## ABSTRACT

RAJO GOMEZ, EDWIN BENJAMIN. Optimizing Efficiency and Productivity in Beef Cattle Exposed to Adverse Environments: The Role and Interplay of Physiological and Nutritional Parameters (Under the direction of Dr. Daniel H. Poole).

Fescue toxicosis is a multifaceted disease that affects grazing cattle in the Southeastern U.S. Symptoms include reduced nutritional intake, suppressed growth, winter hair coat retention, impaired thermoregulation, and vasoconstriction. These issues negatively impact cattle productivity, highlighting the need for improved selection strategies to generate more resilient and efficient cattle populations. Chapter 2 explores the effects of ergot alkaloid exposure on the pulmonary arterial pressure (PAP) and other physiological parameters in beef cattle. Yearling Angus heifers were fed an endophyte-free (EF) or endophyte-infected ration (EI) for 49 days. The EI heifers showed a reduction in their caudal artery diameter ($P < 0.01$) due to ergot alkaloid consumption. However, no differences in mean PAP values were observed between groups. An interaction between PAP results and heat stress exposure was noted, as both groups showed increased PAP as ambient temperatures decreased to thermoneutrality. Additionally, the EI heifers maintained greater body temperatures ($P < 0.01$) than the EF heifers throughout the study, often surpassing the fever threshold. Chapter 3 presents a two-year evaluation of the effects of ergot alkaloid exposure on nutritional intake, feed efficiency, and performance in yearling heifers, followed by an evaluation of these parameters as pregnant heifers consuming an EF ration. In Year I, the EI heifers had lower weight gains (ADG; $P < 0.01$), body weights (BW; $P < 0.05$), body condition scores (BCS; $P < 0.01$), and greater hair shedding scores (HSS; $P < 0.01$) than the EF heifers. A thermographic assessment revealed impaired thermoregulation in EI heifers, as their skin ($P < 0.01$) and ocular globe temperatures ($P < 0.01$) were greater. The EI heifers also had lower feed intake (FI; $P < 0.01$) and greater water intake (WI; $P = 0.01$) and water intake per body

weight (WI/BW; $P = 0.04$). A post hoc analysis showed that low residual feed intake (LRFI) EF heifers had lower FI ($P < 0.01$) than high-RFI (HRFI) heifers, whereas no differences were observed between EI RFI subgroups. Conversely, the EI LRFI had lower WI ($P = 0.02$) than HRFI heifers, and no differences were observed between EF RFI subgroups. The residual ADG assessment showed that low-RADG EI heifers had greater WI than high-RADG heifers ($P = 0.03$). In Year II, pregnant (PREG) heifers tended ($P = 0.09$) to have greater BW and BCS ($P = 0.04$) than the non-pregnant (OPEN) heifers, but no differences were observed in ADG and HSS. No differences were observed in FI, WI and WI/BW between groups. Further, the LRFI PREG heifers had lower FI (88.2±1.6 kg DMI vs 99.1±2.2 kg DMI; $P < 0.01$) than HRFI heifers, whereas no differences were observed between the OPEN subgroups. No differences were observed in WI using RFI or RADG as covariates. A weak correlation ($r = 0.18$; $R^2 = 0.03$) was found for RFI scores between years, and a moderate correlation ($r = 0.32$; $R^2 = 0.10$) was observed for RADG scores. Lastly, no differences were observed in the CALFBW between calves born from LRFI and HRFI dams (26.5±1.4 kg vs 29.8±1.7 kg; $P = 0.10$). These findings highlight the complexity of fescue toxicosis, as EI heifers exhibited suppressed growth, impaired thermoregulation, and altered nutritional intake, negatively impacting efficiency. Despite having lower FI, LRFI PREG heifers delivered calves with similar weights as the HRFI heifers, indicating efficiency benefits for cow-calf operations. Nonetheless, the weak correlations between years suggest that environmental stressors, such as fescue toxicosis and heat stress, complicate feed efficiency estimations. Further research is needed to better understand the impact of these stressors and develop improved selection strategies for cattle producers in the Southeastern U.S.

Optimizing Efficiency and Productivity in Beef Cattle Exposed to Adverse Environments:
The Role and Interplay of Physiological and Nutritional Parameters

by
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# DEDICATION

I want to dedicate this dissertation to God Almighty, for guiding me through this journey with immeasurable Grace and Love. To my wife Carolina, my perfect partner and biggest source of inspiration and strength. None of my achievements would have been possible without you by my side. To my sons, Benjamin Andres and Martin Eduardo, who inspire me daily to become a better man and a role model you can be proud of. To my parents, Edwin Rajo and Rosaura Gomez, for your sacrifices to provide my siblings and I with an education that transformed our lives, and for the constant support you both have given me and my family throughout this journey. To my wife's parents, Martin Fortin and Miriam Matute, who have embraced me as their own and supported our family with boundless care. Lastly, I dedicate this dissertation to the loving memory of my Grandpa Mincho, Dr. Isidro Matamoros, and Don Rey Caballero. Your passion for agriculture, beef cattle, and life continues to illuminate my path. Your wisdom and dedication will forever inspire those whose lives you touched.

This work is not just an academic milestone, but a tribute to those who believe in me.

## BIOGRAPHY

Edwin Benjamin Rajo Gomez was born in Siguatepeque, a small town located in the central region of Honduras, Central America, where he lived with his father, Edwin Rajo, mother, Rosaura Gomez, and siblings Lindelly and Eduardo Rajo Gomez. Benjamin was raised in an agricultural environment, as his mother has a small farm where she produces coffee, beans, and citrus fruit, and his Grandfather Benjamin owns a larger farm where he produces cattle, coffee, avocado and hardwood trees. Evidently, this exposure led Benjamin to become more interested in agriculture and motivated him to pursue a Bachelor's degree in Agricultural Sciences from Zamorano Panamerican Agricultural University, which he began in January of 2009.

Following the obtention of his degree in 2012, Benjamin worked as a farm manager for eight years in the northern region of Honduras. The different challenges faced motivated him to develop research modules inside the farm, with the objective of increasing forage productivity utilizing a silvopasture model, evaluating different diet results on crossbred steers and heifers, and reducing mortality rates during calving season. The positive results obtained motivated him to resume his academic career and apply to Graduate school. In the fall of 2021, Benjamin started his Master's degree at North Carolina State University under the direction of Dr. Daniel Poole, focusing on studying the impact of fescue toxicosis on beef cattle physiology, but also collaborating on other projects related to ruminant production. Upon the completion of his Master's degree, Benjamin continued his studies at North Carolina State University to pursue a Ph.D. degree in Animal Science.

Benjamin is currently a Ph.D. candidate, with expected graduation in 2025. Benjamin's research integrates technology platforms to evaluate metabolic and thermographic responses in beef cattle exposed to environmental stressors, specifically ergot alkaloids and heat stress. His work aims to determine their impact on nutritional efficiency and productivity, ultimately improving beef producer’s decision-making abilities to enhance performance and genetic progress in their herds. Benjamin's results have been disseminated in seven publications, and he has had a significant role in securing extramural funding (over $118k) for his program. Additionally, Benjamin plays a significant role in outreach and teaching efforts for our NC Beef System, and his efforts have been recognized across the state.

Following completion of his Doctoral degree, Benjamin intends to continue investigating the impact of environmental stressors on the nutritional efficiency and productivity of beef cattle. His objectives are to develop data-driven selection strategies that enhance the adaptability, productivity and efficiency of beef cattle in adverse environments, and to translate these innovations to producers to improve the profitability and sustainability of their operations.

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# CHAPTER 1

Literature Review

### *Current Challenges for the Beef Cattle Industry in the United States*

Beef cattle production represents one of the most important agricultural commodities in the U.S. In 2023, cash receipts for cattle and calves accounted for $101.4 billion, which represents 40.5% of the total cash receipts for animals and animal products. However, cash receipts for cattle are expected to decrease approximately 0.2% in 2025 (USDA-ERS, 2023). This economic reduction is associated with the observed reduction in cattle inventory, as the latest estimation indicated that the total population of cattle in the U.S. decreased to 86.7 million head, approximately 1% less than in 2024. From the total population, it is estimated that 27.9 million head correspond to the beef cows inventory, which makes it the lowest since 1961 (USDA-NASS, 2025). These estimations imply a significant challenge to the industry, as the U.S. beef industry is one of the major suppliers to the national and global food systems, but will be required to meet the expanding market demands with a reduced animal inventory (Rotz et al., 2019).

The global population is expanding at an accelerated rate and is expected to increase by approximately 2 billion people in the upcoming 25 years, from the current 8 billion to 9.7 billion in 2050 and reaching nearly 10.4 billion by 2080 (United Nations, 2024). Per capita beef consumption in the U.S. was estimated at 25.8 kg in 2017 and is expected to remain stable or increase slightly through 2027, which will support stability in domestic demand for beef products. Additionally, international markets also maintain a strong demand for U.S. beef products, and these exports consist primarily of high value cuts and variety meats, depending on destination (Drouillard, 2018). As a consequence of the predicted increase in global population, the total demand for protein of animal origin is expected to experience a comparable increase and is forecasted to become one of the main endeavors for livestock production, as this agricultural sector provides an estimated 33% of the protein in human diets (Havlík et al., 2014).

Furthermore, the increasingly variable precipitation patterns, extended periods of high ambient temperatures, and more recurrent extreme weather events are some of the changes in global climate that negatively impact livestock performance and productivity (Cheng et al., 2022). For instance, Rodziewicz et al. (2023) estimated that drought events have had variable impact levels in at least 50% of the continental U.S. since 2020, and these events had intensified and spread to more than 85% of the U.S. by 2022. The estimated financial losses associated with large drought events over the last two decades are estimated over $190 billion (Rodziewicz et al., 2023). Additionally, extreme weather events also affect pasture and crop productivity, which decreases the availability of affordable feed alternatives and consequently increases feed costs.

Moreover, when comparing the major animal protein sources that are demanded by consumers on a life-cycle basis, beef has two major advantages over pork and chicken: 1) consumers generally prefer beef due to its sensory qualities and 2) beef cattle are considered as *'up-cyclers'* due to their ability to consume and convert large amounts of lower-cost and lower-quality forages and byproducts into valuable and nutritious protein (Nielsen et al., 2013). However, an assessment of the cost per unit of product indicates that beef has a greater cost per edible unit than pork and chicken, and beef cattle also have the poorest feed-to-meat conversion efficiency (Terry et al., 2020). Additionally, the provision of feed may account for up to three-quarters of the total direct costs of a beef operation, which can have a tremendous impact on the profitability of cattle enterprises (Nielsen et al., 2013).

Based on these facts, the U.S. beef industry is facing multiple challenges to maintain productivity and supply an increasing demand. Nonetheless, it is important to acknowledge that beef production systems bring value to society by transforming solar energy, carbon dioxide, carbohydrates, nitrogen and water into high-quality nutrients for human growth and development, and in a form that has superior market value and demand (Wickersham and Sawyer, 2017). Additionally, beef operations bring value to marginal land, where factors such as topography, soil quality, location and water availability reduce the possibility for crop production or urbanization, but maintain sufficient forage available for grazing livestock production. However, the improvement of efficiency in beef production requires further investigation, as it would not only increase the feed-to-meat conversion rates but could also lead to more competitive prices and greater accessibility for consumers.

### ***Distribution of Beef Cattle Production Systems Across the United States***

Operationally, the beef cattle industry of the U.S. can be categorized into the following segments: cow-calf, stocker, and feedlot (Herring, 2014). Over 40% of its territory is used for activities related to beef production systems, which are mainly pasture-based for cow-calf and stocker enterprises found in all 50 states. Feedlot establishments are concentrated in the Central region of the country due to their proximity to different crop and by-products sources that provide the necessary ingredients to formulate high energy and protein concentrate-based diets (Greenwood, 2021). Around 55% of the total beef cows are also found in the Central states, due to the extensive grasslands and plains that can be used for cattle grazing. Approximately 20% of the national cow herd is established in the Western region, and an additional 20% is located in the Southeastern region. The remaining 5% of the beef cows are dispersed all through the Northeastern states, Hawaii and Alaska (Drouillard, 2018).

The U.S. cattle inventory is very dynamic, and changes in its numbers are related to the phases that the cattle herd experience over time, which follow the biological nature of beef cattle production and the producers' responses to fluctuations in multiple variables, such as cattle prices, supplemental feed and other operational costs, and climatic conditions (USDA-ERS, 2024). Consequently, it is important to consider the development of improved selection and management strategies that facilitate the identification of cattle capable of effectively adapting to challenging environments while improving nutritional efficiency. These strategies will not only enhance the genetic composition and phenotypic traits of their offspring, but also secure the long-term productivity and sustainability of cattle operations across the country.

### ***Beef Cattle Production and its Relevance to the Southeastern United States***

The Southeastern region of the United States is highly diverse in terms of climate and natural ecosystems, including variable temperature, precipitation, topography and edaphic distributions, which offer optimal conditions for agricultural production. The total land area of the Southeast is estimated to be 135 million ha, which represent 14.9% of the United States. From this amount, approximately 11 million ha are used as grassland pastures for grazing species, representing around 4.5% of the national grassland area (Ingram et al., 2013). In terms of productivity, it was estimated in 2016 that the Southeast beef industry generated over $13.4 billion in gross sales, from which $4.3 billion correspond to on-farm production of beef cattle (English et al., 2020).

The principal type of cattle operations established in the Southeastern U.S. are small cow-calf production units that depend on improved pastures (Drouillard, 2018). It has been estimated that the average cow herd is 44 head, and most of these operations are either part of a larger system of agricultural enterprises or serve as a secondary source of income and resources to their owners (USDA-ERS, 2022a). Among the attributes facilitating cow-calf production in this region are extended grazing periods due to the warmer environmental temperatures throughout the year, which lead to a reduction in the requirements of winter supplementation feed. Additionally, some of the topography found in this part of the country is not suitable for crop production, but is favorable for the establishment of pasturelands (Herring, 2014).

The primary product of cow-calf operations is weaned calves. A percentage of the heifer calves weaned that possess superior traits and desired phenotypes are selected for replacement females and are retained in the herd. The remaining female and male weaned calves usually follow one of two trajectories. Approximately 60% of calves are sold to stocker or backgrounding operations to be raised on grazing or forage-based diets before heading to the feedlots to be finished with high-energy concentrate diets. The remaining 40% of calves are transferred directly to feedlots following the weaning phase (Drouillard, 2018).

Furthermore, the cow-calf operation can also be divided into seedstock and commercial categories, depending on the genetic value of their cattle. The seedstock sector focuses on raising purebred animals, and are commonly registered with a specific breed association. These establishments keep updated and detailed records of their cattle's pedigree and performance, which are valuable assets to their main purpose of selling breeding animals, most distinctly bulls, to commercial producers. The commercial cow-calf operation involves producing animals of lower genetic merit and typically raises crossbred cattle, which benefit from increased heterosis that enhances favorable traits for increasing beef production. The number of commercial establishments considerably surpasses that of seedstock operations. However, it is also common that seedstock owners have a commercial component in their farms (Herring, 2014).

### *Forage Management and Grazing Systems in the Southeastern United States*

Forage systems represent one of the principal feed sources for cattle enterprises across the U.S. In the Southeast, annual grazing systems are common due to the extended mild temperatures and considerable rainfall found in this area throughout the year. In addition, these forages provide farmers with the opportunity to combine cattle production with certain row crops in the same operation (Dillard et al., 2018). Consequently, most of the cow-calf operations established in this region use forages as the main feed source for the herd. Pasture management for grazing cattle implies understanding and analyzing the interface between two different species, where the selected forages need to be maintained under the appropriate conditions in order to provide optimal nutrition to cattle. Considering the external variables that impact this model, different strategies must be considered to form a productive, efficient, and sustainable system (Evers, 2008). Some of the grazing management techniques that are commonly used are continuous, rotational, creep, and strip grazing. From these methods, strip and rotational grazing both offer an improved utilization of the pastures for increasing the quantity and quality of forage that is produced. Producers also rely on seasonal forages that can be classified into warm-season and cool-season species, depending on their respective growth cycles throughout the year. Warm-season perennial forages are used during the late-spring and summer months due to their high yields and drought tolerance, in spite of their more fibrous and less digestible nutritional values (Harmon et al., 2019). These herbages are also combined with row crop rotations as an alternative to combat disease and weed presence in the production lot (Dillard et al., 2018). Moreover, cool-season forages spread their production cycle into a bimodal distribution, with the first peak of production beginning simultaneously with the spring season, then dropping to a hiatus during the warmer temperatures of the summer, to follow the second production peak that begins in the fall and lasts until the beginning of the winter. Both cool-season grasses and legumes produce forage of high nutritional value, and are best utilized for animals with an increased nutrient demand (Evers, 2008). Among the different forages used in the Southeast, tall fescue (*Schedonorus arundinaceus* [Schreb.] Dumort) predominates in most grazing livestock operations (Allen at al., 1996).

### ***Endophyte-Infected Tall Fescue***

Tall fescue (*Schedonorus arundinaceus* [Schreb.] Dumort) is a widely grown cool-season perennial forage that is used in livestock production. It is highly valued in beef grazing operations due to its remarkable biomass production, tolerance to increased stocking rates, and persistence in diverse soil conditions. Tall fescue can be found in different regions of the U.S., including small areas of the Pacific Northwest and lower Midwest, and an extensive area in the Southeast (Ball et al., 1993), and is estimated to cover more than 15 million hectares of grassland in the country (Alfaro and Moisá, 2022). Being a cool-season forage, tall fescue growth periods concentrate in the spring and late fall months, when temperatures range between 4 and 25 degrees Celsius. Low pasture production during the extreme temperatures of summer, which limit both the quantity and quality of the forage, are typically insufficient to meet the animal nutritional requirements (Roberts et al., 2015). Native to the European continent, tall fescue was first introduced to the U.S. in the 19$^{th}$ century but was not extensively planted until 1940 (Fribourg et al., 2009). In 1931, E.N. Fergus from the University of Kentucky evaluated a particularly robust and flourishing variety, which was later released as the Kentucky-31 cultivar in 1943. This new tall fescue strain was better adapted to poor edaphic conditions, had a broader productive period, and increased pest and drought tolerances (Ball et al., 1993). These traits favored its rapid adoption and establishment in pastures across the country, despite the fact it was not officially certified as a cultivar until 1972 (Fergus and Buckner, 1972), and positioned it as one of the principal forages used for livestock grazing in the U.S. (Alfaro and Moisá, 2022).

In the 1960s, alkaloid compounds identified in forage samples were linked to reports of animal disorders that had similarities to ergotism, a disease caused by consumption of grains infected with ergot alkaloids produced by the *Claviceps* fungi. It was not until the mid-1970s, following a considerable number of analyses, that the endophyte infection in tall fescue was identified (Ball et al., 1993). Bacon et al. (1977) suggested that *Epichloë typhina*, a clavicipitaceous systemic endophyte, could be responsible for producing the alkaloids that caused the toxicosis syndrome. Bush et al. (1979) published several theories after analyzing samples of tall fescue, including as probable causal agents the loline, perloline and perlolidine alkaloids, in addition to other ergot and ergot-like vasoconstrictor alkaloids. Following these studies, Morgan-Jones and Gams (1982) reclassified the endophyte fungus into a separate and unique section, due to its singular taxonomy, and changed its scientific name to *Acremonium coenophialum*. Moreover, in lieu of genus reclassification following phylogenetic studies, the genus *Acremonium* was renamed as *Neotyphodium coenophialum* (Glenn et al., 1996). Lastly, in accordance to changes in international nomenclature rules for fungi that stated a single name to be used for each species, the genus *Neotyphodium* was annexed to the *Epichloë* genus, defining the current scientific name of the tall fescue endophyte as *Epichloë coenophiala* (Leuchtmann et al., 2014).

It is estimated that the symbiotic relationship between the endophyte and tall fescue forage can be found in 90% of the tall fescue pastures established in the U.S., which has been beneficial for both species (Chai et al., 2020). *E. coenophiala* is known to have a subtle mode of asexual reproduction, where instead of using spores as other fungi, it disseminates the next generation through seed-borne inoculation (Tsai et al., 1994). From the perspective of the endophytic fungus, the tall fescue plant offers an appropriate and safe environment to grow, develop and reproduce, assuring the completion of its life cycle. First, the seeds of tall fescue serve as an effective vehicle that stores and transports the mycelial filaments that represent the fungus's vegetative stage. Once the seed germinates, the filaments initiate their growing phase, rapidly extending through the stem and leaf cells of the shoot's green tissue. Following the mature stage of the plant, the endophyte concentrates mostly in the leaf sheaths, and advances towards the flower stem when the reproductive phase approaches. Finally, the endophyte concentrates in the flower heads, where it invades the tissue of the ovaries and ovules, and remains to infiltrate the seed embryo and secure its path towards the next generation (Ball et al., 1993). As a result of the endophyte's metabolic activities, various secondary bioactive compounds classified as alkaloids are generated. These alkaloids play various roles within the plant, such as providing support with active feeding deterrents to herbivorous insects and nematodes that attack its root system. Ergot alkaloids also cause an amplified osmotic adjustment and nutrient accumulation that improves water conservation to aid in developing tolerance to drought. This symbiosis also allows tall fescue to withstand overgrazing conditions (Ball et al., 2003) and intensify its photosynthetic activity in response to the energy requirements of the fungus and its host (Rozpądek et al., 2015).

The alkaloids produced by the *Epichloë* species are divided into four classes: indole-diterpenes, peramines, lolines, and ergot alkaloids. Each of these chemical compounds have been found to have specific functions that are beneficial for the symbiotic relationship. Lolitrem B is identified as the indole-diterpene causal agent of ryegrass staggers in mammalian herbivores. Peramines are recognized as an effective insect deterrent (Bush et al., 1997), and Siegel et al.

(1990) reported a potent insecticidal action from loline alkaloids in an aphid bioassay. Further, ergot alkaloids that infect tall fescue produce a variable level of toxicity to herbivores that graze on it (Young et al., 2014). All naturally-originated ergot alkaloids are characterized for having an ergoline ring system (Flieger et al., 1997), and depending on their tetracyclic structure can be further divided into clavines, ergoamides or ergopeptines. These structures facilitate the interaction with multiple receptors in the central nervous system and adrenergic receptors found in blood vessels, due to their resemblance to neurotransmitter molecules such as dopamine, serotonin and noradrenaline (Wallwey and Li, 2011). Both ergoamides and ergopeptines are derivatives of lysergic acid (Gerhards et al., 2014), but several studies have reported that ergopeptines are the main causative agent of fescue toxicosis in grazing livestock (Strickland et al. 2011). Within these ergopeptines, ergovaline has been identified as the predominant compound (Yates et al., 1985; Lyons et al., 1986). TePaske et al. (1993) found in analyzed endophyte-infected tall fescue samples, ergovaline represented the largest amounts in both forage and seed samples, while ergosine, ergotamine and ergocryptine were also found in smaller portions.

Furthermore, these alkaloids have a variable distribution throughout the plant and change depending on its developmental stage. In the reproductive stage of tall fescue, ergot alkaloid concentration correlates to the mycelial abundance. E. coenophiala mycelia are highly concentrated in the basal stem tissue and in the seed gametes, and found in lesser amounts in the sheath and ligular area. Ergovaline concentrations are found in higher abundance in the seedhead and decrease significantly in the sheath, leaf, and root tissue (Kenyon et al., 2018). In addition, research done by Kenyon et al. (2018) reported differences on the vegetative canopy and vertical distribution of ergot alkaloids in endophyte-infected tall fescue, where the highest concentration of ergovaline and total ergot alkaloids was found in the 0 to 5 cm canopy portion, with a significant alkaloid reduction above that height. Ergot alkaloid concentrations also have seasonal variation that follow the growth curve of the plant. Ergovaline concentration has an ascending pattern that begins in the spring and reaches its highest peak in the fall period. Alternatively, total ergot alkaloid concentrations show a bimodal distribution, reaching an initial peak in late spring to further decrease as the plant enters a lethargy phase during the summer, and repeating an increase to maximum concentration in early fall (Rogers et al., 2011). Further, ergot alkaloid concentration in autumn-stockpiled fescue has been shown to decrease as winter progresses, which provides an opportunity for producers to utilize their pastures for grazing livestock during the winter, reducing negative effects of the alkaloids and increasing profitability by reducing feeding costs (Franzluebbers and Poore, 2021). Alkaloid concentrations may also fluctuate depending on changes in environmental temperature and precipitation. Exposure to warmer environmental temperatures (+3ºC) for extended periods of time has shown to significantly increase ergot alkaloid concentrations by 30-40% in tall fescue. Interestingly, this increase in temperature may also be detrimental to the plant species dominance in an ecosystem, as warming seemed to induce a significant reduction in relative abundance of tall fescue within a specific pasture ecosystem (McCulley et al., 2014). Several variables must be assessed to determine appropriate management strategies for tall fescue pastures, in order to obtain optimal productivity from their utilization.

### *Effects of Fescue Toxicosis on Cattle Performance*

Fescue toxicosis is a disease caused by the consumption of endophyte-infected tall fescue by cattle. In the U.S., it is estimated that the economic impacts associated with this disease account for approximately $2 billion every year (Kallenbach, 2015). The alkaloid metabolites, predominantly ergovaline, produced by the symbiotic relationship cause several unfavorable effects on physiological mechanisms within grazing cattle. These problems vary in mode of action and severity, as the mycotoxins interact with the organism and are degraded through digestive, absorption and metabolic processes.

Oral ingestion is the principal route of exposure for cattle, and the amphipathic properties of ergot alkaloids determine the rate and extent of their absorption (Strickland et al., 2011). Once ingested, the ergot alkaloids present in forage enter the ruminal cavity, where microbial fermentation and digestion processes increase the solubility and availability of the total ergot alkaloids, transforming them into simpler ergopeptine alkaloids, such as ergovaline and lysergic acid (Ayers et al., 2009). De Lorme et al. (2007) also reported that ruminal microbial action in sheep can further degrade ergovaline into lysergic acid. Additionally, Hill et al. (2001) suggested that ergot alkaloids are also metabolized in the reticulum and omasum chambers. Lysergic acid and ergoline are absorbed at a faster pace in the rumen compared to the other ergopeptine alkaloids, which are transported in the digesta to the hind-stomach and the intestinal system to complete the nutrient assimilation process. Previous research indicates that in ruminants and non-ruminants, the small intestine is the primary organ for ergopeptine alkaloids absorption (Strickland et al., 2011).

Following ruminal and enteric absorption, the ergot alkaloids are released into the blood stream and transported to the liver, where metabolization continues and a process of sorting occurs, in which the large ergopeptine alkaloids are carried to the bile for further processing and excretion in fecal matter, while smaller compounds like ergoline and lysergic acid flow to the kidneys to be excreted in the urine (De Lorme et al., 2007). It was previously assumed that ergovaline was the exclusive ergot alkaloid responsible for inducing the symptoms of fescue toxicosis (Yates et al., 1985). However, recent findings contradict this theory, based on identified diversity in types of ergot alkaloids and the previously described metabolic processes that these compounds undergo in the ruminant digestive tract (Hill et al., 2001; De Lorme et al., 2007; Ayers et al. 2009; Strickland et al., 2011).

As the ergot alkaloids are metabolized and transported to different tissues and organs, several negative effects arise affecting cattle performance. Physiologically, an interaction has been identified between ergot alkaloids and different biogenic amine receptors, and these alkaloids can have variable agonist, partial agonist, and antagonist effects (Berde and Stürmer, 1978). These associations are facilitated by the similarities in chemical structure that ergot alkaloids share with endogenous neurotransmitters, such as adrenergic, dopaminergic and serotonergic receptors in different species and environmental conditions (Klotz, 2015). Among the tall fescue alkaloids, ergovaline, ergotamine and ergocryptine have shown similar binding characteristics to D2-dopamine receptors in cell culture, which reduce the availability of receptors and directly inhibit the secretion of cyclic AMP, prolactin, serotonin, and indirectly affect insulin secretion (Larson et al., 1999; Strickland et al., 2011). Consequently, one of the symptoms that cattle affected by fescue toxicosis show is reduced concentrations of serum prolactin (Parish et al., 2013). The reduction in prolactin concentrations is a relevant concern in beef cattle operations because this hormone plays an important role in regulating lactation, body hair coat growth and shedding, and body temperature (Littlejohn et al., 2014). A correlation exists between ergot alkaloid consumption and decreased prolactin production, which is caused by the antagonist effect of ergovaline in the dopamine pathway, disabling proper hormone binding to its receptor. This disruption causes some of the symptoms associated with fescue toxicosis, such as decreased milk production, retention of winter hair coat, and increased body temperatures (Porter and Thompson, 1992).

Among the ergopeptines identified in tall fescue, ergovaline is the most abundant and potent alkaloid, making it the central focus of research done on fescue toxicosis (Guerre, 2015). This ergot alkaloid has vasoconstrictive effects that alter the normal functioning of the circulatory system (Alfaro and Moisá, 2022). Sensitivity to ergovaline varies among ruminant species, with sheep being more tolerant than cattle, and it will also be influenced by health and environmental conditions. A threshold for cattle has been established indicating 400-750 ppb (1 ppb = 1 ng/kg) of ergovaline will induce clinical symptoms (Tor-Agbidye et al., 2001). However, under heat stress conditions this tolerance range can decrease to 200-350 ppb (Hovermale and Craig, 2001). In addition to ergovaline, there are other alkaloids related to vasoconstriction. Pesqueira et al. (2014) found that ergopeptine alkaloids can initiate and sustain contractile responses, with ergovaline and ergotamine causing the most potent and durable contractions in a bovine saphenous vein bioassay. These authors also indicated a possible accumulation of the toxin that might prolong the toxicosis effects, hence delaying the recovery (Pesqueira et al., 2014). Klotz et al. (2007) reported a potential bioaccumulation of ergovaline and ergotamine related to slow alkaloid dissociation from serotonin receptors, which may be the principal cause of vasoconstriction related to fescue toxicosis.

The effects produced by alkaloid-induced vasoconstriction are well documented. Aiken et al. (2009) and Rajo-Gomez et al. (2025) confirmed this via doppler ultrasonography, with a lower heart rate and reduced blood flow reported in caudal arteries of beef heifers that were previously exposed to diets containing ergot alkaloids. One of the major consequences is the reduced blood circulation to peripheral vasculature, which causes lameness and gangrene in the extremities that considerably perturb normal mobility and performance in cattle. The gangrenous ergotism, one of the most visually evident consequences of fescue toxicosis, causes necrosis in ear, tail and hoof tissue, and in severe cases can lead to loss of the affected extremity or the entire animal (Klotz, 2015).

In addition, vasoconstriction has a negative impact on normal ovarian function and pregnancy establishment, which consequently reduces productivity in cow-calf operations. Some of these offsets may be a limited nutrient transport and intake, abnormal functioning of the hypothalamus, hypophysis, and pineal glands, and concomitant alterations on the ovaries and the estrous cycle (Strickland et al., 2011). Lyons et al. (2018) observed a significant reduction in the diameter of arteries and veins that transport blood to the ovary and uterus of beef heifers after consuming a ration containing endophyte-infected fescue seed.

Progesterone concentrations are also reduced in female cattle grazing endophyte-infected fescue pastures, compromising the establishment of pregnancy and increasing the risk of abortion. Mahmood et al. (1994) described a significant reduction on serum progesterone levels in crossbred beef heifers grazing high endophyte-infected fescue pasture trial. Furthermore, an age difference impacted sensitivity to alkaloid exposure, as weaned heifers had lower progesterone concentrations compared to yearling heifers. This decrease in progesterone levels may be linked to local vasoconstriction of blood vessels that limits normal circulation of the hormone in the blood stream.

Moreover, ergot alkaloid effects on the male reproductive tract are not well documented. Pratt et al. (2014) reported that bulls grazing on endophyte-infected fescue pastures had reduced weight gain, lower serum prolactin concentrations, inferior sperm concentration and increased morphological abnormalities in ejaculate samples, reducing the overall semen quality and freezing potential. However, additional research is necessary to fully comprehend the effects of fescue toxicosis on bull reproductive performance, especially in the Southeastern U.S. where 95% of cow-calf operations use natural service during the breeding season (Pratt and Andrae, 2015).

Ergot alkaloids also negatively impact the ruminal and intestinal microbiome. As it was previously described, the level of alkaloids present will vary depending on the maturity stage of tall fescue plants. The ergot alkaloids contained within the forage are exposed through the rumination process and initiate an interaction with different species of microorganisms distributed along the digestive tract. Previous research by Moyer et al. (1993) and Harlow et al. (2017) found alkaloid susceptibility to ruminal degradation in their respective in vitro and ex vivo studies. Despite this degradation, there is evidence that ergot alkaloids, principally ergovaline, can still induce vasoconstriction in the ruminal vessels (Foote et al., 2011) and also limit blood flow to the duodenum, colon, and kidney of cattle (Rhodes et al., 1991).

From a nutritional perspective, sufficient blood flow to the ruminal and gut surfaces is necessary for an adequate absorption of nutrients. During fescue toxicosis, this mechanism is disrupted and compromises volatile fatty acid (VFA) absorption and metabolism, consequently leading to decreased weight gain and growth performance in ruminants. In addition, an augmented microbial interconversion of the VFA may negatively impact feed efficiency, as methanogenesis could be potentially increased in the rumen as a consequence of the decreased absorption of VFA (Foote et al., 2013). Further, Mote et al. (2022) stated that endophyte-infected tall fescue modified the ruminal microbiota in grazing steers, by reducing the population of fungi species that directly engage in cellulose degradation and digestion, which ultimately may affect ruminal passage rates. Ergovaline has also been associated as a disruptor of microbiota diversity in the gut of cattle that grazed on alkaloid infected fescue pastures by reducing the number of microbial species. More notably, the effects of ergovaline worsened under heat stress conditions, indicating an interaction between the toxin and environmental conditions that influences the animal response to the disease (Mote et al., 2022).

There are many repercussions that ergot alkaloids have on the immune system. Cattle that graze on endophyte-infected pastures show reduced body weight and average weight gains (Parish et al., 2013; Poole et al., 2019; Rajo-Gomez et al. 2024), which lead to a decreased intake of nutrients that are necessary for adequate functioning of the immune system. Following exposure to a pathogenic infection or tissue injury, the immune response is activated by the surge of inflammatory mediators, which include various cytokines, chemokines and prostaglandins. These compounds regulate the inflammatory response by stimulating and orienting the activity of immune cells and mediators that eliminate infectious agents and damaged cells, and proceed to restore affected tissue (Sheldon et al., 2014). Most of these mediators, especially cytokines, are soluble factors either released by cells into the circulatory system, directly into tissue, or remain within the cell to act as integral membrane proteins (Dinarello, 2007). Proinflammatory cytokines are known to be associated with reduced levels of prolactin in blood serum, increased body temperature as they are pyrogenic compounds, and may be related to the vasoconstrictive effects of fescue toxicosis due to their inflammatory nature (Filipov et al., 1999). Poole et al. (2019) reported a hyperactive innate immune response in stocker steers exposed to endophyte-infected tall fescue seed, which exhibited increased concentrations of pro- and anti-inflammatory cytokines when compared to the response of steers that were not exposed to the alkaloids. This translates to repercussions on major stressful events, such as weaning and immunization programs, as they become more challenging to overcome for calves that have been exposed to the toxins present in endophyte-infected tall fescue (Poole et al., 2019).

Moreover, the antagonist effects of ergot alkaloids on prolactin pathways trigger several disruptions. In the immune system, it is known that white blood cells have prolactin receptors on their membrane, and lymphocytes are capable of secreting prolactin (Reber, 1993) which may have regulatory functions on prolactin production and secretion for adequate immune responses (Freeman et al., 2000). Additionally, prolactin is also involved in lactogenesis and mammary gland differentiation mechanisms in cattle. As it was previously stated, reduced serum prolactin levels and decreased milk production are common symptoms associated with fescue toxicosis. However, prolactin is not directly responsible for maintaining milk production in lactating cows (Porter and Thompson, 1992). Consequently, the decrease in milk production associated with fescue toxicosis is not directly related to the reduction in prolactin concentration, but may be associated with other detrimental symptoms of the syndrome such as vasoconstriction and reduced feed intake (Poole and Poole, 2019). Further, Wilbanks et al. (2021) reported that pregnant cows grazing on endophyte-infected tall fescue pastures had reduced weight gain during the last trimester, and a lower milk production during the first postpartum month that resulted in depressed calf weight gains.

Depressed prolactin concentrations are also related to the retention of winter hair coats in cattle during the summer. When unaffected, prolactin is a hormone directly associated with photoperiod, meaning that its concentrations increase with increasing daylight hours (Karg and Schams, 1974). Porter and Thompson (1992) reported prolactin's association with winter hair growth in the fall and early winter, as daylight time shortened, and with hair coat shedding in the spring and early summer, as daylight hours extended. More recently, a mutation in prolactin signaling has been found to be a direct modulator of hair length and weight, and also the sweat rate in cattle. Further, cattle carrying these mutant transcripts show an association between prolactin and thermoregulation processes, indicating that the protein hormone might control sweat secretion in addition to coat length (Littlejohn et al., 2014). McClanahan et al. (2008) found that yearling calves grazing on endophyte-infected tall fescue experienced lack of shedding and continued hair growth during the summer months, which could be linked to depressed prolactin concentrations. These calves also had lower weight gains, reduced sweating rates and shorter grazing intervals during periods of high environmental temperature and humidity. Aiken et al. (2011) reported an increased effect of hyperthermia attributed to rough hair coats in steers that grazed toxic fescue during the summer. However, they attribute the coat thickness to hair retention from the winter and excessive hair growth during longer summer days. Despite the cause, heat stress induced by retained hair coats increases the vulnerability of cattle that graze endophyte-infected pastures during the summer.

Ruminants excrete ergot alkaloids through urine and fecal matter. Different hepatic, biliary and renal metabolic processes take place to remove ergot alkaloids from blood circulation and lead to detoxification (Klotz, 2015). According to Eckert et al. (1978), the elimination pathway for ergot alkaloids is dependent on the molecular weight of each compound. Ergot alkaloids under 350 Da are removed via the renal system, whereas compounds weighing over 450 Da, such as ergovaline and other ergopeptine alkaloids, are eliminated via the biliary system and excreted with the feces. Furthermore, ergot alkaloid elimination via milk occurs in isolated cases of extremely high intake of these toxic compounds, and most likely is not a principal route for excretion. Despite the metabolic and physiological efforts of the animal to remove the consumed toxic alkaloids, only a partial elimination is possible (Klotz, 2015). When comparing the ingestion and excretion of ergovaline and lysergic acid in crossbred steers consuming high-alkaloid tall fescue hay, Merrill et al. (2007) reported a higher intake of ergovaline, but a higher excretion of lysergic acid. This partial recovery of the consumed ergovaline from the excreta suggests a possible degradation process converting it to lysergic acid, which might correlate to having a greater amount of excreted lysergic acid than was consumed.

Different researchers have evaluated if detoxification from fescue toxicosis in livestock is possible and what the duration of these timeframes would be. Cattle may recover from most symptoms, excluding gangrene and necrosis of tissue, by removing the source of the ergot alkaloids. Recovery times for body temperatures in cattle will likely be influenced by the environmental temperature and humidity index, and might fluctuate between one and four weeks, whereas prolactin concentrations might increase to normality in a two-week period (Aiken et al. 2013). Vasoconstriction effects on the caudal artery have variated among studies. Klotz et al. (2016) reported changes in peripheral vasoactivity and recovery from vasoconstriction in steers 36 days after being removed from endophyte-infected pastures. In contrast, Aiken and coworkers (2013) indicated that vasoconstriction of the caudal artery caused by ergot alkaloids was not relieved after relocating steers to a non-toxic diet for 30 days (Aiken et al., 2013). These differences might relate to the possibility of alkaloids binding to specific receptors in tissue that impede their removal and could even give rise to bioaccumulation. More specifically, several studies evaluating contractile responses of bovine lateral saphenous vein indicate that the ergoline ring structure found in all ergot alkaloids facilitates the interaction with the different biogenic amine receptors. Further, these results indicate that contractile responses produced by ergopeptine alkaloids are more persistent, implying that compounds like ergovaline might be more difficult to metabolize and extend the recovery period (Klotz, 2015). However, more research is needed to confirm if bioaccumulation of ergot alkaloids in different tissue and body fluids persists for extended periods.

### ***Animal Management Strategies to Reduce the Impact of Fescue Toxicosis***

Animal health and productivity are all parameters affected by fescue toxicosis. To reduce the negative effects caused by this disease, efforts have been made to increase the understanding of the multiple interactions between endophyte-infected tall fescue and cattle that consume it, with the ultimate objective of identifying strategies that can increase animal productivity and welfare. These methods are based on multiple approaches focusing on nutritional supplementation, specific management practices, genetic and breeding management and genomic identification of traits associated with the disease.

Producers have used energy or protein supplementation during grazing with the objectives of increasing animal performance, improving and extending the availability of forage in pastures, increasing stocking rates, and diluting the alkaloid intake from endophyte-infected tall fescue. Effects of supplementing steers grazing endophyte-infected tall fescue pastures with pelleted soybean hulls have been evaluated. After a two-year study, steers consuming soybean hull pellets had greater average daily weight gains, increased serum prolactin concentrations, and tended to have more sleek hair coats than steers without supplementation (Carter et al., 2010). Correspondingly, Aiken et al. (2008) reported that supplementation of soybean hulls to steers grazing endophyte-infected tall fescue pastures increased their average daily weight gain, but did not observe differences in hair coat roughness and serum prolactin concentrations. Nonetheless, the incorporation of nutritional supplements  may be challenging as it represents an increase in production costs.

A complementary approach is to identify the optimal periods in the calendar when maximum benefit can be obtained from the available resources in the operation and to develop specific herd management strategies based on these periods. One strategy that has proven improvements is deferring the calving season from the spring to the fall. Many beef producers develop their breeding plans with the objective of spring calving, coinciding with the increase in forage resources and their capacity to satisfy the increasing nutritional demand of lactating cows. However, this specific timeframe also coincides with the growing and reproductive phases of tall fescue when the ergot alkaloids reach their greatest concentrations in the plant. Consequently, exposing cattle to these ergot alkaloids during the spring increases their susceptibility to heat stress, vasoconstriction, and other previously stated maladies during the higher temperatures of the summer, negatively impacting the herd productivity.

By postponing calving season to the fall, cow-calf grazing operations avoid this exposure to high levels of ergot alkaloids during the spring and are able to maintain productivity. Caldwell et al. (2013) indicated that this strategic adjustment to fall calving may also reduce calving intervals when compared to spring calving, due to the seasonal decrease in environmental temperatures, and to the reduction in toxin concentration of tall fescue during a period when cow nutritional requirements are high. Further, calf performance was also improved in this study, with fall-born calves having higher average daily gains, weaning weights, and overall increased productivity than calves born in the spring (Caldwell et al., 2013). Based on this evidence, producers who utilize tall fescue as the main forage source may profit from implementing fall calving to increase animal performance and productivity in their operations.

Multiple investigations have focused on identifying animals that have reduced susceptibility to ergot alkaloid toxicosis from tall fescue intake. One of the approaches evaluated the performance of *Bos indicus* breed of cattle under the effects of fescue toxicosis, due to their increased resistance to heat stress and ability to thrive in adverse environments. Superior performance has been reported from Brahman-cross cattle compared to British cattle breeds when grazing endophyte-infected tall fescue pastures. Goetsch et al. (1988) stated that Brahman crossbred steers had increased average daily weight gains compared to English crossbred steers (Goetsch et al., 1988). Similarly, Brown et al. (2000) reported that Brahman-cross cows consuming endophyte-infected tall fescue demonstrated better tolerance to the toxin deleterious effects than purebred cows (Brown et al., 2000).

