprivation despite limited or ad libitum access to feed. Decades of genetic selection since the previous work was published may have resulted in the behavioral differences observed in the current study.

Average Bird Water Use

We predicted that SAD-fed birds would have higher water usage on feed days compared to ED-fed birds, specifically that SAD+ADLIB birds would have the highest water usage of all four treatments; however, this was not the case. During the on-feed day, SAD-fed birds in both ADLIB and WR groups used more water at 10 wks, but not at any other age. In fact, ED+ADLIB

birds used more water than SAD+ADLIB birds at both 16 and 22 wks on the on-feed day. These results differ from previous research that observed SAD-fed pullets displayed more drinking behavior than ED-fed pullets at 11, 13, and 17 wks (Morrissey et al., 2014). All pullets in the current study were switched to developer diets 16 wks, so the increase in water use by ED-fed pullets could indicate a greater metabolic demand for water in this dietary phase. Indeed, Nielsen et al. (2011) noted that higher levels of insoluble fiber (ISF) in an ED feeding regimen reduced water consumption in breeders. Dietary fiber levels for the ED-fed pullets was higher in the grower diet compared to the developer diet, which may explain why water use was lower during the grower diet phase. However, it is still not clear why the ED-fed pullets used more water than SAD-fed pullets as the ED diet had higher ISF compared to the SAD diet in the grower and developer phases. Inconsistencies between the results of the current study compared to Nielsen et al. (2011) could be attributed to different genetic strains being used, as genetics is one of many factors that affects water use in chickens (Deeb and Cahaner, 2002). Ogunji et al. (1983) found that, when comparing two common broiler breeder strains, one had increased water intake and fecal moisture despite feed treatment, suggesting that genetics may have a more substantial influence on water use than feeding regimens.

Taken together with the behavior results described above, ED pullets spent less time at drinkers than SAD pullets later in the on-feed day, despite using more water overall. Consequently, ED pullets higher water use is driven by more drinking behavior during HAF than SAD pullets. The smaller feed portions may leave ED-fed pullets initially unsatiated, and higher water use shortly after feeding may be a strategy to increase gut fill. This could be a result of the inclusion of ISF in the ED regimen, which has a higher water-holding capacity and allows for greater gastrointestinal distention with the presence of water (Nascimento et al., 2021).

Alternatively, higher water use may also be an indicator of frustration. Increased drinker use in feed-restricted birds is often regarded as a redirected stereotypic behavior associated with unsatisfied consummatory behavior (de Jong and van Emous, 2017). Although they were not measured in the present study, ED-fed birds displayed greater activity levels prior to feeding and lower performance of comfort behaviors compared to SAD-fed birds in previous research, which further supports potential distress for birds on this feeding regimen (Morrissey et al., 2014; Lindholm et al, 2018).

Water restriction protocols resulted in no differences for water use between SAD and ED pens at 16 and 22 wks. Implementing a water restriction protocol levels out the amount of water use across both feed treatments. This could be concerning from an animal welfare standpoint if pullets cannot consume the amount of water they need, particularly for ED birds at 16 wks where ED+ADLIB used more water than ED+WR. Bennett and Leeson (1989) also noted the limiting effect of water restriction protocols on water use.

At 10 wks, both SAD treatments used more water than both ED treatments during the on-feed day, and both SAD treatments used a similar amount of water as both ED treatments during the off-feed day at this age. The reason for this age-related change in water use is not entirely clear. Previous work has attributed higher drinking behavior SAD-fed pullets to acclimating to their dietary regimen for a longer period of time than ED-fed pullets (Morrissey et al., 2014). At 10 wks, pullets had been on their feeding regimen for 7 wks, and no differences in water use were observed at 4 wks after changing to the grower diet. As a result, the role of acclimation does not seem likely. Further research is needed to investigate why certain ages have increased sensitivity to feeding regimens. There are few prior studies that examine drinking behavior and water use across the entire rearing period. Age specific strategies for breeder feed restriction and

management should be explored. Repeatability of this experiment should also be investigated in an environmentally controlled house.

Litter Moisture

We hypothesized that the SAD feed treatments would have higher litter moisture, particularly under the water line, as a result of water wastage from increased drinker use. We also expected WR treatments to have lower litter moisture levels; however, this was not entirely the case. ED+ADLIB and ED+WR pens had greater WL litter moisture than SAD+ADLIB and SAD+WR pens at 16 wks, while ED+ADLIB simultaneously used more water than SAD+ADLIB pullets at this age. Similar results were reported in Arrazola et al. (2019) showing that birds fed a diet with increased ISF, similar to the ED diet in the current study, had higher litter moisture and worse footpad condition in earlier rearing phases. Although, this previous study did not measure water intake or fecal moisture, so the cause for higher litter moisture and subsequent worsening of footpad condition is not entirely clear. The litter moisture results in the current study combined with average bird water use suggest that the ED diet may have created a greater physiological need for water than the SAD diet at older ages, particularly as they shifted onto the developer diet phase at 16 wks. Water restriction protocols did not mitigate the litter moisture under the waterline.

However, SAD+ADLIB and SAD+WR pens had higher WL litter moisture at 22 wks than both ED treatments, despite ED+ADLIB pullets using more water at this age than SAD+ADLIB pullets. Again, water restriction protocols did not mitigate the litter moisture under the waterline. SAD+ADLIB pullets also displayed longer durations of drinking behavior at 12:00 and 14:30 at this age, so the higher WL litter moisture content could be attributed to water spillage from drinker pecking. The ambient temperature increased at 22 wks as the study entered

into warmer summer months (Figure 1.6). Alongside greater portions of feed given to the SAD birds during on-feed days, the longer drinking durations and higher WL moisture at this age could be related to an increased thermoregulatory need (i.e., using the water to cool off), as higher temperatures and higher feed intake are known to cause increased water usage in chickens (El Sabry et al., 2023).

ADLIB birds had greater GEN litter moisture across all ages. While the WL litter moisture is more indicative of water spillage, the GEN litter moisture is more indicative of fecal moisture content, since this sample was taken 1 m away from the water line. Because ADLIB treatments tended to have increased water intake, particularly on off-feed days, this could cause an increase in fecal moisture which would result in a greater GEN litter moisture. SAD birds also had higher GEN litter moisture at 10 wks, which is congruent with the increase in pullet water use at this age, again possibly causing higher moisture in both feces and litter. Ogunji et al. (1983) found a similar association between increased water intake and higher fecal material. It is worth noting that nearly all GEN estimates in the present study do not exceed the optimal litter moisture of 25%, and none of the GEN estimates reach the concerning moisture range of 35 to 45% for caking (Dunlop et al., 2016). Despite treatment differences for GEN litter moisture, the estimates detected do not indicate animal welfare or environmental concerns.

Footpad Condition Scoring

We anticipated that the treatments with the most water use, and highest litter moisture would have worsened footpad condition scores; however, this was not the case. There were no significant differences in footpad scoring across treatments or ages. In contrast, previous work concluded that quantitative feed restriction caused polydipsia that consequently increased litter moisture and incidence of footpad dermatitis (Riber 2020). There are mixed results on the effect

of qualitative feed restriction on footpad condition, with some observing worsened footpad condition in ED treatments fed a higher concentration of ISF and others observing improved footpad condition in groups fed high concentrations of ISF (Arrazola et al., 2019; Kittelsen et al., 2023). The nonsignificant results in the current study could be attributed to differences in sampling methods, whereas Kittlesen et al. (2023) sampled footpad scores post-mortem using a five-level scoring system and Arrazola et al (2019) scored footpad condition biweekly using a binary (i.e., yes/no) scoring system. In contrast, the current study scored footpads three times throughout the study using a three-level scoring system. Using different scoring systems, reducing the number of collection timepoints, and scoring footpad condition on live birds could potentially cause inconsistent results. Further research is needed to fully understand the implications of feed restriction on the incidence of footpad dermatitis.

Body Weight and Uniformity

We hypothesized that birds under ED feeding and ADLIB water conditions would have higher body weights and lower uniformity. Broadly, there was no clear effect of water treatment on body weight; although, there seemed to be an inconsistent effect of feed treatment with ED treatments having higher body weights than SAD treatments at 8, 12, 13, 17, 18, 19 and 20 wks. This is similar to findings by Sweeney et al. (2022) that also observed higher body weights in ED-fed birds compared to SAD-fed birds. In the current study, drinking behavior and/or water use were measured at 4, 10, 16, and 22 wks. Although body weight is a factor that can affect water use in poultry, with larger birds typically consuming more water than smaller birds (Aggrey et al., 2023), a significant difference in body weights was only noted during the 22 wk data collection. During this time point, SAD treatments had higher body weights than ED treatments, but lower water use. This combined with the lack of significant difference in body

weights between treatments at the other data collection weeks indicate that higher body weights were not a driver of increased water use in the current study.

Lastly, there was broadly no effect of feed treatment and water treatment on uniformity, as CV only differed between treatments for two weeks out of the entire trial. Carneiro et al. (2019) also noted no differences in feed restriction programs on CV; however, in contrast Zuidhof et al. (2015) and Sweeney et al. (2022), found that ED treatments decreased and increased uniformity when compared to SAD diets, respectively. Differences in uniformity results could be attributed to genetics, where Zuidhof et al. (2015) and Sweeney et al. (2022) utilized Ross 308 and Ross 708 strains, respectively, while Carneiro et al. (2019) and the current study used Cobb 500. Further research into effect of strain and feeding regimen on uniformity is needed.

CONCLUSION

Feed and water restriction have a complex effect on the drinking behavior, water use, and overall welfare of broiler breeder pullets during rearing. Our study found that SAD-fed birds performed more drinking behavior later in the day compared to ED birds, who displayed more drinking behavior at an HAF. The type of feeding regimen influences the timing of pullet water usage. Furthermore, ED-fed birds tended to have increased water usage later in rearing compared to SAD birds when water was provided ADLIB. These findings indicate possible differences in physiological and/or behavioral needs between feeding regimens that are challenging to disentangle in the current study, including satiety, hydration, stereotypic pecking behavior, and ambient temperatures. The ED-fed pullets may be initially unsatiated from smaller feed quantities and drinking to increase gut fill during HAF, particularly when switched to developer

feed. The SAD-fed pullets may be pecking at waterlines later in the day and at an older age to stay hydrated from larger feed quantities during hotter temperatures. Litter moisture under the waterline is at the greatest risk at older ages, potentially caused by water spillage from more drinking associated with higher ambient temperatures and larger feed quantities in SAD treatments in particular.

The WR treatments performed increased compensatory drinking behavior when water access resumed. Water restriction may inhibit birds from consuming water when physiologically needed, subsequently causing thirst and discomfort. Although the water restriction protocol leveled out water use at older ages, it did not mitigate effects on litter moisture under the waterline, likely due to water spillage during compensatory drinking behavior. Water restriction does benefit general litter moisture throughout rearing though.

A limitation of this study was that it was conducted in a curtain-sided house, and future pullet research should investigate water use in an environmentally controlled, solid-walled house to mitigate the influence of higher ambient temperatures. Future work should also include metrics of satiety and hydration to disentangle these factors from stereotypic behavior to better understand the underlying mechanisms of water use and drinking behavior.

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Table 1.1 Composition of every-day (ED) pullet diets and calculated analysis.

Ingredients %	Starter	Grower	Developer
Ground Corn	58.00	45.99	54.39
Soybean Meal 48% CP	25.00	3.73	2.00
Wheat Bran	11.12	--	--
Wheat Midds	--	34.40	26.04
DDGS	--	8.44	11.30
Limestone Fine	1.63	2.18	2.16
Mono-Dicalcium Phosphate	1.77	1.00	1.20
Salt (NaCl)	0.25	0.25	0.25
Sodium Bicarbonate	0.25	0.25	0.25
L- Lysine (50% Liquid)	0.48	0.20	0.23
DL- Methionine	0.12	0.20	0.01
L-Threonine 98.5%	0.056	--	--
Mineral Premix[1]	0.20	0.20	0.20
Vitamin Premix[2]	0.05	0.05	0.05
Arbocel® (cellulose)	--	0.80	0.80
Selenium premix[3]	0.05	0.05	0.05
Choline Chloride	0.02	--	--
Choline 60% Dry	--	0.02	0.02
Phytase	--	--	0.05
Poultry Fat	1.00	2.235	1.00
Total	100	100	100
Calculated Analysis:			
Crude Protein, %	19.00	13.50	13.40
Ca, %	1.00	1.09	1.10
P, %	0.82	0.75	0.81
Available P, %	0.45	0.39	0.40
Metabolizable energy, kcal per kg of diet	2850.58	2799.87	2623.50
Digestible lysine, %	1.34	0.70	0.63
Digestible methionine	0.42	0.39	0.23

[1]Mineral premix provides per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt.

[2]Vitamin premix provides per kg of 3diet: 13,200 IU vitamin A, 4000 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 2 mg menadione (K3), 2 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 4 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.

[3]Selenium premix, 1 mg Selenium premix provides 0.2 mg Se (as Na2 SeO3) per kg of diet.

Table 1.2 Composition of skip-a-day (SAD) pullet diets and calculated analysis.

Ingredients %	Starter	Grower	Developer
Ground Corn	58.00	53.52	53.44
Soybean Meal 48% CP	25.00	9.00	8.75
Wheat Bran	11.12	--	--
Wheat Midds	--	23.92	22.15
DDGS	--	9.174	8.00
Limestone Fine	1.63	2.14	2.31
Mono-Dicalcium Phosphate	1.77	1.91	1.32
Salt (NaCl)	0.25	0.25	0.25
Sodium Bicarbonate	0.25	0.25	0.25
L- Lysine (50% Liquid)	0.48	0.06	0.10
DL- Methionine	0.12	0.122	0.07
L-Threonine 98.5%	0.056	--	--
Mineral Premix[1]	0.20	0.20	0.20
Vitamin Premix[2]	0.05	0.05	0.05
Arbocel® (cellulose)	--	--	--
Selenium premix[3]	0.05	0.05	0.05
Choline Chloride	0.02	--	--
Choline 60% Dry	--	0.025	0.02
Phytase	--	--	--
Poultry Fat	1.00	0.05	3.00
Total	100	100	100
Calculated Analysis:			
Crude Protein, %	19.00	15.00	14.80
Ca, %	1.00	1.10	1.18
P, %	0.82	0.75	0.80
Available P, %	0.45	0.42	0.42
Metabolizable energy, kcal per kg of diet	2850.58	2799.87	2799.87
Digestible lysine, %	1.34	0.70	0.68
Digestible methionine	0.42	0.36	0.31

[1]Mineral premix provides per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt.

[2]Vitamin premix provides per kg of 3diet: 13,200 IU vitamin A, 4000 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 2 mg menadione (K3), 2 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 4 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.