Moreover, tolerance traits have also been found in specific *Bos taurus* breeds. The Romosinuano, which are a tropically adapted breed developed in Colombia from Spanish cattle, have been described with increased resistance to heat stress. Cows of this particular breed were utilized in a trial to compare their tolerance to endophyte-infected tall fescue consumption relative to Angus cows. The results indicate that despite having decreased milk production and serum prolactin concentrations attributed to ergot alkaloids, Romosinuano cows had lower rectal temperatures than Angus cows (Burke et al., 2010). Another breed that has been shown to reduce the negative consequences of fescue toxicosis is the Senepol. This composite genetic line was developed from crossing Red Poll and N'Dama animals, and are renowned for their increased capacity to tolerate high environmental temperatures. This heat resistance ability is facilitated by the presence of a slick gene, which is affected by the prolactin receptor mutation, and is expressed phenotypically as a permanently short, slick hair coat that permits a more efficient thermoregulation capacity. This slick trait might have positive implications in offsetting some fescue toxicosis symptoms, such as retained winter hair coat and increased body temperature, and improving reproductive performance. Poole et al. (2019) studied the impact of the slick hair trait in physiological and reproductive parameters in crossbred Angus-Senepol heifers consuming endophyte-infected tall fescue haylage. Their results indicated that heifers with the wildtype hair coat gene had a decreased number of dominant follicles, compared to groups of slick gene heifers consuming endophyte-infected diets (Poole et al., 2019).

Cattle responses to diseases like fescue toxicosis have also been reported to differ depending on the region of origin. Johnson et al. (2015) reported regional differences in stocker cattle responses to consumption of endophyte-infected tall fescue seed. Angus steers from Missouri, a state that is part of the Fescue Belt, had a minor reduction in feed intake when fed toxic tall fescue compared with nontoxic tall fescue, when compared to Angus steers from Oklahoma, which were considered naïve to fescue toxicosis due to the absence of tall fescue in this state (Johnson et al., 2015). This evidence supports the idea that cattle populations might be capable of developing certain level of tolerance to diseases like fescue toxicosis following exposure to the causal agent for extended timeframes. However, a well-defined genetic approach to identify and select these specific groups of animals is yet to be identified and developed to disseminate to beef cattle operations.

### ***Improvements in Genetics to Reduce the Impact of Fescue Toxicosis***

Over the last decade, significant progress has been made to understand genetic properties and mechanisms associated with fescue toxicosis. Two different perspectives have received interest among research objectives: phenotypic selection and genomic selection. Both approaches focus on identifying groups of animals within a population that have demonstrated traits of tolerance to the symptoms of fescue toxicosis, and including them in phenotypic and genotypic evaluations to detect the potential traits conferring this superiority (Smith and Cassady, 2015). Nonetheless, the complexity of this disease and its interaction with diverse environmental factors generates a wide variability within herds and challenges the possibility of developing an accurate and consistent method to identify these fescue-tolerant animals.

One of the phenotypic strategies that has gained acceptance in recent years has been selecting cattle based on their hair shedding capacity. As previously discussed in this review, winter hair coat retention associated with low serum prolactin concentrations is one of the most evident symptoms of fescue toxicosis, which increases the animal susceptibility to heat stress (Porter and Thompson, 1992; McClanahan et al., 2008). Gray et al. (2011) investigated the variation in hair coat shedding of Angus cows following exposure to endophyte-infected tall fescue and developed a method to quantify this shedding ability. Their study utilized 532 Angus cows dispersed in two locations and implemented the use of a numeric scale from 1 to 5 to score hair shedding, with 1 representing a fully-slick, summer hair coat and 5 representing a rough, winter hair coat. The score evaluation was performed every 30 days from March to July for three consecutive years. Following data collection and analysis, a threshold was established for cows averaging a score of 3 or lower by the beginning of June, defining them as “early shedders”, while cows with higher scores were defined as “late shedders”. Additionally, this study also identified an average increase of 11.1 kg in adjusted weaning weight of calves born from early shedding dams. It was determined that hair shedding ability is moderately heritable ($h^2 = 0.35$), and a strong correlation between adjusted weaning weight and dam shedding ability was identified to have a high repeatability at 0.65. Lastly, this study concluded that hair shedding variation within the cow population was still evident subsequent to grazing of endophyte-infected pastures.

Furthermore, calf weaning weight results obtained by Gray et al. (2011) were reported to the American Angus Association to perform calculations and provide adjusted 205d weaning weights, which take into account the age of dam and age of calf to 205 days. Based on data published by this study, the American Angus Association released a new production EPD for Hair Shedding (HS) in May of 2022. The HS EPD is expressed in units of hair shed score, with lower EPD values indicating a more favorable capacity of the animal to shed their winter hair coat earlier in the spring (American Angus Association, 2022). This EPD is an efficient tool to improve the identification of individuals with enhanced efficiency and productivity within their herds, leading to a better selection of replacement females and sires that will produce the next generation of cattle in their operation.

In a different study, Poole et al. (2020) accurately identified tolerant animals to fescue toxicosis within a herd by utilizing phenotypic performance parameters. The experiment consisted of exposing purebred, pregnant Angus cows to endophyte-infected tall fescue pastures for 13 consecutive weeks. The evaluated phenotypic performance parameters included body weight, body condition score, hair coat score, and hair shedding score. Based on results obtained, the Fescue Toxicosis Selection Method (FTSM) was utilized to designate cows into tolerant or susceptible groups. The results showed that cows deemed tolerant had greater average daily weight gains, and lower hair coat and hair shedding scores when compared to susceptible cows (Poole et al., 2020). More recently, the FTSM was also adopted by Rajo-Gomez et al. (2024) to classify cows that grazed on endophyte-infected pastures at two different locations into tolerant and susceptible groups. Comparable to the findings of Poole et al. (2020), the tolerant groups tended to have greater final body weights and daily weight gains, and lower hair shedding scores than the susceptible group (Rajo-Gomez et al., 2024). These studies validate the FTSM as a viable means to identify animals with superior performance in spite of chronic exposure to ergot alkaloids.

In a different environment, the adoption of an EPD for animal selection has proven effective in reducing the negative effects of high-altitude disease. High-altitude disease, commonly referred to as brisket disease, is a frequent condition experienced by cattle in regions with increased altitudes. It was previously thought that it only affected animals in elevations over 1,600 m, but different cases have been reported recently in more moderate elevations between 1,200 and 1,600 m (Pauling et al., 2018). High-altitude disease is exhibited as oxygen concentrations in the atmosphere decline with increased elevation, which causes an increase in effort by the cardiovascular system to compensate for the demand of oxygen supply. This additional stress has negative repercussions in the cardiopulmonary system, as bovine species hyper-reactive to low atmospheric oxygen concentrations. Consequently, the increased stress caused by alveolar hypoxia causes pulmonary arterial vasoconstriction, and with long-term exposure can develop changes in vascular walls, and in more severe cases induce right-side cardiac failure and death of the animal (Veit and Farrell, 1978; Holt and Callan, 2007).

In 2007, Holt and Callan reported a PAP test to identify tolerant animals to high-altitude disease. This test involves introducing a flexible catheter tubing through a large bore needle into the jugular vein and passing it down through the right atrium, into the right ventricle, and finally reach the pulmonary artery. Once in the artery, PAP is measured by a pressure transducer linked to the catheter (Holt and Callan, 2007). This PAP measurement is reflected on a monitor by a change in the pressure wave, which is caused by an increase in the diastolic pressure of the pulmonary artery over the diastolic pressure of the right ventricle. In an animal with normal health conditions, the mean PAP at altitudes between 1,500 and 2000 m should range from 34 to 44 mmHg. Cattle with clinical symptoms of pulmonary hypertension can reach PAP measurements between 48 and 213 mmHg. These results indicate that any animal with a PAP measurement above 50 mmHg should be evaluated to detect any cardiac murmurs or congenital defects. Generally, cattle over 12 months of age that report a PAP measurement below 41 mmHg at an altitude above 1,500 m are expected to maintain an adequate PAP at higher elevations and can be selected as breeding stock for high-altitude operations. Animals with PAP measurements ranging between 41 and 49 mmHg should be utilized with caution at high altitudes due to their performance unpredictability. Further, any cattle that scores a PAP measurement greater than 49 mmHg at any elevation has an increased risk of suffering high-altitude disease and should not be kept or bred at increased elevations (Holt and Callan, 2007).

Moreover, it has been found that susceptibility of cattle to hypoxia-induced pulmonary hypertension is a heritable trait (Holt and Callan, 2017). Based on these indications, Pauling et al. (2018) evaluated the relationship between PAP and elevation, by analyzing if PAP measurements recorded at moderate elevations (below 1,600 m) had a genetic correlation to PAP measurements recorded at high elevations (over 1,600 m). A substantial population ($n$ = 14,665) of purebred Angus cattle was utilized to gather PAP and elevation data. The results obtained from this study indicated that PAP measurements collected from animals located in altitudes between 1,219 m and 1,600 m may be included as a correlated trait in a multi-trait genetic analysis to generate EPDs that can be utilized for selection of animals with increased tolerance to pulmonary hypertension (Pauling et al., 2018). In February 2019, the American Angus Association released a PAP EPD as an indicator of animals with lower risk of developing high-altitude disease. Lower PAP EPDs are more advantageous and indicate the potential of generating progeny with lower PAP measures (American Angus Association, 2019).

The study conducted by Rajo-Gomez et al. (2024) also evaluated the impact of fescue toxicosis on the pulmonary arterial pressure of cattle. Similar vasoconstrictive effects between high-altitude disease and fescue toxicosis, as well as the release of the PAP EPD as genetic selection marker for tolerance to high-altitude disease, supported the hypotheses that ergot alkaloid consumption may increase the mean PAP values of cows, and that the PAP test may be adopted as a phenotypic evaluation to identify animals with increased tolerance to fescue toxicosis. However, both hypotheses were rejected as cows that grazed on endophyte-infected pastures had a decrease in their PAP values. This study also indicated that the mean PAP values may have been influenced by the exposure to different levels of heat stress that these cows experienced (Rajo-Gomez et al. 2024). This hypothesis was supported by the results reported by Rajo-Gomez et al. (2025), who evaluated changes in PAP and various physiological parameters in replacement beef heifers. In this study, two groups of heifers were fed either an endophyte-infected (EI) or an endophyte-free (EF) ration for 49 days. Caudal artery diameter, PAP, and heat stress exposure were assessed. The PAP results did not differ between treatment groups, but an interaction between changes in PAP and heat stress exposure was identified as both groups had increased PAP values when exposed to mild and moderate heat stress conditions. Additionally, heifers consuming the EI ration had lower caudal artery diameters than their EF pairs, which translates to vasoconstriction caused by ergot alkaloid exposure (Rajo-Gomez et al., 2025).

These results indicate that ergot alkaloid intake does not cause vasoconstriction on the pulmonary artery. Instead, changes in PAP may be influenced by the exposure to heat stress. Further investigations should be conducted in regions where changes in PAP are of interest to better understand the effects of heat stress exposure on the cardiopulmonary system of cattle. Moreover, future research in EI fescue-dominant environments should aim to develop strategies to reduce the negative consequences of FT and heat stress and in order improve animal welfare, productivity and profitability in these regions.

### *Optimizing Efficiency Parameters in a Fescue Environment*

As thoroughly discussed in this review, fescue toxicosis is a complex disease that causes multiple detrimental effects on many physiological and performance parameters of beef cattle. Consequently, the efficiency and profitability of cattle operations managing this malady are also impacted due to the increased operational costs associated with implementing strategies to alleviate the toxicosis symptoms and enhance animal performance. Nonetheless, focusing on strategies to improve efficiency metrics has proven beneficial both in the short and long term. For instance, improving feed efficiency is positively correlated to greater feed-to-meat conversion rates, which not only optimizes feed resource use but also improves the financial performance of the operation. In the long term, increasing the number of efficient animals in the herd may reduce the operational costs and boost the profitability of beef cattle operations (Nielsen et al., 2013; Terry et al., 2020).

The parameters of efficiency in beef operations can be assessed from two different perspectives. The general production efficiency evaluation focuses on increasing the output of production using the same amount of input resources. Some examples for this approach include measuring the total weight of calves weaned during a cow's lifetime, improving herd health to reduce mortality losses and to improve reproductive performance, maintaining optimum breeding systems, and harvesting cattle at an optimum weight and age (Terry et al., 2020). A different approach focuses on evaluating feed efficiency. Initially, this parameter was measured on a feed conversion ratio (FCR) or gross feed efficiency basis, which accounted for the amount of feed required to produce one unit of body weight, and are correlated with genetic and phenotypic traits of growth, mature body weight and composition of gain (Kenny et al., 2018). However, increasing selection pressure for FCR over the past decades has resulted in cattle with faster growth rates, and increased mature size and feed intake, along with the associated economic costs (Basarab et al., 2013).

A different standard metric for evaluation of feed efficiency in livestock is residual feed intake (RFI), which is defined as the difference between the animal's actual feed intake and its predicted feed requirement for maintenance of body size and production (Koch et al., 1963). A low (negative) RFI value indicates lower feed intake than predicted whilst maintaining expected body size and growth, thus improved efficiency. Conversely, higher (positive) RFI values represent greater consumption than predicted, hence being less efficient (Basarab et al., 2013; Terry et al., 2020). In consequence, evaluating and predicting RFI has gained relevance as a selection tool to improve efficiency in beef cattle, as individuals with low RFI consume less feed but maintain similar weight gains (ADG) when compared to their high RFI contemporaries (Damiran et al., 2018). Unlike growth rate, FCR, and dry matter intake (DMI), RFI is independent of body size, weight, production stage, sex and age, and is also a moderately heritable trait ($h^2$ = 0.16–0.43; Kenny et al., 2018). Thus, adopting selection for RFI may represent an opportunity to reduce feed costs along the entire beef production chain.

Another approach to evaluate feed efficiency is residual average daily gain (RADG), which is defined as the difference between the actual ADG of an animal and its predicted ADG, based on its DMI, BW maintenance and fat cover. This indicator has been selected by the American Angus Association and a RADG EPD has been released as its latest feed efficiency selection tool. This EPD is a result of research projects performed by North Carolina State University, Iowa State University, and the University of Illinois, where individual intake data were collected over several years (Northcutt and Bowman, 2010). This selection marker provides a comprehensive approach to identifying superior animals in feed conversion by combining individual intake data with genomic information on DMI. Additionally, RADG is moderately heritable trait ($h^2$ = 0.31–0.41; Northcutt and Bowman, 2010), which facilitates the improvement of future generations in cattle production systems. A study conducted by Freetly et al. (2020) compared the relationship between feed intake in growing heifers and mature cows. Heritabilities of 0.21±0.11 in heifers and 0.14±0.10 in cows were reported. This research also identified genetic correlations for RFI (0.41±0.36) and RADG (0.31±0.46) between heifers and cows (Freetly et al., 2020). Contrary to RFI, animals with greater RADG values are more desirable due to increased ADG consuming the same amount of feed. However, it is important to consider that, as with FCR, increasing selection pressure for RADG may result in larger, heavier cows with greater nutritional requirements, which is detrimental for cow efficiency and the production system (Ojo et al., 2024). Thus, it is recommended that producers consider multiple traits of interest and create a balanced selection of EPDs when developing breeding and selection strategies.

Selection for efficiency in cattle must also consider external factors, such as climate and management system. The exposure to extreme thermal stressors directly impacts the energy requirements for maintenance and growth in cattle (NASEM, 2016), and producing beef cattle under controlled environments is not an economic or spatially viable strategy (Terry et al., 2020). Considering the recurrent exposure to heat stress that cattle experience in the Southeastern U.S., traits associated with increased tolerance to this stressor, such as hair shedding, should be considered in the selection model for efficiency (American Angus Association, 2022). Additionally, the majority of cattle operations in this region are cow-calf farms that use pasture management systems. Cattle raised on pasture-based systems commonly have less efficient weight gain due to the variable nutritional quality in pastures and the implied energy losses in the ruminal fermentation processes of forages (Basarab et al., 2013). Incorporating supplement feeds to forage-based diets has proven successful for the improvement of productivity in pasture-based cow-calf operations, but this is also challenging due to the logistic factors required and the increase in production costs (Lancaster and Larson, 2022).

Another challenge for the determination of efficiency in cow-calf operations is the change in nutritional requirements of cattle depending on their growth stage. This variation is caused by the evolution of the animal's body as tissues and organs grow at different rates over time. Consequently, young animals will demand different types and quantities of nutrients than adults (Ojo et al., 2024). In the current beef production system, feed efficiency assessments are typically conducted on young growing cattle after weaning at test stations that measure performance using high-energy feed rations. However, once these animals are relocated to systems with different management schemes, they consume forage-based diets that are higher in fiber and lower in energy. Consequently, low correlations have been observed when evaluating the feed efficiency metrics of growing cattle compared to subsequent evaluations as mature cows, which may be attributed to differences in nutrient metabolism (Ferrel and Jenkins, 1985; Lancaster, 2014).

Moreover, these correlations may also be impacted by changes in the physiological state of the animal and its surrounding environment. Archer et al. (2002) reported strong genetic correlations between postweaning RFI of heifers and FI ($r = 0.64$) and RFI ($r = 0.98$) of mature non-pregnant cows, although the phenotypic correlations for FI ($r = 0.34$) and RFI ($r = 0.40$) were lower. However, these animals were fed the same pelleted ration with high energy and protein content during their evaluations. These findings indicate that RFI evaluated across different production stages in cattle is genetically the same trait, but the expression of its genetic potential may be altered by changes in the physiological state of the animal (Archer et al., 2002; Lancaster, 2014). Further, Durunna et al. (2012) found that crossbred heifers experienced changes in their RFI reranking across two feeding periods, despite receiving the same diet, and the reranking was more severe in heifers with extreme RFI performances in each period (Durunna et al., 2012). These considerations are relevant for the determination of efficiency in cattle raised in the Southeastern U.S. due to the increased exposure to fescue toxicosis and increased ambient temperatures that negatively impact their physiological parameters.

***Incorporating Precision Livestock Farming to Improve Cattle Management Strategies in a Fescue Environment***

Given the challenges that have been discussed in this review, Precision Livestock Farming (PLF) systems may become an effective resource by integrating advanced technologies to improve animal management and monitoring. Traditional methods for information collection are usually labor-intensive and involve time-consuming processes. In contrast, PLF systems encompass various technological tools to collect and analyze information in real-time, which facilitate detailed analysis of various parameters and optimize decision-making and production (Michelena et al., 2024; Ojo et al., 2024)

Several PLF applications that have been developed over the past two decades can be incorporated to cattle operations to improve management strategies. Some of these technologies include electronic identification systems like radio-frequency (RFID) tags, image analysis methods like infrared thermography, movement detection systems including accelerometers and GPS, herd management systems such as virtual fencing and drones, health monitoring systems with the use of implanted sensors, and feeding stall systems to monitor individual feed intake (Tzanidakis et al., 2023; Ojo et al., 2024). These systems can measure and estimate different physiological and behavioral parameters, which allow the collection of accurate measurements that can be analyzed to detect changes in performance or health status. Thus, by continuously evaluating the physiological conditions of animals, these systems facilitate early detection of disease or stress and animal welfare can be improved (Michelena et al., 2024).

The cattle sector seems to incorporate and benefit from a greater variety of PLF applications in comparison to other livestock species (Schillings et al., 2021). Many of these technologies are used simultaneously in confinement management systems to improve data collection. For instance, feed and water station technologies have been developed as an alternative for measuring nutritional intake by an individual animal. These systems typically require the use of RFID tags for animal identification, and some may incorporate specific sensors for monitoring gastrointestinal health, fluctuations in body temperature and estrus activity, or greenhouse gas emissions, as well as smart camera applications that use computer vision combined with deep, automated learning software to monitor frequency and duration of feed and water consumption periods (Schillings et al., 2021; Schaefer et al., 2023). These sophisticated technologies have become research and industry standards for monitoring individual nutritional intake in physiological, metabolic, and nutritional studies in confinement. Additionally, they facilitate the development of rankings based on consumption to determine variations in nutritional efficiency. However, some of the limitations that these systems still present are that they represent an elevated financial investment, require frequent maintenance, have limited animal number capacity, and some applications rely on historical data to rank animal groups according to their efficiency (Schaefer et al., 2023).

Further, PLF systems can also be adapted to improve the utilization of land through enhanced grazing techniques and pasture management. Among the applications developed, RFID tags, boluses, collars, and noseband sensors facilitate grazing behavior measurement and monitoring cardiovascular and respiratory patterns for health and welfare evaluation. Additionally, the use of GPS, GIS, and accelerometers improve individual animal location detection, theft prevention, feeding and ruminating behavior assessment, and reproduction monitoring (Tzanidakis et al., 2023). The data collected by these applications could also be used to improve pasture design and management. For example, virtual fencing incorporates collars equipped with audio signal reproduction attached to each animal and a GPS-controlled virtual fence positioned in multiple areas within the grazing limits, eliminating the need of physical fences and reducing labor requirements (Campbell et al., 2019). Moreover, Sprinkle et al. (2021) reported using 3-axis accelerometers to predict daily activity for cattle with divergent RFI classifications grazing rangelands in Idaho. Their results indicate that accelerometers successfully identified patterns of grazing behavior and differentiated the influences of climate, grazing system, supplementation status, and RFI classification on the duration and frequency of grazing, resting and walking events. According to this methodology, high RFI cattle rest less and walk more under more moderate climate compared to low RFI cattle, and fluctuations in climatic conditions also influenced the daily time of grazing and resting bouts (Sprinkle et al., 2021). These studies provide methodologies that could be adapted to the Southeastern U.S. to improve the understanding of ergot alkaloid effects and heat stress exposure on grazing performance of cattle.

Several PLF applications have been utilized in various studies investigating the effects of fescue toxicosis in beef cattle. Eisemann et al. (2014) and Poole et al. (2018) evaluated changes in the skin temperature of two groups of cattle consuming either an endophyte-infected or endophyte-free diet. While Poole et al. (2018) did not report differences between groups in their study, Eisemann et al. (2014) identified that cattle consuming the endophyte-infected diet had greater skin temperature than their contemporaries. Additionally, Poole et al. (2019) incorporated the use of temperature data loggers in a trial evaluating the effect of supplemental crude protein on physiological parameters in stocker steers experiencing fescue toxicosis. Specifically, the data loggers were subcutaneously inserted in the lateral neck region to record hourly body temperature throughout the study. Their results showed that subcutaneous body temperature was greater in steers consuming an endophyte-infected ration than the temperature recorded from steers consuming an endophyte-free ration (Poole et al., 2019).

More recently, Rajo-Gomez et al. (2025) incorporated several PLF applications in a study that evaluated the influence of heat stress and fescue toxicosis on various physiological parameters in beef heifers. Temperature data loggers were inserted intravaginally to record temperature variation on a 20-minute frequency throughout the study. Their results showed that heifers consuming the endophyte-infected ration maintained greater vaginal temperatures than the endophyte-free heifers throughout the study. Additionally, it was described that the endophyte-infected heifers sustained temperatures that surpassed the established fever threshold during various periods of the study, which provides novel insights about the impact of fescue toxicosis on cattle thermoregulation (Rajo-Gomez et al., 2025). In this same study, the Vytelle Sense ® (https://vytelle.com/vytelle-sense) system was used to monitor individual feed and water intake. It was reported that heifers consuming the endophyte-infected ration had lower feed intake and greater water intake than their endophyte-free pairs (Rajo et al., 2024).

Based on these studies, PLF applications have demonstrated their effectiveness in detecting physiological changes associated with fescue toxicosis. As these technologies become more accessible and cost-effective, opportunities for their adoption and integration into cow-calf operations across the Southeastern U.S. should increase, facilitating the development of more effective strategies to identify productive and efficient animals, and mitigate the impacts of fescue toxicosis in beef cattle operations (Eisemann et al., 2014; Poole et al., 2018; Poole et al., 2019; Rajo-Gomez et al., 2025).

# CHAPTER 2

Influence of Heat Stress and Fescue Toxicosis on the Pulmonary Arterial Pressure of Beef Heifers

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**ABSTRACT**

Peripheral vasoconstriction is a major consequence of fescue toxicosis (FT). However, it remains unclear if major blood vessels, such as the pulmonary artery, are impacted by this disease. This study aimed to evaluate changes in pulmonary arterial pressure (PAP) and different physiological parameters in replacement beef heifers consuming an endophyte-infected (EI) ration. Purebred Angus heifers (216.3 ± 6.1 kg; 9-11 mo of age; $n = 36$) were randomly separated and fed either an endophyte-infected (EI) or endophyte-free (EF) total mixed ration (TMR) for 49 days. On day 1, iButton temperature data loggers were inserted intravaginally to record temperature every 20 minutes throughout the entire study. The cattle comfort index (CCI) was used to assess  comfort and define exposure to heat stress as thermoneutral, mild heat stress (MILD-HS), or moderate heat stress (MOD-HS). PAP was measured every 20 days to assess its response to ergot alkaloid and heat stress exposure. Weekly measurements were recorded to monitor growth and physiological responses of both treatment groups. Data were analyzed using the MIXED procedure of SAS with repeated measures. Both groups were exposed to MILD- and MOD-HS on several occasions. An interaction between PAP results and heat stress exposure was identified, as both groups reported increased PAP values as CCI values decreased. The EF heifers ended the study with greater weight (BW; $P < 0.05$), ADG ($P < 0.01$), body condition score (BCS; $P < 0.01$), and lower hair shedding score (HSS; $P < 0.01$) than EI heifers. In contrast, EI heifers maintained greater vaginal temperatures (VT) throughout the study ($P < 0.01$). Additionally, EI heifers reported lower caudal artery diameters (CAD; $P < 0.01$) than the EF heifers, but no differences were observed in caudal vein diameters (CVD; $P > 0.05$). Lastly, no differences were observed in the packed cell volume (PCV) and prolactin (PRL) concentrations ($P > 0.05$). While the EI group displayed classical symptoms of FT, including

caudal artery vasoconstriction, the PAP results indicate that ergot alkaloid consumption does not induce vasoconstriction on the pulmonary artery, but rather reflect that PAP may be altered by the exposure to heat stress. Additional research should be conducted in regions where changes in PAP are of interest to fully understand the effects of heat stress exposure on the cardiopulmonary system of cattle. Moreover, future investigations in EI fescue-dominant environments should focus on developing strategies to mitigate the negative consequences of FT and heat stress, in order to enhance animal welfare, productivity and profitability in the beef industry.

## INTRODUCTION

Cow-calf operations are the principal type of beef production system found in the Southeastern USA, which typically consists of small productive units that rely on pastures for grazing (Drouillard, 2018). A crucial management aspect of these operations is the selection of replacement heifers, as these females will significantly influence the productivity and genetic pool of the cow herd in the future (Grings and Wright, 2020). This consideration is particularly relevant given the sustained decrease observed in the U.S. cattle inventory since the last century (USDA-NASS, 2024). Hence, identifying highly prolific individuals that efficiently adapt to specific production environments is paramount for the long-term productivity and longevity of these females.

The majority of cattle grazing operations across the Southeast are dominated by endophyte-infected (EI) tall fescue (*Schedonorus arundinaceus* [Schreb.] Dumort), which is naturally infected with the endophytic fungus *Epichloë coenophiala* (Leuchtmann et al., 2014). This symbiotic relationship generates various secondary bioactive compounds classified as ergot alkaloids, which play different beneficial roles within the plant such as improving the tolerance to overgrazing and harsh climatic conditions, and producing active feeding deterrents to insects and nematodes (Ball et al., 2003). In contrast, these ergot alkaloids produce variable levels of toxicity for grazing livestock which is referred to as fescue toxicosis (FT; Strickland et al., 2011). Given the agronomic attributes observed in EI tall fescue, it is expected that this forage may extend across greater areas of the world as a result of changes in global climate (Poole et al., 2020).

Reduced intake, average daily weight gains (ADG) and growth rate, vascular constriction, and the retention of thick winter hair coat into late spring and summer are all trademark symptoms caused by FT. Most of these negative signs are exacerbated during periods of heat stress due to the inability of the animal to properly regulate their body temperature (Porter and Thompson, 1992; Aiken et al., 2011; Kallenbach, 2015) Furthermore, these maladies also significantly impact fetal development and calf performance, as pregnant cows grazing on EI pastures tend to have decreased ADG during the last gestational trimester and lower postpartum milk production, leading to a reduction in calf birth weight, ADG and growth (Gray et al., 2011; Wilbanks et al., 2021).

During post-weaning phases, cattle exposed to EI tall fescue pastures sustain the reductions in ADG and also report lower serum prolactin (PRL) concentrations (Gunter and Beck, 2004). From a reproductive performance standpoint, ergot alkaloid consumption causes several issues as it decreases circulating hormone concentrations and blood flow to reproductive organs (Pratt et al., 2015; Poole and Poole, 2019). In females, a reduction in the diameter of the arteries and veins is observed due to the vasoconstrictive effects triggered by the metabolism of ergot alkaloids. This limits nutrient transport and lead to abnormal functioning of the hypothalamus, hypophysis, and pineal glands, as well as concomitant alterations in the ovaries and the estrous cycle (Strickland et al., 2011; Poole et al., 2018). Additional consequences of vasoconstriction include the reduced blood circulation to peripheral vasculature, which may cause gangrenous ergotism in ear, tail, and hoof tissue, and in severe cases can lead to loss of the affected extremity or the entire animal (Klotz, 2015).

More recently, a novel approach explored the effects of FT on the pulmonary arterial pressure (PAP) of beef cows (Rajo-Gomez et al., 2024). Based on the pulmonary arterial vasoconstriction observed in high-altitude disease caused by a lack of oxygen in regions above 1,200 meters above sea level (MASL) (Holt and Callan, 2007), Rajo-Gomez et al. (2024) hypothesized that exposure to ergot alkaloids could increase the PAP of cattle grazing on EI fescue pastures. Interestingly, an unexpected decrease in the PAP of cattle consuming EI fescue, as well as an inversely proportional effect of ergot alkaloid concentration on mean PAP values, were observed. However, it remains unclear whether the PAP of cattle exposed to ergot alkaloids may vary depending on changes in environmental conditions and physiological stages of the animal. Therefore, the objective of this study was to evaluate changes in PAP and various production and physiological parameters in replacement beef heifers consuming an EI or an endophyte-free (EF) ration.

## MATERIALS AND METHODS

This experiment was conducted at the Upper Piedmont Research Station (UPRS; altitude: 262 MASL) in Reidsville, NC. All animal procedures were approved by the North Carolina State University Institutional Animal Care and Use Committee (NCSU IACUC #22-160-A).

## Animal Performance and Treatment

Heifer performance data were collected from early August to late September 2023 (Figure 1). Purebred Angus heifers (*n* = 36) in an age range between nine and eleven months at UPRS were randomly divided into two groups to be fed either an EI (*n* = 18) or EF (*n* = 18) total mixed ration (TMR) for 49 days, utilizing the Vytelle® Sense system to measure individual feed and water intake. Before the beginning of the study, all heifers were introduced to the system for a 14-day acclimation period and were fed the EF-TMR. Heifers had *ad libitum* access to water throughout the entire study. Each treatment group was housed in a designated pen equipped with a shaded area measuring 32.9 m x 3.7 m, four feed bunks on a concrete floor, and access to a rear paddock measuring 87 m x 32 m, which contained two automatic water troughs. On day 1, temperature data loggers (iButton, Alpha Mach Inc., Quebec, Canada) were inserted intravaginally (IVDL) to record temperature (VT) on a 20-minute-frequency throughout the entire study.

To evaluate the physiological responses of the heifers, body weight (BW), body condition score (BCS; Richards et al., 1986), hair shedding score (HSS; Gray et al., 2011) and jugular blood samples were collected on a weekly basis throughout the experiment. The BCS and HSS were collected by two trained evaluators to calculate an average score for every animal on any given week. Weekly BW were recorded utilizing a digital livestock scale and were utilized to calculate average daily gain (ADG) considering the difference between two BW measurements within their respective week of collection. Doppler ultrasonography (M-Turbo, SonoSite Inc., Bothell, WA) was used to assess differences in the caudal artery and vein blood flow to determine the extent of vascular constriction that occurs in response to fescue toxicosis. Blood samples were collected from each heifer via jugular venipuncture using 20-gauge needles and sterile, additive-free 10.0 mL vacutainers and K2 EDTA 5.4 mL vacutainers to prevent blood clotting (Becton Dickinson, Franklin Lakes, NJ). After collection at the chute side, blood samples in K2 EDTA tubes were gently inverted to mix the blood, and all samples were stored in a cooler with ice and then transported to the laboratory for processing. Upon arrival, additive-free blood samples were centrifuged for 20 minutes at 2,500 xg at 4°C to facilitate serum collection. Two serum samples were collected from each centrifuged tube to be stored in one glass dram vial and one plastic micro-centrifuge tube, respectively, at -80° C until analyses were conducted. Furthermore, blood samples in K2 EDTA tubes were used to measure packed cell volume (PCV). Blood from each sample was transferred to heparinized microhematocrit tubes, which were sealed with a clay mixture to prevent outflow. Further, the microhematocrit tubes were centrifuged for 8 minutes at a maximum speed of 12,700 xg. After centrifuging, a micro-capillary reader (International Equipment Company, Needham Heights, MA) was utilized to quantify the PCV for each sample.

Additionally, PAP measurements were collected on all heifers every 20 days, beginning on the second day of the trial and following the procedures described by Holt and Callan (2007) and Rajo-Gomez et al. (2024; Figure 1). In brief, after each heifer was secured within a chute and the lateral jugular furrow in the neck was exposed, a catheter was inserted in the jugular vein and advanced into the right ventricle of the heart, where the pressure changes and characteristics of the pressure waves were evaluated using a patient monitor (Datascope Passport 2, Mindray North America, Mahwah, NJ) to confirm the catheter location. After this assessment, the catheter was advanced from the right ventricle through the pulmonary valve and then into the main pulmonary artery. The PAP reading was obtained by observing the previously described changes and characteristics on the patient monitor, and once constant wavelengths and pressure were verified, the mean, systolic and diastolic pressure values were recorded for each animal. After these measurements were collected, the heifers were monitored daily for signs of pain or distress for 72 hours following this procedure.

**Feed Ration Measurements**

Two different feed rations were utilized in this study. Both rations were formulated to meet the National Academy of Sciences (NASEM, 2016) nutrient requirements for CP, TDN, Ca, and P for a heifer weighing 226.8 kg and gaining 0.7 kg/day. Each ration was prepared and delivered using a designated mixer wagon to avoid cross-contamination. The EI-TMR was formulated to contain 500 µg/kg of ergovaline and induce the fescue toxicosis symptoms, and it consisted of 85.9% corn silage, 10.5% Kentucky-31 tall fescue seed, 1.9% soybean meal, 1.3% ground corn, 0.1% trace-mineral salt, 0.01% ADE vitamins, and 0.29% limestone. All the fescue seed used in this study was sourced from the same seed lot. The fescue seed was ground using a hammer mill with a 1.1 cm screen (Meadow Mills, North Wilksboro, NC) prior to being incorporated to the EI-TMR. The EF-TMR was comprised of 86.1% corn silage, 10.3% soybean hulls, 3.0% soybean meal, 0.2% ground corn, 0.1% trace-mineral salt, 0.01% ADE vitamins, and 0.29% limestone. Two samples of each TMR were collected every two weeks starting on week 1 of the study. One pair was submitted to be analyzed for nutrient content (North Carolina Department of Agriculture – Food and Drug Protection Division Laboratory, Raleigh, NC; Table 1). The second pair was freeze-dried in a general-purpose freeze drier (VirTis-ATS, Warminster, PA), ground in a sample mill (Cyclotec 1093, Foss Tecator, Hilleroed, Denmark) with a 1-mm particle screen, and submitted to a laboratory for ergot alkaloid analysis via High Performance Liquid Chromatography (HPLC) (Veterinary Medical Diagnostic Laboratory, University of

Missouri, Columbia, MO) to determine alkaloid type and concentration (μg/kg) in the EI-TMR and confirm the absence of ergot alkaloids in the EF-TMR (Table 2).

**Cattle Comfort Index**

The cattle comfort index (CCI) calculation includes the air temperature, relative humidity, wind speed and solar radiation, and is used as an indicator of cattle comfort under the exposure to different levels of thermal stress, based on the comprehensive climate index developed by Mader et al. (2010). This comprehensive climate index defines the following CCI categories: the thermoneutral range is set between 0 ºC and 25 ºC; MILD-HS between 25 ºC and 30 ºC; MOD-HS between 30 ºC and 35 ºC; severe heat stress between 35 ºC and 40 ºC; and extreme heat danger above 40 ºC. The CCI values were imported from the online database of the environmental station located at UPRS, property of the Environment and Climate Observing Network of North Carolina State University (NCSU ECONet, 2024). To improve the evaluation of the environmental fluctuations that occurred throughout the day, each day was divided into four periods of six hours and the average CCI for each period was calculated. Period 1 (P-1) represented 00:01 to 06:00, period 2 (P-2) represented 06:01 to 12:00, period 3 (P-3) represented 12:01 to 18:00, and period 4 (P-4) represented 18:01 to 00:00. Further, the average CCI was also calculated for the specific timeframe when PAP evaluations were realized. Using these values, a weekly average was calculated for each defined period of the day. Average weekly CCI values are reported in ºC, and values below 25 ºC were considered as thermoneutral, in the range between 25 ºC and 30 ºC were defined as mild heat stress (MILD-HS), and moderate heat stress (MOD-HS) was defined between 30 ºC and 35 ºC (Figure 2).

## Prolactin Assay and Analysis

Serum prolactin (PRL) concentrations were analyzed on all the heifers at weeks 1, 4 and 7, which represent the beginning, midpoint, and end of the study. Concentrations were determined by a commercially available Bovine Prolactin ELISA assay (MyBioSource, San Diego, CA, USA), as previously described by Poole et al. (2019). Concentrations were reported in ng per mL, and the interassay and intraassay variation was 4.5% and 6.7%, respectively.

## Statistical Analysis

All data were analyzed using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC, 1996) with repeated measures. In the current study, individual animal was utilized as the experimental unit and the model for PAP, CAD, CVD, BW, ADG, BCS, VT, HSS, PCV, and PRL concentration included treatment (EI vs. EF) and time (Weeks 1-7; measurements 1-3; dependent on model). While the heifers were in pens containing the Vytelle Sense System, we cannot separate the pen error from the individual experimental error due to the design of the barn and short period in which the study was conducted. The barn structure runs North/South with Vytelle Sense System on the west side of the barn (Appendix A; Suppl. Figure 1). The barn's overhang structure creates equal shade area on the pens (125 $m^2$ per pen) throughout the entire day. Additionally, heifers are not solely confined to the barn and have access to an exercise lot per pen (1,175 $m^2$) which allows for animal measurements to be analyzed as individuals (Appendix A; Suppl. Figure 1). Prior to the start of the study, iButton temperature data loggers were placed above the feed bunks underneath the barn and in the water system for each pen to record temperature every 20 minutes throughout the entire study. There was no difference in environmental temperature between pens ($P = 0.9086$) throughout the study, thus ensuring that heifers experienced equal environmental conditions within each pen. (Appendix A; Suppl. Figure

2). There was no difference in water temperature between pens (P = 0.9531) throughout the study (Appendix A; Suppl. Figure 3). Results were recorded as least square means ± SEM, where statistical significance was determined at $P \leq 0.05$ and statistical tendency at $0.05 < P \leq 0.10$.

## RESULTS

### Feed Ration Characteristics and Ergot Alkaloid Concentration

The overall nutritional value of EF-TMR and EI-TMR remained consistent throughout the study, except for some specific parameters. CP values tended to differ between treatments, specifically in Wk 5 and Wk 7 where the EI-TMR reported 12.8 and 13.1 percent DM, and the EF-TMR had 11.7 and 12.6 percent DM, respectively ($P = 0.08$). Results for NDF differed over time ($P = 0.02$), with the EI-TMR values being 41.3, 38.6, 37.8, and 35.7 percent DM for Wk 1, 3, 5, and 7, respectively, and the EF-TMR values being 45.0, 44.5, 47.9, and 46.2 percent DM for the same periods. The EI-TMR also had lower ADF results over time, as it reported 21.7, 22.5, 22.0, and 21.9 percent DM for Wk 1, 3, 5, and 7, respectively, whereas the EF-TMR values were 30.3, 28.7, 32.0, and 29.4 percent DM for the same periods ($P < 0.01$). In contrast, the EI-TMR had greater TDN values over time, reporting 79.3, 78.3, 78.9, and 79.1 percent DM for Wk 1, 3, 5, and 7, respectively, whereas the EF-TMR values were 71.5, 72.8, 69.9, and 72.3 percent DM for the same periods ($P < 0.01$). Additionally, differences were observed in specific minerals, as phosphorus (P) and zinc (Zn) concentrations tended to differ ($P = 0.06$ and $P = 0.07$, respectively), whereas the EI-TMR had lower potassium (K) concentrations than the EF-TMR in all weeks except for week 1 ($P = 0.04$; Table 1).

Furthermore, different ergot alkaloids were identified in the EI-TMR samples and their concentrations fluctuated throughout the study. Ergovaline and ergotamine had different concentrations over time. Ergovaline was reported at 356.0 µg/kg in Wk 1, 268.0 µg/kg in Wk 3, 682.0 µg/kg in Wk 5, and 220.0 µg/kg in Wk 7, whereas ergotamine concentrations were 541.0, 137.0, 477.0, and 421.0 µg/kg for the same periods. Other ergopeptine alkaloids, including ergosine, ergocornine, ergocryptine, and ergocristine, were also identified in the EI-TMR samples. Considering all the identified alkaloids, the total ergot concentrations varied from 1,938.0 µg/kg in Wk 1 to 848.0 µg/kg in Wk 3, 2,680.0 µg/kg in Wk 5 and 1229.0 µg/kg in Wk 7. Lastly, no relevant concentrations of ergot alkaloids were detected in the EF-TMR samples, confirming the absence of these toxins in this specific feed ration.

**Cattle Comfort Index**

Environmental conditions varied based on the timeline utilized for this study, as it coincided with the transition from summer to fall. The results indicate that both groups of heifers were exposed to MILD-HS and MOD-HS on several occasions until Wk 6 of the study. Specifically, MILD-HS events occurred during P-2, P-3 and P-4 of Wk 1 through Wk 6, whereas MOD-HS events occurred during P-3 of Wk 1, 2, 3 and 5. In the final two weeks, as the season transitioned from late summer into early fall, both groups of heifers remained in the comfortable CCI throughout the day, except for a MILD-HS exposure event on P-3 of Wk 6 (Figure 2).

**Pulmonary Arterial Pressure**

Overall, the mean PAP results tended to differ between groups ($P = 0.09$). Differences in PAP were observed in the initial measurement, as the EI heifers had greater mean PAP values than the EF group (30.6 ± 0.9 mmHg vs. 26.9 ± 0.9 mmHg; $P < 0.05$; Figure 3). However, the mean PAP results between groups in the other two evaluations did not differ ($P > 0.05$). Throughout the study, the mean PAP values of both groups of heifers remained lower than the established range of normal mean PAP values—estimated to be between 34 and 44 mmHg—for cattle at high altitude. Interestingly, the results also indicate a potential interaction between PAP and exposure to heat stress, as an increase in the mean PAP of both groups was observed during the last evaluation, which coincided with a decrease in the CCI results (22.2 ± 1.9 ºC vs. 15.6 ± 1.9 ºC; $P < 0.05$; Figure 3).

**Heifer Performance and Physiological Assessment**

No differences were observed in the initial BW of heifers between groups ($P > 0.05$), but the EF heifers had greater final BW than their EI pairs (256.2 ± 8.8 kg vs. 237.1 ± 8.8 kg; $P < 0.05$; Table 3). These differences in BW are directly associated with changes in ADG, as the EF group maintained greater gains throughout the study (0.9 ± 0.1 kg/d vs. 0.3 ± 0.1 kg/d; $P < 0.01$; Table 3). Similar results were observed for BCS, as the EF heifers finished the study with greater BCS than their EI pairs (6.0 ± 0.1 vs 5.0 ± 0.1; $P < 0.01$; Table 3). When evaluating differences in HSS, the EF heifers displayed a better ability to shed their hair coats and ended the study with lower HSS than the EI heifers (3.4 ± 0.2 vs. 4.8 ± 0.2; $P < 0.01$; Table 3). For the VT assessment, EI heifers maintained greater body temperatures than their EF pairs throughout the entire study ($P < 0.01$), and both groups surpassed the established fever threshold on several occasions (Figure 4). The overall retention rate of the IVDL was 87% (EI: 17/18; EF: 14/18). Moreover, EI heifers reported lower CAD values than the EF heifers (35.8 ± 0.6 mm vs. 40.0 ± 0.6 mm; $P < 0.01$; Table 3), but no differences were observed in CVD values between groups ($P > 0.05$; Table 3). Lastly, no differences were detected between groups when analyzing the PCV and PRL concentrations ($P > 0.05$; Table 4).

# DISCUSSION

The data presented here offer insights into the physiological alterations when beef cattle are exposed to EI feed rations, emphasizing the complexity of FT. As previously discussed, one of the most detrimental effects of this disease is vasoconstriction, a symptom also seen in high-altitude disease among cattle raised in regions with increased elevation. The reduced concentrations of oxygen in these environments leads to constriction of the pulmonary artery, which alters the normal pattern of blood flow and imposes stress on the cardiovascular system of the animal. Prolonged exposure to these conditions significantly raises the risk of right-sided heart failure and can ultimately result in the death of the affected animal (Thomas et al., 2018). To mitigate these consequences and identify animals that maintain appropriate PAP values despite the challenging environment, a catheterization procedure has been developed as an effective technique for assessing tolerance and facilitating the selection of individuals to propagate these desirable traits in future offspring (Holt and Callan, 2007; Pauling et al., 2018).

Given these similarities, it was hypothesized that ergot alkaloid consumption might also have constrictive effects on the pulmonary artery and increase the PAP of cattle. This suggests that these differences could be detected through catheterization and that the procedure may serve as a phenotypic assessment to identify animals with greater tolerance to FT. The mean PAP results observed in this study indicate that this vascular measurement is not impacted by ergot alkaloid exposure. Contrary to our previous results (Rajo-Gomez et al., 2024), differences in mean PAP values were only noted during the initial measurement, which took place in the first week of the study. It is important to consider that at this time, the EI heifers had just begun consuming ergot alkaloids, as both groups were fed the EF ration during the acclimation period before the study started. The onset of symptoms of FT in cattle after ergot alkaloid intake is variable and depends on multiple factors, including toxin concentration, absorption rate, health and physiological status of the animal, and their interaction with the environment (Strickland et al., 2011). Further, as the study progressed and the EI heifers began to display symptoms of FT, such as increased VT and reduced CAD, no differences in mean PAP values between groups were observed. Therefore, it is unlikely that the changes in mean PAP were caused by ergot alkaloid consumption, as its effects may not have been sufficient to overcome the homeostatic adjustments of these heifers.

In contrast, the results observed in the CAD between groups are evident signs of FT-induced vasoconstriction. Previous research have demonstrated that ergot alkaloid intake can alter cardiovascular function and cause peripheral vascular constriction (Klotz, 2015). Aiken et al. (2009) indicated that the luminal cross-sectional area of the caudal artery decreased in beef heifers that were fed different ergot alkaloid concentrations, while Poole et al. (2018) reported that vasoconstriction was observed in the caudal artery, but not in the caudal vein, in heifers consuming EI fescue seed. These results are comparable to the CAD and CVD observed in this study, as the area of the caudal vein did not differ between EI and EF heifers. Ergot alkaloids share similar structural and binding characteristics with various neurotransmitters, such as serotonin and dopamine, facilitating binding to these biogenic amine receptors in different vascular tissues (Berde and Stürmer, 1978; Dyer, 1993; Snider et al., 2018). More specifically, Bakunzi and Eyre (1983) reported that dopamine and apomorphine induced concentration-dependent contractions in the bovine pulmonary artery. Considering the differences observed in the CAD, CVD, and PAP results, a potential explanation may hinge on the presence or absence of specific receptors in the tissue, which is directly related to the contractile responses observed in these vasculatures.

Furthermore, the changes observed in the mean PAP results of both treatment groups indicate a potential interaction with thermal stress exposure. Based on the specific timeline utilized for this study, both groups of heifers were significantly exposed to heat stress conditions. Particularly, heat caution events, which occurred during P-3 (12:01-18:00 h) until Wk 6 of the study, are very frequent throughout the summer months and negatively impact livestock production in the Southeastern U.S (Ingram et al., 2013). During the first and second PAP evaluations, the registered average CCI was 24.3 ºC and 22.2 ºC, respectively, whereas a reduction in the CCI was observed for the last PAP evaluation, coinciding with an increase in the mean PAP values of both groups. Even though all CCI values registered while PAP was being evaluated remained below the heat caution threshold, these results indicate that the mean PAP of the heifers may be influenced by the CCI. As previously stated, PAP evaluations are typically conducted in high-altitude areas, especially above 1,200 MASL, to identify cattle with greater tolerance to high-altitude disease. In the context of this study, the influence of altitude is considered unlikely due to the significantly lower elevation found at UPRS. Further, Rajo-Gomez et al. (2024) suggested that the interaction between ergot alkaloid concentrations and variation in environmental conditions may affect mean PAP values. However, the results presented herein contradict this hypothesis, as no differences were observed between groups after the EI heifers were exposed to ergot alkaloids. Additionally, the mean PAP values of both groups of heifers remained in a similar pressure range—between 25 and 35 mmHg—as those reported for the cows used in that study, in spite of the maturity and physiological differences, and the variation in ergot alkaloid concentrations (Rajo-Gomez et al., 2024). Instead, mean PAP values appear to be more influenced by environmental variation. Holt and Callan (2007) stated that after performing PAP evaluations at temperature ranges of -40 ºC to 41 ºC, it has been observed that cold environmental temperatures can induce pulmonary hypertension in beef cattle; however, no effects of heat stress conditions are documented. Potentially, the observed changes in the mean PAP of both groups may be associated with the preceding exposure to MILD- and MOD-HS episodes and the consequent increase in respiration rates. Previous studies have documented that exposure to ergot alkaloid and heat stress raises respiration rates in cattle as a mechanism for heat dissipation (Scharf et al., 2010; Eisemann et al., 2014). Additionally, it has been reported that elevated respiratory rates in hyperthermic animals are associated with a reduction in mean PAP of cattle (Whittow, 1965). Considering these results and their implications, future investigations should consider analyzing the effects of heat stress exposure and heat dissipation mechanisms on the mean PAP of cattle across different altitudes and physiological stages.

The intensive monitoring of VT performed in this study demonstrated how core body temperature and the thermoregulatory abilities of cattle are influenced by FT and the changes in environmental conditions throughout the day. Previous research has determined specific ranges to make more accurate and comprehensive assessments of thermal stress (Mader et al., 2010). Moreover, animals utilize various routes for heat exchange between their body and the surrounding environment, including radiation, convection, conduction, which depend on thermal differences both within the animal and between the animal and its environment, and evaporation that is influenced by relative humidity (Hansen, 2004; Shephard and Maloney, 2023). These processes are highly dynamic and interact with different physiological mechanisms, such as increased respiration rates, sweat rates, peripheral vasodilation and blood flow, to enhance heat dissipation under thermal stress conditions (Eisemann et al., 2014). The interplay of several environmental factors, including the air temperature, relative humidity, solar radiation and wind speed, activate these mechanisms (Mader et al., 2010). The VT results generated by this study demonstrate how environmental fluctuations impacted the body temperature of these heifers. During the periods of MOD-HS exposure, both groups exceeded the fever threshold (39.5 ºC) established for cattle (Burfeind et al., 2012), indicating an imbalance in thermoregulation. Additionally, it is evident that the EI heifers maintained greater VT than their EF pairs throughout the study. Particularly, during Wk 2 and 3, the lowest VT of the EI group was still greater than the greatest VT recorded for the EF group during those specific periods. These results highlight the severity of FT effects on the thermoregulatory abilities of cattle.

Additional detrimental effects caused by heat stress exposure include decreased feed intake and metabolic rates, increased water intake, panting, drooling and sweating, and suppressed production and reproductive performance (Shephard and Maloney, 2023). The evaluation of certain hematological parameters, such as PCV, can serve as methods to analyze the oxygen transport capacity and dehydration levels in animals under thermal stress (Roland et al., 2014). In the current study, no differences in PCV were observed between the groups. Interestingly, the PCV results obtained in this study fall within the same range as those reported by Poole et al. (2018), who also compared this parameter in heifers consuming either an EI or EF ration. Further, the negative consequences caused by heat stress are aggravated in animals suffering from FT, as its vasoconstrictive effects and the retention of thick winter hair coats reduce their ability for heat exchange via peripheral vasodilation (Aiken et al., 2011; Klotz, 2015). The HSS results observed in this study support this statement, as the EI heifers were unable to properly shed their hair coats, whereas the EF heifers ended the study with lower HSS. In this regard, previous studies have demonstrated that superior shedding ability is positively correlated with increased productivity in cattle grazing EI fescue pastures (Gray et al., 2011;

Poole et al., 2019; Rajo-Gomez et al., 2024). The retention of winter hair coats during the summer has been thoroughly explored in cattle exposed to FT and is associated with reduced PRL production (Porter and Thompson, 1992; Parish et al., 2013). This hormone plays a fundamental role in the regulation of body hair coat growth and shedding (Littlejohn et al., 2014), and decreased serum concentrations are often used as a marker of FT (Strickland et al., 2011). Nonetheless, there are other factors that may also alter its concentrations, including extended photoperiod and increased ambient temperatures (Schams & Reinhardt, 1974; Wettemann & Tucker, 1974). The lack of differences observed in serum PRL between the EI and EF groups in this study was unexpected, but may be associated with the timeframe utilized as the heifers were exposed to greater CCI and daylight duration.

The performance results observed portray the detrimental effects of FT. In spite of starting the study with similar BW, the EI heifers ended the study with considerably lower BW and BCS than the EF heifers, as a consequence of maintaining a decreased ADG throughout the study. Ergot alkaloid consumption has a negative impact on the ruminal and intestinal microbiome, and in spite of the degradation, these compounds can still induce vasoconstriction in the ruminal vessels (Foote et al., 2011) and also limit blood flow to the duodenum, colon, and kidney of cattle (Rhodes et al., 1991). From a nutritional perspective, sufficient blood flow to the ruminal and gut surfaces is necessary for an adequate absorption of nutrients. During FT, this mechanism is disrupted and compromises volatile fatty acid (VFA) absorption and metabolism, consequently leading to decreased weight gain and growth performance in ruminants (Foote et al., 2013). The results of this study are comparable to those reported by Poole et al. (2018), indicating that heifers consuming an EF ration had greater ADG and tended to have lower BCS than those fed an EI ration. Furthermore, the EI-TMR had lower NDF and ADF and greater TDN values, suggesting greater energy and fiber quality contents that should support animal growth (NASEM, 2016). In contrast, the suppressed growth rates observed in EI heifers may be associated with the negative nutritional impact caused by the presence of ergot alkaloids in the ration. Additionally, intensified exposure to heat stress and the subsequent increase in body temperature may have also affected feed consumption and digestibility. Altogether, these negative effects on performance also have a significant economic impact on the beef industry. It is estimated that 15.7%—about $370 million—of the annual economic losses in the US beef industry can be attributed to heat stress (Key et al., 2014), while the total financial losses generated by FT account for more than $1 billion each year (Strickland et al., 2011; Kallenbach, 2015). Therefore, optimizing management strategies that mitigate these negative effects and improve animal welfare, productivity, and efficiency in beef operations is of utmost importance.

## TABLES

**Table 2.1.** Nutritive value [1] of feed ration samples.

| | EI-TMR [2] | | | | EF-TMR [3] | | | | SEM | P-value |
|---|---|---|---|---|---|---|---|---|---|---|
| | Wk 1 | Wk 3 | Wk 5 | Wk 7 | Wk 1 | Wk 3 | Wk 5 | Wk 7 | | |
| DM (%) | 36.8 | 38.7 | 40.5 | 36.8 | 37.3 | 37.2 | 36.4 | 34.7 | 0.7 | 0.15 |
| CP (%DM) | 12.6 | 12.8 | 12.8 | 13.1 | 12.4 | 12.5 | 11.7 | 12.6 | 0.1 | 0.08 |
| NDF (%DM) | 41.3 [a] | 38.6 [b] | 37.8 [b] | 35.7 [b] | 45.0 [c] | 44.5 [c] | 47.9 [d] | 46.2 [c] | 1.2 | 0.02 |
| ADF (%DM) | 21.7 [a] | 22.5 [a] | 22.0 [a] | 21.9 [a] | 30.3 [b] | 28.7 [c] | 32.0 [d] | 29.4 [c] | 0.6 | < 0.01 |
| TDN (%DM) | 79.3 [a] | 78.3 [a] | 78.9 [a] | 79.1 [a] | 71.5 [b] | 72.8 [c] | 69.9 [d] | 72.3 [c] | 0.5 | < 0.01 |
| Ash (%DM) | 7.3 | 7.5 | 9.6 | 4.8 | 8.1 | 7.1 | 9.9 | 5.5 | 0.2 | 0.29 |
| Ca (%DM) | 0.7 | 0.8 | 0.9 | 0.6 | 0.9 | 0.9 | 0.6 | 0.5 | 0.1 | 0.84 |
| P (%DM) | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.2 | 0.2 | 0.2 | 0.02 | 0.06 |
| S (%DM) | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.0 | 0.99 |
| Mg (%DM) | 0.3 | 0.2 | 0.3 | 0.2 | 0.3 | 0.3 | 0.3 | 0.3 | 0.02 | 0.18 |
| Na (%DM) | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.0 | 0.99 |
| K (%DM) | 1.0 [a] | 0.9 [b] | 0.7 [c] | 0.8 [d] | 1.1 [e] | 1.0 [a] | 1.0 [a] | 1.0 [a] | 0.03 | 0.04 |
| Cu (ppm) | 16.5 | 27.3 | 26.4 | 23.7 | 24.6 | 22.8 | 17.0 | 12.7 | 3.1 | 0.41 |
| Fe (ppm) | 184.2 | 174.3 | 139.8 | 192.0 | 209.2 | 193.4 | 243.7 | 196.8 | 18.6 | 0.73 |
| Mn (ppm) | 114.6 | 157.1 | 162.5 | 134.3 | 183.5 | 186.8 | 142.6 | 100.9 | 16.6 | 0.66 |
| Zn (ppm) | 66.8 | 95.7 | 90.8 | 80.3 | 147.8 | 146.6 | 112.6 | 92.8 | 11.0 | 0.07 |

[a-e] Within the row, means with a different superscript differ ($P \leq 0.05$); [1] Values are reported as percentage on a dry matter basis (%DM) and parts per million (ppm); [2] EI-TMR: Endophyte-infected total mixed ration; [3] EF-TMR: Endophyte-free total mixed ration.

**Table 2.2.** Ergot alkaloid concentration in endophyte-infected and endophyte-free total mixed rations.

| Ergot alkaloid (μg/kg) [3] | EI-TMR [1] | | | | EF-TMR [2] | | | |
|---|---|---|---|---|---|---|---|---|
| | Wk 1 | Wk 3 | Wk 5 | Wk 7 | Wk 1 | Wk 3 | Wk 5 | Wk 7 |
| Ergovaline | 356.0 | 268.0 | 682.0 | 220.0 | N.D | N.D | N.D | N.D |
| Ergosine | 574.0 | 210.0 | 825.0 | 286.0 | N.D | N.D | N.D | N.D |
| Ergotamine | 541.0 | 137.0 | 477.0 | 421.0 | N.D | N.D | N.D | N.D |
| Ergocornine | 214.0 | 104.0 | 384.0 | 135.0 | N.D | N.D | N.D | N.D |
| Ergocryptine | 381.0 | 260.0 | 566.0 | 271.0 | N.D | N.D | N.D | N.D |
| Ergocristine | 228.0 | 137.0 | 428.0 | 116.0 | N.D | N.D | N.D | N.D |
| Ergot Total | 1938.0 | 848.0 | 2680.0 | 1229.0 | N.D | N.D | N.D | N.D |

[1] EI-TMR: Endophyte-infected total mixed ration; [2] EF-TMR: Endophyte-free total mixed ration; [3] HPLC analysis at the University of Missouri Veterinary Medical Diagnostic Laboratory (Columbia, MO, USA); N.D: Not detected via HPLC.

**Table 2.3.** Physiological and performance parameters for Angus heifers consuming either endophyte-infected (EI) or endophyte-free (EF) total mixed rations at UPRS1 from early August to late September 2023.

| Measurement [2] | Treatment | | SEM | P-Value |
|---|---|---|---|---|
| | EI | EF | | |
| Initial BW, kg | 222.3 | 210.4 | 8.8 | 0.17 |
| Final BW, kg | 237.1 [a] | 256.2 [b] | 8.8 | 0.03 |
| ADG, kg/d | 0.3 [a] | 0.9 [b] | 0.1 | < 0.01 |
| BCS [3] | 5.0 [a] | 6.0 [b] | 0.1 | < 0.01 |
| HSS [4] | 4.8 [a] | 3.4 [b] | 0.2 | < 0.01 |
| CAD [5], mm | 35.8 [a] | 40.0 [b] | 0.6 | < 0.01 |
| CVD [6], mm | 43.1 | 42.6 | 0.7 | 0.67 |

[a-b] Within the row, means with a different superscript differ ($P \leq 0.05$); [1] UPRS: Upper Piedmont Research Station, Reidsville, NC; [2] Values reported as least square means for the entire experiment; [3] BCS: body condition score (1-9 scale); [4] HSS: hair shedding score (1-5 scale); [5] CAD: caudal artery diameter; [6] CVD: caudal vein diameter.

**Table 2.4.** Packed cell volume (PCV) and serum prolactin (PRL) concentrations for Angus heifers consuming either endophyte-infected (EI) or endophyte-free (EF) total mixed rations at UPRS1 early August to late September 2023.

| Measurement [2] | Treatment | | SEM | P-Value |
|---|---|---|---|---|
| | EI | EF | | |
| PCV, % | 34.9 | 34.8 | 0.7 | 0.91 |
| PRL, ng/mL | 37.3 | 42.1 | 4.8 | 0.49 |

[1] UPRS: Upper Piedmont Research Station, Reidsville, NC; [2] Values reported as least square means for the experiment.

## FIGURES

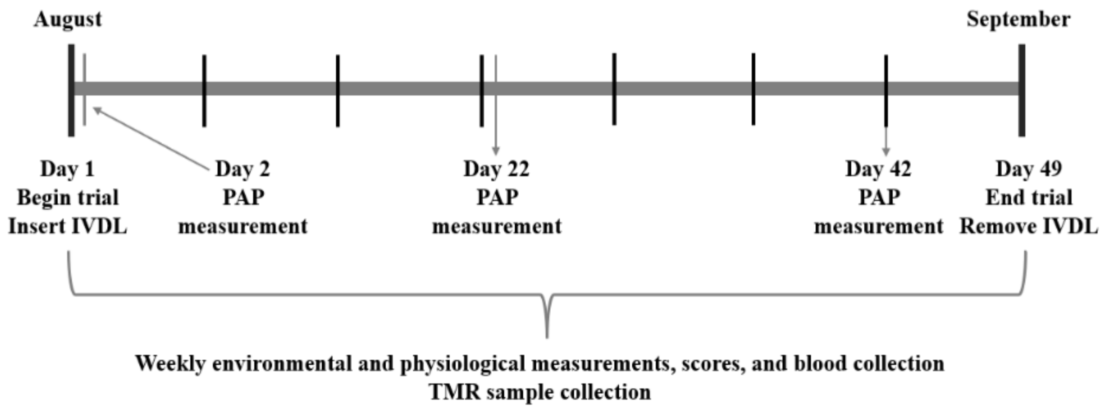


**Figure 2.1.** Experimental timeline used during phase of data collection from early August to late September 2023.

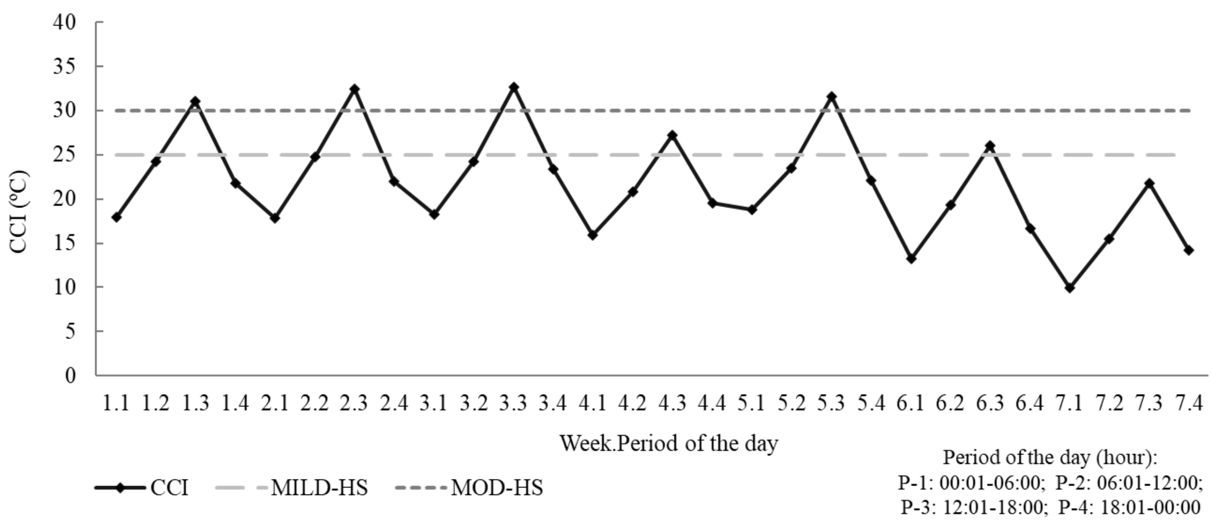


**Figure 2.2.** Cattle Comfort Index (CCI) over time (weeks by period of the day): Both groups of heifers were exposed to different levels of heat stress throughout the study.

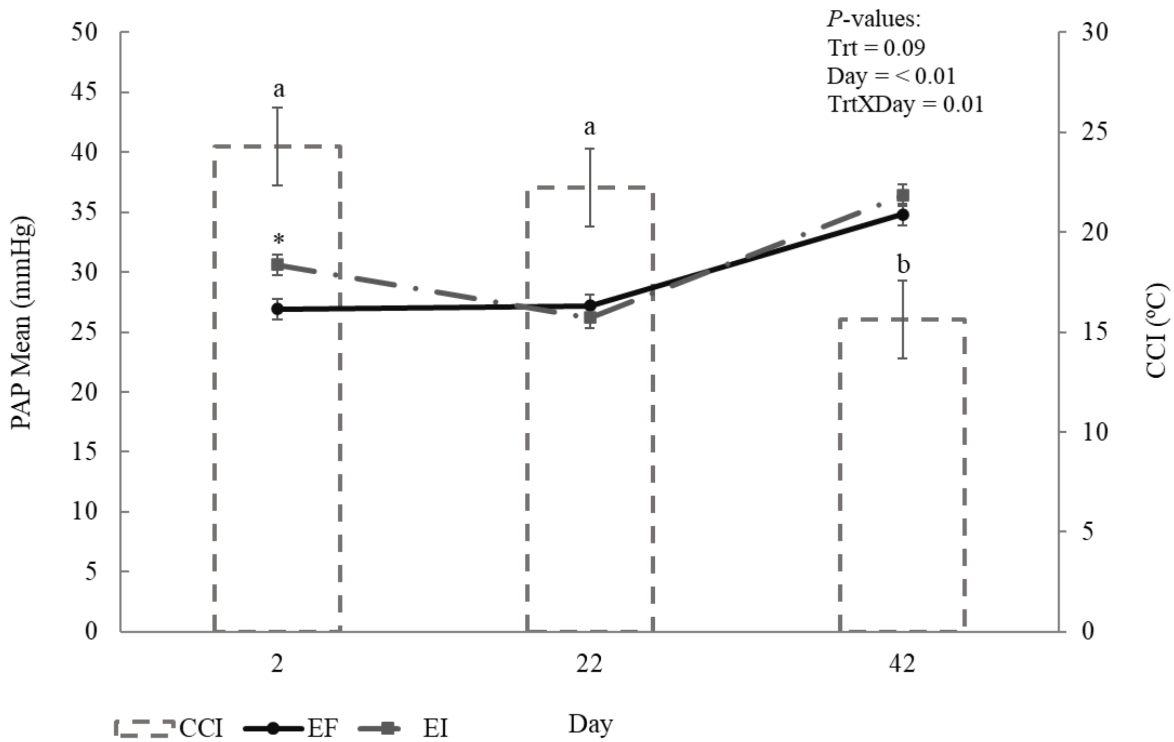


**Figure 2.3.** PAP by treatment (LINES) and heat stress exposure (BARS) over time (days): Mean PAP values between treatment groups did not differ except on day 2, and the values increased as heat exposure decreased. The model tended to differ by treatment, and was significant by day and treatment X day by period of the day (trt P < 0.01; day P < 0.01; trt X day P < 0.01).

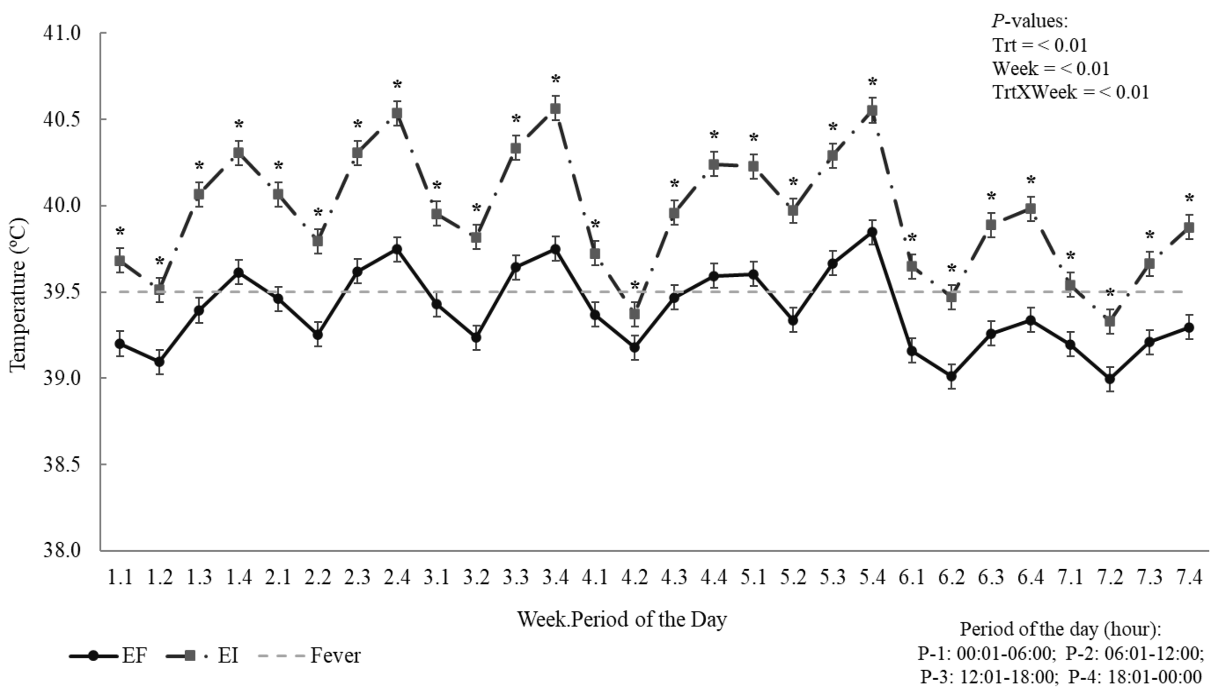


**Figure 2.4.** VT by treatment over time (weeks by period of the day): Heifers consuming the endophyte-infected (EI) ration maintained greater VT throughout the study compared to heifers that consumed the endophyte-free (EF) ration. The model was significant by treatment and treatment X week by period of the day (trt P < 0.01; trt X week P < 0.01).

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# CHAPTER 3

Assessing Performance Metrics, Physiological Parameters and Nutritional Intake to Measure Efficiency in Angus Heifers Across Different Production Stages

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## ABSTRACT

Fescue toxicosis represents a significant challenge for cow-calf operations, as exposure to this disease decreases feed intake (FI), weight gain (ADG), and growth rates, while increasing the susceptibility to heat stress in cattle. Consequently, the productivity and efficiency of the herd are negatively impacted. However, the effects of ergot alkaloid and heat stress exposure on feed efficiency classifications in beef cattle remain unclear. This two-year study aimed to evaluate the effects of ergot alkaloid exposure on nutritional intake, feed efficiency metrics, and various physiological and performance parameters of Angus yearling heifers, as well as to determine changes in these parameters that may be associated with pregnancy status. In Year I, Purebred Angus heifers (216.3 ± 6.1 kg; 9-11 mo of age; n = 36) were stratified by weight and randomly divided into two groups to be fed either an endophyte-infected (EI; 500 µg/kg of ergovaline; n = 18) or endophyte-free (EF; 0 µg/kg of ergovaline; n = 18) total mixed ration (TMR) for 49 days using the Vytelle® Sense system. Thermal images of the ocular globe (OGT) and rib skin area (RIBT) were collected from all heifers to evaluate changes in body temperature. In Year II, the same heifers utilized in the Year I study were exposed to a transrectal ultrasonography assessment for pregnancy status determination. Pregnant (PREG; 439.4±7.1 kg; 17-19 mo of age; n=29) and non-pregnant (OPEN; 408.7±15.7 kg; 17-19 mo of age; n=7 ) heifers were reintroduced to the Sense system and were fed an EF-TMR for 56 days. In both studies, intravaginal data loggers were inserted on day 1 to record vaginal temperature (VT) on a 20-minute-frequency throughout the trial, and body weights (BW), ADG, body condition (BCS), and hair shedding scores (HSS) were collected on a weekly basis to evaluate physiological changes. The Sense system recorded individual FI and water intake (WI), and the cattle comfort index (CCI) was used to assess  comfort and define exposure to heat stress as thermoneutral,

mild heat stress (MILD-HS), or moderate heat stress (MOD-HS) in both studies. Following completion of the Year II study, all heifers were relocated to EI pastures, and calf birth dates, birth weights (CALFBW), and sex information were collected as parturitions occurred. Additionally, a post hoc analysis was conducted to assess nutritional efficiency metrics of all heifers across both studies. Physiological and performance data of heifers were analyzed using PROC MIXED with repeated measures, whereas a PROC CORR estimated Pearson correlations for residual feed intake (RFI) and residual average daily gain (RADG) and the level of reranking across studies, and a PROC UNIVARIATE and TTEST evaluated normality and differences in calf birth weight. In Year I, the EF heifers ended the study with greater BW ($P < 0.05$), ADG ($P < 0.01$), BCS ($P < 0.01$), and lower HSS ($P < 0.01$) than EI heifers. In contrast, EI heifers maintained greater VT, RIBT and OGT throughout the study ($P < 0.01$). Both groups were exposed to MILD- and MOD-HS on several occasions. The EF heifers reported greater FI than the EI heifers (50.7±1.5 kg DMI vs 35.0±1.5 kg DMI; $P < 0.01$), and these differences were maintained throughout the study ($P < 0.01$; Figure 3.19). In contrast, the EI heifers had greater WI ($P = 0.01$) and WI per BW (WI/BW; ($P = 0.04$) than their EF pairs over time. Based on the post hoc analysis, the RFI and RADG rankings were used as covariates to assess differences in nutritional intake. The low-RFI (LRFI) EF heifers had lower FI than the high-RFI (HRFI) EF heifers (46.8±2.0 kg DMI vs 54.2±2.0 kg DMI; $P < 0.01$) and both EI subgroups, whereas no differences were observed in FI between LRFI and HRFI EI heifers. In contrast, the EI LRFI heifers had lower WI than their HRFI pairs (212.6±20.3 L vs 289.4±20.3 L; $P = 0.02$), but no differences were observed in the WI of LRFI and HRFI EF heifers. Further, the low-RADG (LRADG) and high-RADG (HRADG) EF heifers had a greater FI than their EI pairs ($P < 0.01$), and the LRADG EI heifers had greater WI than the HRADG EI heifers (295.6±28.3 L vs

229.7±22.5 L; $P$ = 0.03), whereas no differences were observed between the subgroups of EF heifers. In Year II, the PREG heifers tended to have greater BW (471.2±7.0 kg vs 442.5±15.4 kg; $P$ = 0.09) and had greater BCS ($P$ = 0.04) than their OPEN pairs, but no differences were observed in ADG and HSS between groups. The CCI remained in the thermoneutral range and exposure to MILD-HS only occurred towards the end of the study, which may have influenced the differences in VT over time ($P$ < 0.01) between groups. No differences were observed in FI ($P$ = 0.15), WI ($P$ = 0.87) and WI/BW ($P$ = 0.27) between PREG and OPEN heifers. Following the same covariate analysis as in Year I, the LRFI PREG heifers had lower FI (88.2±1.6 kg DMI vs 99.1±2.2 kg DMI; P < 0.01) than the HRFI heifers, whereas no differences were observed between the OPEN subgroups. Additionally, no differences were observed in WI using RFI as a covariate, nor in FI and WI using RADG as a covariate. The post hoc analysis also reported a weak correlation ($r$ = 0.18; $R^2$ = 0.03) for the RFI scores and a moderate correlation ($r$ = 0.32; $R^2$ = 0.10) for the RADG scores between Year I and Year II. Lastly, no differences were observed in the CALFBW between calves born from LRFI and HRFI dams (26.5±1.4 kg vs 29.8±1.7 kg; P = 0.10). The results presented herein demonstrate the complexity of fescue toxicosis, as the EI heifers had a suppressed growth and winter hair coat retention, impaired thermoregulatory abilities, and an altered nutritional intake that negatively impacted their nutritional efficiency rankings. Further, the observed results in Year II indicate that despite having lower FI, the LRFI PREG heifers delivered calves with similar weights than their HRFI pairs, suggesting a positive indicator of efficiency that could benefit cow-calf operations. Nonetheless, the weak correlations observed between years imply that environmental stressors, such as fescue toxicosis and heat stress, impose challenges in feed efficiency estimations. More investigations are needed to better understand the impact of these burdens on cattle efficiency and to develop improved animal selection strategies that can be adopted by cattle producers across the Southeastern U.S.

## INTRODUCTION

Globally, the beef industry faces significant challenges in meeting an increased product demand while improving cattle productivity and efficiency (Terry et al., 2020). Some factors impacting cattle production are a rapid growth in urban and suburban populations, and reductions in land availability, which also aggravate the competition for feed and water resources. Further, fluctuating precipitation patterns, prolonged periods of high ambient temperatures, and more recurrent extreme weather events are among the global climate changes that negatively impact livestock performance and productivity (Havlík et al., 2014). Based on this context, it is crucial to develop improved selection and management strategies that facilitate the identification of highly productive individuals capable of effectively adapting to challenging environments while improving nutritional efficiency, as a means of increasing economic and environmental sustainability of this industry (Kenny et al., 2018).

Various perspectives exist for assessing the parameters of efficiency in beef operations. One approach is a general production efficiency evaluation, which focuses on increasing the output of production using the same amount of input resources. This approach includes measuring the total weight of calves weaned during a cow’s lifetime, improving herd health to reduce mortality losses and to improve reproductive performance, maintaining optimum breeding systems, and harvesting cattle at an optimum weight and age (Terry et al., 2020). A different perspective focuses on evaluating feed efficiency. Initially, this parameter was measured using the feed conversion ratio (FCR), which accounted for the amount of feed required to produce one unit of body weight, and is associated with genetic and phenotypic traits of growth, mature body weight and composition of gain (Kenny et al., 2018). However, increasing selection pressure for FCR has resulted in cattle with faster growth rates, and increased mature size and feed intake, along with the associated economic costs (Basarab et al., 2013).

A different strategy for evaluating feed efficiency in livestock focuses on estimating residual feed intake (RFI), which is defined as the difference between the animal's actual feed intake and its predicted feed intake for maintenance of body size and production (Koch et al., 1963). A low, negative RFI value in growing animals indicates lower feed intake than predicted whilst maintaining expected body size and growth, thus improving their efficiency. Conversely, higher, positive RFI values represent animals that consume more feed than predicted, hence being less efficient (Basarab et al., 2013; Terry et al., 2020). Thus, evaluating and predicting RFI has gained relevance as a selection tool to enhance efficiency in beef cattle, as individuals with low RFI consume less feed but maintain similar weight gains (ADG) when compared to high RFI animals (Damiran et al., 2018). Unlike growth rate, FCR, and dry matter intake (DMI), RFI is independent of body size, weight, production stage, sex and age, and is also a moderately heritable trait ($h^2$ = 0.16–0.43; Kenny et al., 2018). Considering that the provision of feed may account for up to 75% of the total direct costs of a beef operation (Nielsen et al., 2013), adopting selection for RFI may become a viable method to reduce feed costs and increase the profitability of beef operations.

Furthermore, the nutritional requirements and subsequent efficiency evaluations in beef cattle may be influenced by various factors, including environmental variables and the physiological status of the animal. These considerations must be accounted for in specific regions like the Southeastern U.S., where the majority of beef operations depend on seasonal forages to graze and manage their herds. The principal forage utilized by cattle producers in this region is tall fescue (*Schedonorus arundinaceus* [Schreb.] Dumort), due to its exceptional yields, even under challenging climatic and edaphic conditions, and its tolerance to high animal stocking rates (Ball et al., 2003). This superior performance is the result of a symbiotic relationship between the plant and the endophytic fungus (*Epichloë coenophiala*), which generates different ergot alkaloids that improve the plant resistance to pests and drought (Leuchtmann et al., 2014). Nonetheless, these chemical compounds are toxic to cattle and lead to different negative responses, commonly known as fescue toxicosis (FT) (Strickland et al., 2011). The negative effects caused by FT in cattle include reduced intake, ADG and growth rate, vascular constriction, and the retention of thick winter hair coat into late spring and summer. Most of these symptoms are exacerbated during periods of heat stress due to the inability of the animal to properly regulate their body temperature (Porter and Thompson, 1992; Aiken et al., 2011; Kallenbach, 2015; Poole et al., 2020; Rajo-Gomez et al., 2024).

The effects of ergot alkaloid and heat stress exposure on feed efficiency classifications in beef cattle have yet to be elucidated. Most research efforts have focused on evaluating RFI classifications in young, growing animals that are acclimatized to feed intake measurement systems, which record accurate individual feed consumption in a confined environment due to the methods and technologies used for data collection (Basarab et al., 2007; Kenny et al., 2018; Terry et al., 2020). In addition to evaluating feed consumption to determine efficiency, various studies have focused on measuring body surface temperature through infrared thermography to predict radiant heat dissipation and use these differences to predict relative biological efficiency in beef cattle (Montanholi et al., 2009; Colyn, 2013; Thompson et al., 2018). Considering the symptoms induced by FT, the implementation of thermographic assessments may provide novel insights into thermoregulation and efficiency determination in cattle managed in a fescue environment.

Moreover, rankings for feed intake and feed efficiency may fluctuate when females reach a maturity stage and have to maintain adequate pregnancy and calving rates under grazing conditions (Lahart et al., 2020), especially in regions like the Southeastern U.S. where endophyte-infected pastures and extended periods of heat stress exposure are common (Meyer et al., 2008). Consequently, interesting opportunities exist for comprehensive research to explore performance and nutritional efficiency in beef cattle exposed to these stressors. Therefore, the objectives of this two-year study were to evaluate the effects of ergot alkaloid exposure on nutritional intake, feed efficiency metrics, and various physiological and performance parameters of Angus yearling heifers, as well as to determine changes in these parameters that may be associated with pregnancy status.

## MATERIALS AND METHODS

These experiments were conducted at the Upper Piedmont Research Station (UPRS; altitude: 262 MASL) in Reidsville, NC. All animal procedures were approved by the North Carolina State University Institutional Animal Care and Use Committee (NCSU IACUC #22-160-A).

### Animals and Experimental Design

The animals and experimental design used in Year I of this study have been previously described in Chapter 2. Briefly, heifer performance and physiological data were collected from early April to late September 2023 (Figure 2.1). Purebred Angus heifers (216.3 ± 6.1 kg; 9-11 mo of age; *n* = 36) were stratified by weight and randomly divided into two groups to be fed either an endophyte-infected (EI; 500 µg/kg of ergovaline; *n* = 18) or endophyte-free (EF; 0 µg/kg of ergovaline; *n* = 18) total mixed ration (TMR) for 49 days, utilizing the Vytelle® Sense system to measure individual feed and water intake. Before the beginning of the study, all heifers were introduced to the system for a 14-day acclimation period and were fed the EF-TMR. Heifers had *ad libitum* access to water throughout the entire study.

On day 1, temperature data loggers (iButton, Alpha Mach Inc., Quebec, Canada) were inserted intravaginally (IVDL) using hormone-free progesterone controlled internal drug release (CIDR; Zoetis LLC, Parsippany, NJ) devices to record temperature (VT) on a 20-minute-frequency throughout the entire study. To evaluate the physiological responses of the heifers, body weight (BW), body condition score (BCS; Richards et al., 1986), and hair shedding score (HSS; Gray et al., 2011) were collected on a weekly basis throughout the experiment. Weekly BW were recorded utilizing a digital livestock scale and were utilized to calculate average daily gain (ADG) considering the difference between two BW measurements within their respective week of collection. While each animal was in the chute, thermal images of the ocular globe (OGT) and rib skin area (RIBT) posterior to the left scapula were collected using a FLIR T540 (Teledyne FLIR, LLC., Wilsonville, OR) thermal camera located 1 m from the head gate or side gate, and positioned perpendicular to the chute. The emissivity was set at 0.98 and the background temperature of the camera was updated for each animal using a calibrated Fisherbrand remote thermometer (Thermo Fisher Scientific, Goteborg, Sweden) as reference. Prior to collecting the first RIBT image on day 1, each heifer had a 10x15 cm square clipped posterior to the left scapula using a #10 blade. Each week after, the thermal images were taken within the square and then it was clipped again for hair growth removal. All thermal images were processed using the FLIR Thermal Studio Software (Teledyne FLIR, LLC., Wilsonville, OR) and the highest, lowest and average temperatures were recorded.

In Year II, all the heifers that were utilized in the Year I study were exposed to a transrectal ultrasonography assessment with a SonoSite M-Turbo ultrasound system equipped with a 10-5 MHz transducer (FUJIFILM SonoSite, Inc., Bothell, WA) for pregnancy status determination. It was estimated that the pregnant heifers were entering the second trimester of gestation prior to the beginning of the study. The heifers confirmed as pregnant (PREG; 439.4±7.1 kg; 17-19 mo of age; n=29) along with the non-pregnant (OPEN; 408.7±15.7 kg; 17-19 mo of age; n=7 ) heifers were reintroduced to the Vytelle® Sense system for a 14-day acclimation period. Upon completion of this period, all heifers were fed an EF-TMR for 56 days, and animal performance data were collected from early April to late May 2024 (Figure 3.1). As in Year I, IVDL were inserted on day 1 to record VT on a 20-minute-frequency throughout the study, and BW, ADG, BCS, and HSS were collected on a weekly basis to evaluate physiological changes. The Vytelle® Sense system recorded individual feed and water intake. Following completion of the trial, all heifers were relocated to EI pastures, and calf birth dates, birth weights (CALFBW), and sex information were collected as parturitions occurred between September and November 2024.

**Nutritional Intake and Efficiency Metrics Evaluation**

Following completion of each study, a post hoc analysis was performed to evaluate nutritional efficiency metrics of all heifers across both years. Total feed intake (FI) and water intake (WI), and WI per BW (WI/BW) were calculated for each animal and determine the treatment or pregnancy effects, depending on the year. The residual ADG (RADG) was calculated as the difference between the animal’s actual weight gain and its predicted gain, based on its DMI and body weight maintenance. The RFI was calculated for each animal as the difference between actual DMI and expected DMI, as described by Koch et al. (1963). Based on the results obtained during Year I, all heifers within each treatment group were ranked based on their respective RFI result, and negative RFI values were categorized as low-RFI (LRFI) and positive RFI values as high-RFI (HRFI) (Table 3.2). For the RADG ranking assessment, all heifers within each treatment group were ranked based on their RADG result, and negative RADG values were categorized as low-RADG (LRADG) and positive RADG values as high-RADG (HRADG) (Table 3.3). The same approach was utilized to analyze the RFI (Table 3.4) and RADG (Table 3.5) results obtained during Year II based on pregnancy status.