[3]Selenium premix, 1 mg Selenium premix provides 0.2 mg Se (as Na2 SeO3) per kg of diet.

Table 1.3 Footpad scoring system modified from Hunter et al. (2017).

Score	Definition
0	No blemish or discoloration of footpad
1	Small to moderate lesion (<50% of footpad) with black coloring or keratosis of footpad
2	Severe lesions (≥50% of footpad). Black coloring may extend to toes.

Table 1.4 Mean waterline (WL) litter moisture (%) for each feed treatment by water treatment[1] at 10, 16, 22 wks.[2]

	10 wk estimate (95% CI)	16 wk estimate (95% CI)	22 wk estimate (95% CI)
ADLIB			
ED	35.5 (30.8, 40.6)[a]	52.6 (47.3, 57.8)[a]	28.3 (24.1, 32.9)[b]
SAD	21.7 (18.1, 25.7)[b]	34.6 (29.9, 39.5)[b]	35.2 (30.5, 40.2) [a]
WR			
ED	17.8 (14.6, 21.4)[a]	37.9 (33.1, 43.0)[a]	22.0 (18.4, 26.1)[b]
SAD	19.7 (16.3, 23.6 [a]	25.2 (21.3, 29.6)[b]	29.2 (24.9, 33.8)[a]

[1]Feeding treatments consisted of skip-a-day (SAD) and every-day (ED) feeding programs, while water provision treatments consisted of water restriction for 3 h during the 8 h photoperiod (WR) or *ad libitum* (ADLIB) access to water during the 8 h photoperiod.
[2]Superscripts differing within a column within each water treatment indicate statistically significant differences identified with a Tukey's pairwise comparison posthoc test ($p<0.05$).

Table 1.5 Mean general (GEN) litter moisture (%) for each feed treatment[1] at 10, 16, and 22 wks.[2]

Treatment	10 wk estimate (95% CI)	16 wk estimate (95% CI)	22 wk estimate (95% CI)
ED	14.7 (12.8, 16.9)[b]	28.0 (25.4, 30.9)[a]	19.5 (17.3, 22.0)[a]
SAD	18.7 (16.5, 21.1)[a]	25.1 (22.6, 27.8)[a]	21.0 (18.7, 23.5)[a]

[1]Feeding treatments consisted of skip-a-day (SAD) and every-day (ED) feeding programs.
[2]Superscripts differing within a column indicate statistically significant differences identified with a Tukey's pairwise comparison posthoc test ($p<0.05$).

Table 1.6 Percentage of footpad scores of 0 for each feed+water[1] treatment at 16 and 22 wks.[2]

Treatment	16 wk estimate (95% CI)	22 wk estimate (95% CI)
ED + ADLIB	95.3 (86.3, 98.5)	93.2 (83.6, 97.3)
SAD + ADLIB	95.1 (85.6, 98.5)	92.9 (81.6, 97.5)
ED + WR	91.6 (80.0, 96.8)	88.0 (75.1, 94.7)
SAD + WR	91.3 (79.1, 96.7)	87.6 (72.2, 95.0)

[1]Feeding treatments consisted of skip-a-day (SAD) and every-day (ED) feeding programs, while water provision treatments consisted of water restriction for 3 h during the 8 h photoperiod (WR) or *ad libitum* (ADLIB) access to water during the 8 h photoperiod.
[2]No statistical differences identified with a Tukey's pairwise comparison posthoc test ($p<0.05$).

Table 1.7 Percentage of footpad scores of 1 for each feed+water[1] treatment at 16 and 22 wks.[2]

Treatment	16 wk estimate (95% CI)	22 wk estimate (95% CI)
ED + ADLIB	5.5 (1.8, 15.8)	6.9 (2.5, 17.3)
SAD + ADLIB	4.8 (1.6, 14.0)	6.0 (2.1, 16.3)
ED + WR	8.8 (3.3, 21.5)	10.8 (4.5, 23.9)
SAD + WR	7.7 (2.9, 19.2)	9.5 (3.6, 22.6)

[1]Feeding treatments consisted of skip-a-day (SAD) and every-day (ED) feeding programs, while water provision treatments consisted of water restriction for 3 h during the 8 h photoperiod (WR) or *ad libitum* (ADLIB) access to water during the 8 h photoperiod.
[2]No statistical differences identified with a Tukey's pairwise comparison posthoc test ($p<0.05$).

Table 1.8 Mean body weight (kg) estimates for each feed+water treatment[1] during 0-22 wks.[2]

	Treatment					P-Values		
Week	**ED+ADLIB**	**ED+WR**	**SAD+ADLIB**	**SAD+WR**	**SEM[3]**	**Feed**	**Water**	**FeedxWater**
0	0.040	0.040	0.041	0.040	0.00033	0.0709	0.1409	0.0895
1	0.16	0.15	0.17	0.18	0.0047	0.5317	0.1397	0.0514
2	0.36^{b}	0.34^{b}	0.40^{a}	0.40^{a}	0.011	0.0341*	0.1381	0.2716
3	0.56	0.54	0.55	0.55	0.019	0.8376	0.5629	0.8279
4	0.59	0.57	0.58	0.58	0.017	0.8418	0.6283	0.8309
5	0.64	0.62	0.63	0.62	0.019	0.8354	0.5723	0.8883
6	0.69	0.68	0.69	0.68	0.021	0.9423	0.7980	0.8655
7	0.75	0.76	0.76	0.76	0.021	0.7819	0.7994	0.7774
8	0.85^{a}	0.89^{a}	0.80^{b}	0.85^{b}	0.016	0.0464*	0.0893	0.9939
9	0.94	0.95	0.92	0.93	0.024	0.6635	0.6397	0.8188
10	1.02	1.04	1.02	1.01	0.027	0.9688	0.5977	0.6179
11	1.11	1.10	1.09	1.07	0.025	0.6257	0.9241	0.7134
12	1.23^{a}	1.23^{a}	1.15^{b}	1.20^{b}	0.019	0.0117*	0.8764	0.1819
13	1.33^{a}	1.34^{a}	1.23^{b}	1.32^{b}	0.021	0.0073*	0.5891	0.1383
14	1.43	1.41	1.34	1.41	0.027	0.0527	0.6941	0.1454
15	1.54	1.60	1.53	1.51	0.037	0.8576	0.2441	0.2628
16	1.69	1.75	1.67	1.64	0.038	0.7155	0.2340	0.2423
17	1.89^{a}	1.86^{a}	1.67^{b}	1.71^{b}	0.024	<0.0001*	0.4181	0.1370
18	2.08^{a}	1.95^{b}	1.79^{d}	1.87^{c}	0.024	<0.0001*	0.0023*	0.0010*
19	2.18^{a}	2.16^{a}	1.95^{b}	2.08^{b}	0.037	0.0008*	0.6945	0.0606
20	2.30^{a}	2.35^{a}	2.09^{b}	2.19^{b}	0.050	0.0110*	0.5196	0.6008
21	2.41^{b}	2.54^{ab}	2.63^{a}	2.57^{ab}	0.043	0.0035*	0.0516	0.0442*
22	2.58^{b}	2.71^{b}	2.72^{a}	2.75^{a}	0.042	0.0403*	0.0534	0.2705

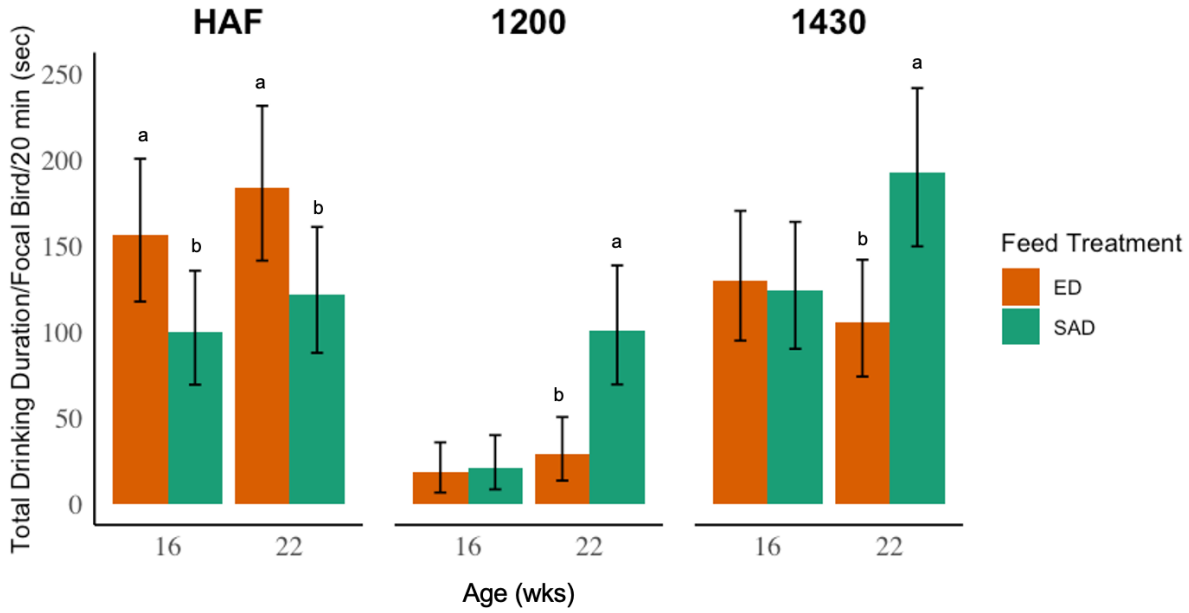
[1]Feeding treatments consisted of skip-a-day (SAD) and every-day (ED) feeding programs, while water provision treatments consisted of water restriction for 3 h during the 8 h photoperiod (WR) or *ad libitum* (ADLIB) access to water during the 8 h photoperiod.
[2]Superscripts differing within a row indicate statistically significant differences ($p<0.05$).
[3]SEM: Standard Error of the Mean

Table 1.9 Mean coefficient of variation (%) estimates for each feed+water treatment[1] during 3-22 wks.[2]

	Treatment					P-Values		
Week	**ED+ADLIB**	**ED+WR**	**SAD+ADLIB**	**SAD+WR**	**SEM[3]**	**Feed**	**Water**	**FeedxWater**
3	9.32	11.57	10.08	10.24	1.85	0.7753	0.4069	0.5827
4	9.32	10.49	10.34	8.81	1.70	0.6778	0.6336	0.4411
5	10.23	10.98	10.78	9.88	1.19	0.7536	0.6670	0.5055
6	11.36	10.74	11.28	10.12	1.51	0.9698	0.7770	0.8595
7	13.04	11.09	11.59	7.84	0.85	0.2509	0.1313	0.3113
8	14.62	11.06	11.33	10.6	1.23	0.0822	0.0623	0.2552
9	14.06[a]	10.96[b]	12.32[a]	8.55[b]	0.88	0.1895	0.0292*	0.7070
10	13.87	11.30	12.85	9.49	1.18	0.5549	0.1512	0.7430
11	15.50	12.60	16.27	13.59	2.84	0.8505	0.4856	0.9712
12	13.38	12.36	15.09	12.64	1.68	0.4866	0.6742	0.6783
13	10.05	12.15	13.62	12.19	1.24	0.0655	0.2562	0.1823
14	16.60	13.12	15.79	11.03	1.38	0.6834	0.1001	0.6537
15	11.49	12.68	12.16	11.71	1.23	0.7083	0.5077	0.5172
16	11.96	12.78	12.08	11.14	1.33	0.9503	0.6713	0.5222
17	11.74	15.48	16.03	13.59	2.43	0.2350	0.2976	0.2271
18	16.12	16.64	15.77	16.82	2.33	0.9190	0.8762	0.9127
19	10.05[b]	13.13[bc]	14.50[a]	9.72[c]	1.36	0.0396*	0.1356	0.0137*
20	10.42	11.27	12.81	10.01	2.06	0.4276	0.7740	0.3927
21	10.87	12.09	10.70	10.38	1.34	0.9299	0.5314	0.5746
22	11.44	11.73	10.15	11.89	1.75	0.6122	0.9103	0.6864

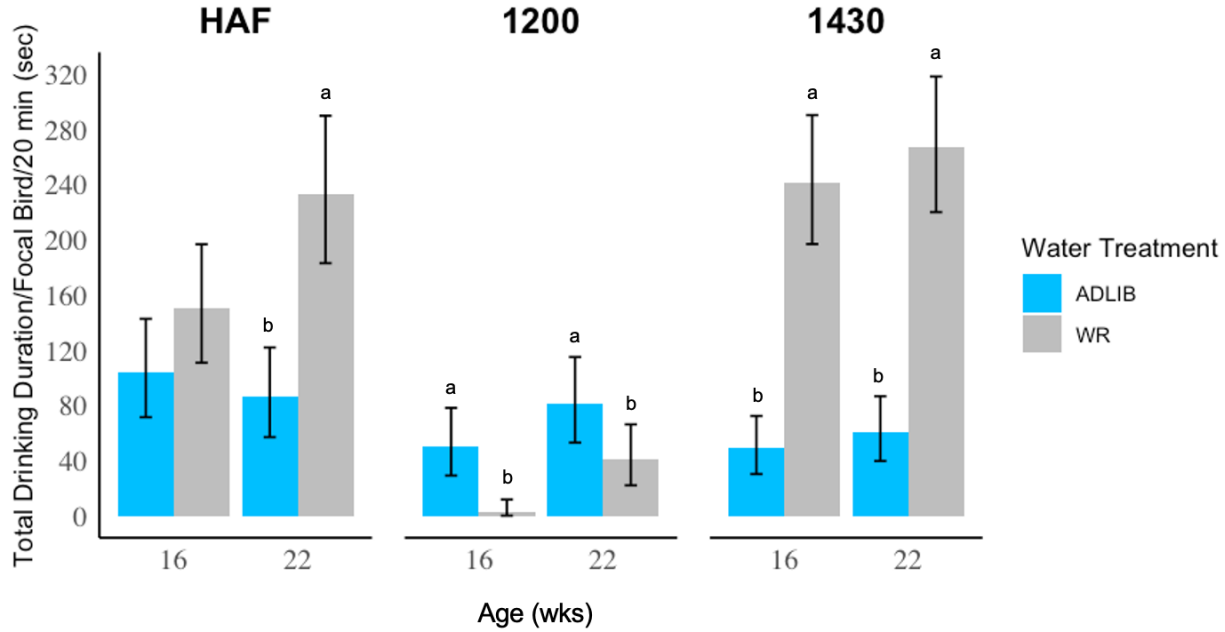
[1]Feeding treatments consisted of skip-a-day (SAD) and every-day (ED) feeding programs, while water provision treatments consisted of water restriction for 3 h during the 8 h photoperiod (WR) or *ad libitum* (ADLIB) access to water during the 8 h photoperiod.
[2]Superscripts differing within a row indicate statistically significant differences ($p<0.05$).
[3]SEM: Standard Error of the Mean