**Feed Ration Measurements**

As described in Chapter 2, two different feed rations were utilized in Year I. Both rations were formulated to meet the National Academy of Sciences (NASEM, 2016) nutrient requirements for CP, TDN, Ca, and P for a heifer weighing 226.8 kg and gaining 0.7 kg/day. Each ration was prepared and delivered using a designated mixer wagon to avoid cross-contamination. Two samples of each TMR were collected every two weeks starting on week 1 of the study. One pair was submitted to be analyzed for nutrient content (North Carolina Department of Agriculture – Food and Drug Protection Division Laboratory, Raleigh, NC; Table 2.1). The second pair was processed and submitted to a laboratory for ergot alkaloid analysis via High Performance Liquid Chromatography (HPLC) (Veterinary Medical Diagnostic Laboratory, University of Missouri, Columbia, MO) to determine alkaloid type and concentration (μg/kg) in the EI-TMR and confirm the absence of ergot alkaloids in the EF-TMR (Table 2.2).

In Year II, all animals were consuming an EF TMR. This ration was formulated to meet the NASEM nutrient requirements for CP, TDN, Ca, and P for a pregnant replacement heifer in her fourth month of pregnancy, weighing 430.9 kg and gaining 0.4 kg/day. The EF TMR consisted of 40% corn silage, 25% cotton gin byproduct, and 35% of a commercial protein pellet feed. One composite sample of the TMR was collected starting on week 1 of the trial, and then every two weeks starting on week 2 of the trial which was submitted to be analyzed for nutritional content (North Carolina Department of Agriculture – Food and Drug Protection Division Laboratory, Raleigh, NC; Table 3.1).

**Cattle Comfort Index**

In both years, the cattle comfort index (CCI) calculation was used as an indicator of cattle comfort under the exposure to different levels of thermal stress, based on the comprehensive climate index developed by Mader et al. (2010). This comprehensive climate index defines the following CCI categories: the thermoneutral range is set between 0 ºC and 25 ºC; MILD-HS between 25 ºC and 30 ºC; MOD-HS between 30 ºC and 35 ºC; severe heat stress between 35 ºC and 40 ºC; and extreme heat danger above 40 ºC. The CCI values were imported from the online database of the environmental station located at UPRS, property of the Environment and Climate Observing Network of North Carolina State University (NCSU ECONet, 2024). To improve the evaluation of the environmental fluctuations that occurred throughout the day, each day was divided into four periods of six hours and the average CCI for each period was calculated. Period 1 (P-1) represented 00:01 to 06:00, period 2 (P-2) represented 06:01 to 12:00, period 3 (P-3) represented 12:01 to 18:00, and period 4 (P-4) represented 18:01 to 00:00. Using these values, a weekly average was calculated for each defined period of the day. Average weekly CCI values of both studies are reported in ºC, and values below 25 ºC were considered as thermoneutral, in the range between 25 ºC and 30 ºC were defined as mild heat stress (MILD-HS), and moderate heat stress (MOD-HS) was defined between 30 ºC and 35 ºC (Figure 3.2).

**Statistical Analysis**

All heifer physiological and performance data collected in both years were analyzed using a MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, 1996) with repeated measures. Individual animal was utilized as the experimental unit and the model for BW, ADG, BCS, HSS, VT, OGT, RIBT, FI, WI, and WI/BW included treatment (EI vs. EF), RFI ranking (LRFI vs HRFI) and time (Weeks 1-7 for Year I; weeks 1-8 for Year II). While the animals in both studies were managed in pens with the Vytelle Sense installed, the pen experimental error cannot be separated from the individual error due to the design of the barn and short duration of both trials. The barn structure was built North/South with the Vytelle Sense on the west side of the barn. The overhang structure of the barn facilitates an equal shade area on the pens (125 $m^2$ per pen) throughout the day. Additionally, the animals were not solely confined in concrete pens, as access to an exercise lot (1,175 $m^2$ per pen) was provided. Consequently, these specifications allow for animal measurements to be analyzed as individuals. To evaluate differences in CALF BW associated with dam RFI, a UNIVARIATE procedure was used as a test for normality and PROC TTEST was used to compare means in SAS. Results were recorded as least square means ± SEM, where statistical significance was determined at $P \leq 0.05$ and statistical tendency at $0.05 < P \leq 0.10$. Moreover, A CORR procedure of SAS estimated Pearson correlations for RFI and RADG to determine the level of re-ranking among individuals within treatments across the two years. Correlations were deemed as ‘strong’ where values were equal to or greater than 0.60, ‘moderate’ where values were between 0.30 and 0.59, and ‘weak’ where values were equal to or less than 0.29.

## RESULTS

### Feed Ration Characteristics and Ergot Alkaloid Concentrations

The majority of nutritional values of the feed rations utilized remained consistent in Year I and II studies, except for some specific measurements. The nutritional quality and ergot alkaloid concentration results of the feed samples collected during Year I are described in Chapter 2 (Table 2.1). As previously mentioned, all heifers consumed an EF-TMR throughout the Year II study. The mean CP results (12.0±0.7 %DM) and met the requirement for pregnant heifers. The mean TDN results (69.8±5.7 %DM) also remained consistent in all measurements except in Wk 4, when its value decreased to 59.5 %DM. In contrast, the mean NDF and ADF values (43.8±4.9 %DM and 32.2±8.7 %DM, respectively) increased in their values during Wk 4 (52.2±4.9 %DM and 47.3±8.7 %DM, respectively). In terms of mineral concentrations, the mean Fe concentrations showed the greatest variation (554.0±158.8 ppm), whereas the Ca, Cu, and Zn mean values indicate a reduced variation (1.3±0.6 %DM, 16.0±1.9 ppm, and 73.7±4.3 ppm, respectively). The rest of the mineral concentrations did not vary throughout the study.

### Cattle Comfort Index

The environmental conditions fluctuated throughout Year I and II studies, as different timelines were utilized and the trials were conducted during different seasonal changes. During Year I, all heifers were exposed on several occasions to periods of MILD-HS and MOD-HS until Wk 6 of the study. The exposure to MILD-HS occurred between P-2 and P-4, and heifers experienced an increase to MOD-HS during P-3. However, the CCI declined to the thermoneutral zone during P-4 throughout the study. During Year II, all heifers remained in the thermoneutral zone for the majority of the study. The exceptions occurred during Wk 6 and 8, when the CCI increased and reached the MILD-HS conditions (Figure 3.2).

**Heifer Performance and Physiological Evaluation**

The performance and physiological results obtained during Year I are thoroughly detailed in Chapter 2. In Year II, the PREG heifers tended to have greater BW than their OPEN pairs (471.2±7.0 kg vs 442.5±15.4 kg; $P$ = 0.09; Figure 3.3), and maintained greater BW over time ($P$ < 0.01; Figure 3.4). However, no significant differences ($P$ = 0.84) were observed in ADG between groups (Figure 3.5). Further, the PREG heifers had greater BCS than the OPEN heifers (6.1±0.07 vs 5.8±0.15; $P$ = 0.04; Figure 3.6), but no differences were observed over time as both groups were able to increase their BCS by the end of the study ($P$ = 0.67; Figure 3.7). Similarly, no differences between groups were observed in the HSS results over time ($P$ = 0.65; Figure 3.8), as both groups had a similar ability to shed their winter hair coats and finished the study with lower HSS than the ones reported in the beginning. For the VT, significant differences were observed between groups over time, as the PREG heifers had lower VT during the first week of the study, but then maintained similar VT than the OPEN heifers until week 7, when the PREG heifers had greater VT than the OPEN heifers ($P$ < 0.01; Figure 3.9).

**Thermographic Assessment**

As it was previously described, thermal images were collected from specific regions of the body of all heifers during the Year I study. Significant differences were observed between groups, as the EI heifers had greater RIBT than EF heifers (36.6±0.1 ºC vs 35.5±0.1 ºC; $P$ < 0.01; Figure 3.10), and similar results were observed over time ($P$ < 0.01; Figure 3.11). Comparable results were observed for the OGT measurements, as the EI heifers had greater temperature (34.9±0.1 ºC vs 34.5±0.1 ºC; $P < 0.01$; Figure 3.12) than the EF heifers, and these differences were maintained over time ($P < 0.01$; Figure 3.13).

Furthermore, these data were analyzed using the RFI and RADG rankings as a covariate to identify any potential associations between the thermal images results and feed efficiency classification. The RIBT by RFI ranking analysis maintained the differences between the EI and EF groups ($P < 0.01$), but no differences were observed between the LRFI and HRFI heifers within treatment groups (Figure 3.14). However, the OGT by RFI ranking analysis indicated that the EF-HRFI subgroup had lower temperatures than both EI subgroups, but the EF-LRFI heifers tended to have similar temperatures to both EI subgroups ($P = 0.07$), whereas no differences were observed between the EI subgroups (Figure 3.15).

Similar results were obtained for the RADG covariate analysis, as the differences in RIBT between EI and EF groups were maintained ($P < 0.01$), but no differences were reported between the LRFI and HRFI heifers within the treatment groups (Figure 3.16). For the OGT by RADG analysis, the EF-HRADG subgroup had lower temperatures than both EI subgroups, but the EF-LRADG heifers tended to have similar temperatures to both EI subgroups ($P = 0.07$), and no differences were observed between the EI subgroups (Figure 3.17). No thermal images were collected during Year II.

**Nutritional Intake and Efficiency Metrics Evaluation**

Significant differences were observed in feed and water consumption between treatment groups during Year I. The EF heifers reported greater FI than the EI heifers (50.7±1.5 kg DMI vs 35.0±1.5 kg DMI; $P < 0.01$; Figure 3.18), and these differences were maintained throughout the entire study ($P < 0.01$; Figure 3.19). In contrast, the EI heifers had greater WI than EF heifers over time ($P$ = 0.01; Figure 3.20). Additionally, the EI heifers also had a greater WI/BW than the EF heifers (1.1±0.07 L/kg vs 0.9±0.07 L/kg; $P$ = 0.04; Figure 3.21) and maintained these differences over time ($P$ = 0.01; Figure 3.22).

Based on the results obtained from the post hoc analysis, eight EI heifers had a negative RFI and were ranked as LRFI, whereas ten EI heifers had a positive RFI and were ranked as HRFI. In contrast, the EF heifers showed equilibrated results, with nine heifers were ranked as both LRFI and HRFI (Table 3.2). Further, seven EI heifers had a negative RADG and were ranked as LRADG, whereas eleven EI heifers had a positive RADG and were ranked as HRADG. For the EF group, their RADG results were also equilibrated and nine heifers were ranked as both LRADG and HRADG (Table 3.3).

Using the RFI ranking as a covariate, the nutritional intake results were analyzed to identify any potential associations between the feed efficiency classification and the overall intake. Significant differences were observed in the FI of EF heifers, as the LRFI subgroup had a lower FI than their HRFI pairs (46.8±2.0 kg DMI vs 54.2±2.0 kg DMI; $P < 0.01$), whereas no differences were observed between the EI subgroups (Figure 3.23), and similar results were observed over time ($P < 0.01$; Figure 3.24). In contrast, differences were observed within the EI group, as the LRFI heifers had lower WI than their HRFI pairs (212.6±20.3 L vs 289.4±20.3 L; $P$ = 0.02), whereas no differences were observed between the EF subgroups (Figure 3.25), and similar results were reported over time ($P$ = 0.04; Figure 3.26).

The same analysis was performed using the RADG ranking as a covariate. Differences were observed between treatments, as both LRADG and HRADG subgroups of EF heifers had a greater FI when compared to their EI pairs ($P < 0.01$; Figure 3.27), and these differences were maintained over time ($P < 0.01$; Figure 3.28). However, no differences were observed between the subgroups within each treatment. In contrast, the LRADG heifers in the EI group had greater WI than their HRADG pairs (295.6±28.3 L vs 229.7±22.5 L; *P* = 0.03), whereas no differences were observed between the subgroups of EF heifers (Figure 3.29), and these results were maintained over time (Figure 3.30).

In Year II, no differences were observed in FI between PREG and OPEN heifers (91.5±1.6 kg DMI vs 97.0±3.4 kg DMI; *P* = 0.15; Figure 3.31) and similar results were observed over time (*P* = 0.25; Figure 3.32). Comparably, the WI results between PREG and OPEN heifers did not differ (291.9±6.2 L vs 289.4±13.7 L; *P* = 0.87; Figure 3.33), and no differences were observed over time (*P* = 0.56; Figure 3.34). In addition, the WI/BW results did not differ between PREG and OPEN heifers (0.63±0.01 L/kg vs 0.66±0.03 L/kg; *P* = 0.27; Figure 3.35).

Based on the post hoc analysis results, 20 PREG heifers had a negative RFI and were ranked as LRFI, whereas nine PREG heifers had a positive RFI and were ranked as HRFI. For the OPEN group, one heifer was ranked as LRFI and six heifers had a positive RFI that categorized them as HRFI (Table 3.4). Moreover, 12 PREG heifers had a negative RADG and were ranked as LRADG, whereas 16 PREG heifers had a positive RADG and were ranked as HRADG. For the OPEN heifers, four animals had negative RADG values and were categorized as LRADG, whereas three had positive RADG values and were ranked as HRADG (Table 3.5).

Following the same covariate analysis as in Year I, the nutritional intake results were assessed based on the pregnancy status and the RFI and RADG rankings. The LRFI PREG heifers had a lower FI than their HRFI pairs (88.2±1.6 kg DMI vs 99.1±2.2 kg DMI; P < 0.01), whereas no differences were observed between the RFI subgroups of OPEN heifers (Figure 3.36), and similar results were reported over time (*P* = 0.17; Figure 3.37). For the WI results, no differences were observed between the subgroups nor between treatments (*P* = 0.55; Figure

3.38). Furthermore, no differences were observed in the FI of the RADG subgroups between treatments (*P* = 0.61; Figure 3.39). Similarly, no differences were observed in the WI of LRADG and HRADG heifers in both treatment groups (*P* = 0.61; Figure 3.40).

Based on the RFI and RADG results, a correlation analysis was performed to determine the reclassification of efficiency rankings among individuals across the two years. No statistical differences and a weak correlation ($r = 0.18$; $R^2 = 0.03$; $P = 0.28$) were reported for the RFI scores between Year I and Year II (Figure 3.41). Additionally, a tendency and a moderate correlation ($r = 0.32$; $R^2 = 0.10$; $P = 0.06$) were observed for the RADG scores between Year I and Year II (Figure 3.42). For the RFI repeatability analysis, 60% ($n = 9$) of the 15 heifers that reported a negative RFI score in Year II had also scored a negative RFI in Year I, whereas 42.9% ($n = 9$) of the 21 heifers that had a positive RFI score in Year II had similar results in Year I (Figure 3.43). In terms of RADG, equilibrated results were observed in Year II as the negative and positive RADG subgroups were comprised of 18 heifers each, and 66.7% ($n = 12$) of the animals scored similar values in Year I (Figure 3.44).

**Calf Birthing Evaluation**

Calving dates ranged from late September and to late November 2024. From the 29 PREG heifers that finished the Year II study, two were sold as pregnant females; four are assumed to have lost their pregnancies as they were confirmed as PREG when the study started, but did not calve; and three gave birth to dead calves. Thus, 10 bull calves and 10 heifer calves were registered during this calving season and their respective CALFBW was recorded. Based on the dam RFI ranking, no differences were observed in the CALFBW between calves born from LRFI and HRFI dams (26.5±1.4 kg vs 29.8±1.7 kg; P = 0.10; Figure 3.45).

# DISCUSSION

Ergot alkaloid intake is detrimental to cattle, as it induces fescue toxicosis (FT) which negatively impacts animal growth and performance (Strickland et al., 2011). Based on the results obtained during Year I, the EI heifers had classical symptoms of FT, as this group ended the study with lower BW, ADG and BCS than their EF pairs. These results are directly associated with the decreased FI that EI heifers maintained throughout the study. Previous studies have described the negative effects of ergot alkaloid consumption on FI (Parish et al., 2003; Drewnoski et al., 2009; Johnson et al., 2014), although other publications have not reported reductions in the FI of cattle consuming EI rations in confinement (Eisemann et al., 2014; Poole et al., 2018). It is widely known that FT reduces dry matter intake in grazing livestock (Strickland et al., 2011; Kallenbach, 2015) but the exact mechanisms behind the decrease in intake remain to be determined. One possibility may be associated with the vasoconstrictive effects that are induced by ergot alkaloid consumption, which limit blood flow to the ruminal and gut surfaces and reduce the absorption rate of volatile fatty acids (Foote et al., 2013). Additionally, the observed reductions in FI may also be associated with the exposure to different levels of thermal stress that the heifers experienced during Year I. The interaction between ergot alkaloids and environmental conditions is well documented, as the susceptibility to the symptoms of FT is incremented under heat stress (Hovermale and Craig, 2001; Mote et al., 2022; Rajo-Gomez et al., 2024). During the majority of the Year I study, both groups of heifers were exposed to several periods of MILD-HS and MOD-HS. More specifically, the last period of MOD-HS exposure occurred in Wk 5, and by this point the EI heifers had only increased their average FI by 4.3±1.7 kg DM/Wk. In the same period, the EF heifers had increased their average FI by 17.1±1.7 kg DM/Wk. Furthermore, as exposure to thermal decreased in the last two weeks of the study, the EI heifers were able to compensate and reported an increase in their average FI of 16.7±1.7 kg DM/Wk, while the EF heifers reported an average FI of 15.3±1.7 kg DM/Wk. These results are comparable to those reported by Spiers et al. (2012) and Johnson et al. (2014), and demonstrate the significant effects of thermal stress exposure in FI of cattle.

Additional negative effects induced by heat stress exposure include increased sweating, panting and drooling, which consequently increase water intake (Shephard and Maloney, 2023). Water is considered as the most important nutrient due to its involvement in growth, digestion, metabolic processes, excretion and temperature regulation (NASEM, 2016). However, few studies have evaluated individual water consumption in beef cattle and the influence that environmental factors have on this specific parameter (Ahlberg et al., 2018). Furthermore, to this date there are no scientific publications that describe the effects of ergot alkaloid consumption on individual water intake in beef cattle. The results presented herein may provide an initial perspective of the impact that FT has on water consumption of beef heifers, as the EI group consumed greater volume of water over time, and also had greater WI per BW than their EF pairs. However, it is difficult to determine the exact influence that exposure to ergot alkaloids had on the water consumption as these heifers were also exposed to varying levels of heat stress throughout the study. The interaction between climatic variables, animal physiological stage and breed, and type of diet complicates the estimation of the daily water intake requirements in cattle (Arias and Mader, 2011). As mentioned earlier, the events of MOD-HS occurred predominantly during the first five weeks of the study, except during Wk 4 when the thermal levels only reached the MILD-HS threshold. The recorded WI over time shows a similar trend, as both groups registered a decrease in their WI during Wk 4. Moreover, the exposure to thermal stress was also decreased during the last two weeks of the study, and similar reductions were observed in the WI of both groups during the same period. The physiological mechanisms for thermoregulation in response to heat stress include increased skin temperature, increased peripheral vasodilation, increased respiration rates, and increased sweat rates (Scharf et al., 2010). As the rate of these mechanisms is incremented, WI is also expected to increase due to the direct involvement of water in these processes. Based on these data, it can be concluded that the exposure to varying levels of thermal stress had a significant impact on the WI of both groups of heifers in this study. These results are similar to those reported by Ahlberg et al. (2018) and Arias and Mader (2011), who described that increases in ambient temperature were associated with increases in WI of beef cattle.

Moreover, WI has been found to be positively correlated with dry matter intake (or FI) in cattle (Winchester and Morris, 1956; Arias and Mader, 2011). The relationship between WI and FI is also dependent on the environmental conditions, as WI will generally increase and FI will generally decrease during the summer, and the opposite is expected during the winter. However, differing relationships may exist among WI and FI depending on the season, and these variations may complicate the accuracy of WI predictions based on FI (Sexson et al., 2012). Considering the timeline for the Year I study included the last period of summer and the following transition to fall, various periods of increased ambient temperature were observed throughout the trial. However, the reported WI results were not positively correlated to the FI results in both groups of heifers. As previously described, the EF heifers maintained significantly greater FI than the EI heifers throughout the study. Yet, opposite results were observed for WI as EI heifers had a greater consumption over time. A potential explanation may reside on the differences reported in the VT of both groups. As mentioned in Chapter 2

, both groups of heifers exceeded the fever threshold (39.5 ºC) established for cattle (Burfeind et al., 2012) during the period of MOD-HS exposure, and the EI heifers maintained greater VT than their EF pairs throughout the study. Additionally, the EI group also had greater retention of their winter hair coats, as shown by the HSS results, which negatively impacts on thermoregulation. Considering the increased VT that EI heifers sustained during this study and their greater susceptibility to the increased thermal stress, it seems likely that the observed differences in WI may have also been influenced by the symptoms of FT that these heifers experienced.

The observed differences in the thermoregulatory abilities between treatment groups also had an effect on the infrared thermography results. The RIBT analysis showed a difference of 1.1±0.1 ºC between the groups, with the EF heifers having significantly lower skin temperatures. In addition, these differences were maintained throughout the study, except in Wk 1 when the EI heifers reported similar RIBT than the EF heifers. These results are associated with the differences observed in VT results between groups. Similar results were documented by Eisemann et al. (2014), who fed EI or EF diets to steers and recorded the changes in skin temperature using a thermography assessment. The steers that consumed the EF diet had lower skin temperature than those consuming the EI diet, suggesting a greater vasodilatory capacity that facilitated the transfer of body heat to the skin surface (Eisemann et al., 2014). Considering the results presented in Chapter 2 describing the vasoconstrictive effects observed in the CAD of EI heifers, and that peripheral vasoconstriction is a trademark symptom of FT (Klotz, 2015; Poole et al., 2018), it may be possible that the EI group also experienced limited peripheral blood flow and vasodilation that negatively impacted their ability for heat exchange. Further, the variations in RIBT may have also been influenced by observed differences in HSS, as studies have found that a greater retention of their winter hair coats is associated with a limited potential for heat transfer to the skin surface in animals suffering from FT (Aiken et al., 2011; Klotz, 2015).

Moreover, the OGT results were similar to those observed for the RIBT as EI heifers had greater OGT than the EF heifers, but the temperature difference between groups was not as large (0.4±0.1 ºC), and the analysis over time showed that both groups had similar OGT during specific weeks. Giro et al. (2019) utilized infrared thermography to monitor body temperature changes of beef cattle managed on pastures, and their analysis included the surface temperature of the ocular globe and ear base of the cows. Their results showed significant differences between the mean ear, OGT, and rectal temperatures. More specifically, the mean OGT (37.4±0.06 ºC) reported by Giro et al. (2019) was greater than the mean OGT of the EI and EF heifers (34.9±0.1ºC vs 34.5±0.1 ºC, respectively) observed in this study. However, the animals used in this study were of a composite breed (⅝ Charolais × ⅜ Zebu), the experimental period was considerably longer (January – June), and the animals were also exposed to different levels of thermal stress (Giro et al., 2019). Evaluating the thermal status of animals is important, as early detection of thermal stress may improve decision-making processes aimed at protecting animal health and productivity. Nonetheless, body temperatures may vary depending on the anatomical site used to collect these measurements. The rectal temperature is considered as the primary measure and reference to body temperature in cattle. Yet, collection of this measurement often requires moving the animals to handling facilities (Taylor et al., 2014). Therefore, technologies that facilitate a more practical thermal evaluation using different body regions, such as the skin, the ocular globe and other regions near the head, may favor improved livestock management strategies and reduce production losses associated with impaired thermoregulation (Giro et al., 2019).

The incorporation of precision technologies into livestock operations has also become a reliable and powerful alternative for monitoring the nutritional intake of individual animals and developing subsequent rankings of animal groups based on levels of consumption and efficiency (Schaefer et al., 2023). Given the challenges that agricultural and livestock enterprises are currently facing, such as a greater demand for animal protein from a rapidly growing global population, increasingly variable climatic patterns, and a reduction in land availability, the identification of animals with superior nutritional efficiency may serve as a means to increase the productivity, profitability, and sustainability of these operations (Havlik et al., 2014; Ojo et al., 2024). The use of the Vytelle Sense ® (https://vytelle.com/vytelle-sense) system in these studies allowed for the monitoring and evaluation of individual FI and WI in all the heifers, as well as the development of feed efficiency rankings based on the RFI and RADG assessment.

Significant differences in the FI results of Year I were observed following the covariate analysis based on the RFI and RADG rankings. The decreased FI that the EF-LRFI heifers reported serves as an indicator of superior nutritional efficiency. Residual feed intake (RFI) is defined as the difference between the actual feed intake of an animal and its predicted feed requirement for maintenance of body size and production (Koch et al., 1963). Thus, the reduced FI that the EF-LRFI had compared to their HRFI pairs indicates a reduction in dry matter consumption whilst maintaining their expected body size and growth, which translates to enhanced efficiency (Basarab et al., 2013; Terry et al., 2020). However, the lack of differences between the LRFI and HRFI subgroups of EI heifers may indicate an impact of ergot alkaloid exposure on the efficiency assessment.

Moreover, the RADG results in Year I showed differences between treatment groups, as both EF-LRADG and EF-HRADG subgroups had greater FI than the EI heifers, but no differences were observed within treatments. Residual average daily gain (RADG) is defined as the difference between the actual ADG of an animal and its predicted ADG, based on its DMI, BW maintenance and fat cover. Contrary to RFI, animals with greater RADG values are more desirable due to increased ADG consuming the same amount of feed (Northcutt and Bowman, 2010; Ojo et al., 2024). As described for the RFI evaluation, the reported decrease in the FI results of EI heifers, as well as the lack of differences among the LRADG and HRADG subgroups within this treatment, may suggest an impact of ergot alkaloid consumption on feed efficiency. Sarich et al. (2023) conducted a study to evaluate the effects of increasing dietary concentrations of ergot alkaloids from cereal grains on health, welfare, and growth performance of feedlot cattle. According to their results, increasing concentrations of ergot alkaloids altered feeding behavior and reduced FI, which consequently caused lower weight gains in the steers consuming greater ergot alkaloid concentrations (Sarich et al., 2023).

Furthermore, Reynolds et al. (2024) evaluated the effect of continuous or intermittent feeding of ergot contaminated grain on the performance and health of beef steers. Their results also indicated a reduction in FI, ADG, and final BW in the groups exposed to ergot alkaloids, regardless of the continuous or intermittent feeding strategies. In addition, a negative impact of increasing concentrations of ergot alkaloids on carcass weight, dressing percentage and quality grade was also documented (Reynolds et al., 2024). However, these studies did not report any feed efficiency metrics, in spite of utilizing the Vytelle Sense ® system (Sarich et al., 2023) and the Calan Data Ranger (Reynolds et al., 2024) to monitor feed intake. In fact, and to this date, there are no published studies documenting the effects of ergot alkaloid consumption on feed efficiency parameters, such as RFI and RADG, in beef cattle. As previously described, ergot alkaloids are known to interact with specific receptors in smooth muscle tissue, including serotonergic receptors that regulate gut motility (Klotz et al., 2015). These negative effects, along with potential vasoconstriction of ruminal vasculatures caused by ergot alkaloid intake, may reduce rumen and gut motility and impair digestion, which consequently affect weight gain and growth (Foote et al., 2011; Sarich et al., 2023). Further, DMI and ADG are also affected by variation in day-to-day intake, which is observed in animals adjusting their FI in response to short-term aversions associated with stimuli that induce discomfort (Beck et al., 2024). As mentioned earlier, the EI heifers displayed classical symptoms of FT and showed an increased susceptibility to heat stress. Even though the FI of EI heifers remained relatively consistent between Wk 1 and Wk 5, the suppressed ADG observed during the same period may have impacted the feed efficiency metrics. More investigations are needed to improve the understanding of the effects that the exposure to environmental burdens, such as FT and heat stress, have on the estimation of feed efficiency parameters.

The covariate analysis based on the RFI and RADG rankings was also adopted to assess the WI results of Year I. The EI-HRFI subgroup had a greater WI than the other three subgroups, whereas no differences were observed in the WI of EF-LRFI and EF-HRFI subgroups. Interestingly, the EI-LRFI heifers had similar WI than the EF-LRFI heifers. For the RADG analysis, similar results were observed as the EI-LRADG, which are categorized as less efficient, had greater WI than the other subgroups of heifers. In contrast, the EI-HRADG had similar WI when compared to both EF subgroups. Few methodologies for the calculation and establishment of water efficiency have been published in scientific literature. Ahlberg et al. (2019) calculated water efficiency metrics for cattle and evaluated their relationship to growth, FI, and feed efficiency. Their results indicated that animals with low WI had lower FI, ADG, and were more efficient with water utilization, as defined by their calculated water to gain ration and residual water intake (RWI). Additionally, a genetic correlation between WI and ADG was identified, suggesting that selecting for cattle with low WI should have a minor impact on animal growth (Ahlberg et al., 2019). The results observed in the covariate analyses using the RFI rankings are similar to those reported by Ahlberg et al. (2019), as the groups that were categorized as feed efficient had lower WI than those deemed as feed inefficient. The EI heifers in the efficient categories maintained similar WI as the EF heifers in spite of the exposure to stressors. Based on these results, selection of animals with superior feed efficiency may also contribute to improve water efficiency, which may be beneficial in regions exposed to droughts, increased competition for water, and stricter regulations for water use (Klopatek and Oltjen, 2022).

The thermography results were also subjected to covariate analysis based on the RFI and RADG rankings. Previous studies have aimed to predict RFI and metabolic efficiency in cattle by measuring radiated heat loss using infrared thermography assessments. The variation in RFI is largely influenced by differences in energy partitioning, with heat production being one of the major contributing factors (Nkrumah et al., 2006). Montanholi et al. (2009) reported moderately positive correlations between RFI and the surface temperature on the back of the left ($r$ = 0.38) and right ($r$ = 0.43) hooves in beef bulls, as well as significant relationships between RFI and eye and cheek temperature regions in yearling beef bulls (Montanholi et al., 2009). Additionally, Colyn (2013) described significant relationships between RFI and cheek temperatures in yearling heifers. In contrast, no differences were observed in the RIBT results of LRFI and HRFI heifers within the treatment groups, although both EF subgroups reported lower RIBT than their EI pairs. Further, the EF-HRFI had significantly lower OGT than both EI subgroups, but the EF-

LRFI tended to differ from the other subgroups of heifers. These differences may be associated with the influence of the increased exposure to thermal stress experienced by these heifers. Thompson et al. (2018) documented that the exposure to cold stress may have biased their results in infrared thermography measurements and their correlations to the RFI ranking in beef bulls. In the Year I study, both groups of heifers were exposed to different levels of heat stress, and the EI heifers displayed symptoms associated with FT. These findings indicate that climatic variables and physiological disorders, such as thermal stress and FT, may hinder the accurate estimation of RFI classification and metabolic efficiency in cattle by using infrared thermography.

The performance and physiological results observed in Year II were expected, as the PREG heifers had greater BW and BCS than the OPEN heifers. In contrast, no differences were observed in ADG between groups, which may be associated with the EF-TMR nutritional quality and the lack of differences in FI and WI. Proper nutritional management of beef females during pregnancy is critical for ensuring the health status of both dam and calf, as well as future fertility (Macrae et al., 2021). As gestation progresses, a variety of physical and physiological changes related to nutritional consumption and digestion may occur, and a gradual reduction in feed intake is expected as gestation reaches the final developmental stages due to the decreased ruminal capacity caused by the expansion of the gravid uterus (Rotta et al., 2015). During the Year II study, the ultrasonography evaluation determined that the PREG heifers were entering the second trimester of gestation. Therefore, limitations in ruminal capacity induced by fetal growth at this point are unlikely, and this may also explain the observed FI results.

Moreover, the increased ability that both groups had to shed their winter hair coats is demonstrated by the HSS results. Hair growth and shedding in cattle is regulated by prolactin (Littlejohn et al., 2014), and its concentrations are influenced by environmental factors, such as the duration of the photoperiod and increased ambient temperatures (Schams & Reinhardt, 1974; Wettemann & Tucker, 1974). Considering the experimental timeline used for the Year II study, daylength duration and thermal conditions increased towards the end of the study, as the season transitioned from spring to summer. Additionally, the lack of ergot alkaloid consumption throughout the study also facilitated the hair shedding ability of these heifers, as it is well known that FT decreases serum prolactin concentrations and induces the retention of winter hair coats (Strickland et al., 2011; Gray et al., 2011; Poole et al., 2019; Rajo-Gomez et al., 2024).

The environmental conditions recorded during the Year II study also favored proper thermoregulation in both heifer groups. As demonstrated by the VT, the PREG and OPEN heifers maintained their body temperatures below the fever threshold (39.5 ºC) established for cattle (Burfeind et al., 2012). These results are associated with the recorded CCI values, as the heifers only experienced partial exposure to MILD-HS during specific periods of the day in Wk 5 and 6, and the exposure increased in Wk 8 as the CCI values approached the MOD-HS threshold. Consequently, an increase in the VT of both groups of heifers was observed during the same period.

Furthermore, the observed differences in FI between LRFI and HRFI PREG heifers demonstrate that efficiency rankings may be established for cattle across different physiological and production stages. In beef cattle operations, one of the major production costs is feed, and it is estimated that the cow herd consumes approximately 70% of the annual feed resources (Freetly et al., 2020). Thus, increasing feed efficiency within the herd could provide a significant benefit to the financial status of the operation (Callum et al., 2014). Feed efficiency metrics, such as RFI and RADG, are extensively evaluated in growing animals, but limited information is available for mature cows (Freetly et al., 2020). The Year II RFI results agree with previous studies that have identified favorable relationships between intake-related traits evaluated postweaning and in adult animals. Hafla et al. (2013) found that LRFI pregnant females consumed 17% less forage compared to the HRFI females. Basarab et al. (2007) reported that pregnant cows that produced progeny with LRFI consumed 12% less forage than cows that produced HRFI progeny. Considering that RFI is a moderately heritable trait (h2 = 0.16–0.43), adopting selection for RFI may represent an opportunity to decrease feed costs along the beef production chain (Kenny et al., 2018).

In the current beef production system, the feed efficiency evaluations are commonly performed in young growing cattle after weaning in performance test stations using high-energy feed rations. However, these animals are then relocated to different management schemes and consume forage-based diets that are higher in fiber and lower in energy. Consequently, low correlations have been identified between the RFI of growing cattle with the RFI of mature cows, which may be related to differences in feed composition and quality and variations in nutrient metabolism (Ferrell and Jenkins, 1985; Lancaster, 2014). In contrast, Archer et al. (2002) reported strong genetic correlations between postweaning RFI of heifers and FI ($r$ = 0.64) and RFI ($r$ = 0.98) of mature non-pregnant cows, although the phenotypic correlations for FI ($r$ = 0.34) and RFI ($r$ = 0.40) were lower. However, these animals were fed the same pelleted ration with high energy and protein content during their evaluations. Collectively, these results indicate that RFI evaluated across growing and mature stages in cattle is genetically the same trait, but the expression of its genetic potential may be altered by changes in the physiological state of the animal (Archer et al., 2002; Lancaster, 2014). Further, Durunna et al. (2012) found that crossbred heifers reported changes in their RFI ranking across two feeding periods, despite receiving the same diet, and the reranking was more severe in heifers with extreme RFI performances in each period (Durunna et al., 2012). Similar results were observed in this study, as changes in RFI ranking were observed when comparing the Year I and Year II results. However, the weak RFI correlation reported between Year I and Year II may have not only been impacted by the metabolic changes associated with pregnancy, but also by the differences in diet composition. As previously stated, the exposure to heat stress that all heifers experienced during Year I, in addition to the ergot alkaloids present in the ration that the EI heifers consumed, may have imposed challenges for an accurate estimation of feed efficiency. These factors should be considered in future nutritional efficiency evaluations conducted in the Southeastern USA, as more than 11 million head of cattle are raised in this region (USDA-NASS, 2024) and are frequently exposed to environmental stressors such as FT and heat stress (Kallenbach, 2015; Poole et al., 2020; Rajo-Gomez et al., 2024).

Moreover, the relationship between dam RFI and calf birth weight (CALFBW) was also evaluated in this study, and no differences were observed in CALFBW of LRFI and HRFI dams. Similar results were reported by Basarab et al. (2011), Damiran et al. (2018), and Callum et al. (2019) , who stated that females divergently ranked for RFI had no significant differences in CALFBW, pre-weaning growth, actual weaning weight, and 200-d weaning weight. Additionally, Parsons et al., (2021) analyzed various performance traits, including BW, BCS, CALFBW, and longevity in the herd, and indicated that RFI rankings did not impact cow-calf production parameters in a forage-based environment. Previous studies have demonstrated that RFI is independent of body weight and growth (Arthur et al., 2005; Basarab et al., 2007; Manafiazar et al., 2016). Thus, these results indicate that selection for feed efficient replacement females should have no impact on their productivity as mature cows.

Overall, these findings suggest that varying physiological stages, such as growing, pregnant, or lactating, can significantly impact the feed efficiency relationships in cattle. As stated, the decreased correlations in RFI comparisons across different stages are likely due to changes in nutrient metabolism as the animal grows. As the production cycle progresses, the cow must be able to mobilize nutrients from body reserves for milk production during lactation and fetal growth during gestation, whilst satisfying its nutritional maintenance demands to uphold adequate productivity rates (Lancaster, 2014). Therefore, the changes in RFI rankings between production phases may be influenced more by the physiological stage than by the type of diet (Lancaster et al., 2021). Nonetheless, future investigations are needed to enhance the understanding of the effects that ergot alkaloid consumption have on the determination of nutritional efficiency metrics.

## TABLES

**Table 3.1.** Nutritive value [1] of feed ration samples

| | EF-TMR [2] | | | | | | |
|---|---|---|---|---|---|---|---|
| | Wk 1 | Wk 2 | Wk 4 | Wk 6 | Wk 8 | Mean | Std. Dev. |
| DM (%) | 42.2 | 48.3 | 55.0 | 49.9 | 45.4 | 48.2 | 4.3 |
| CP (%DM) | 12.5 | 11.5 | 11.0 | 11.9 | 12.9 | 12.0 | 0.7 |
| NDF (%DM) | 37.2 | 41.4 | 52.2 | 43.2 | 45.1 | 43.8 | 4.9 |
| ADF (%DM) | 20.3 | 29.6 | 47.3 | 31.0 | 32.9 | 32.2 | 8.7 |
| TDN (%DM) | 76.7 | 71.6 | 59.5 | 71.6 | 69.7 | 69.8 | 5.7 |
| Ash (%DM) | 5.9 | 10.3 | 20.2 | 9.3 | 9.1 | 11.0 | 4.9 |
| Ca (%DM) | 0.6 | 1.1 | 2.4 | 1.2 | 1.1 | 1.3 | 0.6 |
| P (%DM) | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.0 |
| S (%DM) | 0.2 | 0.2 | 0.2 | 0.2 | 0.3 | 0.2 | 0.0 |
| Mg (%DM) | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.0 |
| Na (%DM) | 0.2 | 0.2 | 0.2 | 0.1 | 0.2 | 0.2 | 0.0 |
| K (%DM) | 1.0 | 1.1 | 1.0 | 1.1 | 1.1 | 1.1 | 0.0 |
| Cu (ppm) | 18.7 | 17.0 | 16.7 | 14.1 | 13.5 | 16.0 | 1.9 |
| Fe (ppm) | 292.9 | 784.5 | 519.9 | 614.6 | 558.3 | 554.0 | 158.8 |
| Mn (ppm) | 63.7 | 78.9 | 95.2 | 80.5 | 71.1 | 77.9 | 10.5 |
| Zn (ppm) | 78.4 | 79.4 | 71.8 | 68.8 | 70.3 | 73.7 | 4.3 |

[1] Values are reported as percentage on a dry matter basis (%DM) and parts per million (ppm); [2] EF-TMR: Endophyte-free total mixed ration.

**Table 3.2.** Residual feed intake (RFI) results of heifers based on treatment and category for Year I study.

| Treatment [1] | EI | | EF | |
|---|---|---|---|---|
| Category [2] | LRFI | HRFI | LRFI | HRFI |
| | -1.72 | 0.13 | -1.41 | 0.13 |
| | -1.46 | 0.26 | -1.17 | 0.18 |
| | -1.17 | 0.29 | -1.12 | 0.53 |
| | -0.64 | 0.33 | -1.01 | 0.60 |
| | -0.33 | 0.62 | -0.60 | 0.66 |
| | -0.20 | 0.66 | -0.44 | 0.73 |
| | -0.18 | 0.68 | -0.26 | 0.90 |
| | -0.15 | 0.90 | -0.13 | 0.95 |
| | | 0.97 | -0.10 | 1.94 |
| | | 0.99 | | |

[1] Treatment based on total mixed ration consumed by the animals (EI: endophyte-infected; EF: endophyte-free); [2] Category based on RFI results, where negative scores were classified as low-RFI (LRFI) and positive scores as high-RFI (HRFI).

**Table 3.3.** Residual average daily gain (RADG) results of heifers based on treatment and category for Year I study.

| Treatment [1] | EI | | EF | |
|---|---|---|---|---|
| Category [2] | LRADG | HRADG | LRADG | HRADG |
| | -0.54 | 0.01 | -0.38 | 0.06 |
| | -0.47 | 0.01 | -0.34 | 0.10 |
| | -0.19 | 0.01 | -0.20 | 0.11 |
| | -0.16 | 0.06 | -0.17 | 0.15 |
| | -0.12 | 0.08 | -0.15 | 0.18 |
| | -0.10 | 0.09 | -0.15 | 0.18 |
| | -0.10 | 0.20 | -0.12 | 0.25 |
| | | 0.22 | -0.06 | 0.43 |
| | | 0.24 | -0.03 | 0.52 |
| | | 0.37 | | |
| | | 0.41 | | |

[1] Treatment based on total mixed ration consumed by the animals (EI: endophyte-infected; EF: endophyte-free); [2] Category based on RADG results, where negative scores were classified as low-RADG (LRADG) and positive scores as high-RADG (HRADG).

**Table 3.4.** Residual feed intake (RFI) results of heifers based on pregnancy status and category for Year II study.

| Status [1] | PREG | | OPEN | |
|---|---|---|---|---|
| Category [2] | LRFI | HRFI | LRFI | HRFI |
| | -5.71 | 0.04 | -0.79 | 0.13 |
| | -3.59 | 0.35 | | 1.21 |
| | -3.53 | 0.48 | | 1.85 |
| | -2.23 | 0.62 | | 3.90 |
| | -2.05 | 0.75 | | 4.23 |
| | -1.68 | 0.82 | | 4.34 |
| | -1.61 | 2.49 | | |
| | -1.50 | 3.40 | | |
| | -1.37 | 3.73 | | |
| | -1.08 | | | |
| | -1.06 | | | |
| | -0.97 | | | |
| | -0.93 | | | |
| | -0.64 | | | |
| | -0.55 | | | |
| | -0.42 | | | |
| | -0.31 | | | |
| | -0.26 | | | |
| | -0.13 | | | |
| | -0.09 | | | |

[1] Status based on the ultrasonography assessment result (PREG: Pregnant; OPEN: Non-pregnant); [2] Category based on RFI results, where negative scores were classified as low-RFI (LRFI) and positive scores as high-RFI (HRFI).

**Table 3.5.** Residual average daily gain (RADG) results of heifers based on pregnancy status and category for Year II study.

| Status [1] | PREG | | OPEN | |
|---|---|---|---|---|
| Category [2] | LRADG | HRADG | LRADG | HRADG |
| | -0.53 | 0.01 | -0.69 | 0.04 |
| | -0.52 | 0.05 | -0.54 | 0.28 |
| | -0.49 | 0.10 | -0.16 | 0.99 |
| | -0.47 | 0.10 | -0.11 | |
| | -0.39 | 0.11 | | |
| | -0.28 | 0.13 | | |
| | -0.28 | 0.18 | | |
| | -0.20 | 0.24 | | |
| | -0.17 | 0.25 | | |
| | -0.14 | 0.26 | | |
| | -0.07 | 0.28 | | |
| | -0.06 | 0.29 | | |
| | | 0.30 | | |
| | | 0.31 | | |
| | | 0.34 | | |
| | | 0.58 | | |

[1] Status based on the ultrasonography assessment result (PREG: Pregnant; OPEN: Non-pregnant); [2] Category based on RADG results, where negative scores were classified as low-RADG (LRADG) and positive scores as high-RADG (HRADG).