Figure 1.1 Average drinking duration (sec) of focal birds by feed treatment[1] during an on-feed day at different times during 16 and 22 wks.[2,3]



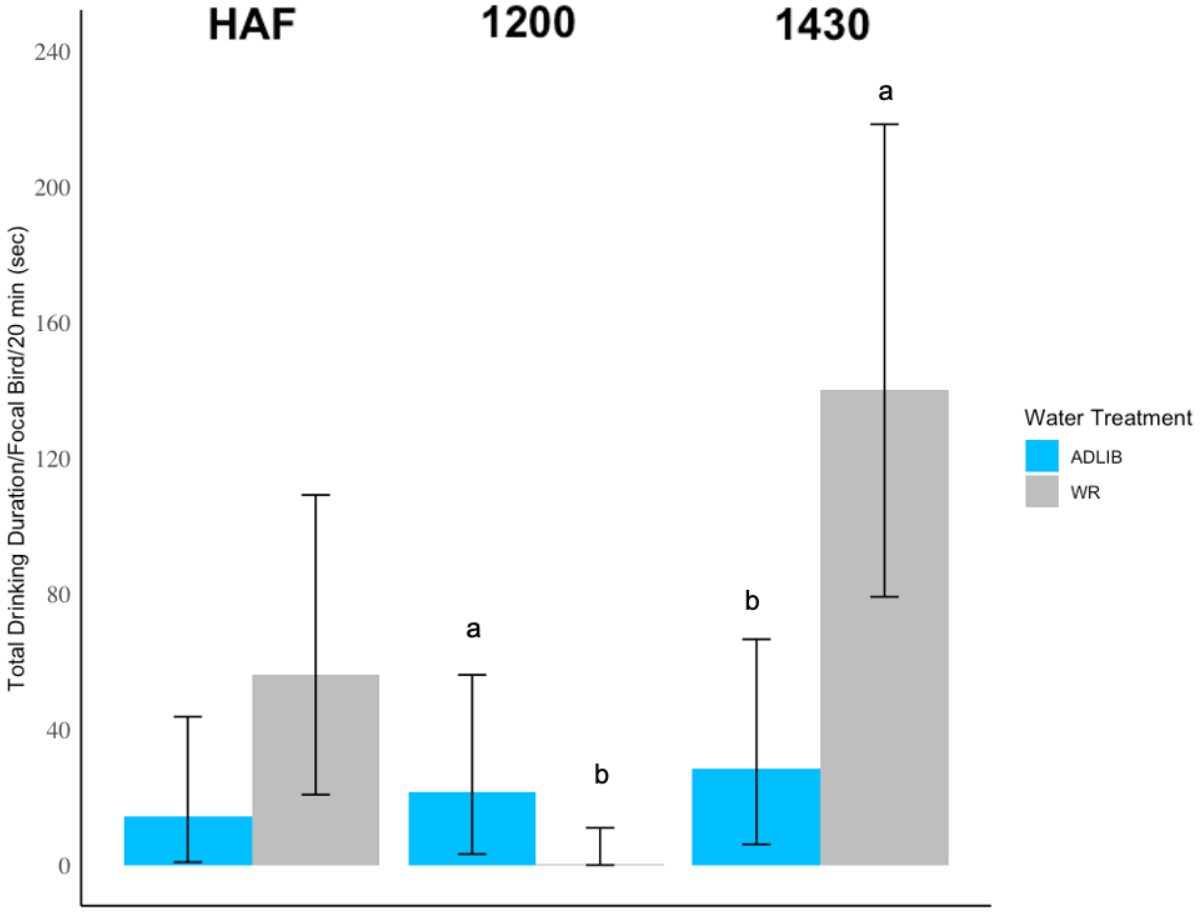
[1]Feeding treatments consisted of skip-a-day (SAD) and every-day (ED) feeding programs.
[2]Times points were sampled at 20-minute segments at an hour after feeding (HAF), during the water restriction period for water restricted treatments (1200), and when the water access resumed for restricted treatments for an hour prior to the photoperiod ending (1430).
[3]Letters indicate statistically significant differences identified with a Tukey's pairwise comparison posthoc test ($p<0.05$). Model estimated means +/- 95% CI. Separate models ran for each time point.

Figure 1.2 Average drinking duration (sec) of focal birds by water treatment[1] during an on-feed day at different times during 16 and 22 wks.[2,3]



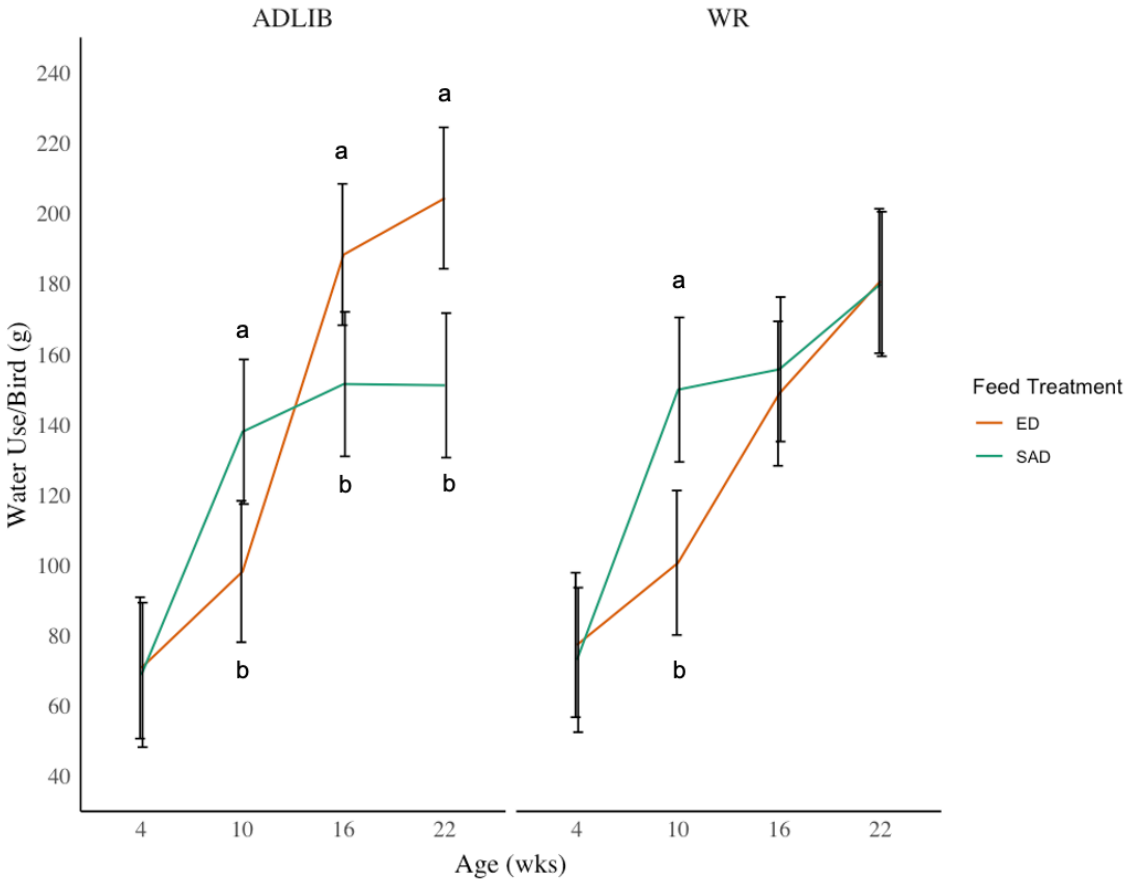
[1]Water treatments consisted of water restriction for 3 h during the 8 h photoperiod (WR) or *ad libitum* (ADLIB) access to water during the 8 h photoperiod.
[2]Times points were sampled at 20-minute segments at an hour after feeding (HAF), during the water restriction period for water restricted treatments (1200), and when the water access resumed for restricted treatments for an hour prior to the photoperiod ending (1430).
[3]Letters indicate statistically significant differences identified with a Tukey's pairwise comparison posthoc test ($p<0.05$). Model estimated means +/- 95% CI. Separate models ran for each time point.

Figure 1.3 Average drinking duration (sec) of skip-a-day (SAD) focal birds by water treatment[1] during an off-feed day at 16 wks.[2,3,4]



[1]Water treatments consisted of water restriction for 3 h during the 8 h photoperiod (WR) or *ad libitum* (ADLIB) access to water during the 8 h photoperiod. Only the skip-a-day (SAD) feed treatment is represented.
[2]Times points were sampled at 20-minute segments at an hour after feeding (HAF), during the water restriction period for water restricted treatments (1200), and when the water access resumed for restricted treatments for an hour prior to the photoperiod ending (1430).
[3]Letters indicate statistically significant differences identified with a Tukey's pairwise comparison posthoc test ($p<0.05$). Model estimated means +/- 95% CI. Separate models ran for each time point.
[4]Only week 16 data is shown due to data loss at week 22.

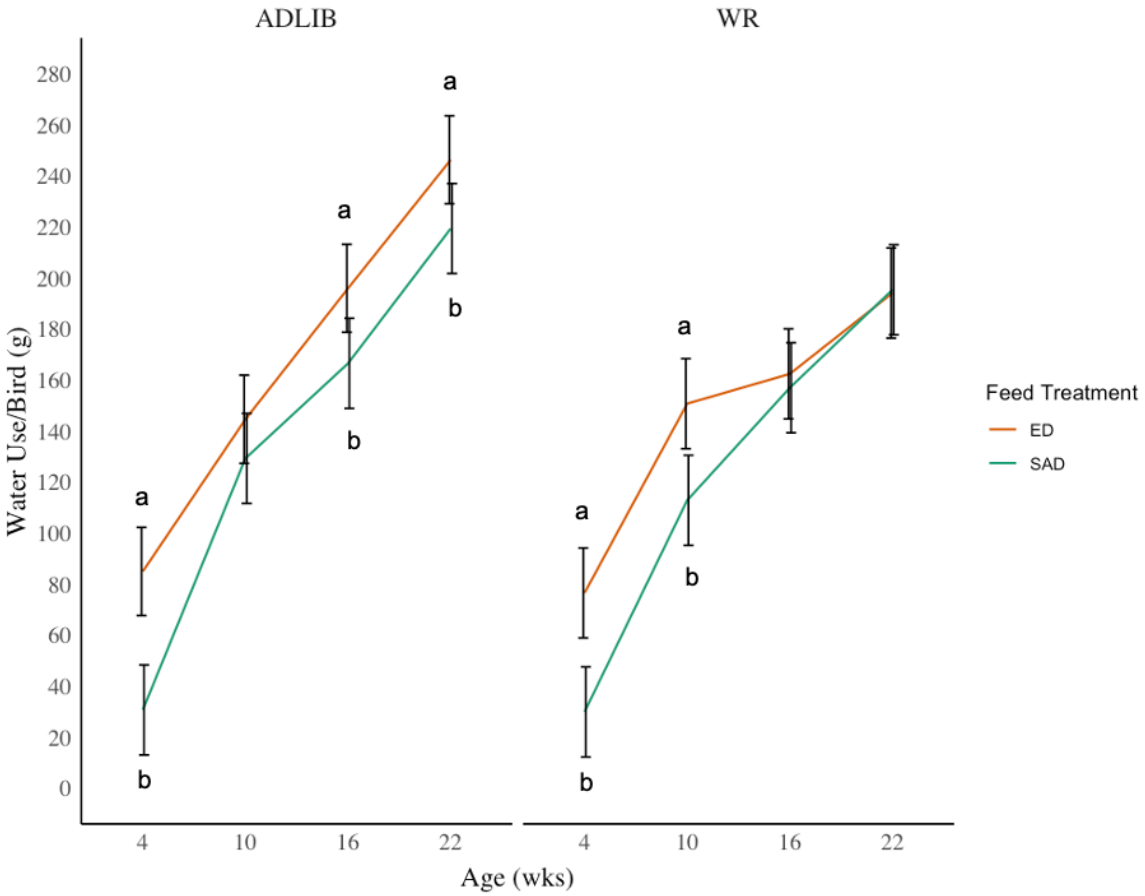
Figure 1.4 Average bird water use (g) by feed+water treatment[1] during an on-feed day at 4, 10, 16, and 22 wks.[2]



[1]Feeding treatments consisted of skip-a-day (SAD) and every-day (ED) feeding programs, while water provision treatments consisted of water restriction for 3 h during the 8 h photoperiod (WR).

[2]Letters indicate statistically significant differences identified with a Tukey's pairwise comparison posthoc test ($p<0.05$). Model estimated means +/- 95% CI.

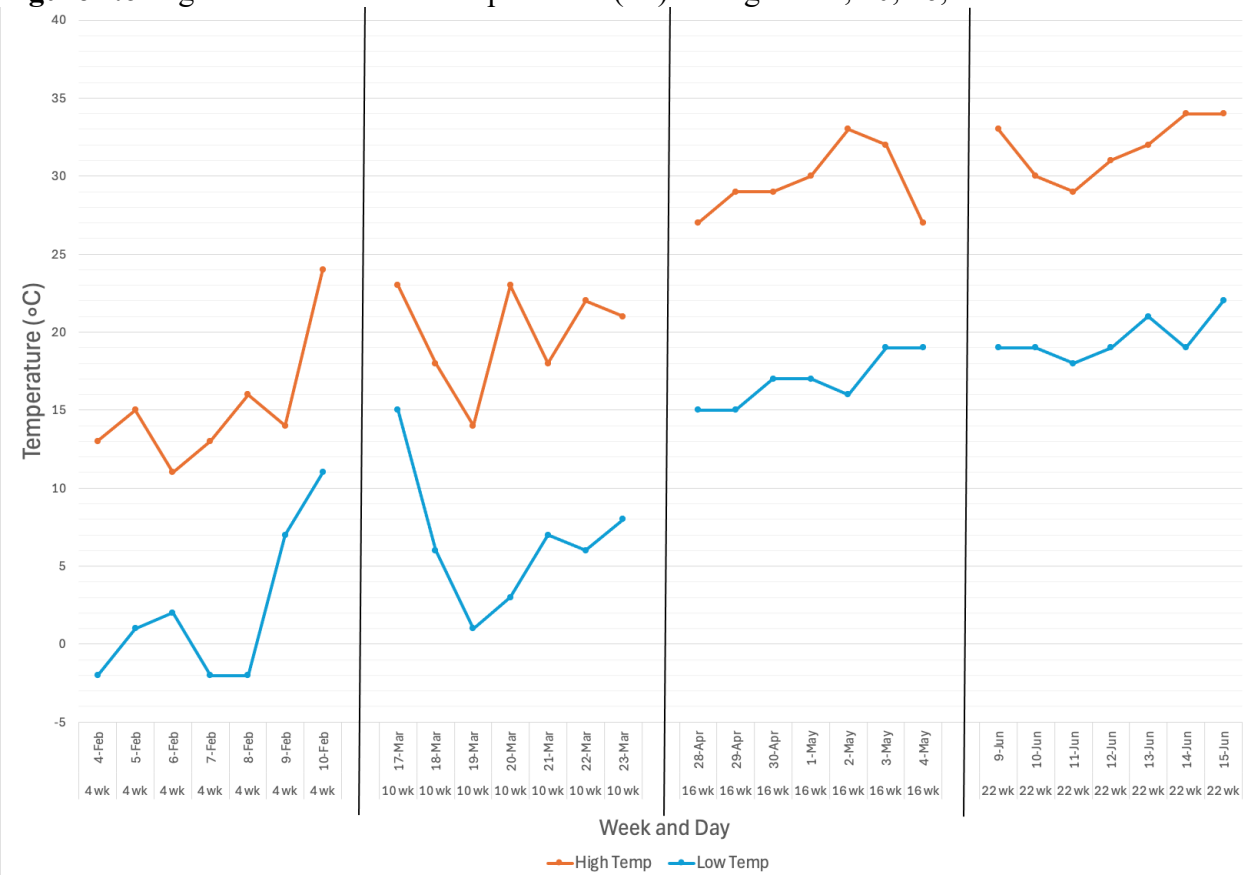
Figure 1.5 Average bird water use (g) by feed+water treatment[1] during an off-feed day at 4, 10, 16, and 22 wks.[2]



[1]Feeding treatments consisted of skip-a-day (SAD) and every-day (ED) feeding programs, while water provision treatments consisted of water restriction for 3 h during the 8 h photoperiod (WR).

[2]Letters indicate statistically significant differences identified with a Tukey's pairwise comparison posthoc test ($p<0.05$). Model estimated means +/- 95% CI.

Figure 1.6 High and low external temperatures (°C) during wks 4, 10, 16, and 22.[1]



[1]Wks separated by vertical lines

CHAPTER 3

Validating the Use of RFID Technology for Monitoring Drinking Behavior in Broiler Breeder Pullets

ABSTRACT

Water is a crucial indicator of health status and welfare in poultry, particularly in broiler breeder pullets where drinker use is significantly altered by feed restriction practices commonly implemented in the industry. Identifying individual drinking behavior in broiler breeder pullets could be beneficial as a genetic selection practice for decreasing water use, maintaining dryer litter, and improving sustainability and welfare outcomes in commercial and research settings. Radio frequency identification (RFID) technology is a potential tool that could help identify drinker use. The objective of this study was to assess the efficacy of RFID technology for monitoring drinking behavior in breeder pullets by validating various reader and tag placement methods and measuring the accuracy of RFID across different time points. Nine hundred sixty Cobb500 FF pullets were placed in a 16-pen curtain sided house (3.81 x 2.44 m, L x W; 60 birds/pen). A 2x2 factorial design was used, with two feed treatments (skip-a-day, SAD and every-day, ED feeding; 8 pens/treatment) and two water treatments (ad libitum water and 3h water restriction; 8 pens/treatment). Ultra-high frequency (UHF) RFID readers (GAO Inc., Manhattan, NY) were used to monitor drinking behavior, and tags were placed on 10 uniquely colored focal birds in each pen. To validate RFID, video cameras were placed in each pen and recorded drinking behavior simultaneously. Trained observers then coded the video recordings to acquire actual drinking durations to compare to RFID. For the first section of the study, four reader and tag placement methods were assessed, including two readers on the water line and one leg tag/focal bird (Placement 1), two readers placed on the ground and one leg tag/focal bird

(Placement 2), one reader placed on the ground and one leg tag/focal bird (Placement 3), and one reader placed on the water line and one wing tag/focal bird (Placement 4). One hour of data was sampled for each placement and drinking durations from video and RFID were summed for each focal bird and compared using correlation and regression analyses. Placement 4 had the highest correlation coefficient; however, regression criteria were not met for any of the placements. For the next section of the study, drinking behavior was recorded for 7 d at 4, 10, 16, and 22 wks of age. Placement 1 was utilized during week 4, but readers and tags were shifted to Placement 4 for the following wks after initial validation. Forty-five min samples of data were sampled on a randomly selected day for each pen during each week. For SAD treatments, on-feed and off-feed days were equally represented, except for 22 wks due to a video hard drive malfunction. High drinkers, (HD), average drinkers (AD), and low drinkers (LD) were identified from the summed drinking durations from both RFID and video recordings and compared using a confusion matrix to calculate overall accuracy, as well as precision, sensitivity, and specificity of each drinker type. Overall accuracy and the precision and sensitivity for classifying HD and LD pullets increased with age and were slightly improved in SAD treatments compared to ED treatments. The results from this study suggest that the utilization of one UHF RFID reader on the water line and one wing tag/focal bird was the most accurate method for monitoring drinking behavior in breeder pullets, especially with older birds in the SAD treatment. This is potentially due to decreased tag and reader interference and more consistent drinking patterns.

INTRODUCTION

Drinking behavior is a critical component of broiler breeder management as water is a key indicator of overall bird health and well-being due to its crucial role in several physiological

processes including thermoregulation, nutrient assimilation, and waste removal (Manning et al., 2007). The behavioral pathway of drinking is primarily motivated by hydration (i.e., the physiological requirement for water); therefore, changes in drinking behavior are often caused by changes in physical status (Nicol, 2015). Hydration in chickens is predominately controlled by intracellular and extracellular fluid volume, which are regulated by various hormones within the endocrine system, such as arginine vasotocin and angiotensin II (McKinley and Johnson, 2004; Fournier et al., 2012). These mechanisms can be altered by factors such as age, sex, genetics, disease, feed quality and intake, water quality, drinker types, and ambient temperature, causing subsequent changes to drinking behavior (Deeb and Cahaner, 2002). For example, excessive drinking (i.e., polydipsia) is an abnormal repetitive behavior often associated with unsatisfied foraging and chronic hunger caused by feed restriction, a practice commonly used in the broiler breeder industry (de Jong and van Emous, 2017). As a result, understanding drinking behavior of broiler breeder pullets has become increasingly important. Identifying individualized drinker use, particularly at extreme ends of the spectrum (i.e., high and low drinking birds), can have beneficial applications for research, genetic selection, and commercial use.

Most commonly, water use in poultry is measured by placing water meters on drinker lines, where meters record the total volume of water used by an entire flock in a certain period of time (McCreery, 2015). This strategy provides flock-level information that may inform whether there is a large-scale threat to flock health, such as an infectious disease, thermal stress, or malfunctioning drinker lines. However, water meters on drinker lines cannot account for individual bird drinking activity. Individualized water measurement systems have been developed and involve a nipple drinker connected to a pressurized reservoir that is filled and weighed to measure water use through weight change; however, this can be quite labor intensive

and requires birds to be housed individually (Hiltz et al., 2021). While this system does measure individual water use, it does not allow birds to be placed in a typical commercial system that consists of a floor house with multiple birds. Radio frequency identification (RFID) technology is a potential solution to provide a more efficient method of measuring individual drinking behavior in a multi-bird system. RFID has been previously used to measure resource use in poultry, such as range use in free-range layers and feeding and drinking behavior in broilers; however, it has never been implemented as a tool to measure drinker use in broiler breeder pullets undergoing feed restriction (Larsen et al., 2017; Li et al., 2019).

The objective of this study was to assess the efficacy of ultra-high frequency (UHF) RFID technology for measuring drinker behavior in broiler breeder pullets. We examined the correlations between RFID and video footage for quantifying drinking durations across four different reader and tag placements. We also investigated the accuracy, precision, sensitivity, and specificity of the RFID system for identifying individual drinker classifications (i.e., high drinkers, average drinkers, and low drinkers).

MATERIALS AND METHODS

This study was approved by the North Carolina State University Institutional Animal Care and Use Committee (Protocol #23-417) and followed the guidelines of the 4th edition of the Guide for the Care and Use of Agricultural Animals in Research and Teaching.

Animals and Housing

Birds in this study were housed in a curtain-sided house at the North Carolina State University Chicken Education Unit from January to June of 2024. Nine hundred sixty Cobb500 FF (Cobb-Vantress Genetics, Siloam Springs, AR) pullets were placed in the house at 1 d of age

and randomly assigned to one of 16 floor pens (3.81 x 2.44 m, L x W; 60 birds/pen, 1549.4 cm^2/bird). Supplemental feeders and waterers were provided for the first 7 d in addition to 4 pan feeders (36.83 cm diameter; 2.46 cm/bird) and a *Ziggity Systems Inc.*© water line with 5 nipples (12 birds/nipple) that remained for the entirety of the trial. Two additional trough feeders (50.80 cm) were placed in each pen at 15 wks to increase feeder space (2.31 cm/bird total). A standard starter diet was provided *ad libitum* for all pens during the first 3 wks, and *ad libitum* access to water was provided for the first 6 wks. Mortalities and culls were replaced with extra birds from the same flock for 2 wks after placement. The lighting program used was in accordance with the Cobb Genetics (2020). The photoperiod was 23 h at placement, then gradually decreased to an 8 h photoperiod (07:00 to 15:00) by 14 d of age and throughout the remainder of the trial (i.e., 23 wks of age).

Feeding and Water Treatments

This experiment followed a 2x2 factorial design (4 pens/treatment) with the following treatments: skip-a-day feeding + *ad libitum* water (SAD+ADLIB), every-day feeding + *ad libitum* water (ED+ADLIB), skip-a-day feeding + water restriction (SAD+WR), and every-day feeding + water restriction (ED+WR). SAD treatments and ED treatments were maintained on separate sides of the house to minimize the effect of visual and auditory stimuli from ED birds on SAD birds during their off-feed days. Due to the infrastructure of the house, feeding treatment blocks were further divided into a WR and ADLIB side to align with water timers attached to quadrants of the house. At 3 wks of age, birds were switched to grower diets and started their respective feed treatments. They were fed a developer diet at 16 wks of age through the end of the trial.

Behavioral Measurements

At placement, 10 birds/pen were randomly selected as focal birds for behavior analyses. The birds received unique color markings from livestock markers for individual identification (All-Weather Twist-Stik, LA-CO Markal, Elk Grove Village, IL). One color video camera (4K Smart IP Fixed Bullet Camera, Lorex Corporation, Irvine, CA) was placed in each pen, and all cameras were connected to a single network video recorder (4K 16-channel Wired NVR System, Lorex Corporation, Irvine, CA). Cameras were directed at the water line to continuously record video footage. The recordings were then coded by 5 trained observers using Behavioral Observation Research Interactive Software (BORIS, version 8.19.3, http://www.boris.unito.it/, Friard and Gamba, 2016) to quantify drinking durations for each focal bird. Drinking behavior was defined as when the bird's neck is extended towards the nipple and the beak is in contact with the nipple. The drinking bout ends when beak contact with nipple pauses for >3 seconds, and/or when the bird turns and its head reaches 1 bird length away from the drinker (~1.5 inches on the computer screen) or reaches the end of the drinker line. The specific ages and times of day for video recordings are described below for each validation method. Inter-rater reliability was performed using Microsoft Excel 2016 (version 16.0.5465.1000, Microsoft Corporation, Redmond, WA) to ensure ≥90% between the observers and the trainer.

UHF RFID readers (GAO Inc., Manhattan, NY) and tags (GAO Inc., Manhattan, NY) were also used to record drinking behavior. RFID tags were placed on 10 focal birds/pen described above. RFID readers were connected to a power source and a singular laptop with the GAO Livestock Tracking System software (version 3.4.7282. GAO Inc, Manhattan, NY) via CAT-5 ethernet cables. Data were exported from the GAO software to Microsoft Excel files (version 16.0.5465.1000, Microsoft Corporation, Redmond, WA). Both R Studio (version 4. 3. 2; R Core Team, 2023) and Microsoft Excel were used to condense and summarize the RFID

duration data. R Studio condensed the files into one Excel spreadsheet, where drinking durations for each focal bird was summed and organized by pen.

Placement Validation

Drinking durations from four RFID readers and tag placements were compared to drinking durations from video recordings to determine which RFID placement strategy had the strongest correlations with video. The placement method included two readers on the water line and one leg tag/focal bird (Placement 1), two readers placed on the ground and one leg tag/focal bird (Placement 2), one reader placed on the ground and one leg tag/focal bird (Placement 3), and one reader placed on the water line and one wing tag/focal bird (Placement 4; Figure 2.1). Drinking durations were collected from video footage and RFID readers for 60 min at an hour after feeding (HAF) on random days between 4 and 10 wks of age. Only pens in the ED treatment were used.

Real-Time Behavior Validation

Video cameras and RFID readers simultaneously recorded drinking behavior data for 7 d at 4, 10, 16, and 22 wks of age. Placement of RFID readers and tags differed at 4 wks compared to the other three time points, as the original setup (i.e., two RFID readers on the water line and one leg tag/focal bird) was adjusted to improve validity. Placement at 10, 16, and 22 wks consisted of one RFID reader on the water line and one wing tag/focal bird. At each age, two pens were randomly selected from each feed+water treatment (i.e., SAD+ADLIB, SAD+WR, ED+ADLIB, ED+WR; 8 pens total). One day of video recording was randomly selected for each pen from the 7 d of available recordings/age. On-feed and off-feed days were equally represented in the SAD treatments, except for 22 wks of age, where only on-feed days were represented due to missing video recordings for the off-feed days. For each pen, three 15 min segments of video

were coded on the selected day at HAF, 12:00 h, and 14:30 h. At 12:00 h, water had been turned off for 60 min in the WR treatments, and at 14:30, water had been turned on for 30 min in the WR treatments. Drinking durations from coded footage and corresponding RFID recordings were summed across the three time points for each day. High drinkers (HD; three birds with the longest drinking durations), low drinkers (LD; three birds with the shortest drinking durations and/or birds that had a drinking duration of zero seconds), and average drinkers (AD; birds in between HD and LD) were identified for video recordings and RFID recordings.

Statistical Analysis

For the placement validation, drinking durations for each focal bird determined from video footage were compared to drinking durations determined from RFID readers with correlation (*cor* function) and regression (*lm* function) analyses in R statistical software (version 4.4.2; R Core Team, 2021) using RStudio (version 2022.12.0+353) for macOS Sonoma 14.7.1. For regression, RFID was considered to accurately estimate drinking behavior if three criteria were met: adjusted $R^2 \geq 0.90$, slope not statistically different from 1 ($p > 0.05$), and intercept not statistically different from 0 ($p > 0.05$).