## FIGURES

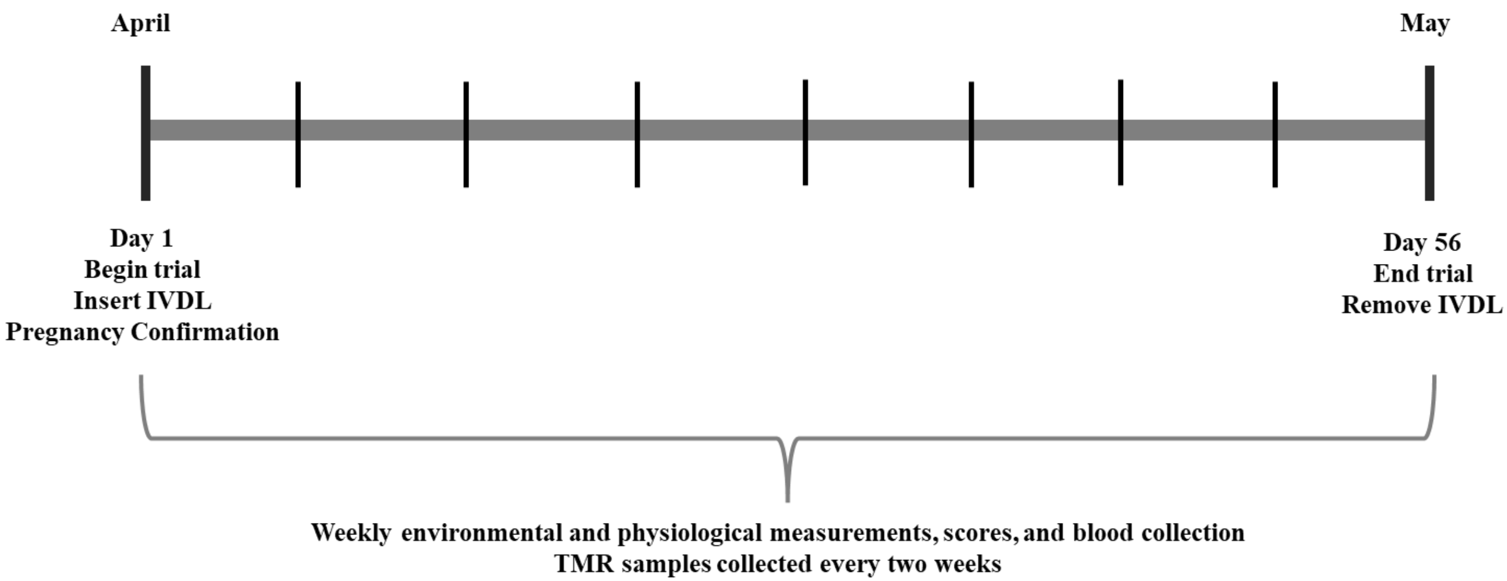


**Figure 3.1.** Experimental timeline used during phase of data collection from early April to late May 2024.

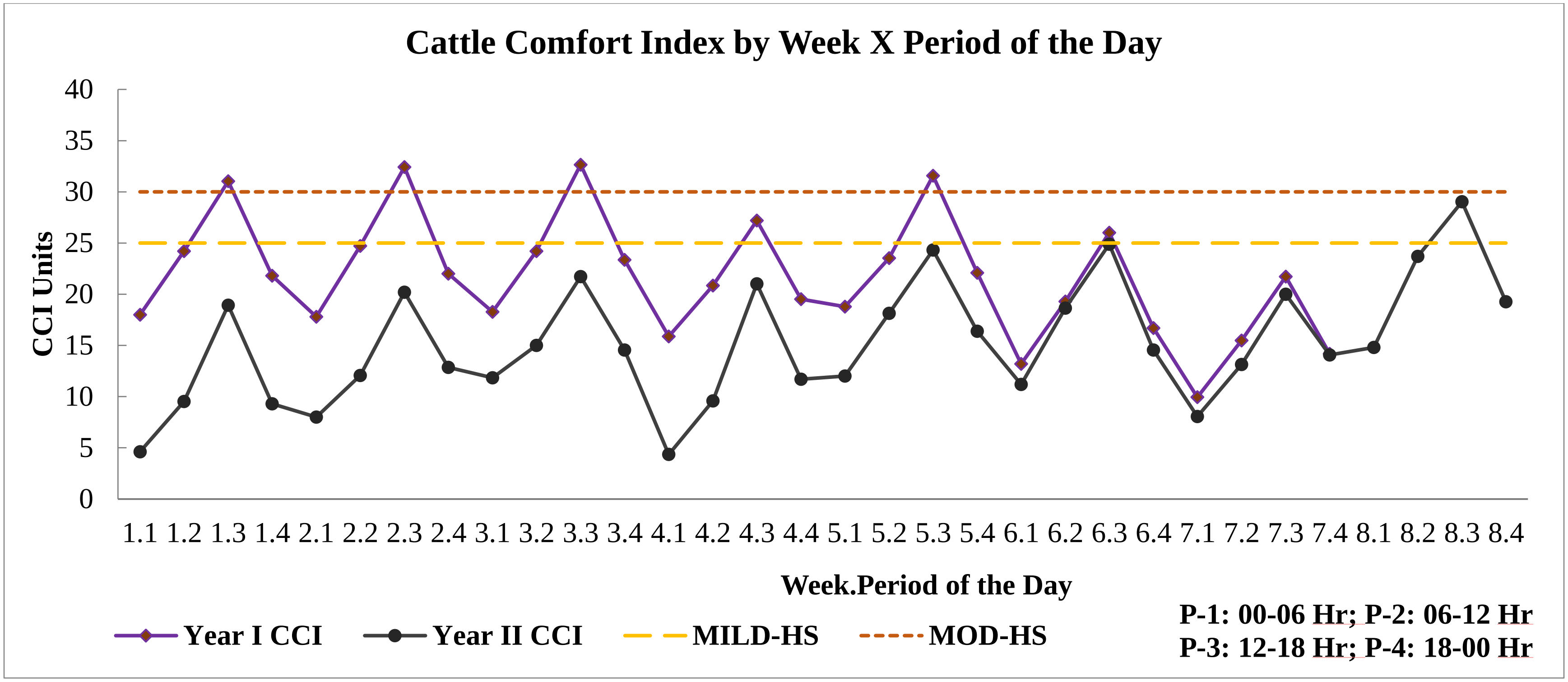


**Figure 3.2.** Cattle Comfort Index (CCI) over time (weeks by period of the day) for Year I and Year II studies: In Year I, both groups of heifers were exposed to different levels of heat stress throughout the study. In Year II, the exposure to heat stress decreased and only occurred in the last two weeks of the study.

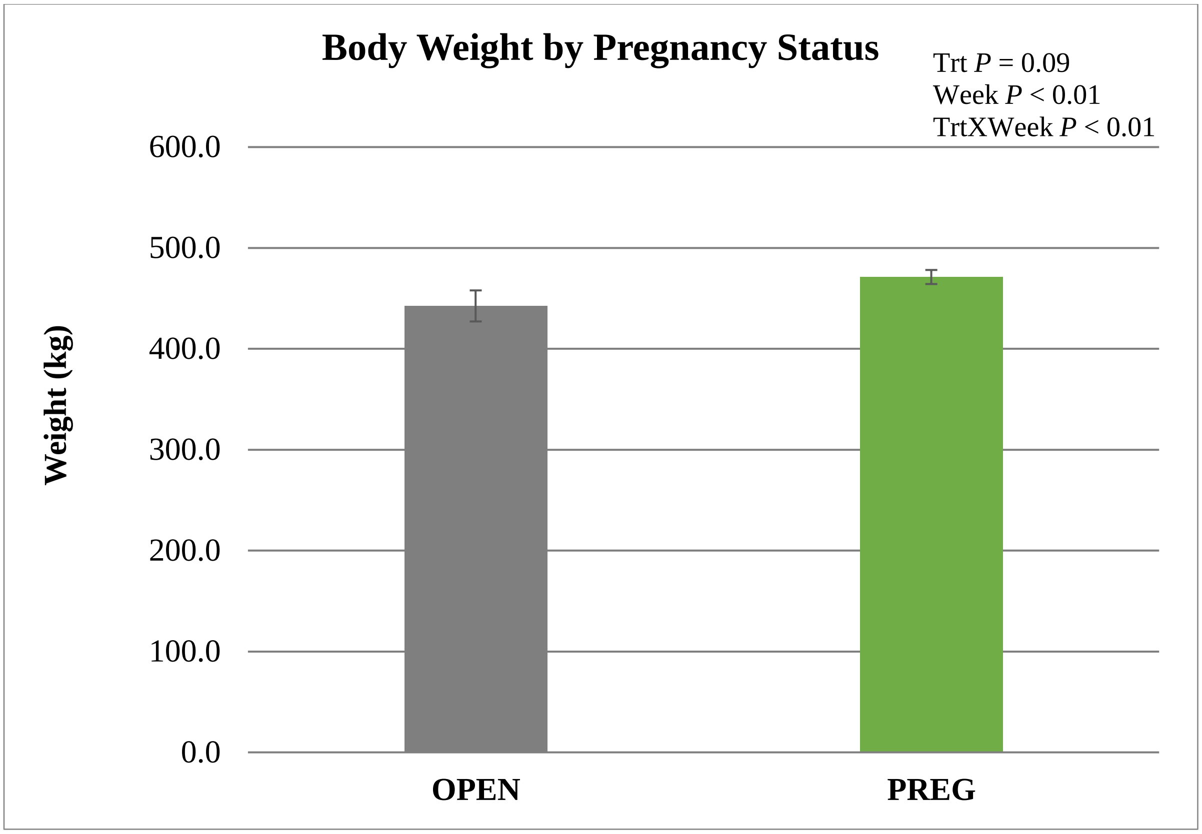


**Figure 3.3.** Body Weight by Pregnancy Status: PREG heifers tended to have greater weights (471.2±7.0 kg vs 442.5±15.4 kg) than their OPEN pairs. Model tended to be significant by treatment, and was significant by time and treatment over time (Trt *P* = 0.09; Week *P* < 0.01; TrtXWeek *P* < 0.01).

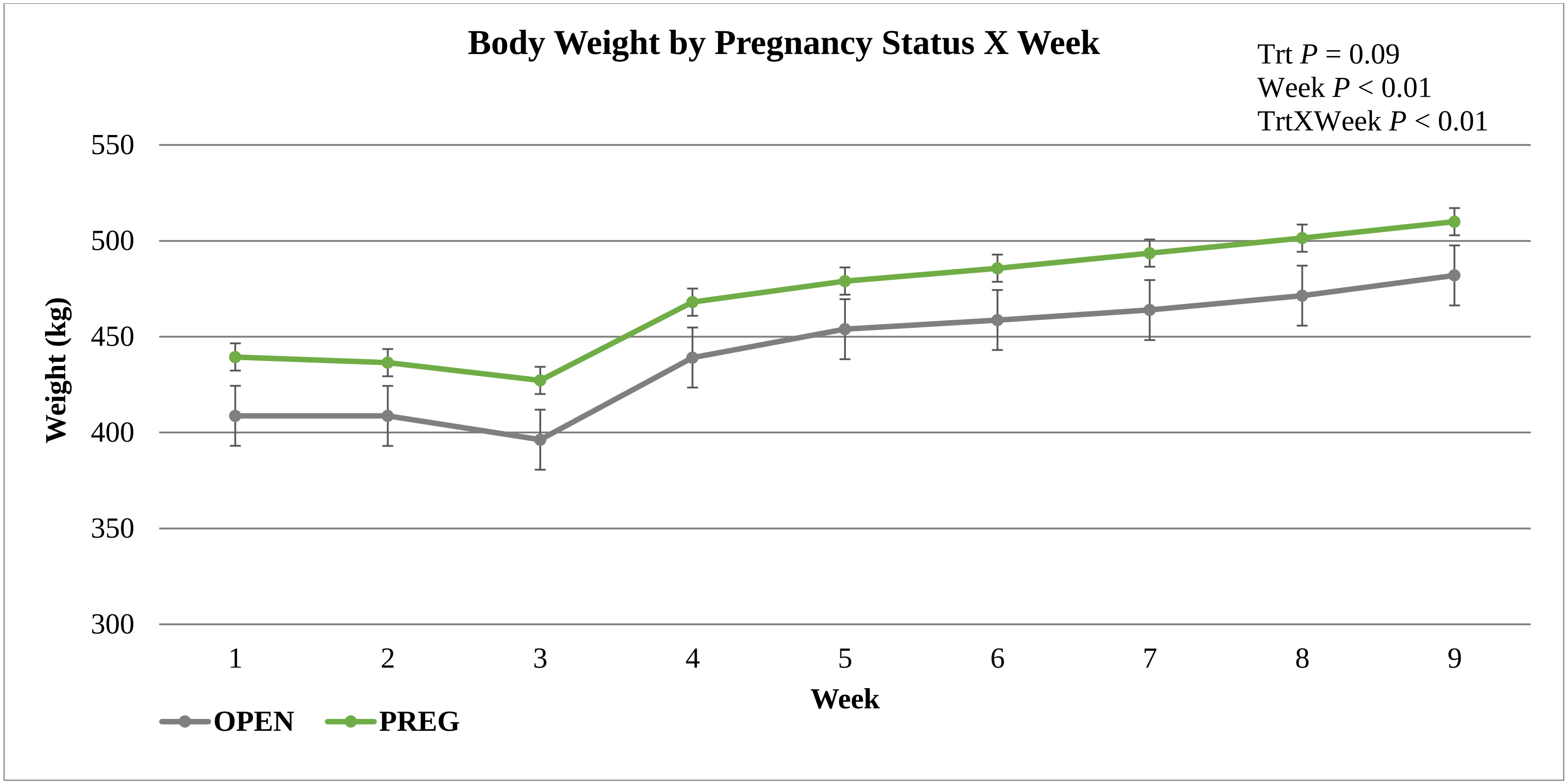


**Figure 3.4.** Body Weight by Pregnancy Status Over Time: PREG heifers maintained greater weights than the OPEN heifers throughout the study. Model tended to be significant by treatment, and was significant by time and treatment over time (Trt $P = 0.09$; Week $P < 0.01$; TrtXWeek $P < 0.01$).

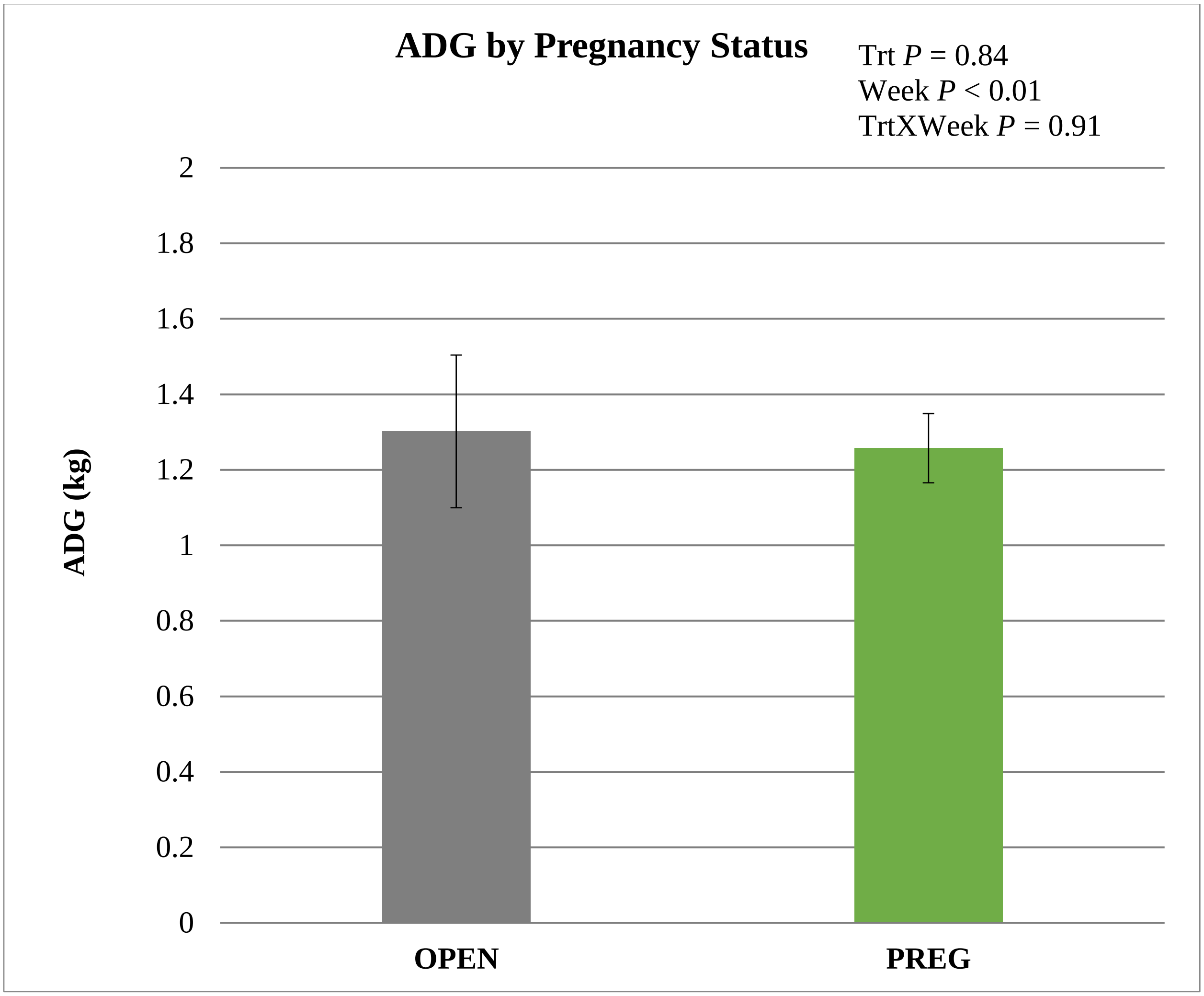


**Figure 3.5.** ADG by Pregnancy Status: No significant differences ($P = 0.84$) were observed between the OPEN and PREG heifers. Model was only significant by week (Trt $P = 0.84$; Week $P < 0.01$; TrtXWeek $P = 0.91$).

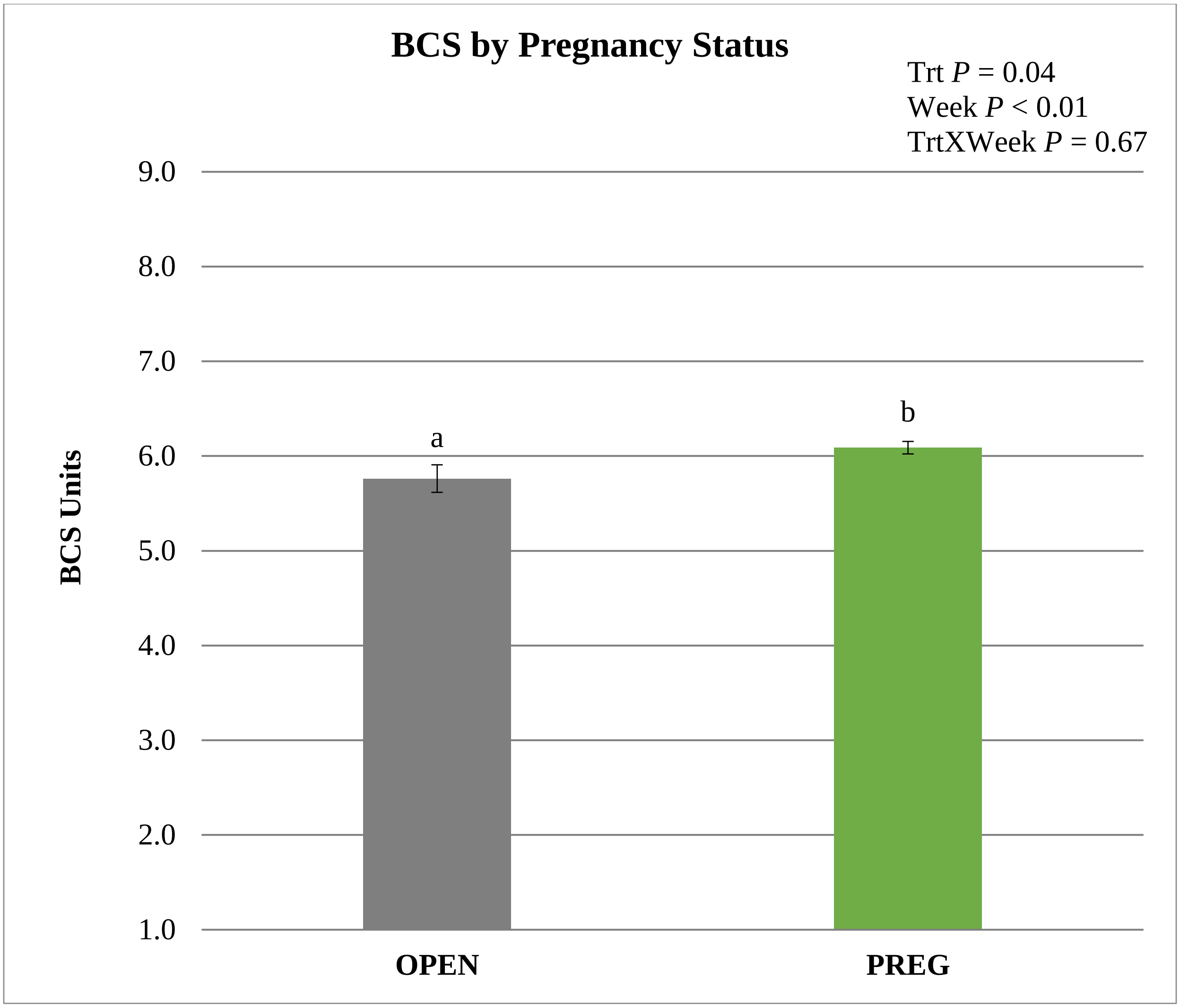


**Figure 3.6.** BCS by Pregnancy Status: The PREG heifers had greater BCS than the OPEN heifers (6.1±0.07 vs 5.8±0.15). The model was significant by treatment and week (Trt $P = 0.04$; Week $P < 0.01$; different letter superscripts indicate statistical difference).

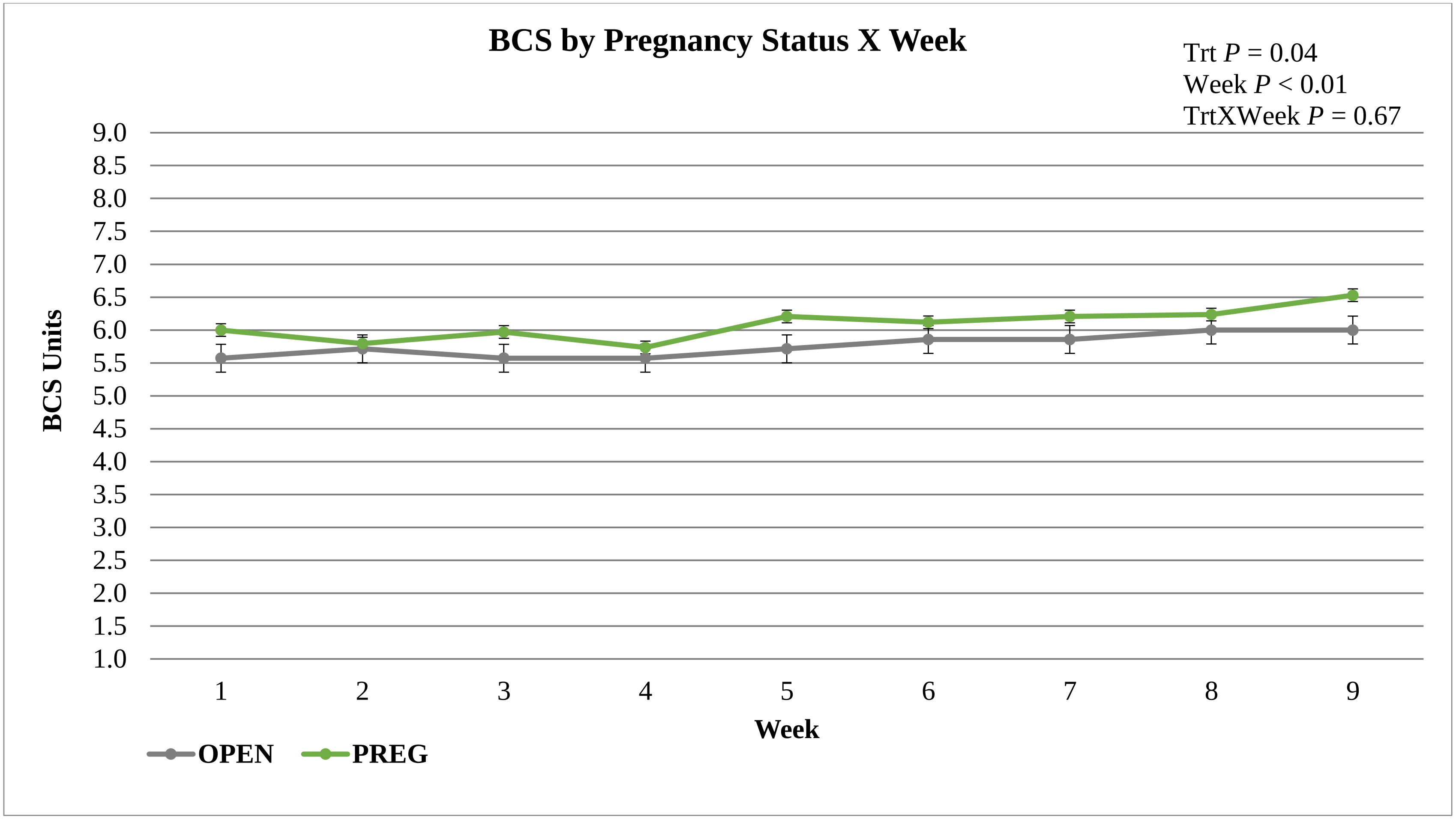


**Figure 3.7.** BCS by Pregnancy Status Over Time: No significant differences were observed in BCS of both heifer groups over time. The model was significant by treatment and week (Trt $P = 0.04$; Week $P < 0.01$).

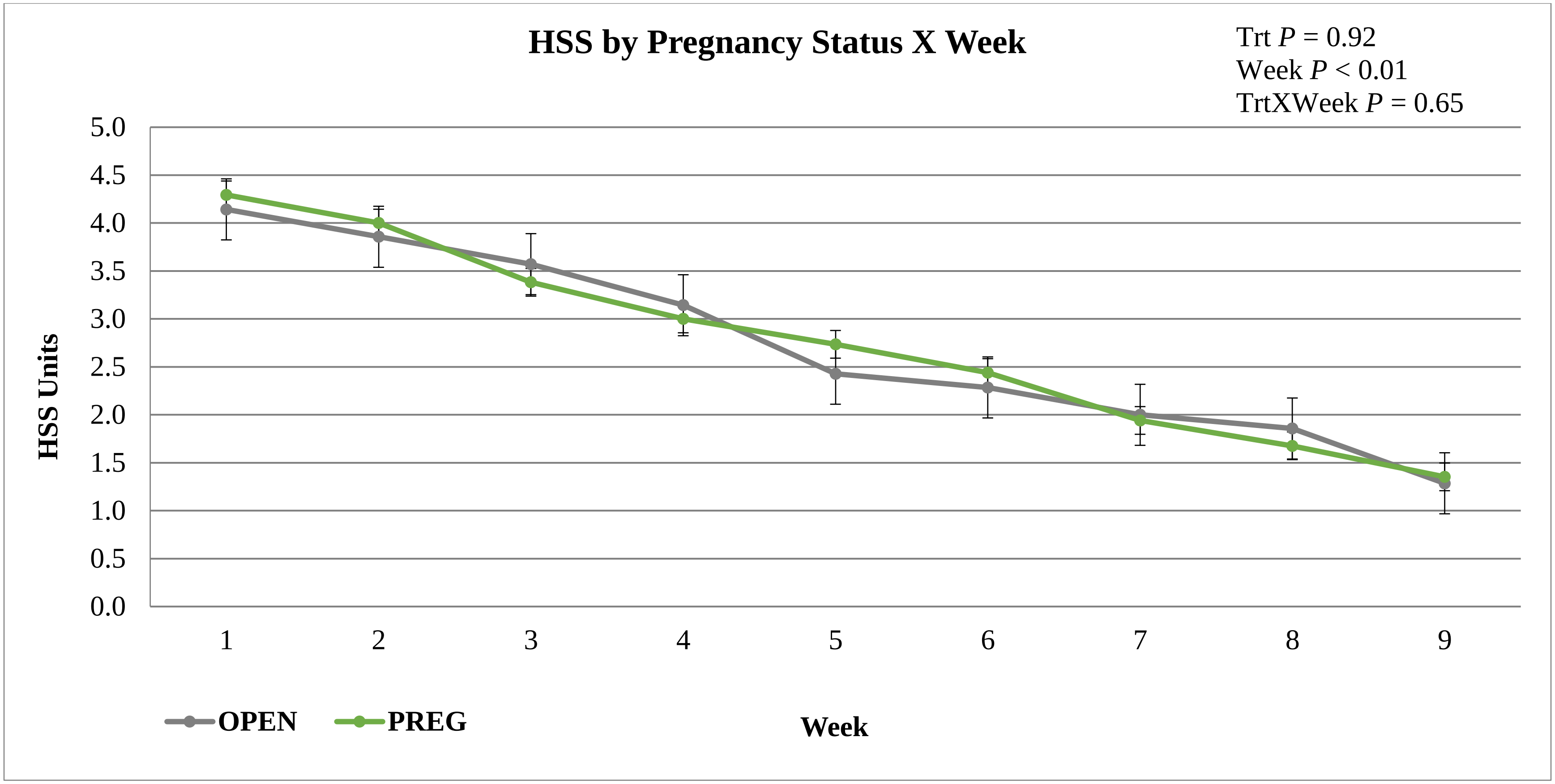


**Figure 3.8.** HSS by Pregnancy Status Over Time: No significant differences were observed in HSS of both heifer groups over time. The model was only significant by week (Week $P < 0.01$).

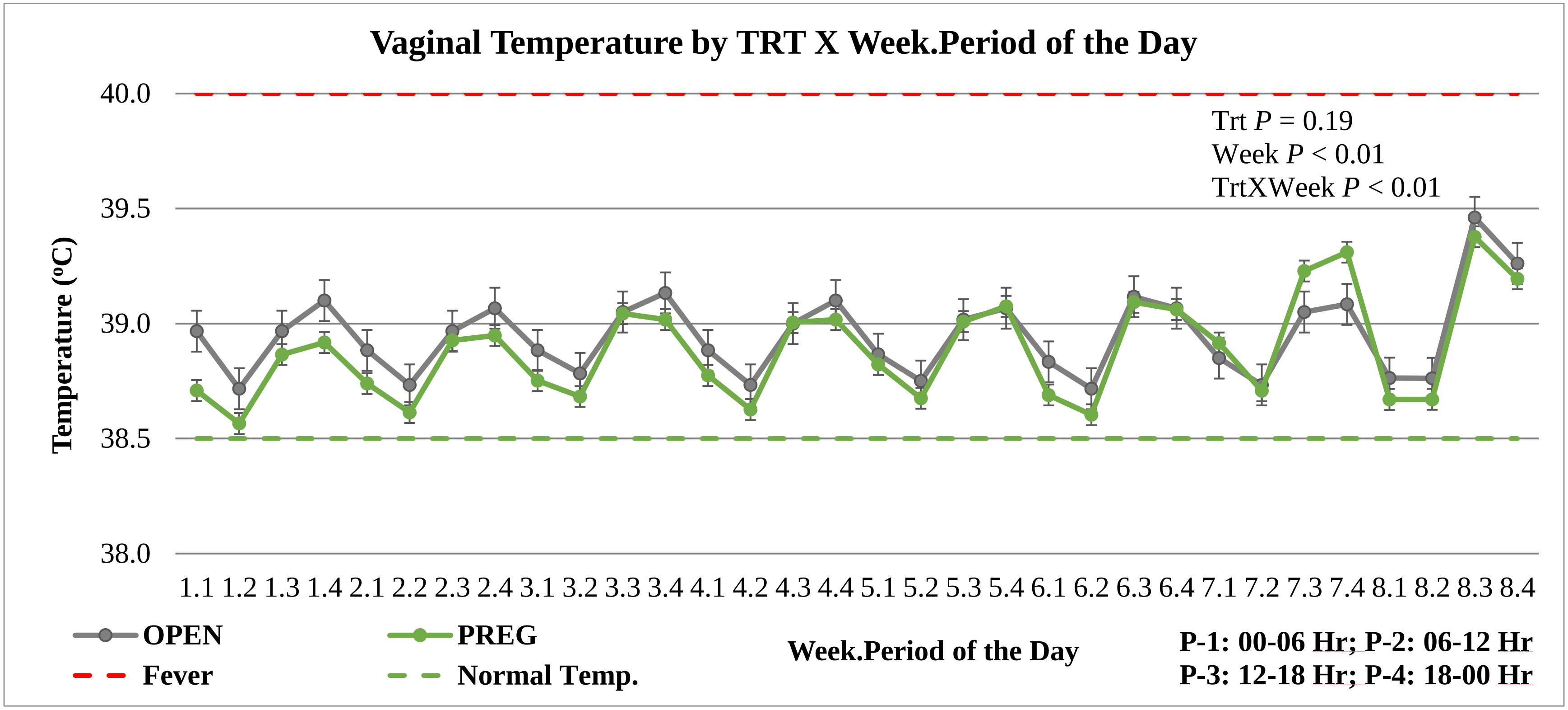


**Figure 3.9.** VT by Pregnancy Status Over Time: Differences in the VT of both groups were observed throughout the study. The model was significant by week and treatment over time (Week $P < 0.01$; TrtXWeek $P < 0.01$).

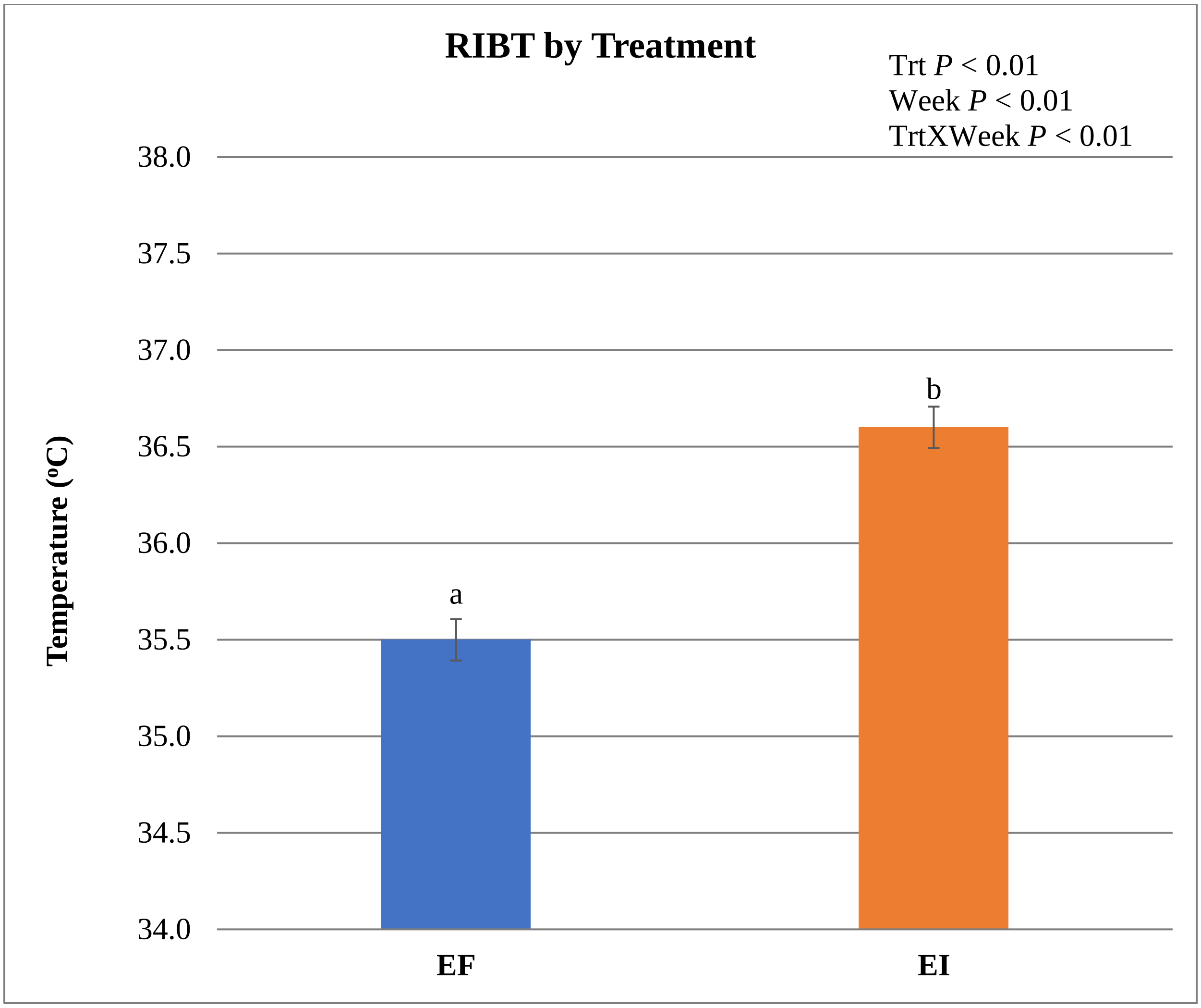


**Figure.3.10.** RIBT by Treatment: The EI heifers had greater RIBT than their EF pairs (36.6±0.1 ºC vs 35.5±0.1 ºC). The model was significant by treatment, week, and treatment over time(Trt $P < 0.01$; Week $P < 0.01$; TrtXWeek $P < 0.01$; different letter superscripts indicate statistical difference).

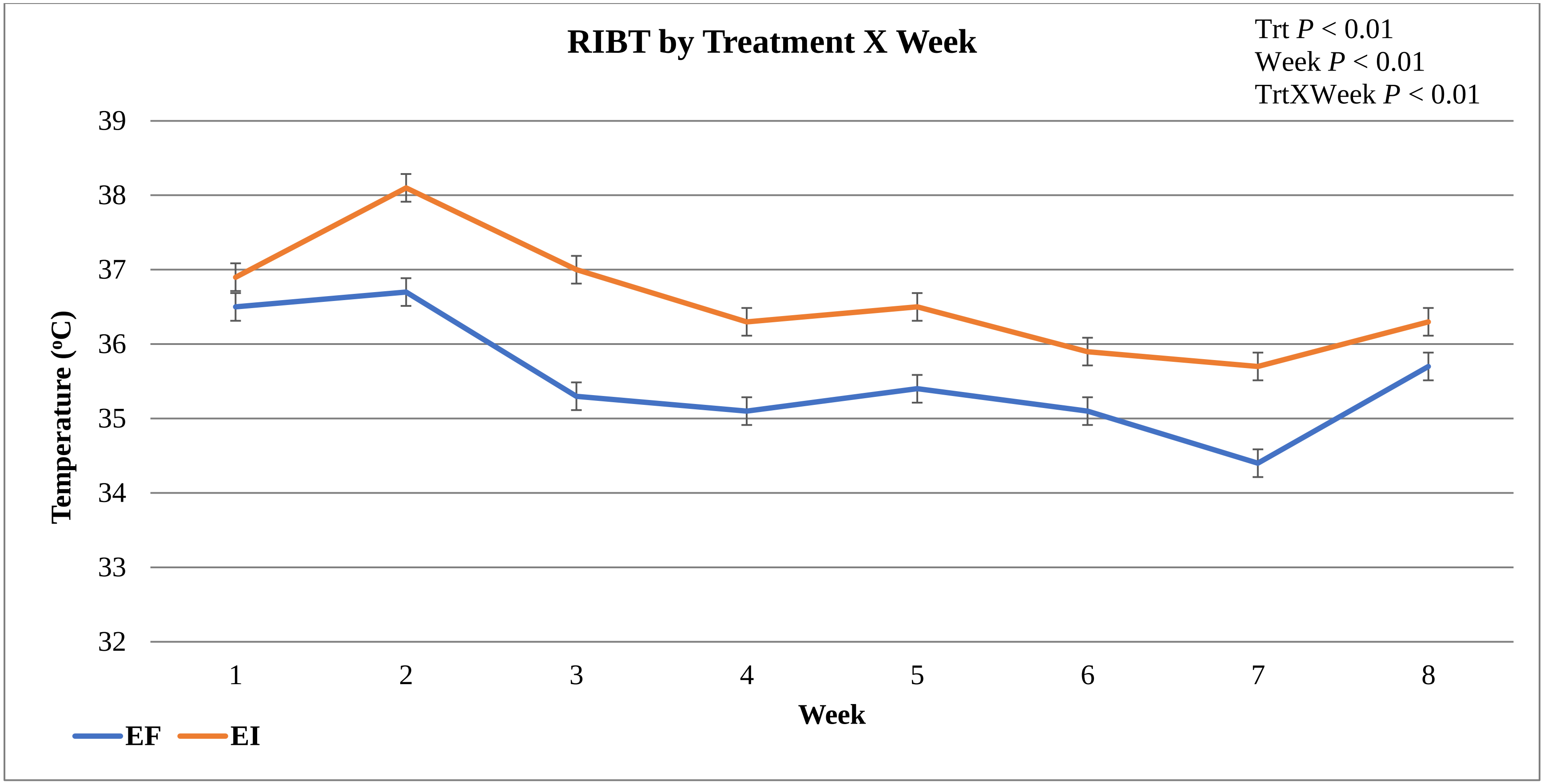


**Figure 3.11.** RIBT by Treatment Over Time: The EI heifers maintained a greater RIBT than the EF heifers throughout the Year I study. The model was significant by treatment, week, and treatment over time(Trt $P < 0.01$; Week $P < 0.01$; TrtXWeek $P < 0.01$).

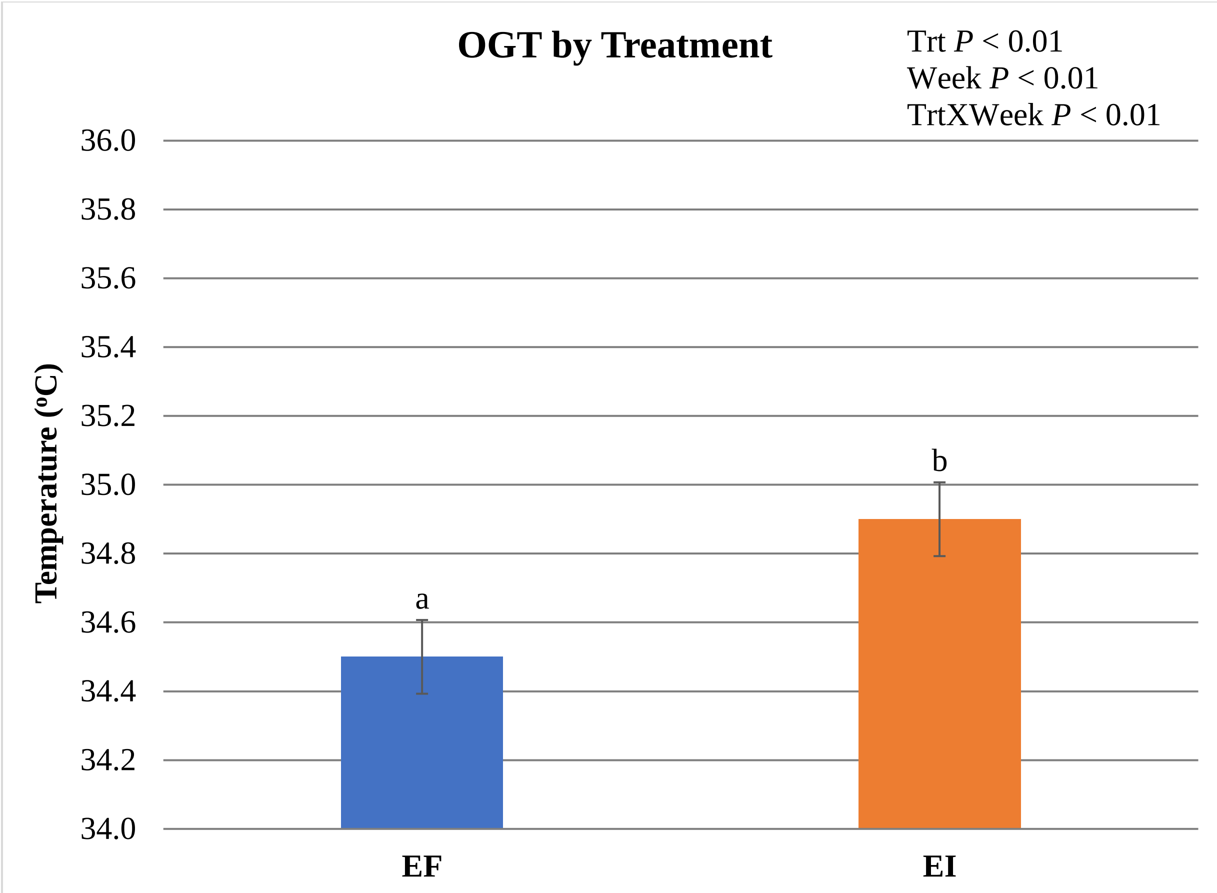


**Figure.3.12.** OGT by Treatment: The EI heifers had greater OGT than their EF pairs (34.9±0.1 ºC vs 34.5±0.1 ºC). The model was significant by treatment, week, and treatment over time(Trt $P < 0.01$; Week $P < 0.01$; TrtXWeek $P < 0.01$; different letter superscripts indicate statistical difference).