For the real-time behavior validation, the HD, AD, and LD focal birds identified from video recordings and RFID recordings at each age were compared using a confusion matrix (Table 2.1.). True positives and negatives were summed according to the acronyms in Table 2.1. Overall accuracy and the precision, sensitivity, and specificity for each drinker type were calculated using the following formulas:

Overall accuracy: (HH+AA+LL)/(HH+AH+LH+HA+AA+LH+HL+AL+LL)

HD Precision = (HH)/(HH+HA+HL)

HD Sensitivity = (HH)/(HH+AH+LH)

HD Specificity= (AA+LA+AL+LL)/(AA+LA+AL+LL+HA+HL)

AD Precision = (AA)/(AA+AH+AL)

AD Sensitivity = (AA)/(AA+HA+LA)

AD Specificity= (HH+LH+HL+LL)/(HH+LH+HL+LL+AH+AL)

LD Precision = (LL)/(LL+LH+LA)

LD Sensitivity = (LL)/(LL+HL+AL)

LD Specificity= (AA+HA+AH+HH)/(AA+HA+AH+HH+LH+LA)

RESULTS

Placement Validation

Placement 4 had the highest correlation coefficient of all RFID reader and tag placements compared to video footage, indicating a moderate positive relationship between the two recording methods (Table 2.2). Regression criteria were not met for any of the placements, demonstrating that RFID readers did not accurately predict drinking durations (Table 2.2.). Drinking durations predicted by RFID readers tended to be underestimated, as indicated by slopes < 1.

Real-Time Behavior Validation

Overall accuracy, HD precision, and HD sensitivity tended to increase with age, with 4 wks of age displaying the lowest values and 22 wks of age displaying the highest values (Table 2.3.). In addition, SAD treatments during wks 10, 16 and 22 had higher overall accuracy, HD precision, and HD sensitivity values compared to ED treatments, while the opposite was true for week 4. AD and LD precision and sensitivity displayed less of a pattern across ages and

treatments; however, higher AD precision and LD sensitivity values were observed in wks 4 and 22. HD and AD specificity was highest at week 4 while, while LD specificity was the lowest at this time point.

DISCUSSION

We aimed to validate RFID readers as a tool for quantifying drinking behavior in broiler breeder pullets by comparing drinking durations to those obtained from video recordings. Our first objective was to determine the placement of RFID readers and bird tags that correlated with and accurately predicted drinking behavior. Our second objective was to validate RFID technology's classification of individual bird drinker types (i.e., HD, AD, LD) across feed and water treatments across four ages.

Placement Validation

Placement 4 (i.e., one reader on the water line and one wing tag/focal bird) improved drinking duration correlations with video footage compared to all other tested placements. By having the focal bird tag on the wing, Placement 4 has the shortest distance between the focal bird tag and RFID reader on the water line, reducing the likelihood of obstructions between the tag and reader. Similarly, Li et al. (2019) found that moving the UHF RFID antenna closer to tagged birds increases accuracy when monitoring drinking behavior in broilers. Since our readers have a built-in antenna, moving the tag closer to the reader is a similar concept to adjusting reader placement. Another potential reason for Placement 4 having a stronger correlation with video footage is the reduction of RFID readers on the water line from two to one. Having multiple readers in close proximity to each other can result in their inability to read tags, either through the signal of one reader reaching the other and causing interference (i.e., reader-to-reader

interference) or because one tag is within the reading range of multiple readers, resulting in a breakdown of communication between tags and readers (i.e., multiple reader-to-tag interference; Engels and Sarma, 2002; Kim et al., 2009).

While Li et al. (2019) found that adjusting the positioning of the antenna resulted in an improvement of accuracy from 68.4±21.3 to 93.7±6.9%, our results from the real-time behavior validation showed adjusting the RFID system from Placement 1 to Placement 4 resulted in an improvement from 30% to, at best, 50% accuracy. These results are remarkably lower; however, this could be attributed to different tag placements in our study compared to the previous work (i.e., wing versus neck, respectively; Li et al., 2019). In addition, while a UHF RFID reader was used in both studies, they were made by different manufacturers and the UHF reader used in Li et al. (2019) did not have a built-in antenna, increasing flexibility in placement modifications and adjustments. Li et al. (2019) also used drinking durations to determine accuracy, where accuracy in the current study was calculated using drinker classifications instead. While we did use drinking duration for our regression analysis in our placement validation, the results were considerably low as none of the placements could accurately estimate behavior.

A limitation of this study is that we did not evaluate the efficacy of two water line readers with one wing band/focal bird, nor did we evaluate the efficacy of one water line reader with one leg band/focal bird due to time constraints. Anecdotally, when two RFID readers were on the water line for Placement 1, we observed that bird tags were oftentimes not signaling even when birds were directly underneath the reader. By turning one RFID reader off, we observed an increase in the frequency of tag reads. We assumed reader-to-reader interference and/or multiple reader-to-tag interference when there were two RFID readers on the water line, which was used to streamline the troubleshooting process to develop Placement 4 with only one reader on the

water line. Future research is needed to compare the efficacy of a single reader versus multi-reader system using wing tags. In addition, further investigation of different tag designs and reader types (i.e., HF vs UHF) should be explored to improve validity of RFID systems for monitoring drinking behavior in broiler breeder pullets.

Real-Time Behavior Validation

Overall accuracy, HD and LD precision and HD and LD sensitivity tended to increase with age, with week 22 showing the highest values. As birds age and grow wider, the RFID tag may be more likely to be within the vicinity of the RFID reader and fewer birds can be underneath the water line at a time. The latter reduces the likelihood of the number of tags being read simultaneously, which could reduce tag collision or tag-to-tag interference. This type of interference occurs when two tags attempt to communicate with the reader simultaneously, resulting in neither of the tags being read (Sarma et al., 2001). Low accuracy, precision, sensitivity and LD specificity values in week 4 could also be attributed to the different reader/tag placement (i.e., Placement 1), which was shown to be less effective than the placement used in at the other three ages (i.e., Placement 4). The different reader/tag placement at 4 wks of age could also account for the slightly increased accuracy, precision, and sensitivity of the ED treatments compared to the SAD treatments, which was not observed at any other age. While these data may initially suggest that Placement 1 is better at identifying drinker types in ED treatments than SAD treatments, ED accuracy, precision, and sensitivity values were higher in the later wks with Placement 4.

In contrast, RFID demonstrated greater overall accuracy, HD and LD precision, and HD and LD sensitivity in SAD treatments compared to ED treatments during wks 10, 16, and 22. This could be the result of more consistent feeding and drinking patterns in SAD-fed birds

combined with the selected sample times. SAD birds may drink more consistently during the sampled time periods, resulting in a greater opportunity to be registered by the RFID readers. During on-feed days, SAD birds are fed larger portions of feed than ED birds; consequently, they spend a longer time eating in the morning and may drink more consistently in the afternoon (Bennett and Leeson, 1989; Riber, 2020). The ED birds consumed their smaller portion of feed faster than SAD birds and displayed their peak drinking behavior in the morning, then drank more sporadically throughout the day. During 22 wks of age, ED birds spent more time drinking at HAF than SAD birds based off of video footage (181.52 vs 118.44 sec/bird, respectively), while SAD birds spent more time at 12:00 (95.41 vs 36.15 sec/bird) and 14:30 (173.84 vs 100.41 sec/bird) than ED birds. These drinking patterns may also account for why there was increased accuracy during wk 22, where all of the drinking durations for the SAD treatments were sampled from an on-feed day, while the other ages had equal representation of both on-feed and off-feed days. Taken together, these findings indicate that RFID technology may be more effective in identifying drinking types (i.e., HD, AD, LD) in SAD treatments during on-feed days.

Sensitivity can be used to identify true positive rates, and subsequently false negative rates (i.e., Type II error). Overall, HD and LD sensitivity tended to increase with age, showing that Type II error rates decreased as the birds grew older. This further supports our observation of increased accuracy with age, potentially due to bird growth and less room underneath the drinker. While higher LD sensitivity was observed in ED birds during wks 4 and 22, this should be interpreted with caution. The RFID readers registered more birds as having 0 sec of drinker use in the ED treatments compared to the SAD treatments. This is potentially due to displaying less drinking behavior than SAD birds during on-feed days noted by Bennett and Leeson (1989), as well as video recordings from the current study which showed that the SAD treatment on

average spent more time drinking throughout the day at 22 wks of age than the ED treatment (129.23 vs 106.03 sec/bird, respectively). Based on our classification system, birds registered by the RFID with a drinking duration of 0 sec were automatically labeled as LD; therefore, there was a higher number of LD birds in the ED treatments. As a result, there was a higher probability that the LD birds identified by the RFID corresponded to the LD birds identified by the video footage. However, most of the birds registered by the RFID as drinking for 0 sec displayed longer drinking durations in the video footage, indicating that the RFID is underestimating drinker use. Therefore, the ability of RFID to identify LD is not properly represented by the sensitivity.

Specificity can be used to show the true negative rates, and subsequently the false positive rates (i.e., Type I error). HD and AD specificity were the highest in week 4 compared to the other wks, while LD specificity was lowest during this time period. The high HD and AD specificity values coupled with the low sensitivity values in week 4 indicate that birds that were actually HD and AD were not being properly classified. In addition, the low LD specificity values suggest the there was a high Type I error rate, meaning that there were multiple birds from other drinking categories that were incorrectly classified as LD, potentially due to the aforementioned high number of LD during this time point. This is most likely the reason for the low accuracy seen in week 4 and Placement 1, and further indicates the need to interpret the LD sensitivity values with caution.

Although this study suggests there is potential for RFID to be used to classify individual drinker types, particularly for HD birds in SAD treatments, further improvement is needed. While 7 d of RFID data and video footage were recorded, only 45 min was used for each pen due to the time and labor associated with manual video coding. Longer sampling periods may

improve accuracy to capture variations in drinking behavior and offer more opportunities for RFID tag reads. Furthermore, the video was coded based on actual drinker use, but RFID readers do not solely record drinker use. RFID readers also register time spent within the vicinity of the reader and water line when a bird may not actually be drinking. Modifying drinking bout criteria with RFID readers may improve validity, which has been noted in previous studies using RFID to monitor feeding behavior in swine, as Maselyne et al. (2014) found that increasing the minimum duration criterion improved sensitivity. Therefore, adjustments to validation sampling and methodology may also lead to improvements in RFID accuracy, precision, sensitivity, and/or specificity when monitoring drinking behavior in breeder pullets and should be further investigated.

CONCLUSION

RFID technology has the potential to monitor drinking behavior in broiler breeder pullets, with Placement 4 (i.e., one reader on the waterline and wing tags) showing a moderate positive correlation to video coding due closer proximity of the tag and reader, as well as reducing reader-to-reader interference. Using Placement 4, RFID technology was better at identifying birds with extreme drinking frequencies, particularly HD birds in SAD treatments, with increased overall accuracy, HD precision, and HD sensitivity. Placement 4 also had improved LD specificity. Accuracy, precision, and sensitivity also increased with age for HD birds due to reduced tag-on-tag interference. However, utilizing RFID technology as a tool for measuring drinking behavior still needs to be improved to provide accurate results. Future research of different reader types, tag modifications, and validation methodology should be explored.

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Table 2.1 3x3 confusion matrix of drinker types[1] identified by RFID versus drinker types identified by video coding.[2]

	RFID-HD	**RFID-AD**	**RFID-LD**
Video-HD	HH	AH	LH
Video-AD	HA	AA	LA
Video-LD	HL	AL	LL

[1]Drinker types are high drinkers (HD), average drinkers (AD), and low drinkers (LD). Columns represent drinker types identified by the RFID and rows represent drinker types identified by video observation.
[2]Confusion matrix labels are ordered "RFID-Video". For example, a label of "HA" represents a bird that was identified by the RFID as a HD, but identified by the video observation as an AD.

Table 2.2 Comparison of drinking duration values (sec) between RFID and video observations by placement.[1,2]

	Placement 1	Placement 2	Placement 3	Placement 4
Correlation Coefficient (r)	0.28	0.23	-0.06	0.45
Adj. R^2	-0.04	-0.07	-0.12	0.10
Slope	0.29*	0.57*	-0.33*	0.57*
Intercept	24.93	296.70	730.72	381.88

[1]Data analyzed via regression and correlation analyses.
[2]Asterisks indicate values where the slope was statistically different from 1.

Table 2.3 Comparison of drinker types observed from RFID and video observations during wks 4, 10, 16, 22.[1]

	Wk 4			Wk 10			Wk 16			Wk 22		
	All Birds	**SAD Birds**	**ED Birds**	**All Birds**	**SAD Birds**	**ED Birds**	**All Birds**	**SAD Birds**	**ED Birds**	**All Birds**	**SAD Birds**	**ED Birds**
Overall accuracy:	0.300	0.25	0.350	0.375	0.400	0.350	0.400	0.425	0.375	0.500	0.525	0.475
HD Precision:	0.273	0.00	0.375	0.375	0.500	0.250	0.500	0.583	0.417	0.500	0.583	0.417
HD Sensitivity:	0.125	0.00	0.250	0.375	0.500	0.250	0.500	0.583	0.417	0.500	0.583	0.417
HD Specificity:	0.857	0.893	0.821	0.732	0.786	0.678	0.786	0.821	0.750	0.786	0.821	0.750
AD Precision:	0.571	N/A[2]	0.571	0.357	0.308	0.400	0.381	0.400	0.364	0.524	0.500	0.571
AD Sensitivity:	0.125	0.00	0.250	0.333	0.286	0.375	0.250	0.250	0.250	0.344	0.438	0.250
AD Specificity:	0.938	1.00	0.875	0.640	0.654	0.625	0.729	0.750	0.708	0.792	0.708	0.875
LD Precision:	0.274	0.270	0.280	0.393	0.400	0.385	0.343	0.333	0.353	0.486	0.500	0.476
LD Sensitivity:	0.708	0.833	0.583	0.423	0.429	0.417	0.500	0.500	0.500	0.708	0.583	0.833
LD Specificity:	0.196	0.036	0.357	0.685	0.654	0.714	0.589	0.571	0.607	0.679	0.750	0.607

[1]Data analyzed via confusion matrix.