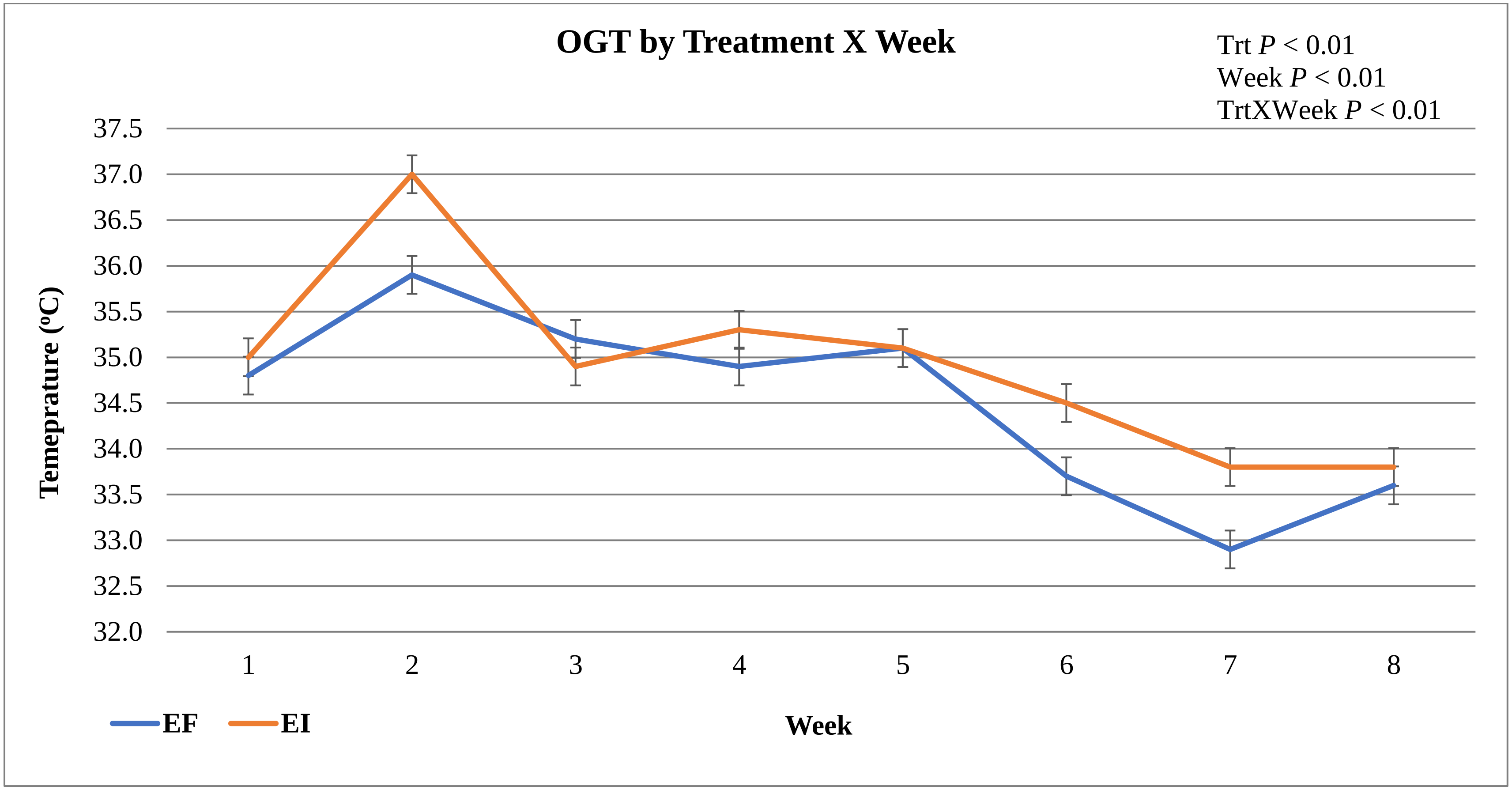


**Figure 3.13.** OGT by Treatment Over Time: The EI heifers maintained a greater OGT than the EF heifers throughout the majority of the study, except in Wk 3 and Wk 5. The model was significant by treatment, week, and treatment over time(Trt $P < 0.01$; Week $P < 0.01$; TrtXWeek $P < 0.01$).

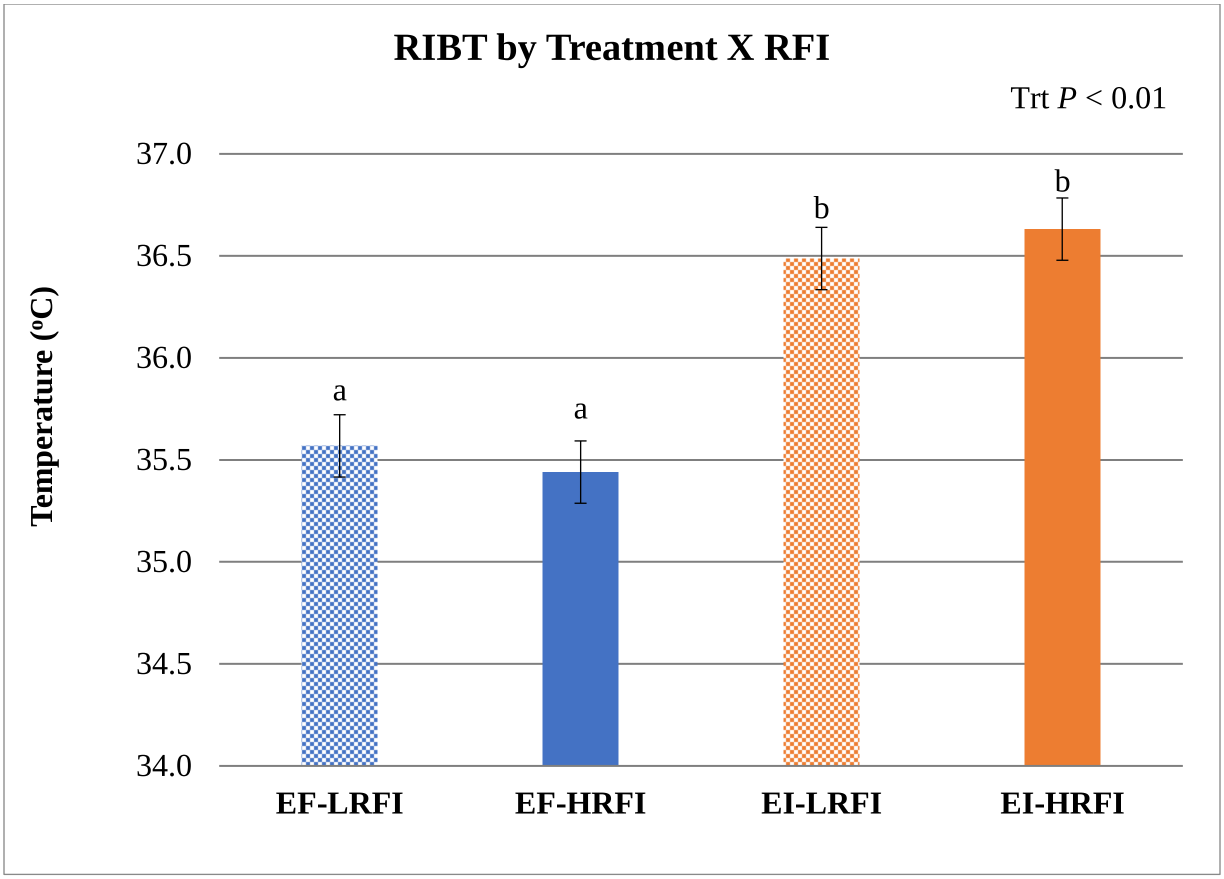


**Figure.3.14.** RIBT by Treatment X RFI ranking: The LRFI and HRFI EI heifers had greater RIBT than their EF pairs, but no differences were observed within treatment groups. The model was significant by treatment (Trt $P < 0.01$; different letter superscripts indicate statistical difference).

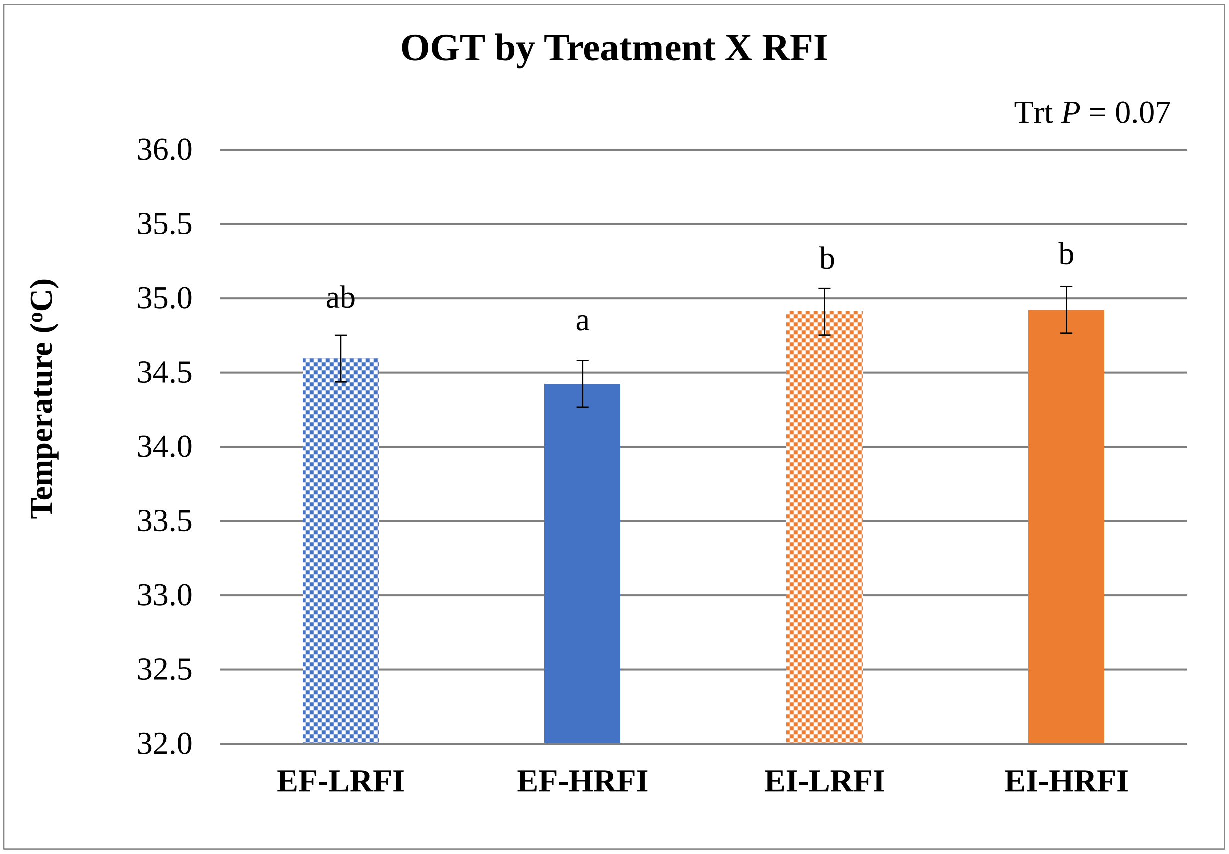


**Figure.3.15.** OGT by Treatment X RFI ranking: The EF-HRFI had lower temperatures than both EI subgroups, whereas the EF-LRFI tended to have similar temperatures to the EI subgroups, and no differences were observed between the EI LRFI and HRFI heifers. The model tended to be significant by treatment (Trt $P = 0.07$; different letter superscripts indicate statistical difference).

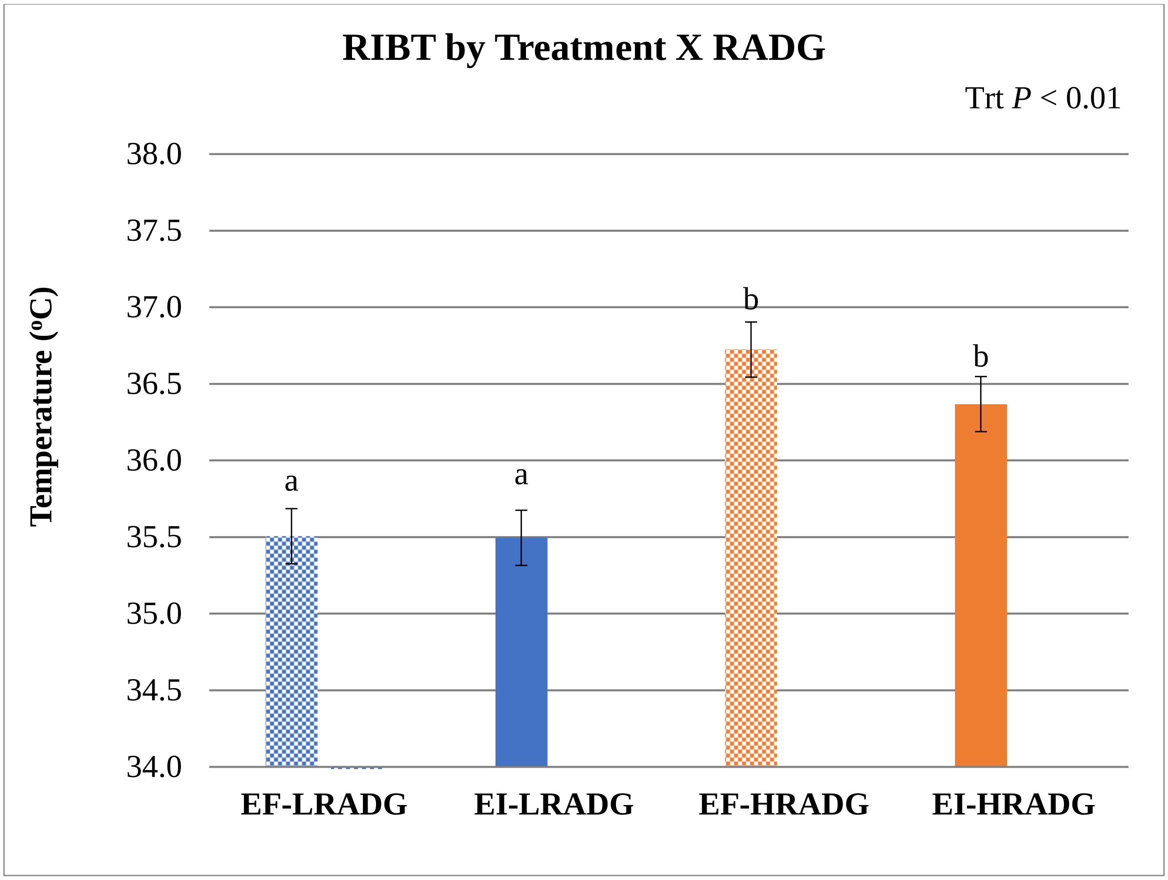


**Figure.3.16.** RIBT by Treatment X RADG ranking: The LRADG and HRADG EI heifers had greater RIBT than their EF pairs, but no differences were observed within treatment groups. The model was significant by treatment (Trt $P < 0.01$; different letter superscripts indicate statistical difference).

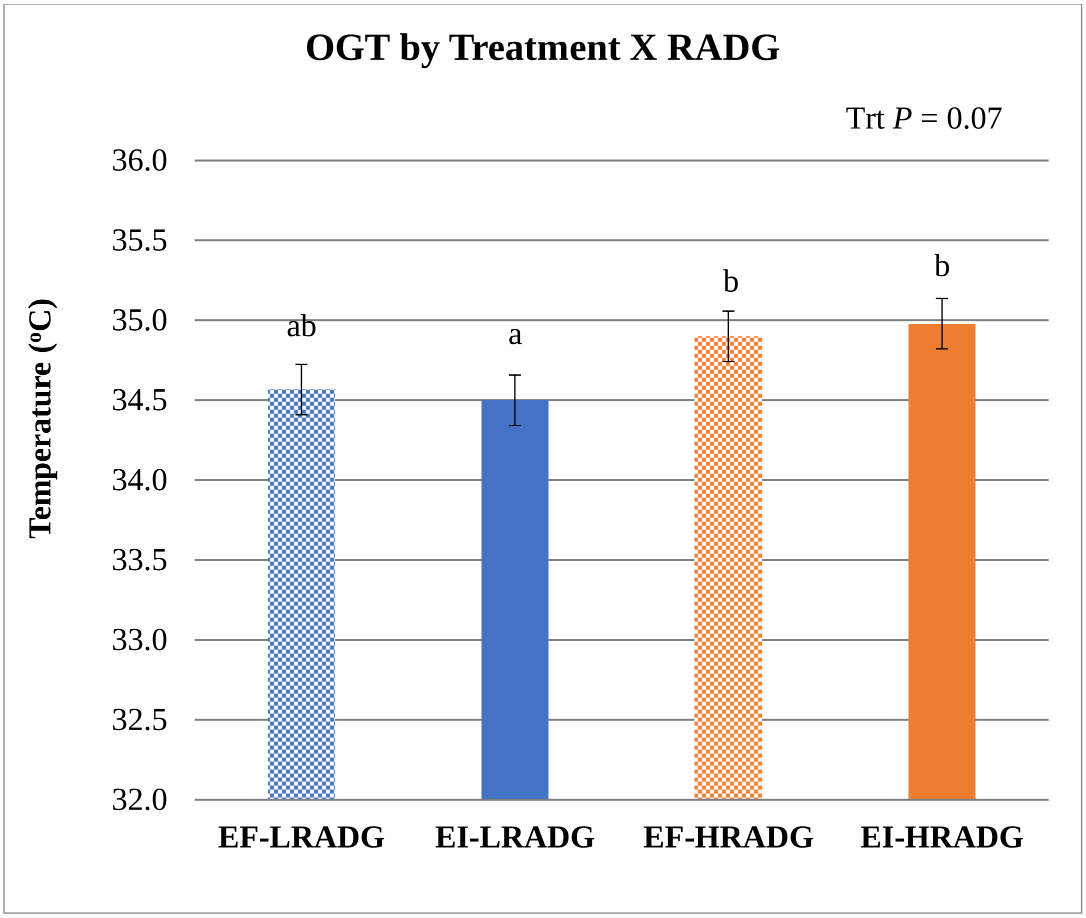


**Figure.3.17.** OGT by Treatment X RADG ranking: The EF-HRADG heifers had lower temperatures than the EI subgroups, whereas the EF-LRADG tended to have similar temperatures to the EI subgroups, and no differences were observed between the EI-LRADG and HRADG heifers. The model tended to be significant by treatment (Trt $P$ = 0.07; different letter superscripts indicate statistical difference).

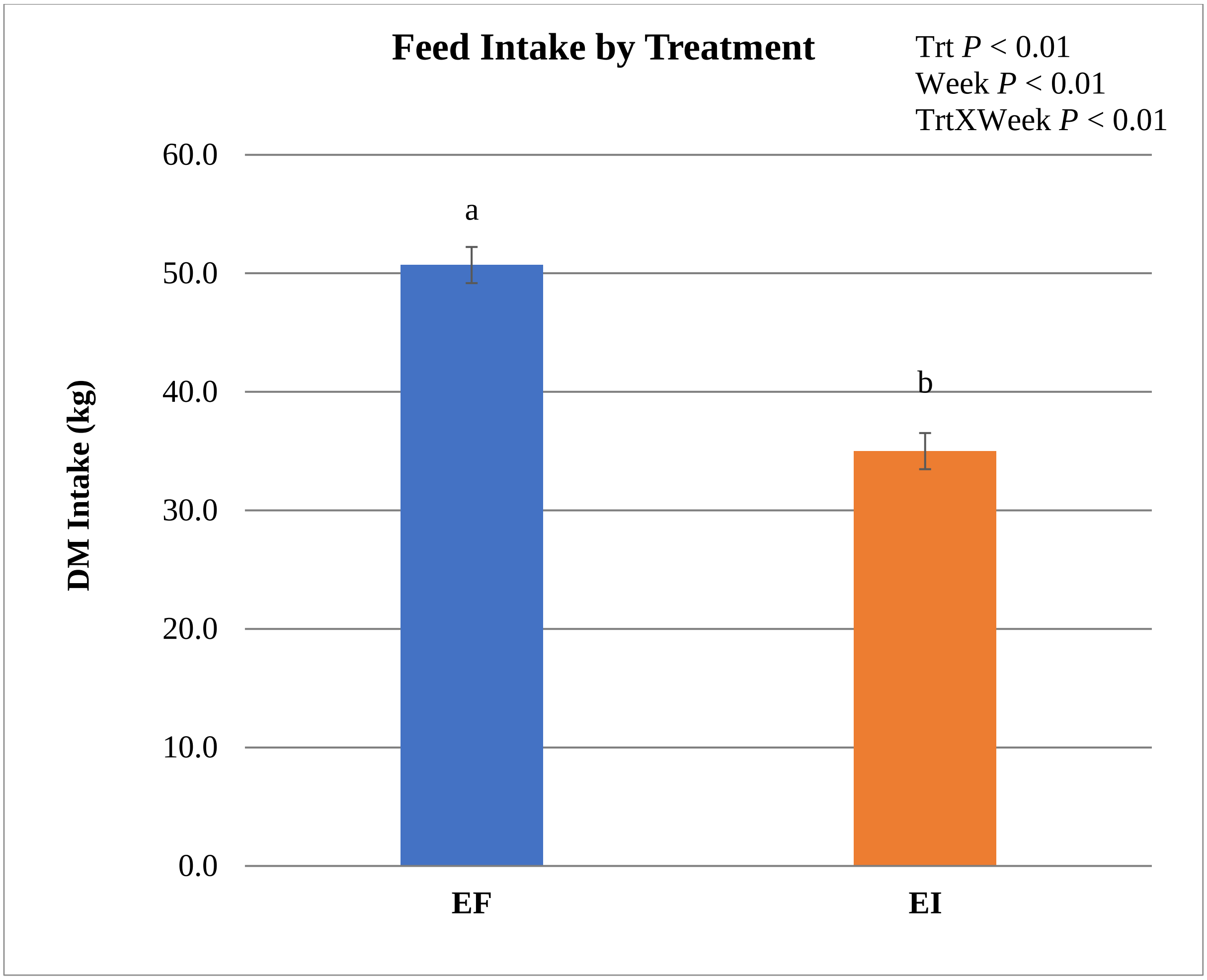


**Figure.3.18.** Feed Intake by Treatment: The EF heifers had greater feed consumption than the EI heifers (50.7±1.5 kg DMI vs 35.0±1.5 kg DMI). The model was significant by treatment, week, and treatment over time (Trt $P < 0.01$; Week $P < 0.01$; TrtXWeek $P < 0.01$; different letter superscripts indicate statistical difference).

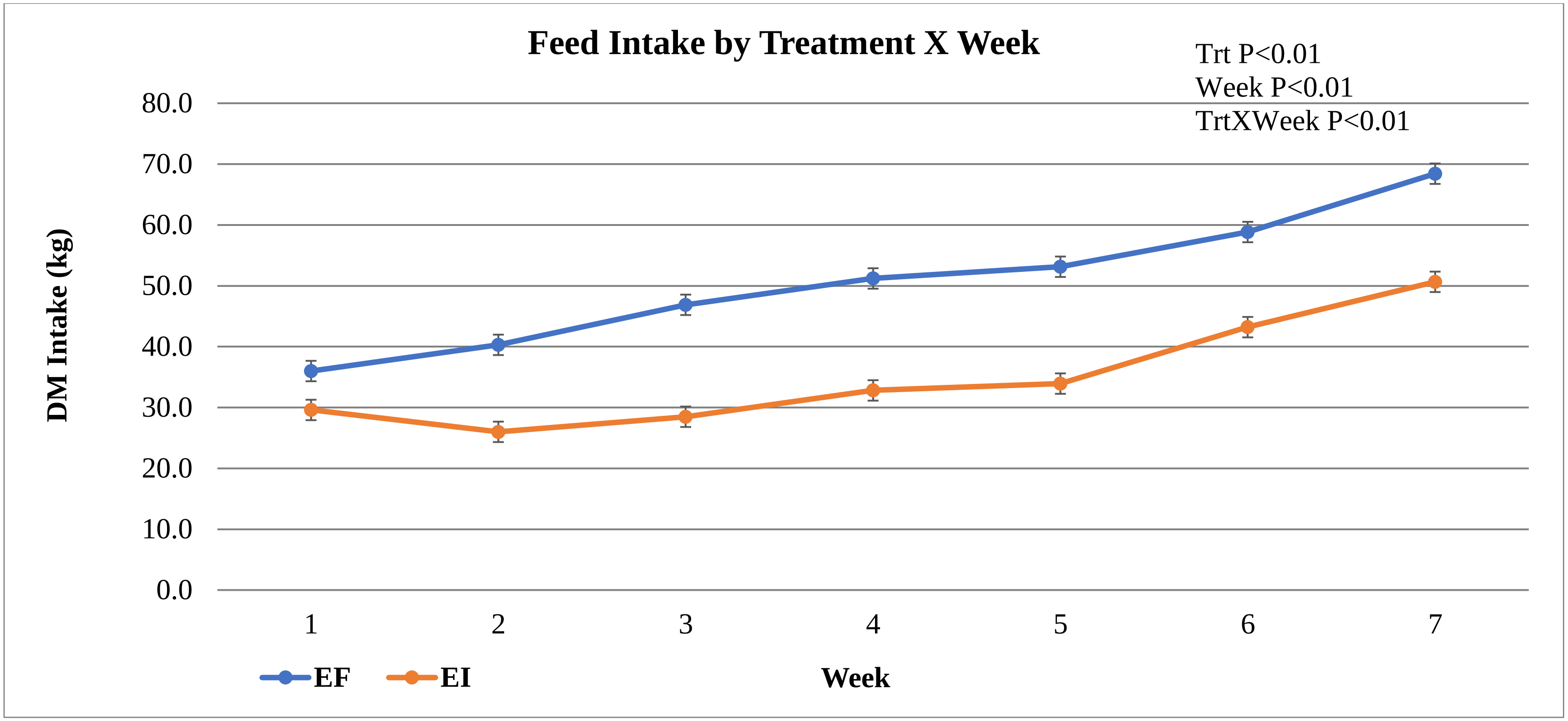


**Figure.3.19.** Feed Intake by Treatment X Week: The EF heifers maintained a greater feed consumption than the EI heifers throughout the entire study. The model was significant by treatment, week, and treatment over time (Trt $P < 0.01$; Week $P < 0.01$; TrtXWeek $P < 0.01$).

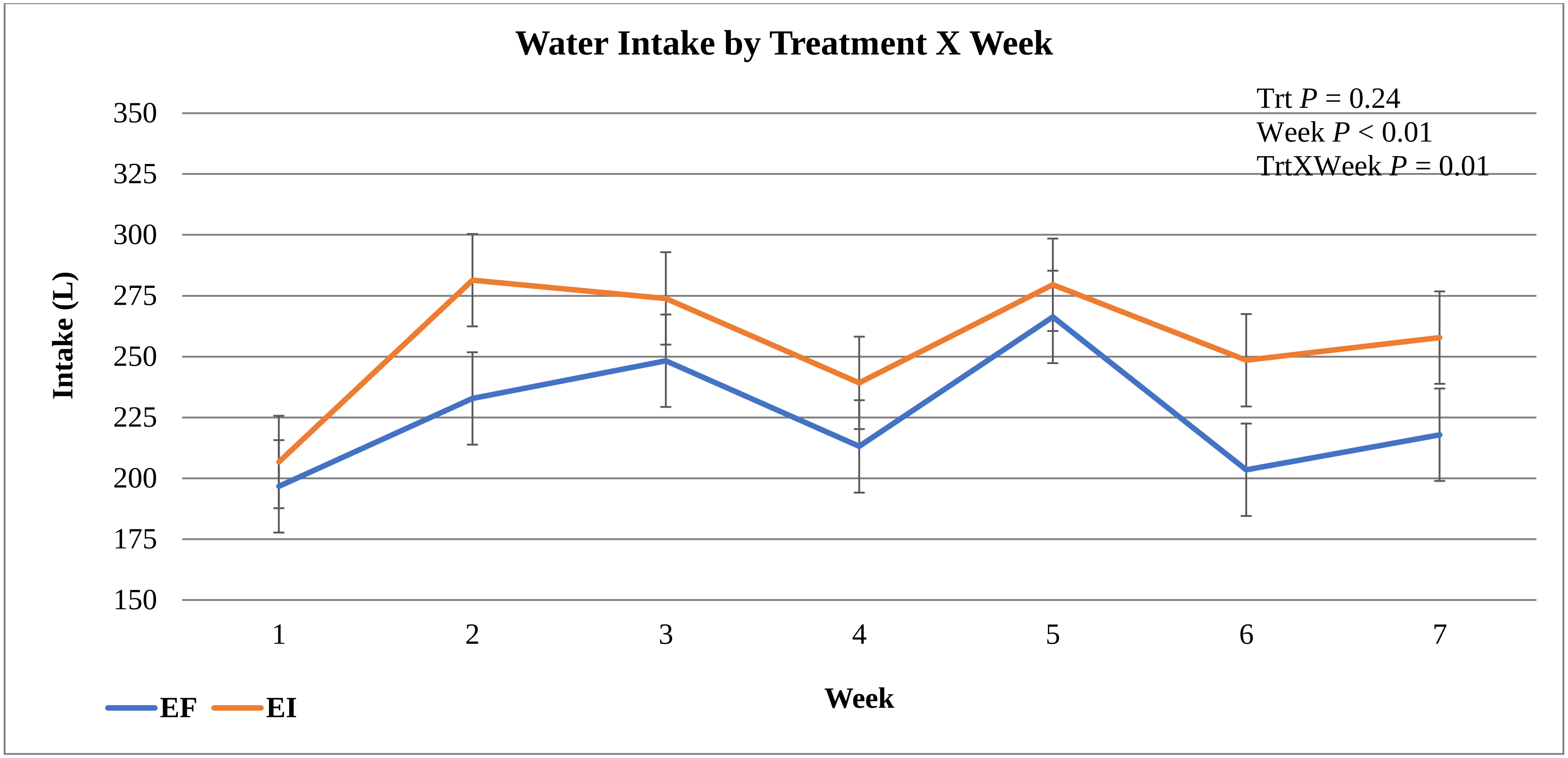


**Figure.3.20.** Water Intake by Treatment X Week: The EI heifers had a greater water consumption than the EF heifers over time. The model was significant by week and treatment over time (Trt $P = 0.24$; Week $P < 0.01$; TrtXWeek $P < 0.01$).

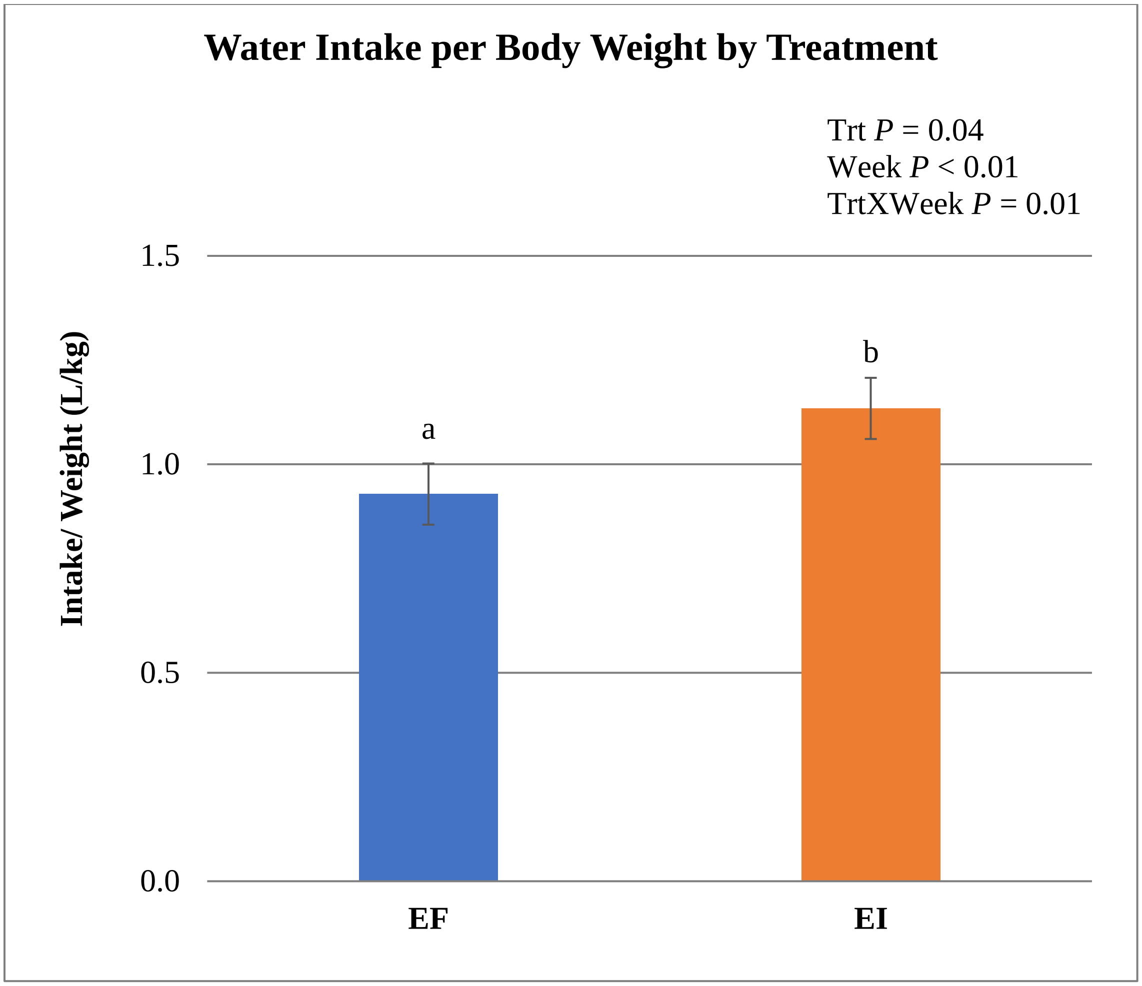


**Figure 3.21.** Water Intake per Body Weight by Treatment: The EI heifers reported a greater WI/BW than the EF heifers (1.1±0.07 L/kg vs 0.9±0.07 L/kg). The model was significant by treatment, week, and treatment over time (Trt $P = 0.04$; Week $P < 0.01$; TrtXWeek $P = 0.01$; different letter superscripts indicate statistical difference).

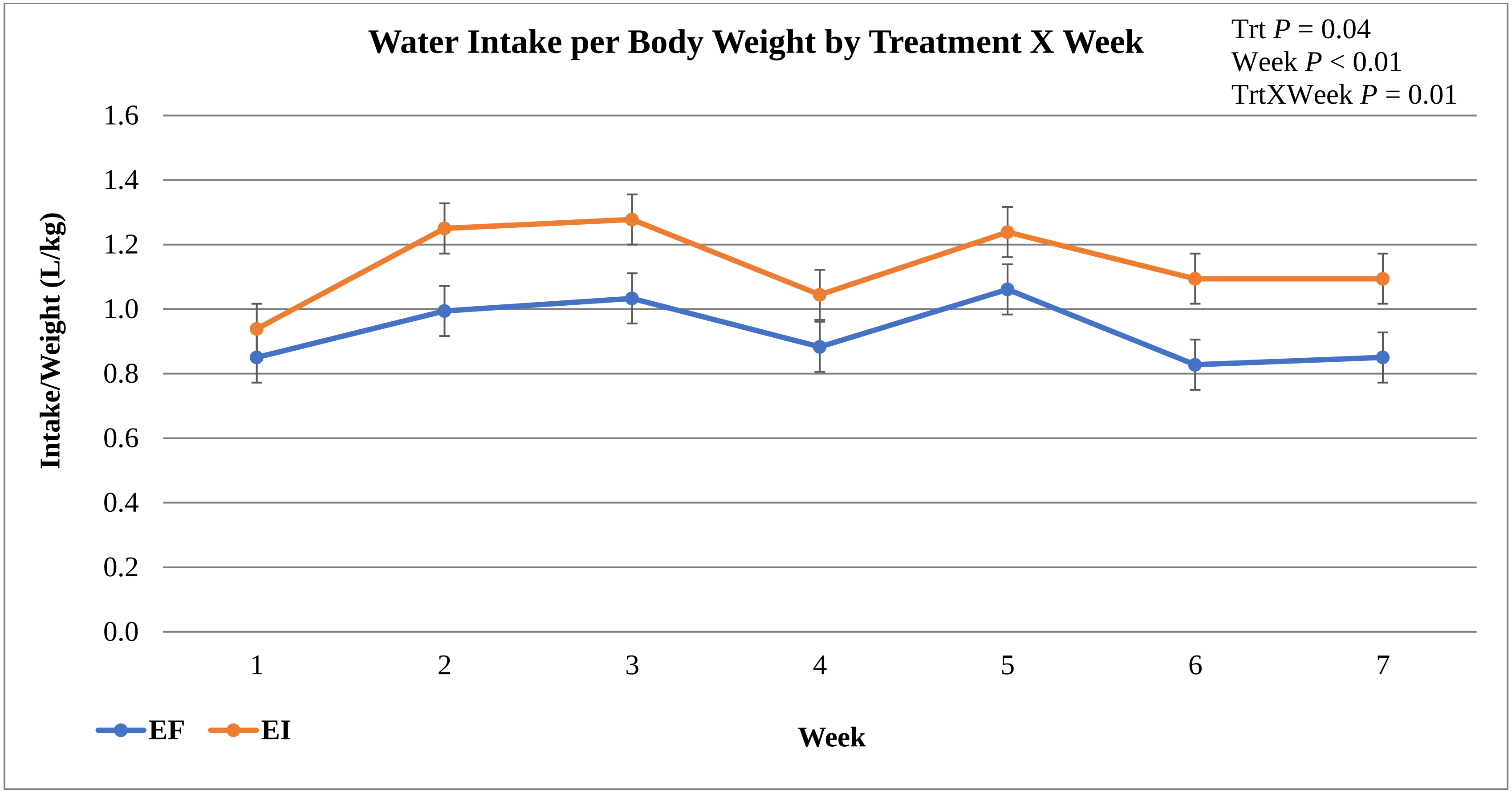


**Figure 3.22.** Water Intake per Body Weight by Treatment X Week: The EI heifers had a greater WI/BW than the EF heifers over time. The model was significant by treatment, week, and treatment over time (Trt $P = 0.04$; Week $P < 0.01$; TrtXWeek $P = 0.01$).

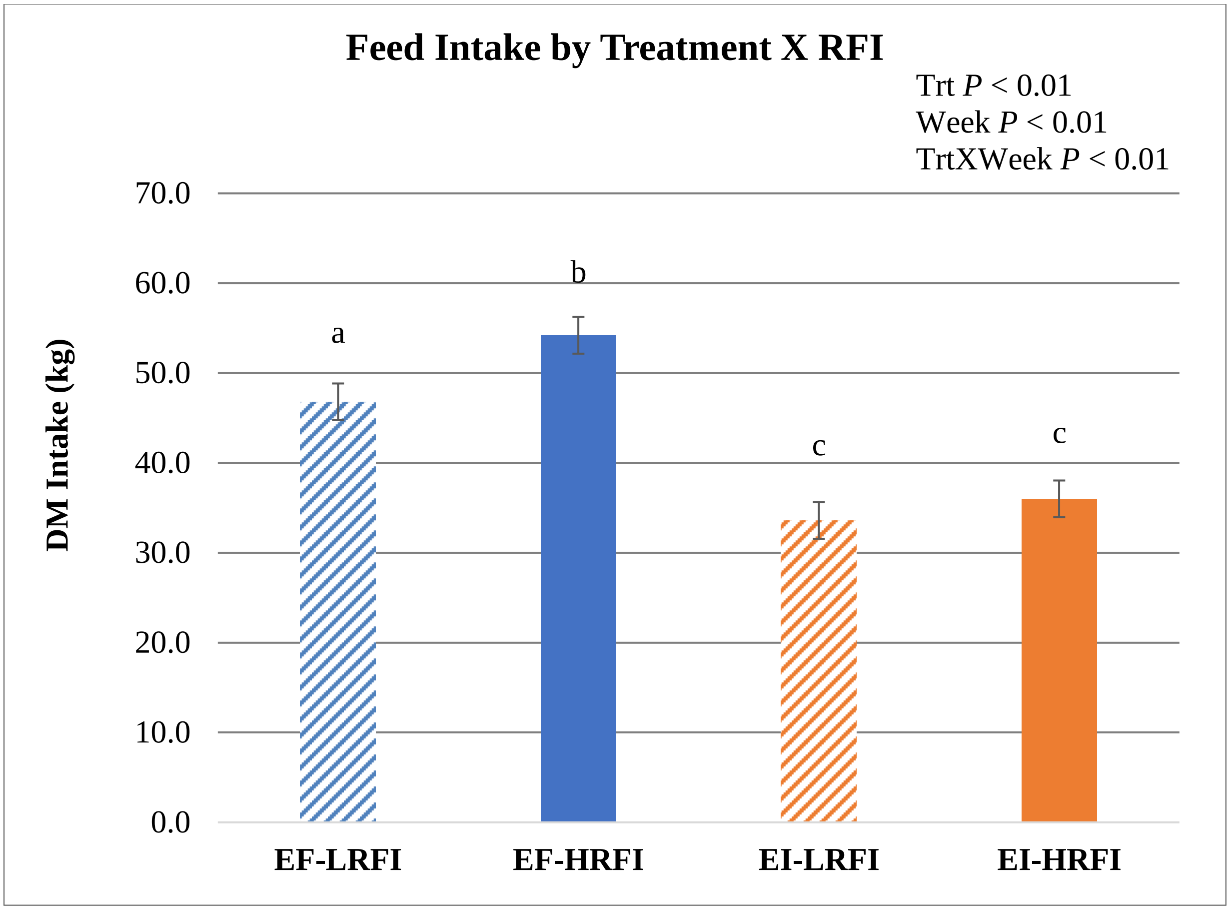


**Figure.3.23.** Feed Intake by Treatment X RFI: The LRFI subgroup of EF heifers had a lower FI than their HRFI pairs (46.8±2.0 kg DMI vs 54.2±2.0 kg DMI), whereas no differences were observed between the EI subgroups. The model was significant by treatment, week, and treatment over time (Trt $P < 0.01$; Week $P < 0.01$; TrtXWeek $P < 0.01$; different letter superscripts indicate statistical difference).

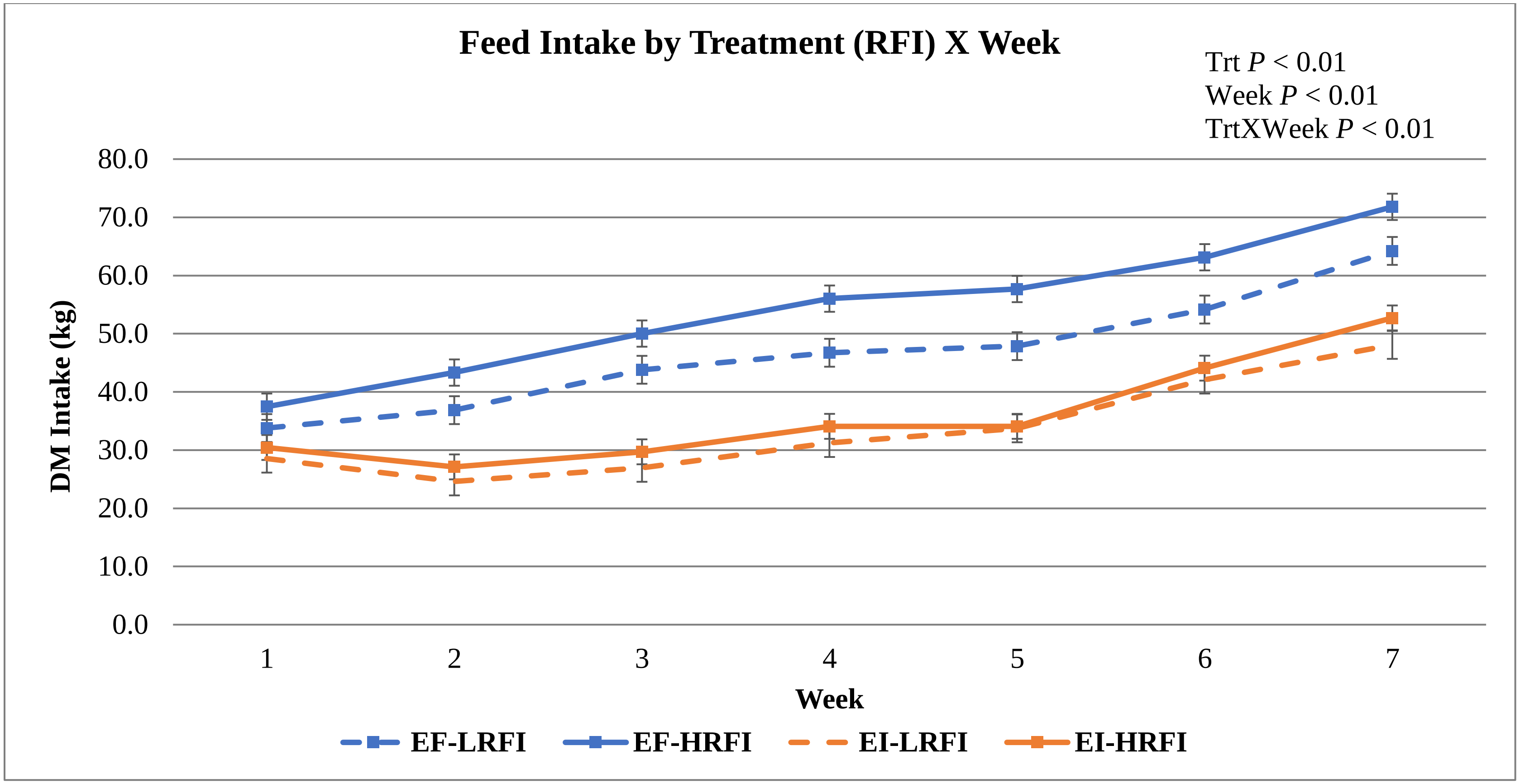


**Figure.3.24.** Feed Intake by Treatment X RFI over time: The LRFI subgroup of EF heifers had a lower FI than their HRFI pairs whereas no differences were observed between the EI subgroups throughout the study. The model was significant by treatment, week, and treatment over time (Trt $P < 0.01$; Week $P < 0.01$; TrtXWeek $P < 0.01$).

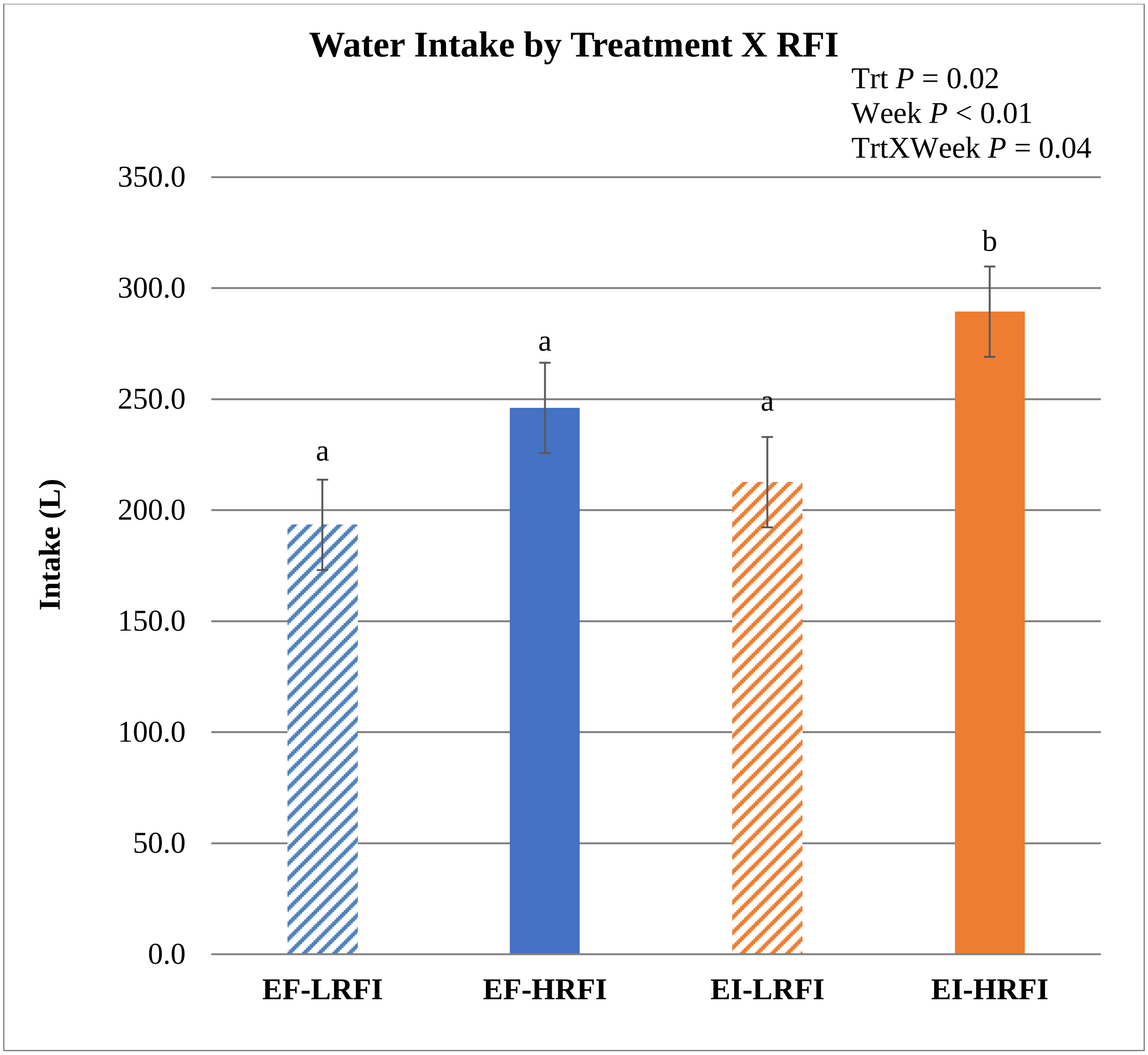


**Figure.3.25.** Water Intake by Treatment X RFI: The LRFI subgroup of EI heifers had a lower WI than their HRFI pairs (212.6±20.3 L vs 289.4±20.3 L), whereas no differences were observed between the EF subgroups. The model was significant by treatment, week, and treatment over time (Trt $P = 0.02$; Week $P < 0.01$; TrtXWeek $P = 0.04$; different letter superscripts indicate statistical difference).

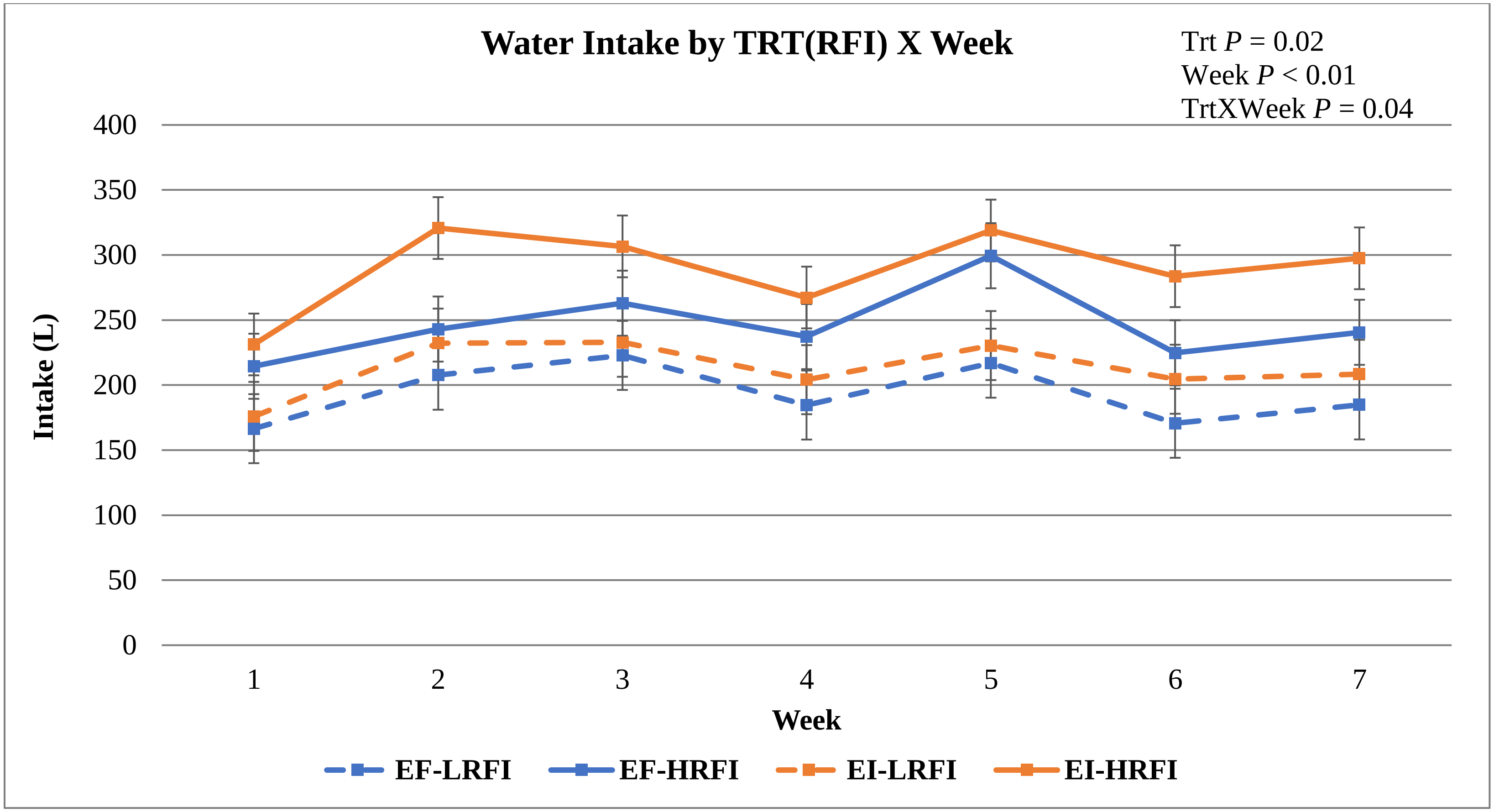


**Figure.3.26.** Water Intake by Treatment X RFI over time: The LRFI subgroups of heifers had lower consumption than their respective HRFI pairs throughout the study. The model was significant by treatment, week, and treatment over time (Trt $P = 0.02$; Week $P < 0.01$; TrtXWeek $P = 0.04$; different letter superscripts indicate statistical difference).

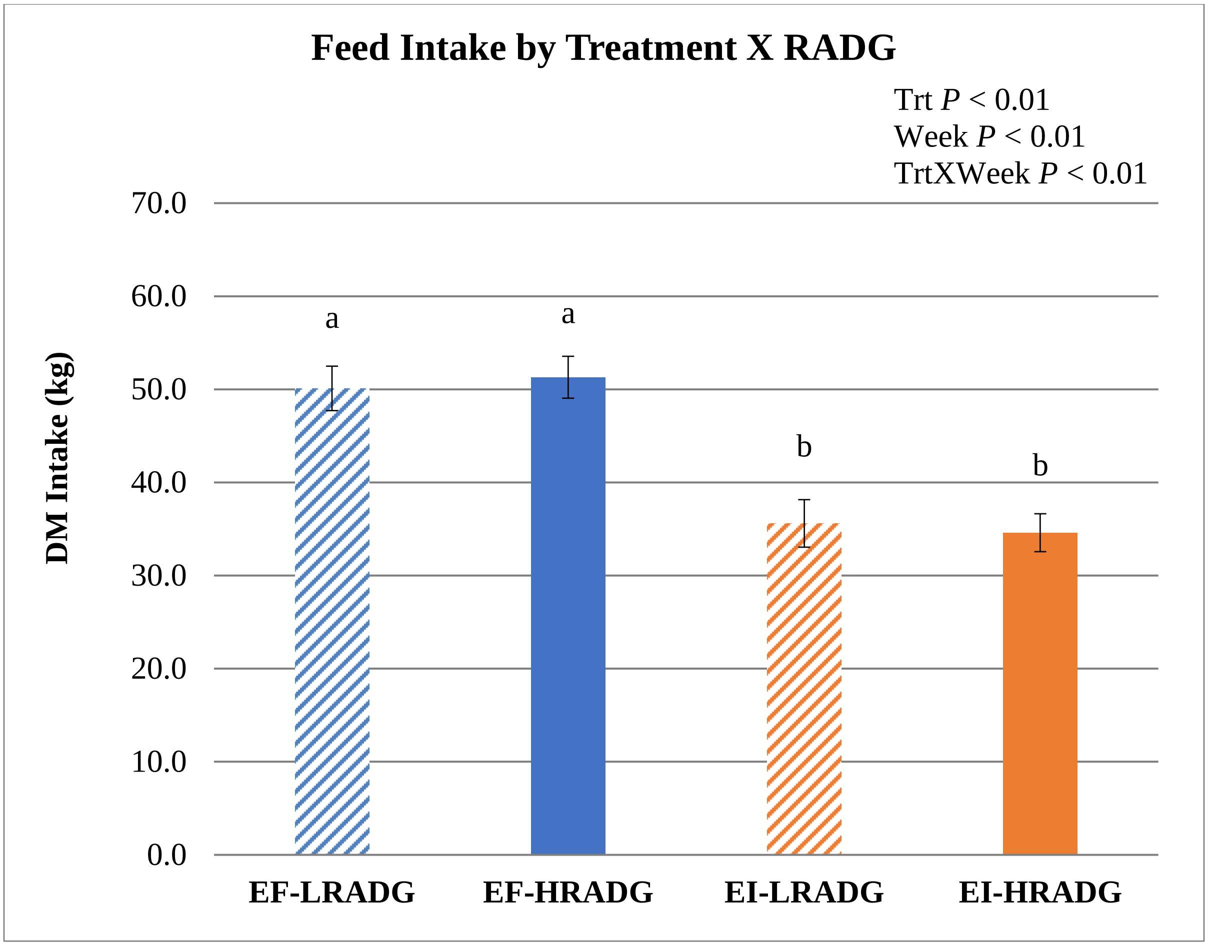


**Figure.3.27.** Feed Intake by Treatment X RADG: The LRADG and HRADG subgroups of EF heifers had a greater FI when compared to their EI pairs, whereas no differences were observed between the subgroups within each treatment group. The model was significant by treatment, week, and treatment over time (Trt $P < 0.01$; Week $P < 0.01$; TrtXWeek $P < 0.01$; different letter superscripts indicate statistical difference).

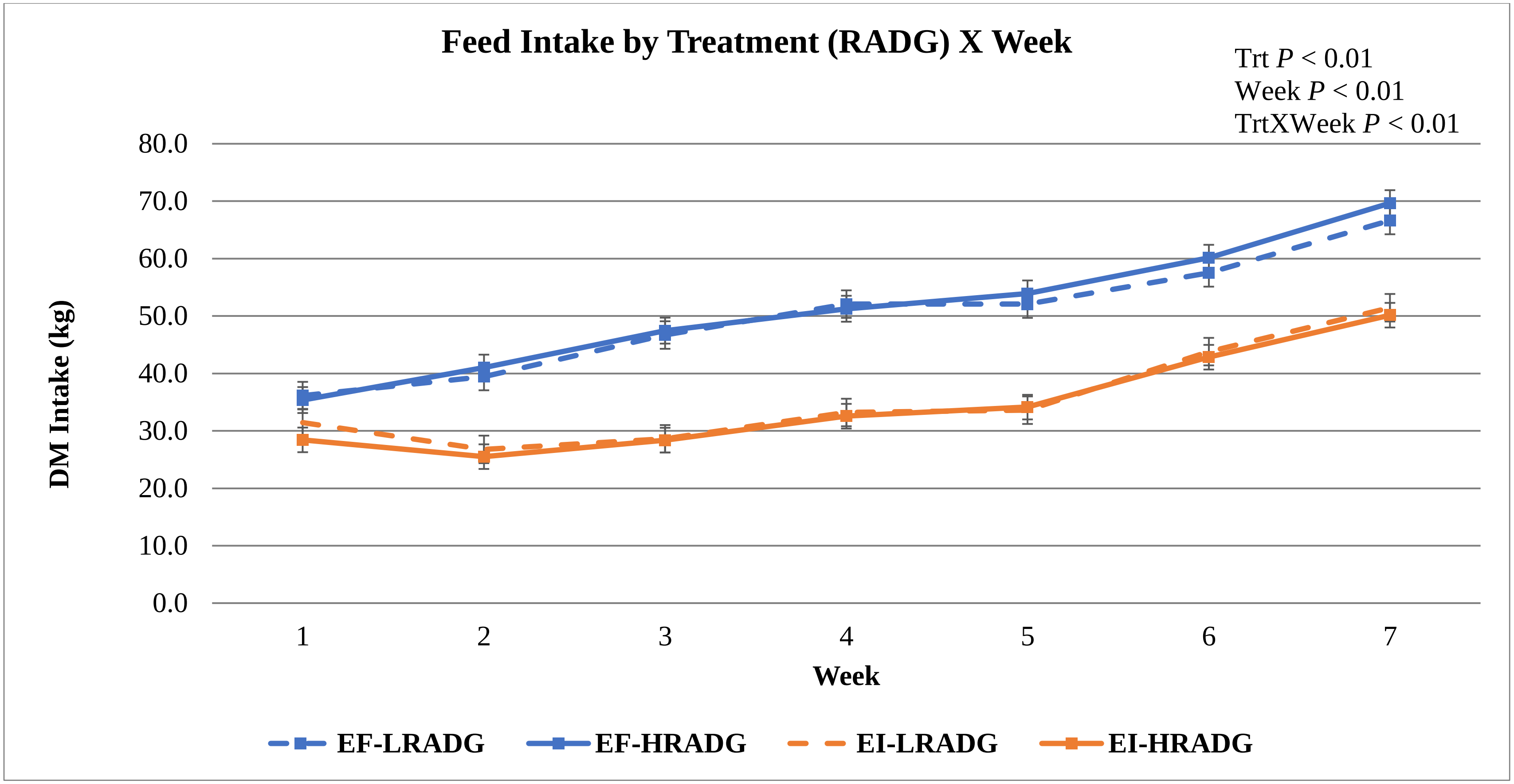


**Figure.3.28.** Feed Intake by Treatment X RADG over time: The LRADG and HRADG subgroups of EF heifers had a greater FI when compared to their EI pairs over time. The model was significant by treatment, week, and treatment over time (Trt $P < 0.01$; Week $P < 0.01$; TrtXWeek $P < 0.01$).

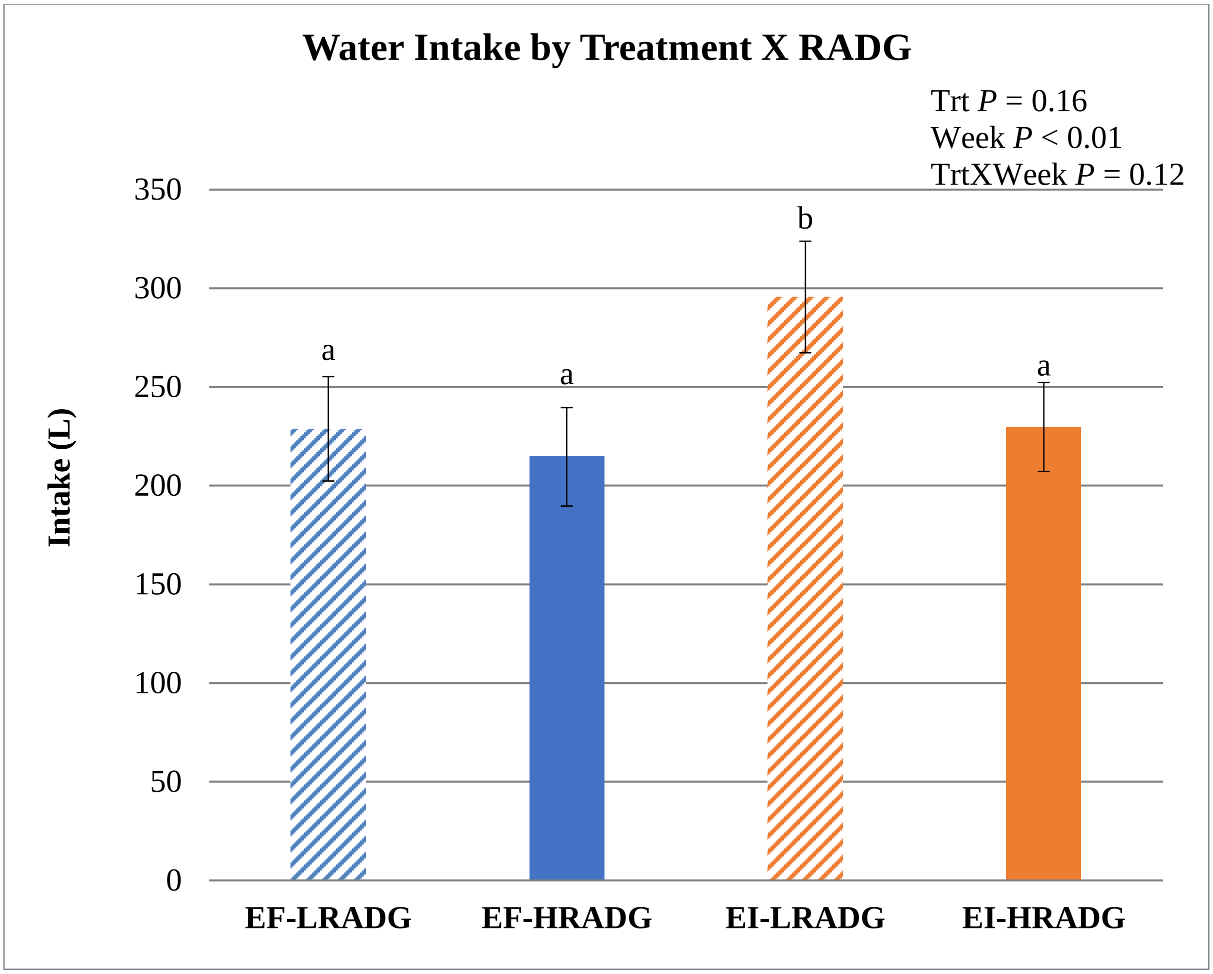


**Figure.3.29.** Water Intake by Treatment X RADG: The LRADG heifers in the EI group had greater WI than their HRADG pairs (295.6±28.3 L vs 229.7±22.5 L), whereas no differences were observed between the subgroups of EF heifers. The model was significant by week (Trt $P$ = 0.16; Week $P < 0.01$; TrtXWeek $P$ = 0.12; different letter superscripts indicate statistical difference).

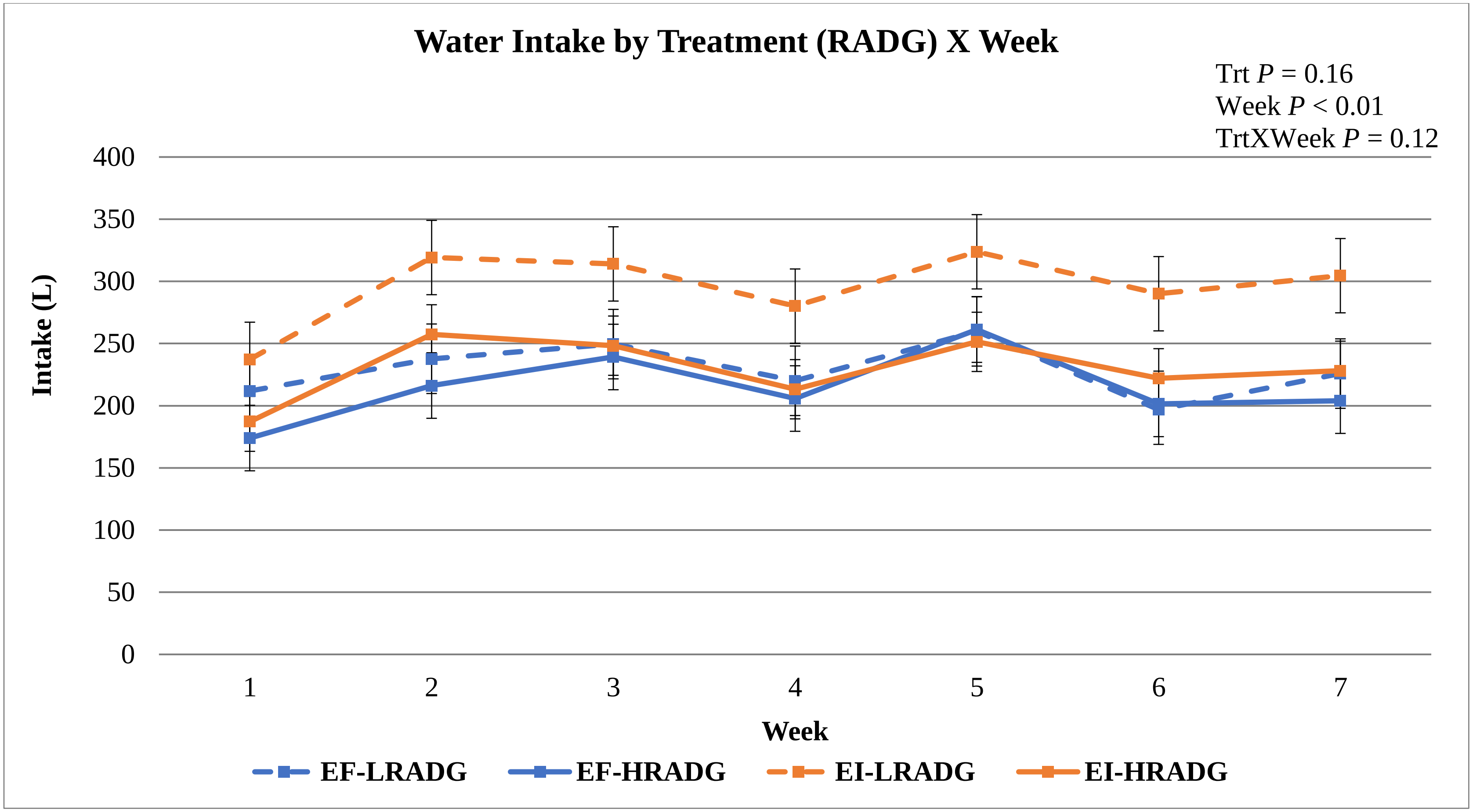


**Figure.3.30.** Water Intake by Treatment X RADG over time: The LRADG heifers in the EI group had greater WI than their HRADG pairs, and the HRADG heifers in the EI group maintained similar WI than both subgroups of EF heifers throughout the study. The model was significant by week (Trt $P = 0.16$; Week $P < 0.01$; TrtXWeek $P = 0.12$).

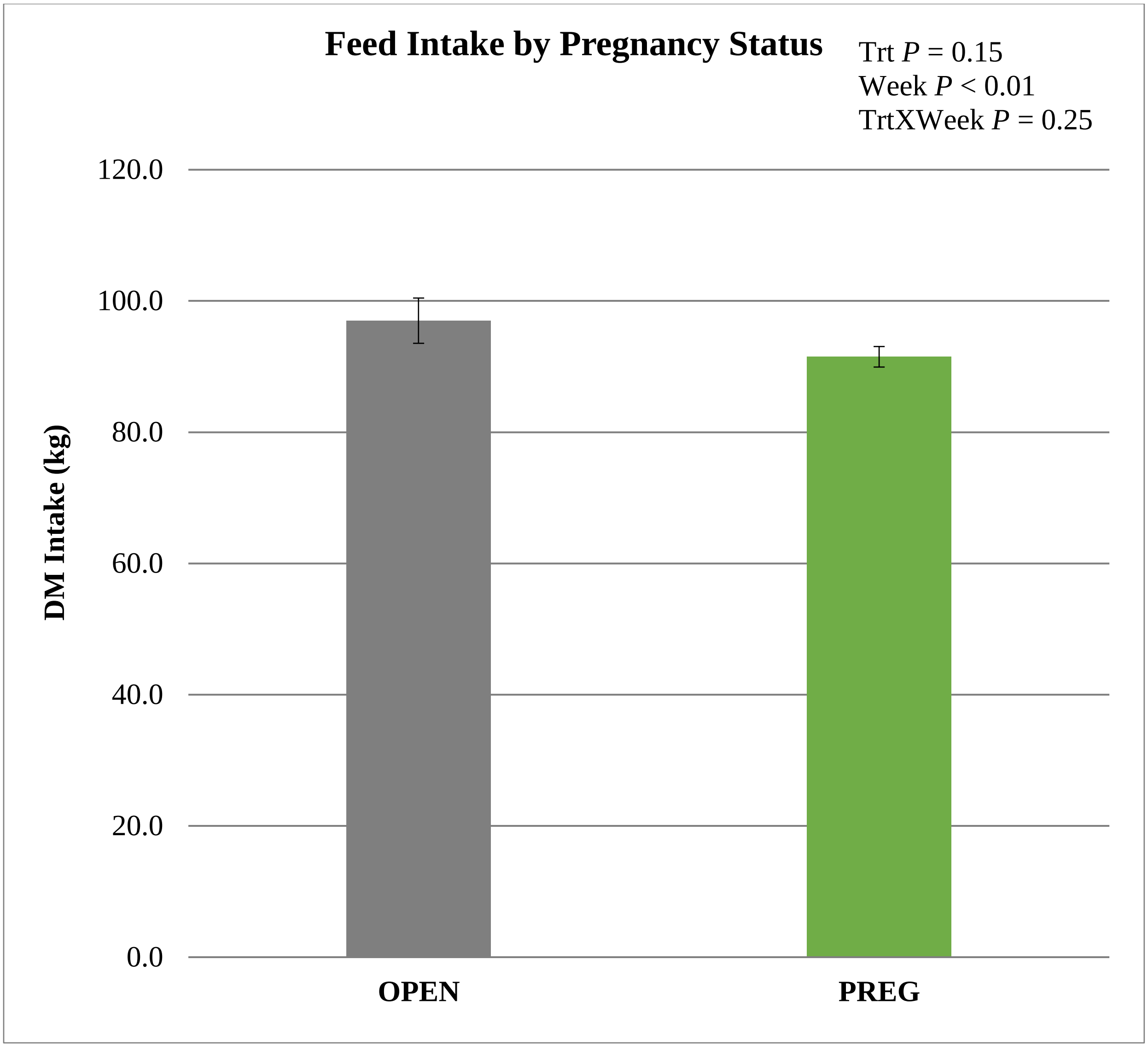


**Figure.3.31.** Feed Intake by Pregnancy Status: No differences were observed in FI between PREG and OPEN heifers (91.5±1.6 kg DMI vs 97.0±3.4 kg DMI). The model was only significant by week (Trt $P = 0.15$; Week $P < 0.01$; TrtXWeek $P = 0.25$).

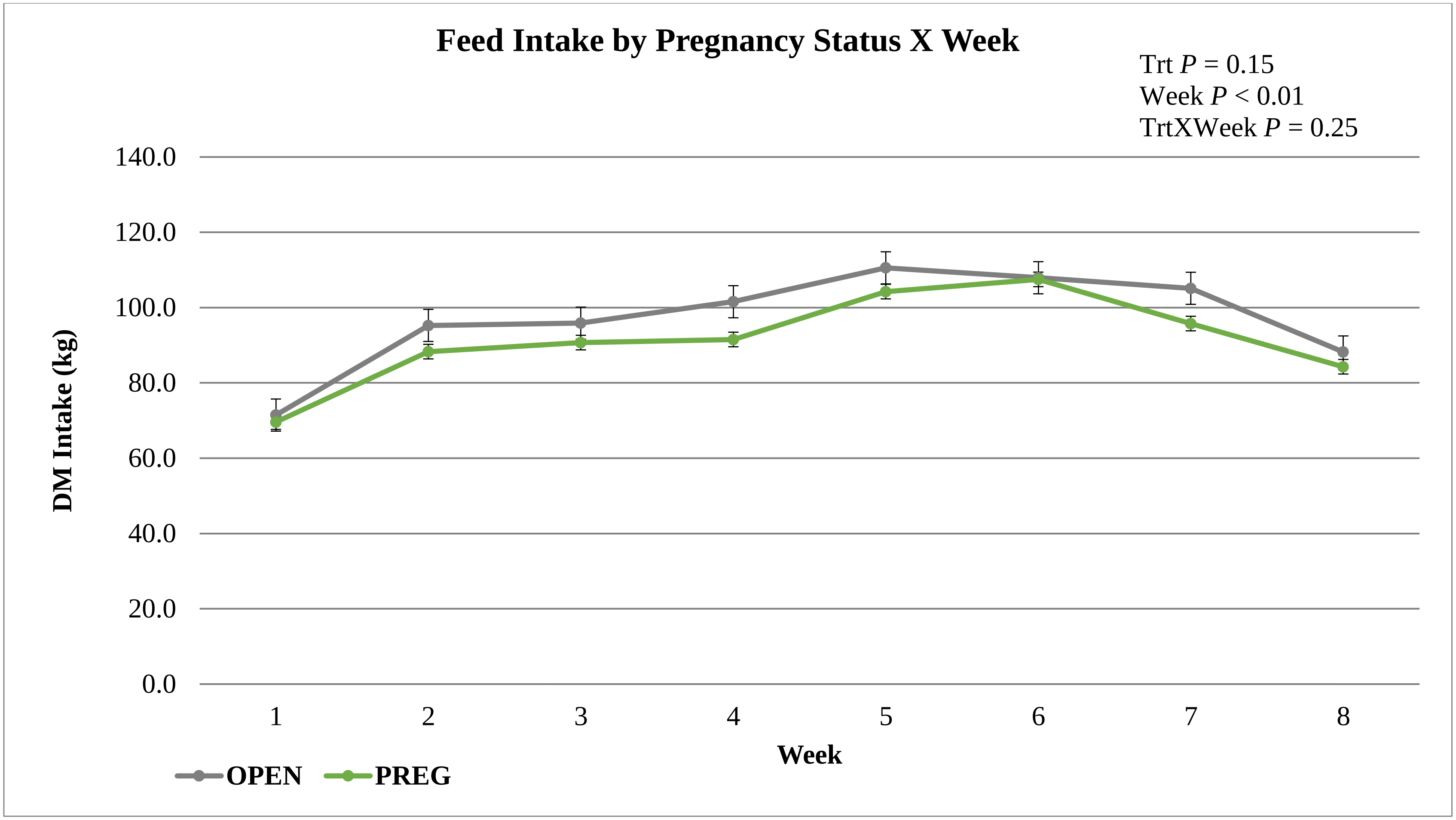


**Figure.3.32.** Feed Intake by Pregnancy Status over time: No differences were observed in FI between PREG and OPEN heifers over time. The model was only significant by week (Trt $P$ = 0.15; Week $P$ < 0.01; TrtXWeek $P$ = 0.25).

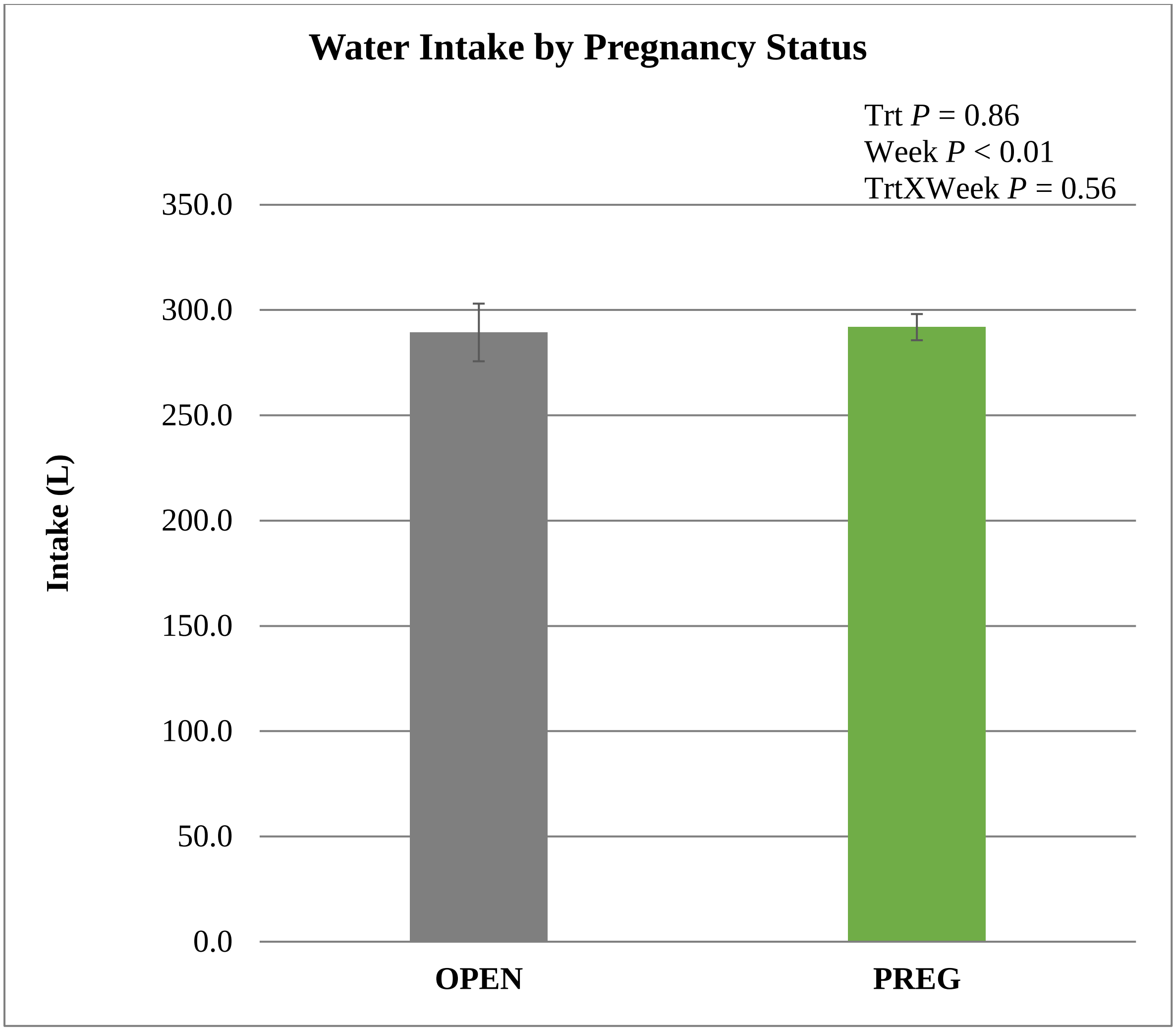


**Figure.3.33.** Water Intake by Pregnancy Status: No differences were observed in WI between PREG and OPEN heifers (291.9±6.2 L vs 289.4±13.7 L). The model was only significant by week (Trt $P = 0.86$; Week $P < 0.01$; TrtXWeek $P = 0.56$).

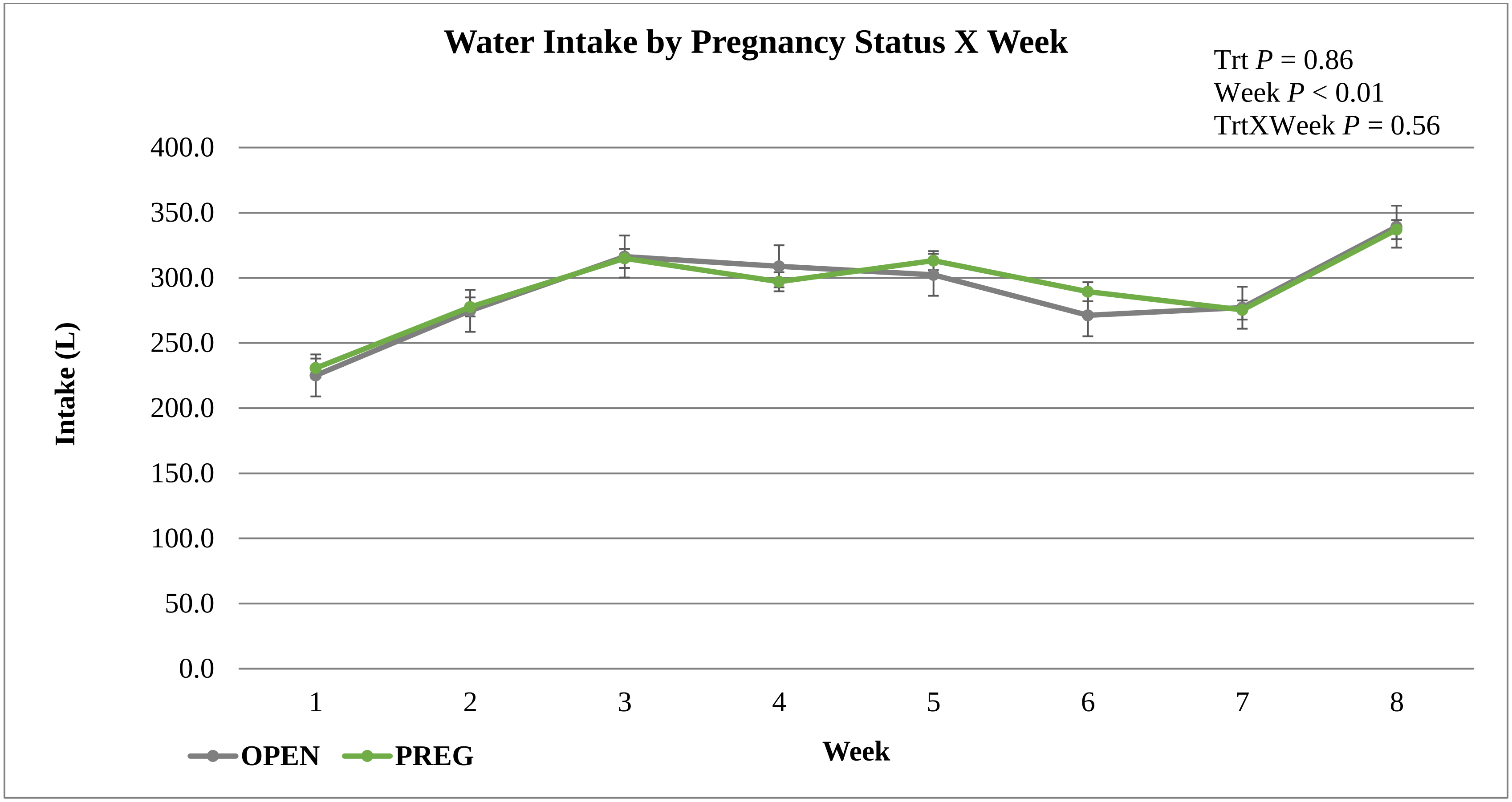


**Figure.3.34.** Water Intake by Pregnancy Status over time: No differences were observed in WI between PREG and OPEN heifers throughout the study. The model was only significant by week (Week $P < 0.01$).