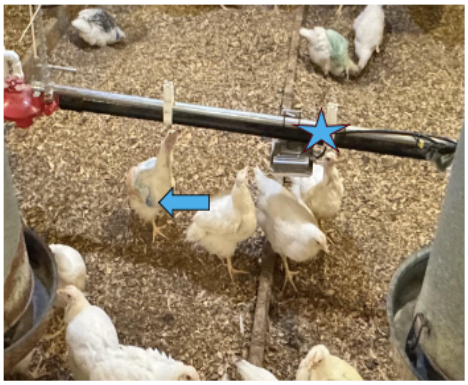
[2]A During week 4, the majority of birds in SAD treatments registered with 0 values for RFID duration and were therefore classified as LD. Therefore, the RFID produced zero AD as output, resulting in an undefined AD precision (i.e., N/A).

Figure 2.1 RFID reader and tag placements.[1]

Placement 1. Two waterline readers with RFID tags placed on focal birds' legs (original setup).

Placement 2. Two ground readers with RFID tags placed on focal birds' legs.

Placement 3. One ground reader with RFID tags placed on focal birds' legs.



Placement 4. One waterline reader with RFID tags placed on focal birds' wings.

[1]Stars represent ultra-high frequency (UHF) readers and arrows represent UHF tags.

CHAPTER 4

Individual Drinking Behavior and Bird Health

ABSTRACT

Feed restriction practices are implemented in the broiler breeder industry to maintain body weight, uniformity, and reproductive performance. These practices often result in increased water use causing increased water spillage, litter moisture, and footpad lesions. The cause of this increased water use may be attributed to abnormal respective pecking behavior towards the drinker line (i.e., polydipsia) possibly due to feeding-related frustration; however, it could also be the result of an underlying physiological issue, such as altered gut health from feeding regimens. Nevertheless, water restriction is sometimes utilized in commercial settings to limit water access and reduce spillage. The objective of this study was to evaluate the effects of feed and water restriction on physiological metrics of intestinal permeability and hydration. Nine hundred sixty Cobb500 FF pullets were placed in a curtain-sided house and were randomly assigned to 16 floor pens. There were four treatments: skip-a-day feeding + ad libitum water (SAD+ADLIB), skip-a-day feeding + water restriction (SAD+WR), every-day feeding + ad libitum water (ED+ADLIB), and every-day feeding + water restriction (ED+WR; 4 pens/treatment). Radio frequency identification (RFID) technology was used to measure drinking behavior and identify high drinkers (HD) and low drinkers (LD) in each pen. One ultra-high frequency (UHF) RFID reader was placed on the water line and RFID tags were placed on the wing of 10 focal birds/pen. Drinking behavior was recorded for 7 d at 10, 16, and 22 wks, and drinking durations were summed for each focal bird to identify drinker type. Two HD and two LD from each pen were inoculated with fluorescein isothiocyanate dextran (FITC-d), a marker of intestinal permeability, 2.5 h prior to blood collection from the brachial wing vein. A subsample

of blood was placed into capillary tubes, centrifuged, and hematocrit was read using a microcapillary reader. The remainder of the blood sample was placed in a plain collection tube, centrifuged to remove serum, and stored at -14 °C and FITC-d assays were performed at a later date. During blood collection, birds from the same treatment and drinker type were placed in transport crates and fecal samples were collected in plastic sample bags, weighed, dried at 70 °C for 7 d, and reweighed to determine fecal moisture. Results were analyzed using generalized and linear mixed effects models, a stepwise backward reduction using ANOVA, and significance was determined at $P \leq 0.05$. Main effects and interactions were determined using Tukey's pairwise comparisons. Hematocrit was significantly higher in HD than LD within the ED+WR treatment ($P=0.026$) and no other significant differences were noted; however, all hematocrit values remained within the recommended levels. Fecal moisture remained consistent between treatments at 10 wks, but SAD treatments decreased at 16 wks and increased at 22 wks, while the ED+WR treatment increased at 22 wks ($P=0.018$). Serum concentration of FITC-d didn't differ by feed, water, or drinker type, but there was a main effect of age with concentrations decreasing as age increased. At 23 wks of age, 30 HD and 30 LD identified from the week 22 RFID data were moved to individual cages and individual water use was measured (g) for 48 h to validate findings. Data were analyzed with a 3-way ANOVA, determining significance at $P \leq 0.05$, and means were separated using student's t test. Results showed that HD had significantly higher water use on the on-feed day and total water use across both days than LD ($P=0.03488$). In conclusion, neither FITC-d concentrations nor hematocrit values differed consistently between treatments and drinker types, indicating that intestinal permeability and hydration are unlikely to be the underlying causes driving differences in drinker use. Other physiological or behavioral factors may be involved, such as other gut health markers or behavioral syndromes and

stereotypic behavior. The effect of age on FITC-d concentration in broiler breeders should be further investigated.

INTRODUCTION

High water use by broiler breeder pullets can result in water spillage, increased litter moisture, and a higher incidence of footpad dermatitis, which creates sustainability and welfare concerns within the broiler breeder industry (Hocking, 1993). Increased water use is associated with feed restriction practices that are implemented in broiler breeder production to maintain ideal body weights (Mench, 2002). The most common type of feed restriction is quantitative, which can be separated into two types: skip-a-day (SAD) feeding, which involves the provision of larger quantities of feed on alternate days, or every-day (ED) feeding, which involves the provision of smaller quantities of feed daily (Riber, 2020). Another type of feed restriction is qualitative, which involves the inclusion of non-nutritive ingredients like insoluble fiber to improve gut fill and satiety by increasing water-holding capacity (D'Eath et al., 2009; Nascimento et al., 2021). Quantitative restriction, particularly SAD, has been shown to increase water use in broiler breeders, while the inclusion of insoluble fiber in qualitative restriction has been shown to reduce water intake (Nielsen et al., 2011; Morrissey et al., 2014).

It is unclear if high water use is linked to underlying physical health issues from feeding practices, or if frequently pecking the drinker lines is an abnormal repetitive behavior (i.e., polydipsia) that is induced by frustration, repeated attempts to cope, and/or central nervous system dysfunction (Mason, 2006). Indeed, frustration from limited foraging/feeding opportunities and chronic hunger are hypothesized to increase exploratory pecking behavior, often directed towards the drinker lines (Riber, 2020). As a result, water restriction protocols,

which involve turning water off for a few hours during the day, are utilized in some parts of the broiler breeder industry to minimize water spillage, but these protocols may exacerbate underlying health issues and/or behavioral restriction (Riber, 2020). The relationship between high water use and frustration has not been evaluated, but there is some evidence that high water use may be driven by underlying physiological indicators of health.

Due to the brain-gut-microbiota axis, there is a strong association between behavior and gut health in chickens (Fu and Cheng, 2024). In laying hens, feather eating and feather pecking, which is another abnormal behavior attributed to redirected foraging behavior, have been associated with increases in keratin hydrolyzing microbial species in the small intestine (Brunberg et al., 2016). In broiler breeders, Sweeney et al. (2022) observed an increase in jejunum villi height in birds fed an ED diet compared to a SAD diet, indicating that feeding regimen could potentially impact nutrient absorption. Feed restriction can also affect paracellular transport within the gut, resulting in increased intestinal permeability, or leaky gut, which can be measured using fluorescein isothiocyanate dextran (FITC-d; Kuttappan et al., 2015; Chasser et al., 2021). Vicuña et al. (2015) found that 24 h feed restriction increased FITC-d concentration in broilers after inoculation, indicating an increase in intestinal permeability. This relationship between feed restriction and gut health could result in greater loss of water, as indicated by higher fecal moisture, and could potentially cause more drinking behavior; however, FITC-d has not been utilized to assess the of feed and water restriction practices on enteric permeability in broiler breeder pullets.

The first objective of this study was to evaluate the effects of feed and water restriction practices on physiological metrics of enteric permeability and hydration. We predicted that SAD feeding and water restriction would have higher FITC-d concentrations, higher hematocrit, and

higher fecal moisture compared to ED feeding and *ad libitum* access to water, indicating that the former feed and water restriction practices result in more enteric permeability and dehydration. Within the feed and water restriction practices, individual birds were classified as "high drinkers" and "low drinkers" using radio-frequency identification technology to also compare these underlying physiological indicators with individual drinker use. We predicted that high drinkers would have higher metrics of FITC-d concentrations, hematocrit, and fecal moisture compared to low drinkers. The second objective of this study was to compare individual water use of "high drinkers" and "low drinkers" using precision water intake equipment to verify the behavior classifications from RFID.

MATERIALS AND METHODS

The protocol for this study was approved by the North Carolina State University Institutional Animal Care and Use Committee (Protocol #23-417). The animals were cared for in accordance with the 4th edition of the Guide for the Care and Use of Agricultural Animals in Research and Teaching.

Animals and Housing

Nine-hundred and sixty Cobb500 FF (Cobb-Vantress Genetics, Siloam Springs, AR) female pullets (*Gallus gallus domesticus*) were placed in a curtain sided house at the North Carolina State University Chicken Education Unit in Raleigh, North Carolina, USA at 1 d of age. The experiment was conducted from January 2024 to June 2024, encompassing the full pullet rearing period (0 to 23 wks of age). Birds were randomly assigned to one of 16 floor pens (3.81 x 2.44 m, L x W; 60 birds/pen) with a maximum stocking density of 1,551.5 cm^2/bird/pen.

The house was divided into four treatment quadrants based on feeding and water protocol: SAD+Water Restricted (SAD+WR), SAD+*Ad libitum* Water (SAD+ADLIB),

ED+Water Restricted (ED+WR), and ED+*Ad libitum* Water (ED+ADLIB). The SAD treatments were on one side of the house, while ED treatments were on the other side with a 3.6 m wide workroom separating the sides from each other (8 pens/feeding treatment). The purpose of this design was to prevent the visual stimuli of feeding ED birds daily from affecting the SAD bird behavior on off-feed days. Feeding treatment pens were further divided into quadrants by water treatment, with WR pens on one side of the hallway in the respective feeding treatment section and ADLIB pens on the other side (8 pens/water treatment; n=4 pens/feeding treatment+water treatment).

At placement, birds received 23 h of light that was reduced by 1 to 2 h per day over a 14-d period until an 8 h photoperiod (07:00-15:00) was reached by 2 wks of age. This photoperiod stayed consistent for the remainder of the trial until 23 wks of age. Birds were provided with four pan feeders (36.83 cm diameter; 15 birds/feeder) and a water line with 5 nipples (12 birds/nipple; Ziggity Systems Inc.©, Middlebury, IN), along with supplemental feeders and waterers that were removed after the first week. Two trough feeders (50.80 cm/each) were added to each pen at 15 wks to increase feeder space (4.15 cm/bird total). For the first two wks of the experiment, mortalities and culls were replaced with extra birds from the same flock that were housed and placed under the same conditions.

Feeding and Water Treatments

Birds were fed a standard starter diet (2850.58 kcal/kg, 19% CP; Table 3.1 and 3.2) until 3 wks of age. Birds were redistributed to achieve similar bird numbers across pens at 3 wks of age prior to being switched to grower diets and beginning their respective feed treatments. Both grower diets had a similar nutritional content (ED: 2262.57kcal/kg, 13.68% CP, 1.18% Ca; SAD: 2581.89kcal/kg, 14.06% CP, 1.19% Ca; Table 3.1 and 3.2), but Arbocel® (JRS Pharma,

Patterson, NY) and additional wheat middling were added into the ED diet to increase fiber and promote gut fill. SAD birds began their treatment on a 4/3-program, with 4 days on feed, 3 days off. This was later switched to a 5/2-program with 5 days on feed, 2 days off at 14 wks of age. Both treatments were switched to a developer diet at 16 wks of age, which again had similar nutritional content (ED: 2623.50 kcal/kg, 13.40% CP, 1.10% Ca; SAD: 2799.87 kcal/kg, 14.80% CP, 1.18% Ca; Table 3.1 and 3.2), but increased the quantity of fiber in the ED treatment. Weekly feed quantities were based on age and average bird weight per pen per recommendations from Cobb Genetics (2020).

Birds had 24-h *ad libitum* access to water until 6 wks of age, when water treatments were gradually applied by nightly water restriction from 15:00-07:00 for a 16 h scotoperiod for all pens. Each water treatment corresponded to a timer that turned water flow on and off. After birds had one week to acclimate to nightly water restriction, daily water restriction was applied to SAD+WR and ED+WR pens at 7 wks of age. Water restricted birds had access to water during and after feeding (07:00-11:00; 4 h total) and 1 h before the photoperiod ended (14:00-15:00).

High Drinker and Low Drinker Identification

Drinking behavior was monitored by ultra-high frequency (UHF) radio frequency identification (RFID) readers (GAO Inc., Manhattan, NY) at 10, 16, and 22 wks of age for a period of 7 d. Prior to the 10 wk data collection, one reader was placed on the water line, and RFID tags (GAO Inc., Manhattan, NY) were placed on the wings of 10 focal birds/pen. RFID readers were connected to a power source and to a singular laptop with the GAO Livestock Tracking System (version 3.4.7282. GAO Inc, Manhattan, NY) via CAT-5 ethernet cables. At the end of the 7-d recording period, data was exported from the GAO software to Microsoft Excel files (version 16.0.5465.1000, Microsoft Corporation, Redmond, WA). R Studio (version

4.3.2; R Core Team, 2023) was used to condense RFID duration data into one excel sheet where drinking duration was summarized for each bird across the entire 7 d period. The drinking duration data collected from the RFID system was used to identify the two highest drinkers (HD) and seventh and eighth lowest drinkers (LD) from each pen. The ninth and tenth lowest drinkers from each pen were removed from the data to exclude outliers from tag malfunctions as several of these values were not biologically appropriate (e.g., 0 sec of drinking behavior over 7 d). The two HD and two LD-classified drinkers at each age were then utilized for physiological measurements.

Hematocrit

Blood samples were collected from the two HD and two LD pullets in each pen at 10, 16, and 22 wks of age to evaluate hematocrit Blood was collected from the brachial wing vein.. Subsamples of blood were collected in hematocrit tubes (Fisherbrand™ Microhematocrit Capillary Tubes, Fisher Scientific, Pittsburgh, PA) and centrifuged (IEC MB Microhematocrit Centrifuge, Damon/IEC Division) immediately after collection. Samples were read using a micro-capillary reader (Damon/IEC Division) to determine hematocrit levels.

Fecal Moisture

During blood sample collection, birds were placed into transport crates with other birds of the same drinker type (HD or LD) and treatment (e.g., all HD pullets from SAD+WR pens in one crate, while all LD pullets from SAD+WR pens were placed into a separate crate). Fecal samples were collected from each crate to determine group level fecal moisture. Samples were collected in plastic sample bags, weighed, dried in an oven at 70° C for 7 d, and reweighed. Fecal moisture was determined by calculating 1- (dry weight/wet weight).

FITC-d Concentration

For analysis of FITC-d levels as a marker of intestinal permeability, blood serum levels were measured from HD and LD pullets at 10, 16, and 22 wks following the methods described in Chasser et al. (2021). The two HD and two LD pullets in each pen were administered FITC-d (MW 3,000–5,000 Da; Sigma-Aldrich Co., St. Louis, MO, USA) by oral gavage 2.5 h prior to blood collection. Blood was then collected from the brachial wing vein of the HD and LD pullets, as well as an additional random bird in each pen that had no FITC-d administered prior to blood collection to represent a negative control. After blood collection, samples were placed in plain collection tubes (Fisherbrand™ Culture Tubes, Fisher Scientific, Pittsburgh, PA) and centrifuged (Sorvall Legend XFR Centrifuge, Thermo Fisher Scientific, MA) at 1000 rpm to separate serum. Serum was then extracted and placed in mini tubes (Axygen™ Mini Tube System, Axygen, Union City, CA) and stored at -14 ° C. Serum from the negative control birds and HD and LD birds were diluted (1:4 in PBS). Fluorescence levels of diluted serum were measured using a FilterMax F5 Multi-Mode MicroPlate Reader (excitation wavelength: 485 nm, emission wavelength:528 nm; Molecular Devices, San Jose, CA) and SoftMaxPro software (version 7.0.3., Molecular Devices, San Jose, CA). FITC-d concentration (µg/ml) of serum was calculated based on a standard curve.

Individual Bird Precision Water Use

At 23 wks of age, 30 HD birds and and 30 LD birds (n=8 HD/treatment, 8 LD/treatment for all treatments except SAD+ADLIB and ED+ADLIB, with 7 HD and 7LD) identified in week 22 were moved and randomly allocated into individual cages. These cages were connected to equipment designed to precisely measure individual water usage. Birds remained on their experimental feed and water treatment during the 24-h acclimation period and the 48-h precision water use recording period, which consisted of an off-feed and on-feed day. The precision water

equipment was manually turned on and off to recreate the same water restriction protocol schedule utilized in the rearing house, and water tanks were weighed after each on-period to measure water usage (g).

Statistical Analysis

Hematocrit, fecal moisture, and FITC-d data were analyzed using R statistical software (version 4.4.2; R Core Team, 2021) using RStudio (version 2022.12.0+353) for macOS Sonoma 14.7.1. Both linear mixed effects models (nlme package, version 3.1.166, Pinheiro et al., 2024) and generalized linear mixed effects models (glmmTMB package, version 1.1.10, Brooks et al., 2017) were used. Linear mixed effects model fits were tested for normality and homogeneity of variance with visual inspection of quantile-quantile plots and plots of residuals versus fitted values (*qqnorm*, *plot*, and *hist* functions in base R). Generalized linear mixed effects model fits were evaluated using DHARMa package (version 0.4.7; Hartig, 2022). A stepwise backward reduction using ANOVA for model comparison was used for all analyses to obtain the final models, where $p>0.05$ was the criterion of exclusion. Differences between specific fixed effects in significant main effects or interactions were determined with Tukey's pairwise comparisons (emmeans package, version 1.10.5, Lenth, 2024).

Hematocrit and fecal moisture were analyzed using generalized linear mixed models with a poisson distribution and a negative binomial distribution, respectively. FITC-d was analyzed using a linear mixed effects model. For all models, feed treatment (factor with two levels: SAD and ED), water treatment (factor with two levels: ADLIB and WR), age (factor with three levels: 10, 16, and 22 wks), and drinker type (factor with two levels: HD and LD) and their 2-way, 3-way, and 4-way interactions were included as fixed effects. Pen was included as a random effect.

For precision water use, statistical analysis was performed with JMP Pro 16 software. Data were analyzed with a 3-way ANOVA. The main effects of drinker type (HD and LD), feed treatment (ED and SAD), and water treatment (ADLIB and WR), as well as their interactions, were included in the model. Significance was determined at $p<0.05$. Means were separated using the student's t-test.

RESULTS

Hematocrit

For hematocrit levels, there was a significant three-way interaction between feed, water, and drinker type, where HD within the ED+WR treatment had a lower hematocrit than LD within that same treatment (χ^2=4.98, df=1, P=0.026; Table 3.3). All other hematocrit levels were similar between HD and LD pullets across feed and water treatments. There was also a main effect of age, with lower hematocrit levels at 16 wks compared to 10 and 22 wks (χ^2=58.72, df=2), P<0.0001; 10 wks: 26.4 [25.1, 27.7], 16 wks: 20.0 [18.8, 21.1], 22 wks: 24.9 [23.7, 26.2]).

Fecal Moisture

Fecal moisture content had a significant three-way interaction between feed, water, and age (χ^2=6.64, df=2, P=0.034; Table 3.4). Fecal moisture was similar for all treatments at 10 wks of age, and ED+ADLIB pullets maintained a consistent fecal moisture as they aged. SAD+ADLIB and SAD+WR fecal moisture dropped at 16 wks of age, then increased at 22 wks of age, with SAD+ADLIB displaying the highest fecal moisture at 22 wks. Similarly, ED+WR remained consistent between 10 to 16 wks, then fecal moisture increased at 22 wks. There was also a main effect of drinker type, with HD having higher fecal moisture on average than LD

(χ^2=5.56, df=1, P=0.018; HD: 70.60 [69.70, 71.50], LD: 69.10 [68.20, 70.00]%). Data were analyzed as proportions but are presented as percentages for readability.

FITC-d Concentration

FITC-d concentrations had no significant four-way, three-way, or two-way interactions (p>0.05). There was a main effect of age, with FITC-d concentrations decreasing over time (Log-Likelihood Ratio (LLR)=202.81, P<0.0001; Wk10: 1.87 [1.79, 1.96], Wk16: 1.41 [1.31, 1.50], Wk22: 0.73 [0.65, 0.82] µg/ml). There were no significant main effects of feed, water, or drinker type (P>0.05; Table 3.5).

Individual Bird Precision Water Use

The three-way and two-way interactions were not significant for individual bird precision water use. There was a main effect of feed treatment with ED birds drinking more on the off-feed day and SAD birds drinking more on on-feed day (P<0.05; Table 3.6); however, there were no differences in average or total water use across the two days (P>0.05; Table 3.6). There was also a main effect of drinker type, with HD having higher water use on the on-feed day and higher average and total water use across both days than LD (P<0.05; Table 3.6). HD and LD used water at similar levels on the off-feed day (P>0.05; Table 3.6). There were no significant differences in water use between ADLIB and WR birds (P>0.05; Table 3.6).

DISCUSSION

The objective of this study was to assess indicators of hydration and gut health in HD and LD broiler breeder pullets in relation to feed and water restriction practices (i.e., SAD versus ED and ADLIB versus WR). We hypothesized that pullets with longer temporal gaps in feed and/or water provision (i.e., SAD and WR) would experience impaired hydration and gut health

compared to ED and ADLIB. Furthermore, we hypothesized that pullets with impaired gut health would display more water use in an attempt to cope with malabsorption issues, such that HD pullets would display higher levels of FITC-d concentrations, hematocrit, and fecal moisture.

Hematocrit

We hypothesized that impaired water absorption and dehydration were underlying physiological factors driving higher water use with SAD feeding, particularly for HD individuals, and that WR protocols may also impair hydration. However, all feed and water treatments and bird drinker types were similarly hydrated according to hematocrit values, except for the ED+WR treatment. In this treatment, the HD pullets displayed lower hematocrit values compared to the LD pullets throughout rearing, indicating that HD pullets were consistently more hydrated than LD pullets due to using more water. Similarly, Chikumba et al. (2013) observed a decrease in hematocrit levels with *ad libitum* consumption of water compared to when water was restricted to 70% and 40% of previous *ad libitum* intake (*ad libitum*: 28.3, 70%: 31.5, 40%: 35.5). This pattern was not observed in the HD pullets in other treatments in the current study though, and it is not entirely clear why. The ED+WR treatment provided the lowest daily quantities of feed and water compared to other treatments, which may create conditions where hematocrit levels are more sensitive to water use. We do not want to overinterpret this result though as the hematocrit ranges in this study (21.5 to 24.7) fall within the range of what is considered normal for a broiler breeder hen (21.3 to 30.8; Martin et al., 2010).

We also found a main effect of age for hematocrit, with birds at 16 wks having a lower hematocrit than birds at 10 and 22 wks. The hematocrit value at 16 wks (20.0) fell outside of the normal hematocrit range for broiler breeder hens (21.3 to 30.8; Martin et al., 2010), possibly indicating a minor risk for anemia at this age. However, a natural variation of hematocrit levels,

up to 15% from the mean, has been noted in domestic chickens and can be caused by several factors that are not the result of anemia, including energy expenditure, nutrition, and genetics (Fair et al., 2007). At 16 wks of age, birds in the current study were switched to the developer phase of their diets, which could have created a nutritional stressor. Indeed, previous work noted that nutritional changes (i.e., a diet with 19% CP and 2800 kcal, compared to diets with 18, 20, and 21% CP and 2800 kcal or diets with 18, 19, 20, and 21% CP and 3000 kcal) were associated with decreased hematocrit levels in chickens (Hafsah et al., 2021).

Fecal Moisture

Similar to hematocrit, we hypothesized that SAD feeding and WR protocols would impair gut health and water absorption, resulting in increased fecal moisture content in these treatments. Interestingly, we found limited support for this hypothesis. Both SAD feeding treatments decreased fecal moisture at 16 wks of age while both ED feeding treatments remained consistent between to 10 to 16 wks of age. Hematocrit also decreased at 16 wks of age for all birds, indicating a possible risk for anemia, and severe anemia is associated with fluid retention in humans (Anand et al., 1993). As pullets transitioned from grower to developer diets in the present study, protein increased in SAD-fed treatments (14.08 to 14.80%), while ED-fed pullets slightly decreased crude protein and total fiber. Typically, increasing nutrients like crude protein and fiber result in increased water intake by birds (Jiménez-Moreno et al., 2016; Alfonso-Avila et al., 2022), but the SAD+ADLIB treatment used less water than the ED+ADLIB treatment at 16 wks (Chapter 2). This lower water use, coupled with possible minor fluid retention per anemic hematocrit values, may explain why both SAD feeding treatments produced lower fecal moisture than both ED feeding treatments at 16 wks.

At 22 wks, both SAD feeding treatments and ED+WR increased fecal moisture, with SAD+ADLIB displaying the highest fecal moisture and ED+ADLIB the lowest in the current study. Higher levels of dietary insoluble fiber typically reduce fecal moisture content (Nielsen et al., 2011; van der Hoeven-Hangoor et al., 2014), which is likely why ED+ADLIB maintained lower fecal moisture than SAD+ADLIB. The ED+WR treatment had higher fecal moisture than ED+ADLIB likely due to increased compensatory water intake from the WR protocol. Although WR treatments may be expected to consume less water because of their limited access, Bennett and Leeson (1989) observed that SAD+WR birds actually drank more than SAD+ADLIB birds on off-feed days. This phenomenon of compensatory intake after water deprivation has also been noted in broilers 3 wks of age or older (Vanderhasselt et al., 2014; El Sabry et al., 2023).

Outdoor temperature was highest at 22 wks as the trial entered into the summer (Figure 3.1), which coincided with increased water intake by all treatments (Chapter 2) and consequently higher fecal moisture levels. Indeed, Ogunji et al. (1983) noted that increased water intake in broiler breeders resulted in higher fecal moisture content. We found higher fecal moisture in HD pullets compared to LD pullets across ages, further supporting that fecal moisture patterns are driven by water use patterns, not malabsorption. Taken together, it is important to note that the average fecal moisture values across all treatments fell within the normal range for chickens (i.e., 65 to 80% fecal moisture; van der Hoeven-Hangoor et al., 2013).

FITC-d Concentration

We hypothesized that FITC-d concentrations would be higher in SAD and WR treatments, indicating increased incidence of paracellular transport within the intestine (i.e., leaky gut). We further hypothesized that birds with this dysfunction in the mucosal barrier would display more drinking behavior (i.e., classified as high drinkers). However, there were no

significant interactions or effects associated with feed treatment, water treatment, or drinker type. In contrast, previous studies have observed increased FITC-d concentrations in broilers after feed restriction ranging from 34 h to as little as 4.5 h (Kuttappan et al., 2015; Vicuña et al., 2015, Gilani et al., 2018a). It is important to note that these trials evaluated broilers that are 6 wks of age or younger, while the current study focused on broiler breeders between 10 and 22 wks of age. In addition, these broilers did not have repeated feed restriction, whereas the pullets in the current study may have been able to adapt to the intermittent feeding schedules that were applied wks prior to the FITC-d measurements. Based on previous research, we anticipated that 24 h feed restriction represented by ED and 48 h feed restriction represented by SAD across an off-feed day would generate differences in FITC-d concentrations.

There is limited research on intestinal permeability of chickens at older ages, although age could have a significant impact on the ability of birds to cope with feed restriction. Broiler studies, which are limited to a maximum of 8 wks, have found that intestinal permeability increases with age (Gilani et al., 2018b; Liu et al., 2021). Wiersema et al. (2021) found similar results in laying hens, although this study examined birds during egg production (i.e., 26 to 70 wks of age). The results from the current study are dissimilar to the findings from both broilers and laying hens, with FITC-d concentrations (i.e., intestinal permeability) decreasing with age; however, differences in age and genetics between broiler breeder pullets, broilers, and laying hens could potentially account for these differences. To our knowledge, there are no other published studies looking at intestinal permeability in breeder pullets at intermediate ages; therefore, further research is needed to evaluate the effect of age on intestinal permeability in broiler breeders, particularly in the rearing stage.

Individual Bird Precision Water Use

We expected different drinker types (HD and LD) to have different levels of water use as well as different patterns of drinking behavior. Though both drinker types used a similar amount of water during the off-feed day, HD displayed higher levels of water use on the on-feed day compared to LD, which consequently inflated the total water use across the two days and the average daily water use for HD pullets. Broiler breeder pullets maintained their individual water use patterns, despite being in a novel environment with minimal social influences. Additionally, regardless of drinker type, the ED birds typically drink more on the off-feed day than the SAD birds, while the SAD birds typically drink up to 150% more on their on-feed day when they received larger quantities of feed than ED birds (Bennett and Leeson, 1989). Being that half of the HD pullets are from the SAD feeding treatments, the differences in water use on on-feed day clearly demonstrate the relationship between feed consumption and water use. The underlying motivation for high water use in individual pullets, whether behavioral or physiological, is unclear.

Although the HD pullets had slightly higher fecal moisture than the LD pullets due to greater water use, the values were still within a normal range. There were minimal differences between the two drinker types for hematocrit and no differences for FITC-d concentrations, suggesting minimal underlying physiological differences. Bird weight can also be a contributing factor of water intake, with larger birds drinking more (Aggrey et al., 2023); however, descriptively, the average weight of HD and LD pullets at 22 wks only differed by 0.06 kg, with LD actually weighing more (LD: 2.75±0.05, HD: 2.69±0.05kg). Therefore, it is unlikely that the difference in water use is driven by heavier body weights in HD.

There may be a behavioral component of increased drinker use, with drinker pecking being a redirected foraging (i.e., exploratory behavior) or an abnormal repetitive behavior that is

associated with frustration of thwarted behavioral opportunities during feed and water restriction practices (Mench, 2002). However, this was not observed at the feed or water treatment level for individual bird water use, as SAD and ED treatments did not differ in the total water use across two days or average daily water use, while ADLIB and WR treatments had no significant differences in water use for any metric. This is in contrast to previous work that reported higher drinker use in SAD feeding programs compared to ED feeding programs (Morrissey et al., 2014) and more compensatory water use in WR treatments (Bennett and Leeson, 1989). Differences between previous studies and the current study may be related to different social or environmental cues (e.g., multi-bird room during the drinking behavior observations in Morrissey et al., 2014 versus individual cages in the current study) or different genetic strains (Hubbard in Bennett and Leeson, 1989 and Cobb 500FF in the current study), as genetics are a known predictor of water consumption and use (Ogunji et al., 1983; Deeb and Cahaner, 2002).

Differences in water use observed between HD and LD pullets may also be due to differences in behavioral syndromes, which is defined as individual behavioral differences that are consistent across time and correlated in other contexts (Wolf and Weissing, 2012). For example, HD pullets may represent a more active or bold behavioral syndrome, while LD pullets may represent a more passive or shy behavioral syndrome (Sih et al., 2004). However, additional research is needed to verify the stability of these possible behavioral syndromes.

CONCLUSION

The lack of association between feed treatment, water treatment, and drinker type with FITC-d levels in the current study suggests that high levels of drinking behavior reported in feed restricted broiler breeder pullets is likely not the result of enteric permeability or malabsorption.

Fecal moisture is primarily associated with water use, which appears to be primarily driven by feed quantity and schedule. ED-fed pullets displayed higher individual water use on the off-feed day, and SAD-fed pullets used more water on the on-feed day. Hematocrit levels decreased at 16 wks of age, which may represent a risk for physiological stressors during nutritional changes to the developer diet; however, the pattern of FITC-d concentrations suggest lower risks for enteric permeability as the birds age. Further research is needed to fully understand the implications of age on enteric permeability and hematocrit in broiler breeders during the pullet stage.

There were also distinct behavioral differences in water use between individual pullets, with HD-classified pullets using significantly more water than LD-classified pullets, resulting in increased fecal moisture across treatments and decreased hematocrit levels within the ED+WR treatment. Since behavioral classification was not associated with a gut health issue, this finding may be a component of distinct broiler breeder behavioral syndromes that requires further investigation.

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Table 3.1 Composition of every-day (ED) pullet diets and calculated analysis.

Ingredients %	Starter	Grower	Developer
Ground Corn	58.00	45.99	54.39
Soybean Meal 48% CP	25.00	3.73	2.00
Wheat Bran	11.12	--	--
Wheat Midds	--	34.40	26.04
DDGS	--	8.44	11.30
Limestone Fine	1.63	2.18	2.16
Mono-Dicalcium Phosphate	1.77	1.00	1.20
Salt (NaCl)	0.25	0.25	0.25
Sodium Bicarbonate	0.25	0.25	0.25
L- Lysine (50% Liquid)	0.48	0.20	0.23
DL- Methionine	0.12	0.20	0.01
L-Threonine 98.5%	0.056	--	--
Mineral Premix[1]	0.20	0.20	0.20
Vitamin Premix[2]	0.05	0.05	0.05
Arbocel® (cellulose)	--	0.80	0.80
Selenium premix[3]	0.05	0.05	0.05
Choline Chloride	0.02	--	--
Choline 60% Dry	--	0.02	0.02
Phytase	--	--	0.05
Poultry Fat	1.00	2.235	1.00
Total	100	100	100
Calculated Analysis:			
Crude Protein, %	19.00	13.50	13.40
Ca, %	1.00	1.09	1.10
P, %	0.82	0.75	0.81
Available P, %	0.45	0.39	0.40
Metabolizable energy, kcal per kg of diet	2850.58	2799.87	2623.50
Digestible lysine, %	1.34	0.70	0.63
Digestible methionine	0.42	0.39	0.23

[1]Mineral premix provides per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt.

[2]Vitamin premix provides per kg of 3diet: 13,200 IU vitamin A, 4000 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 2 mg menadione (K3), 2 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 4 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.

[3]Selenium premix, 1 mg Selenium premix provides 0.2 mg Se (as Na2 SeO3) per kg of diet.

Table 3.2 Composition of skip-a-day (SAD) pullet diets and calculated analysis.

Ingredients %	Starter	Grower	Developer
Ground Corn	58.00	53.52	53.44
Soybean Meal 48% CP	25.00	9.00	8.75
Wheat Bran	11.12	--	--
Wheat Midds	--	23.92	22.15
DDGS	--	9.174	8.00
Limestone Fine	1.63	2.14	2.31
Mono-Dicalcium Phosphate	1.77	1.91	1.32
Salt (NaCl)	0.25	0.25	0.25
Sodium Bicarbonate	0.25	0.25	0.25
L- Lysine (50% Liquid)	0.48	0.06	0.10
DL- Methionine	0.12	0.122	0.07
L-Threonine 98.5%	0.056	--	--
Mineral Premix[1]	0.20	0.20	0.20
Vitamin Premix[2]	0.05	0.05	0.05
Arbocel® (cellulose)	--	--	--
Selenium premix[3]	0.05	0.05	0.05
Choline Chloride	0.02	--	--
Choline 60% Dry	--	0.025	0.02
Phytase	--	--	--
Poultry Fat	1.00	0.05	3.00
Total	100	100	100
Calculated Analysis:			
Crude Protein, %	19.00	15.00	14.80
Ca, %	1.00	1.10	1.18
P, %	0.82	0.75	0.80
Available P, %	0.45	0.42	0.42
Metabolizable energy, kcal per kg of diet	2850.58	2799.87	2799.87
Digestible lysine, %	1.34	0.70	0.68
Digestible methionine	0.42	0.36	0.31

[1]Mineral premix provides per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt.

[2]Vitamin premix provides per kg of 3diet: 13,200 IU vitamin A, 4000 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 2 mg menadione (K3), 2 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 4 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.

[3]Selenium premix, 1 mg Selenium premix provides 0.2 mg Se (as Na2 SeO3) per kg of diet.

Table 3.3 Hematocrit levels for breeder pullets classified as high drinkers (HD) and low drinkers (LD) by feed and water provision treatments.[1,2]

Treatment	HD estimate (95% CI)	LD Estimate (95% CI)
ED+ADLIB	24.6 (22.7, 26.8)	23.2 (21.4, 25.3)
SAD+ADLIB	22.1 (20.2, 24.2)	23.3 (22.3, 26.4)
ED+WR	21.5 (19.6, 23.5)[a]	24.7 (22.8, 26.8)[b]
SAD+WR	23.9 (22.0, 25.9)	24.6 (22.7, 26.6)

[1]Feeding treatments consisted of skip-a-day (SAD) and every-day (ED) feeding programs, while water provision treatments consisted of water restriction for 3 h during the 8 h photoperiod (WR) or *ad libitum* (ADLIB) access to water during the 8 h photoperiod.
[2]Superscripts differing within a row indicate statistically significant differences identified with a Tukey's pairwise comparison posthoc test ($p<0.05$).

Table 3.4 Fecal moisture content (%) of broiler breeder pullets reared on different feed and water treatments at 10, 16, and 22 wks of age.[1,2]

Treatment	10 wks estimate (95% CI)	16 wks estimate (95% CI)	22 wks estimate (95% CI)
ED+ADLIB	69.2 (66.8, 71.4)	67.1 (65.3, 69.0)	69.7 (67.2, 72.0)
SAD+ADLIB	68.4 (65.6, 71.1)[b]	64.5 (62.6, 66.4)[c]	75.5 (73.3, 77.6)[a]
ED+WR	70.1 (67.5, 72.6)[b]	68.8 (66.7, 70.4)[b]	72.5 (70.1, 74.8)[a]
SAD+WR	72.5 (69.7, 75.0)[a]	66.5 (64.6, 68.4)[b]	72.7 (70.5, 74.7)[a]

[1]Feeding treatments consisted of skip-a-day (SAD) and every-day (ED) feeding programs, and water provision treatments consisted of water restriction for 3 h during the 8 h photoperiod (WR) or *ad libitum* (ADLIB) access to water during the 8 h photoperiod.
[2]Superscripts differing within a row indicate statistically significant differences identified with a Tukey's pairwise comparison posthoc test ($p<0.05$).

Table 3.5 FITC-d concentrations (μg/ml) of broiler breeder pullets by feed treatment, water treatment, and drinker type.[1]

Treatment	FITC-d concentration estimate (95% CI)
Feed	
ED	1.35 (1.27, 1.42)
SAD	1.32 (1.25, 1.40)
Water	
ADLIB	1.34 (1.26, 1.41)
WR	1.33 (1.26, 1.40)
Drinker Type	
HD	1.32 (1.24, 1.39)
LD	1.35 (1.28, 1.42)

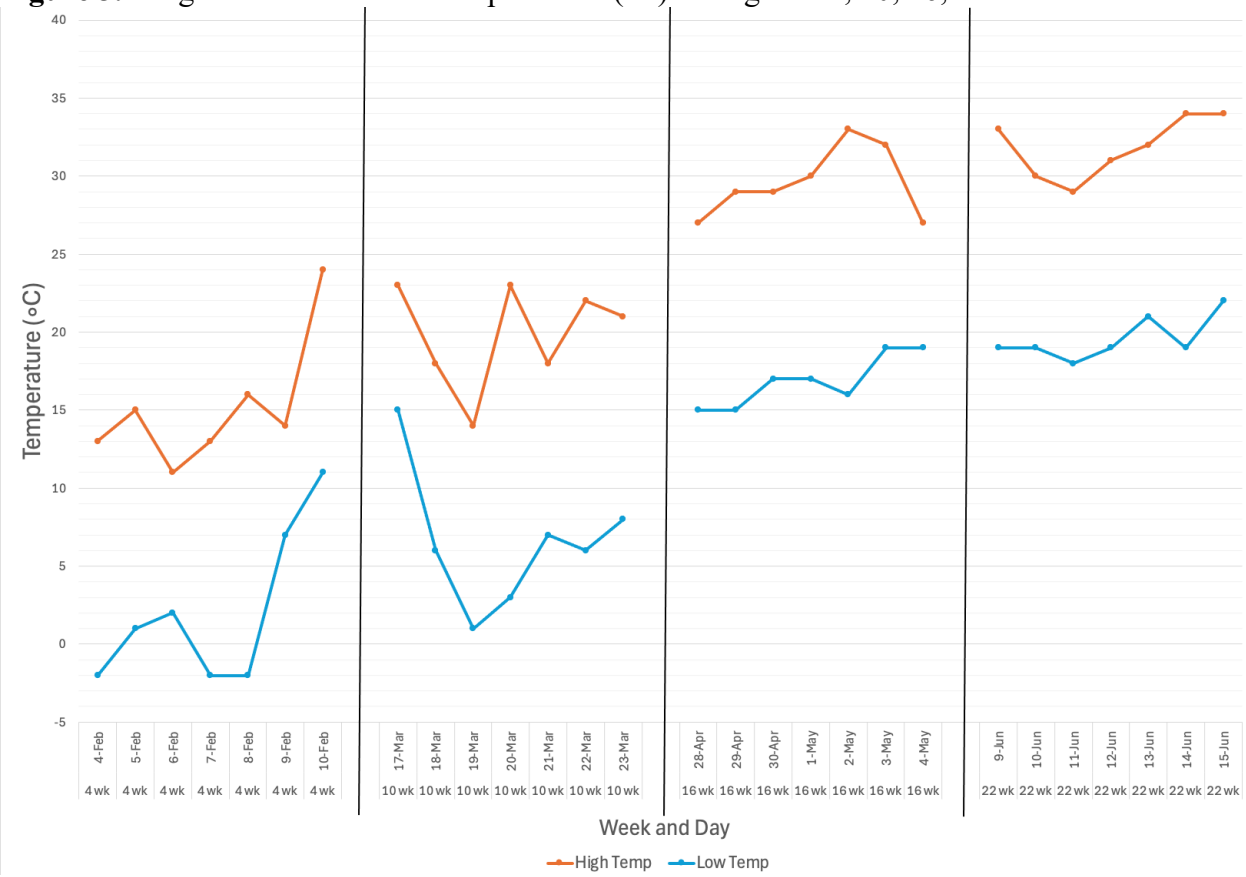
[1]Feeding treatments consisted of skip-a-day (SAD) and every-day (ED) feeding programs, while water provision treatments consisted of water restriction for 3 h during the 8 h photoperiod (WR) or *ad libitum* (ADLIB) access to water during the 8 h photoperiod. Using radio frequency identification, birds were classified as either high drinkers (HD) or low drinkers (LD) based on their drinking durations over a 7-d period.

Table 3.6 Precision water use (g) by drinker type, feed treatment, and water treatment.[1,2]

	HD	LD	ED	SAD	ADLIB	WR
Off-feed day	254.6±16.1	234.7±15.6	271.2±15.3[a]	218.1±16.3[b]	244.1±16.6	245.3±15
On-feed day	322.2±21.3[a]	257.6±20.7[b]	254.3±20.3[b]	325.4±21.7[a]	304.4±22.1	275.4±19.9
Total (sum)	576.8±27.8[a]	492.4±27.0[b]	525.6±26.5	543.6±28.3	548.5±28.8	520.6±26.0
Average/day	288.4±13.9[a]	246.2±13.5[b]	262.8±13.3	271.8±14.1	274.3±14.4	260.3±13.0

[1]Feeding treatments consisted of skip-a-day (SAD) and every-day (ED) feeding programs, and water provision treatments consisted of water restriction for 3 h during the 8 h photoperiod (WR) or *ad libitum* (ADLIB) access to water during the 8 h photoperiod.
[2] Data are presented as mean ± SE. Superscripts differing within a row indicate statistically significant differences identified with a posthoc student's t-test ($p<0.05$).

Figure 3.1 High and low external temperatures (°C) during wks 4, 10, 16, and 22.[1]



[1]Wks separated by vertical line.