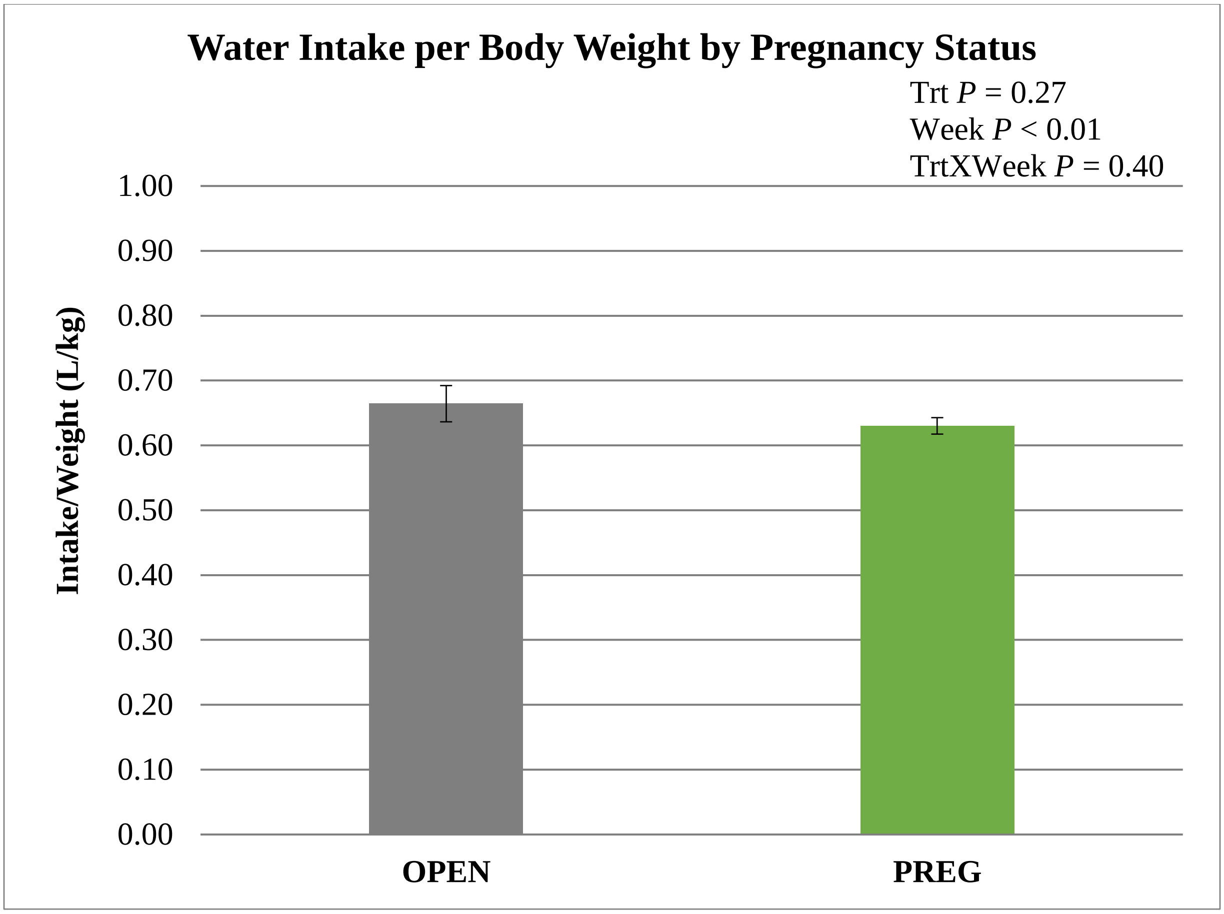


**Figure.3.35.** Water Intake per Body Weight by Pregnancy Status: No differences were observed in WI/BW between PREG and OPEN heifers (0.63±0.01 L vs 0.66±0.03 L). The model was only significant by week (Trt $P = 0.27$; Week $P < 0.01$; TrtXWeek $P = 0.40$).

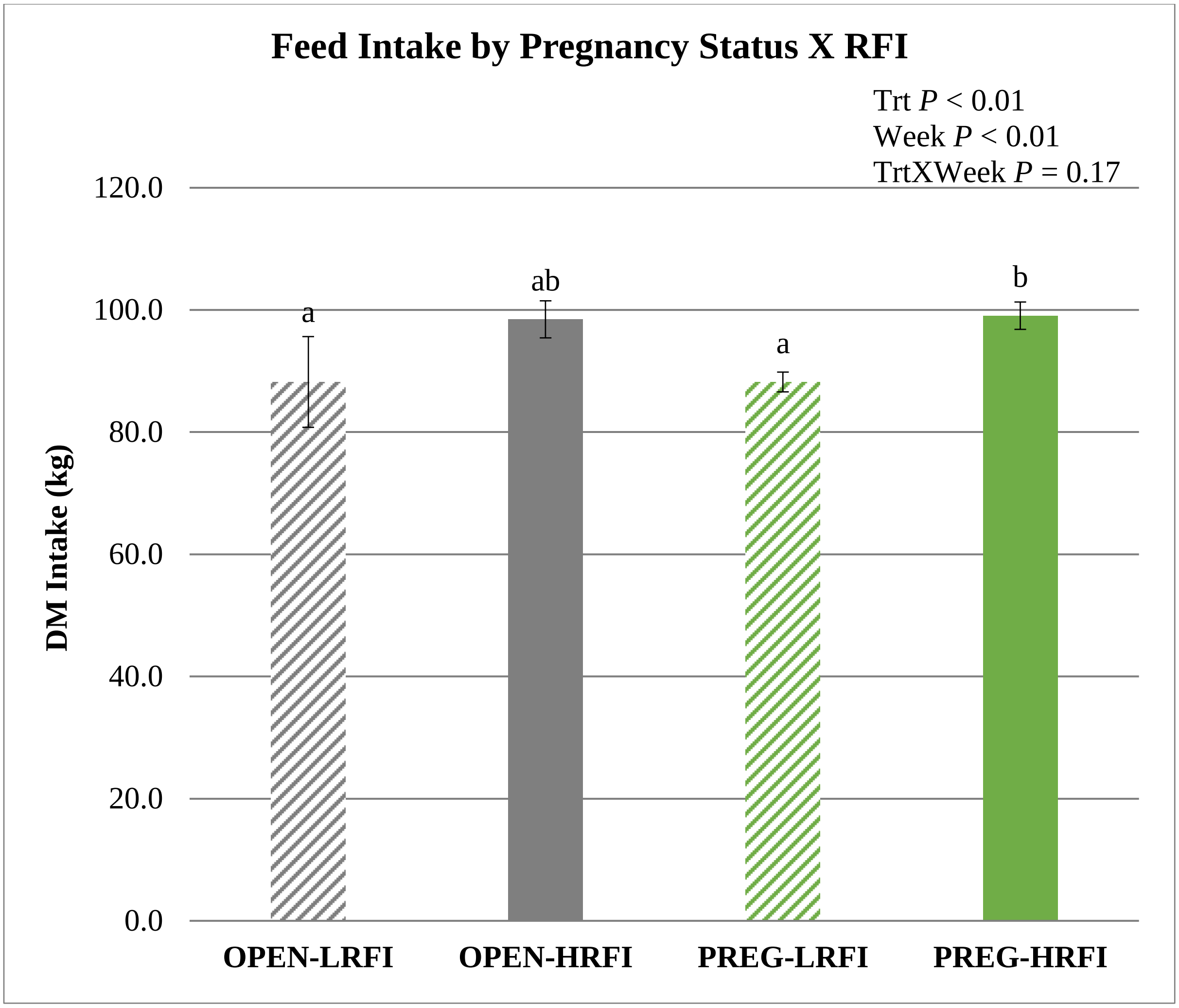


**Figure.3.36.** Feed Intake by Pregnancy Status X RFI: The LRFI PREG heifers had a lower FI than their HRFI pairs (88.2±1.6 kg DMI vs 99.1±2.2 kg DMI), whereas no differences were observed between the RFI subgroups of OPEN heifers. The model was significant by treatment and week (Trt $P < 0.01$; Week $P < 0.01$; TrtXWeek $P = 0.17$; different letter superscripts indicate statistical difference).

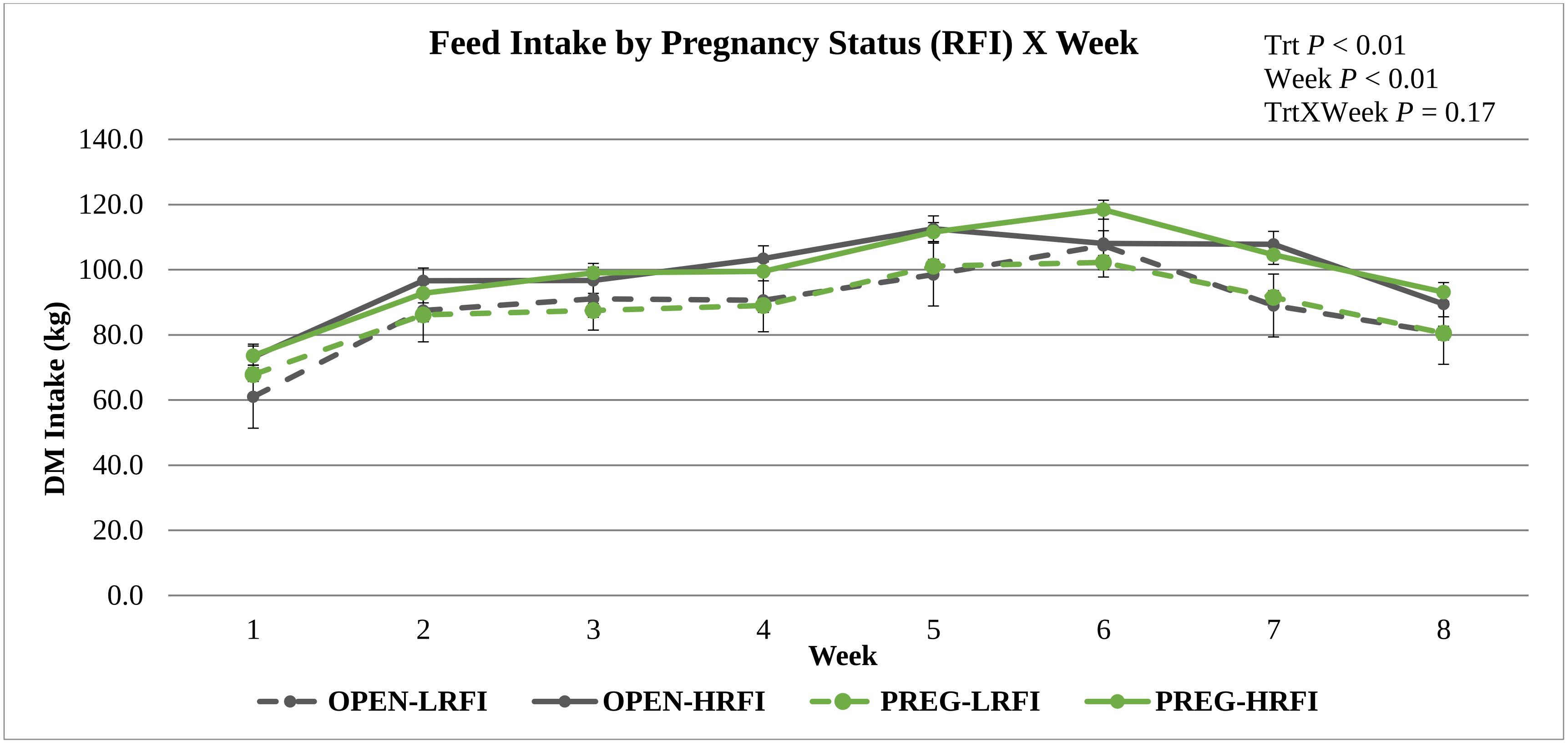


**Figure.3.37.** Feed Intake by Pregnancy Status X RFI over time: The FI of LRFI and HRFI heifers in both treatment groups fluctuated throughout the study. The model was significant by treatment and week (Trt $P < 0.01$; Week $P < 0.01$; TrtXWeek $P = 0.17$).

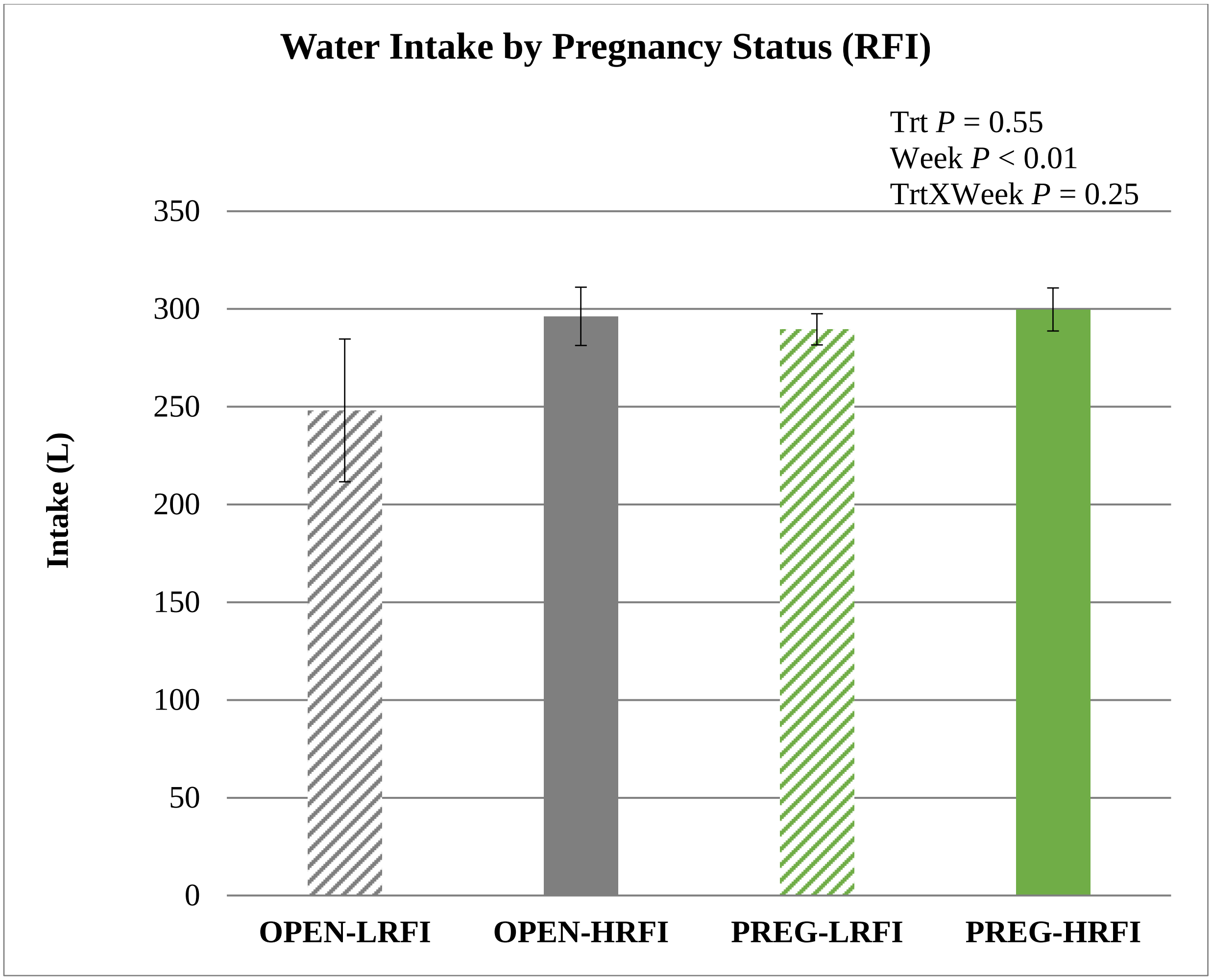


**Figure.3.38.** Water Intake by Pregnancy Status X RFI: No differences were observed in the WI of RFI subgroups between treatments. The model was significant by week (Trt $P = 0.55$; Week $P < 0.01$; TrtXWeek $P = 0.25$).

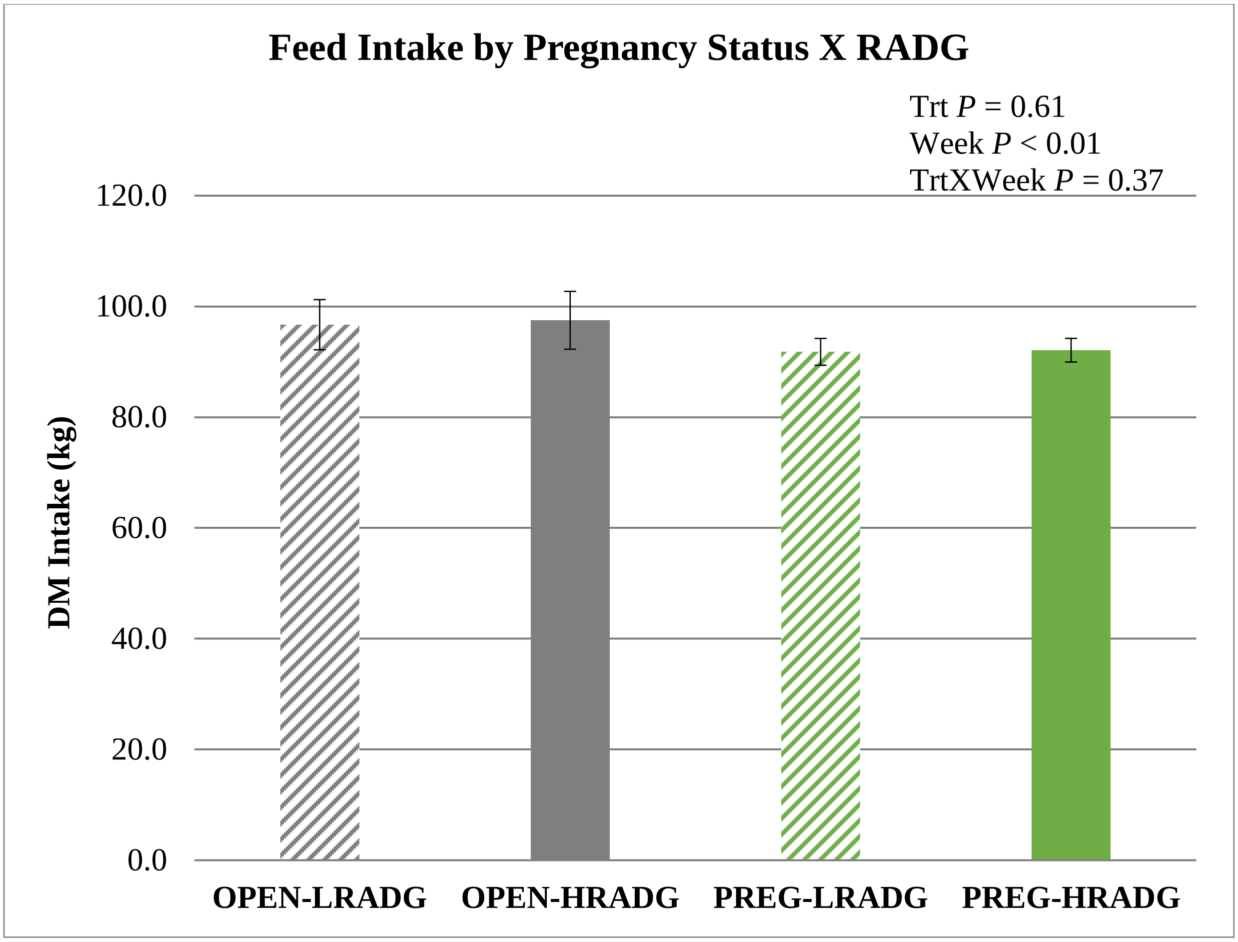


**Figure.3.39.** Feed Intake by Pregnancy Status X RADG: No differences were observed in the FI of the RADG subgroups in both treatments. The model was significant by week (Trt $P = 0.61$; Week $P < 0.01$; TrtXWeek $P = 0.37$).

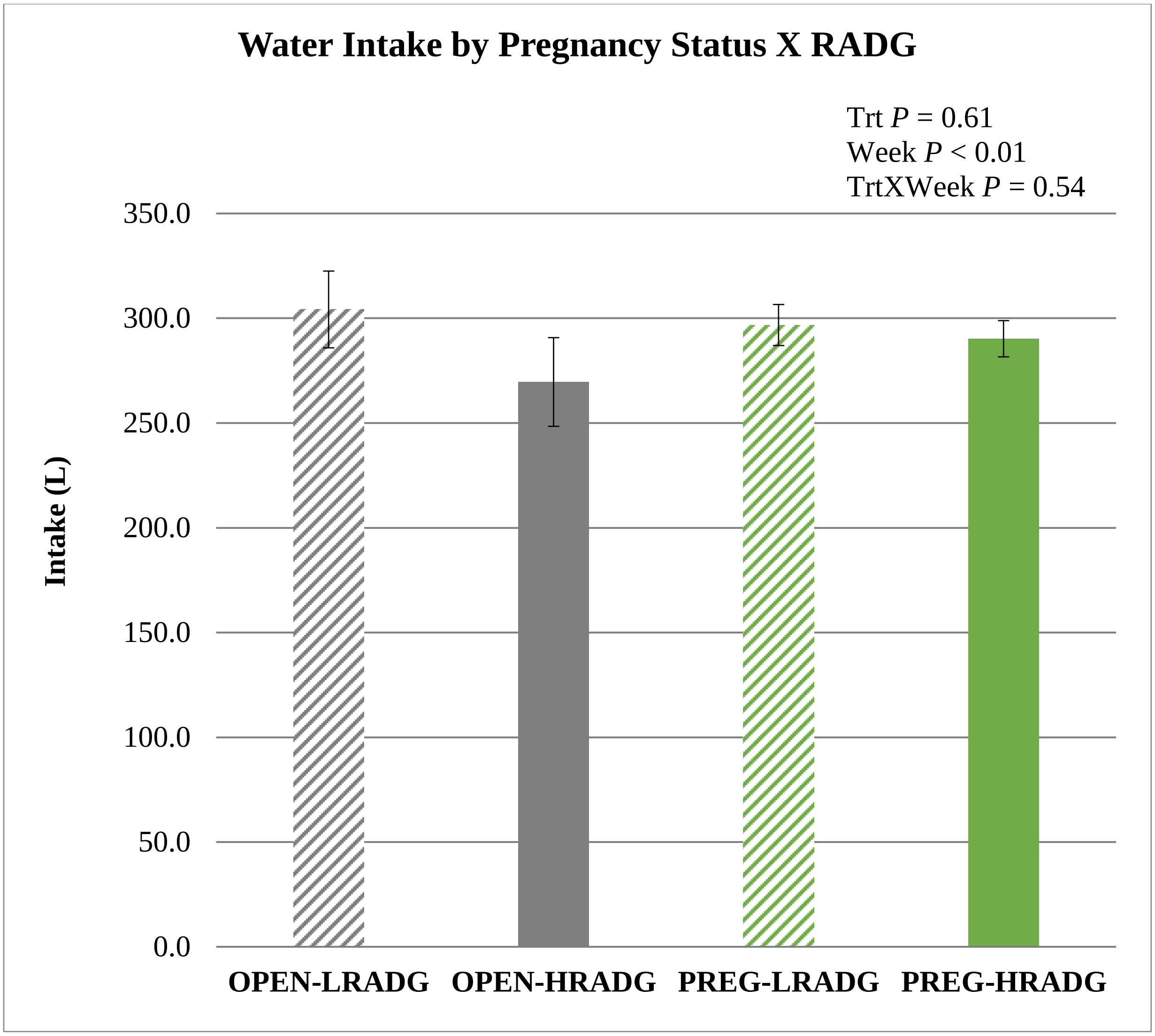


**Figure.3.40.** Water Intake by Pregnancy Status X RADG: No differences were observed in the WI of the RADG subgroups in both treatments. The model was significant by week (Trt *P* = 0.61; Week *P* < 0.01; TrtXWeek *P* = 0.54).

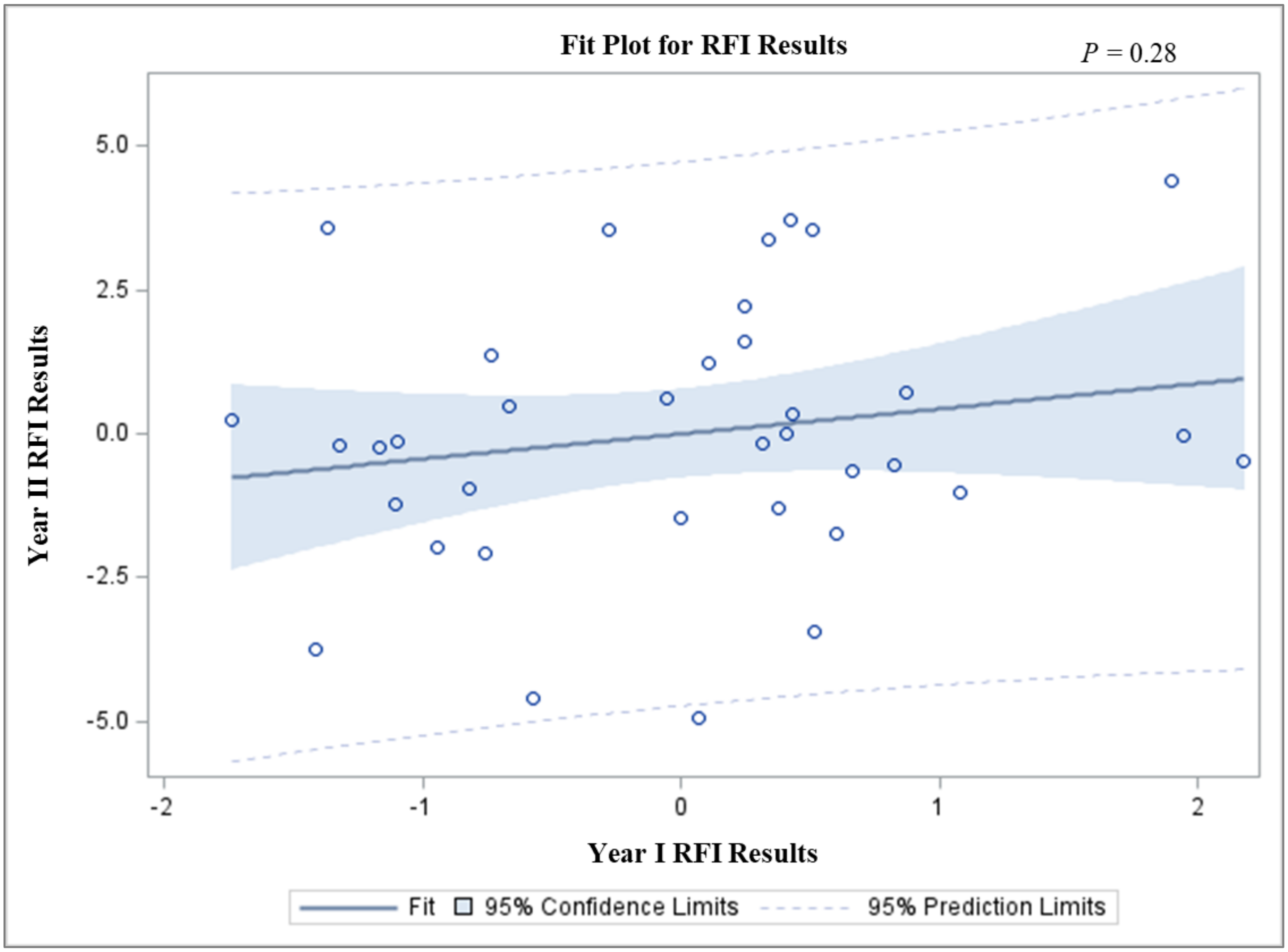


**Figure.3.41.** Fit Plot for RFI Results: A weak correlation ($r$ = 0.18; $R^2$ = 0.03; $P$ = 0.28) was observed for the RFI scores between Year I and Year II results.

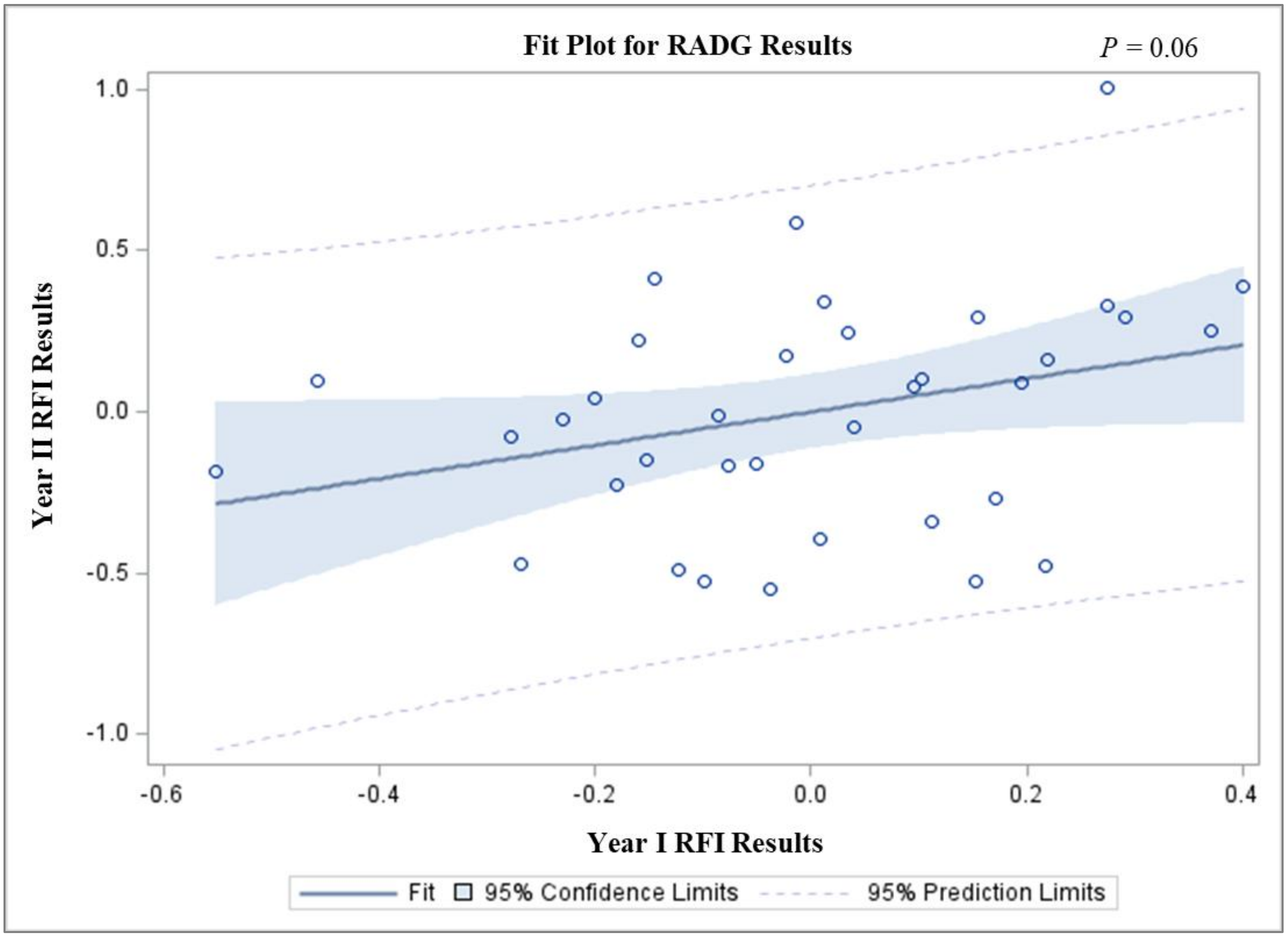


**Figure.3.42.** Fit Plot for RADG Results: A moderate correlation ($r$ = 0.32; $R^2$ = 0.10; $P$ = 0.06) was observed for the RADG scores between Year I and Year II results.

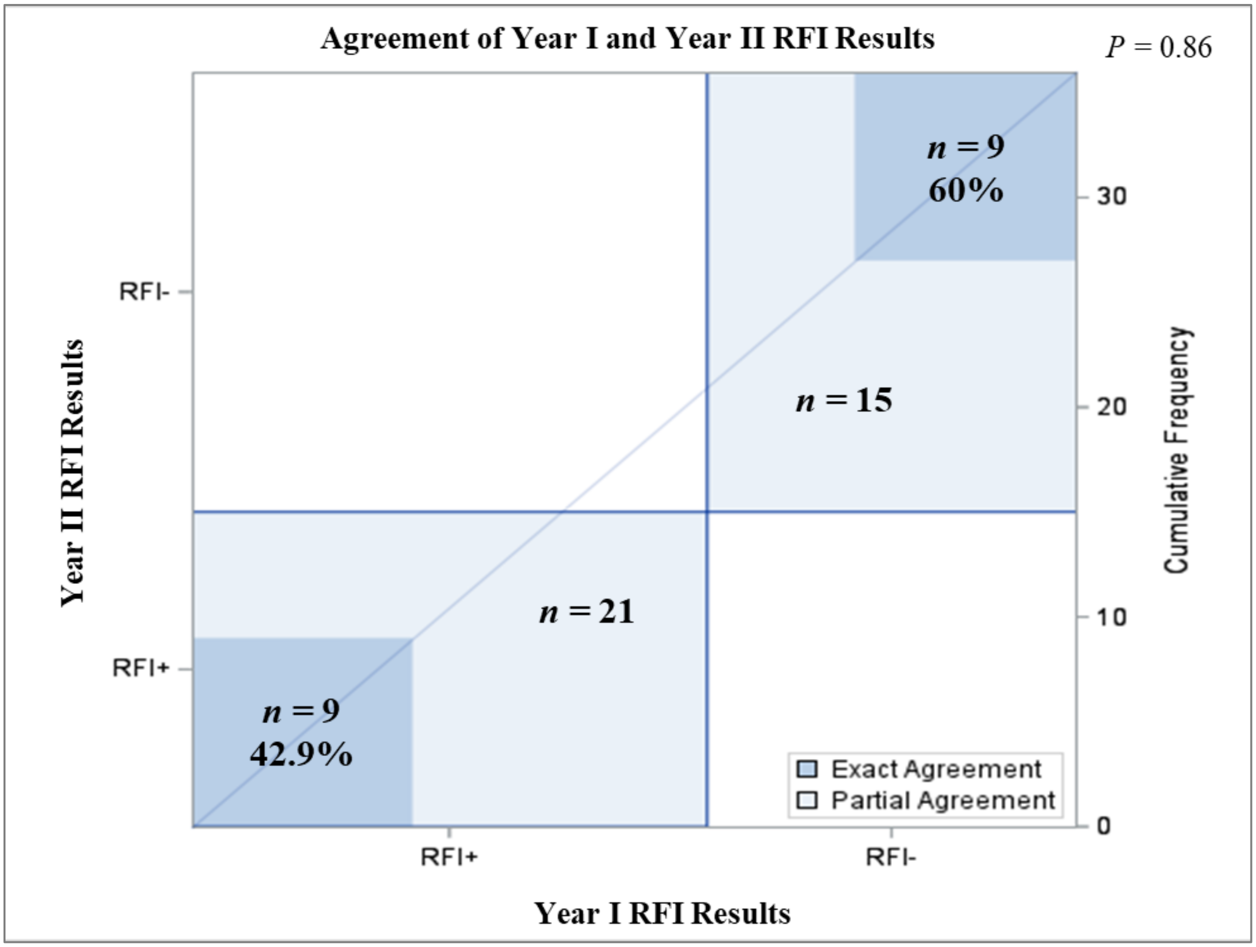


**Figure.3.43.** Agreement of Year I and Year II RFI Results: From the 21 heifers that had a positive RFI score in Year II, 42.9% ($n$ = 9) had similar results in Year I ($P$ = 0.86).

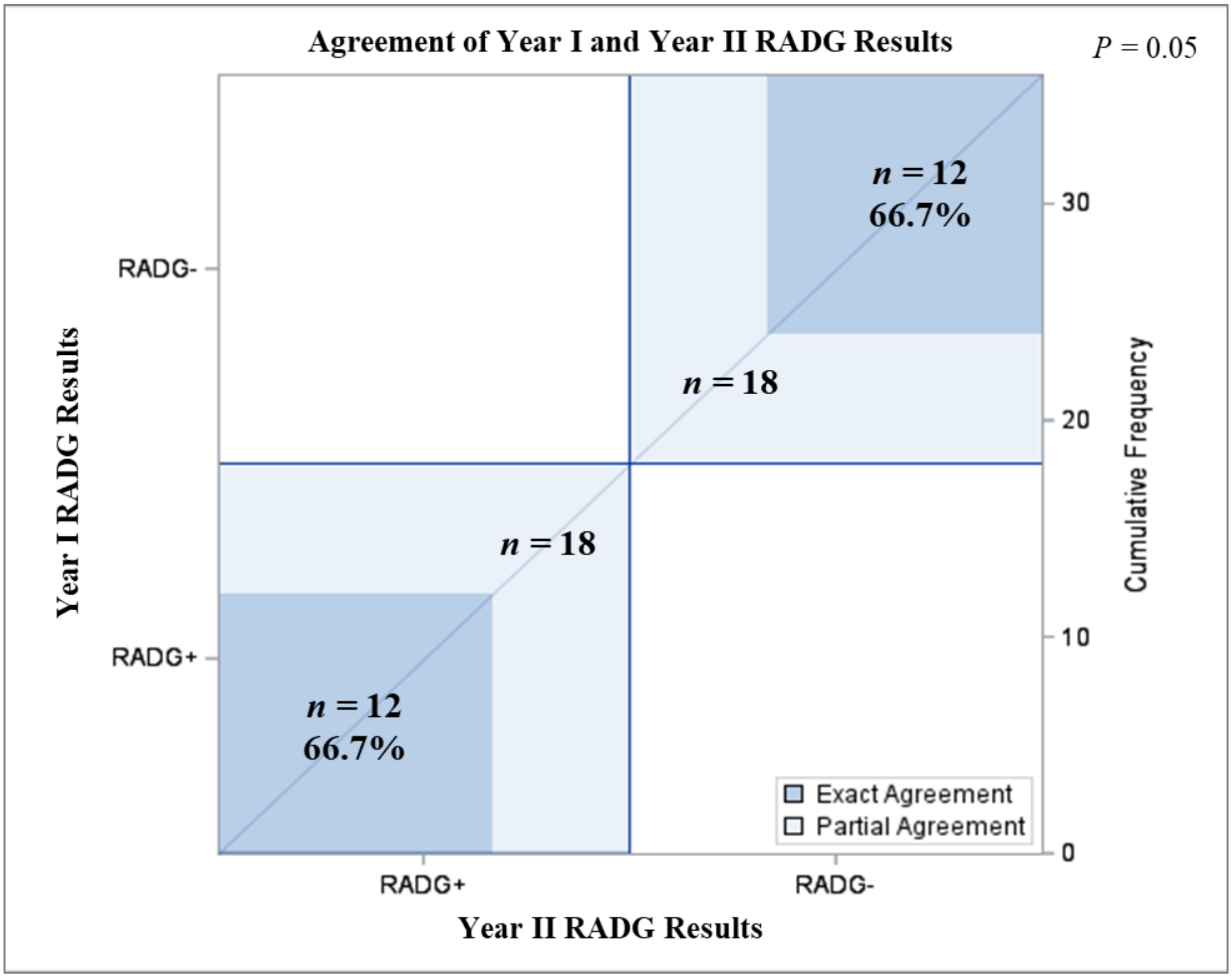


**Figure.3.44.** Agreement of Year I and Year II RADG Results: Equilibrated results were observed in Year II as the negative and positive RADG subgroups were comprised of 18 heifers each, and 66.7% (*n* = 12) of the animals scored similar values in Year I (*P* = 0.05).

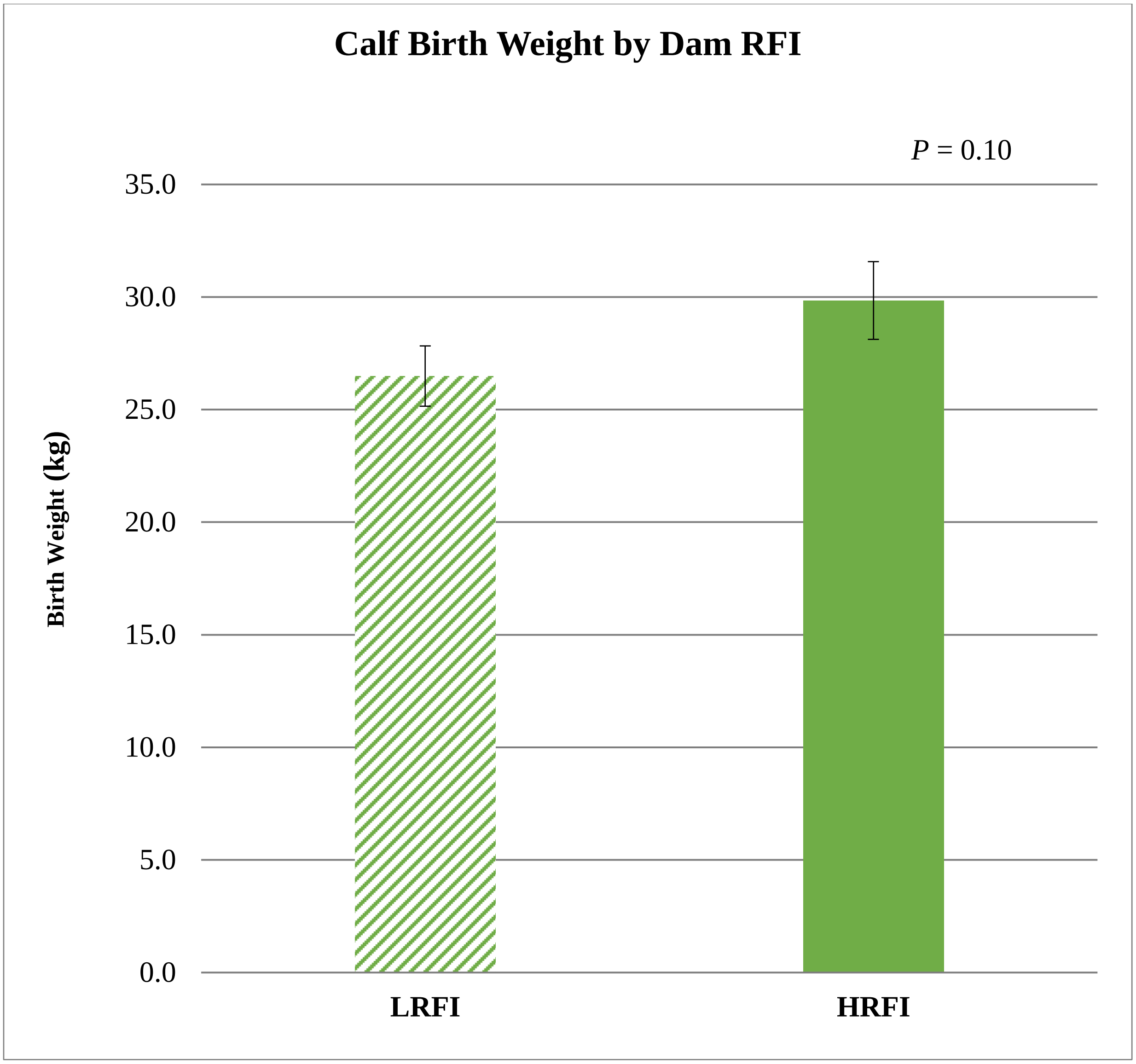


**Figure.3.45.** Calf Birth Weight by Dam RFI: No differences were observed in the CALFBW between calves born from LRFI and HRFI dams. The model was significant by RFI category (Trt *P* = 0.10).

# CHAPTER 4

Interpretative Summary and Future Directions to Determine Efficiency in Beef Cattle Across Different Production Stages in the Southeastern USA

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## INTERPRETATIVE SUMMARY AND FUTURE DIRECTIONS

The U.S. beef industry faces significant challenges in meeting increased global demand. This endeavor will require increasing productivity with reduced cattle inventories and land availability, while competing for feed and water resources with other livestock sectors and expanding urbanization projects, and enduring increasingly variable climatic events that aggravate the exposure to environmental stressors. Therefore, as the necessary resources for production become scarcer, it is important to consider the development of improved selection and management strategies that facilitate the identification of highly productive cattle capable of effectively adapting to challenging environments while improving the efficiency and sustainability of the operation.

As research efforts focus on developing novel selection and management strategies for cow-calf operations, the influence of environmental variables and changes in the physiological status of the animal should be accounted for in regions like the Southeastern USA, where grazing cattle are frequently exposed to environmental stressors due to the presence of endophyte-infected varieties of tall fescue in pastures and increased ambient temperatures during the summer months. The ergot alkaloids produced by endophyte-infected varieties of tall fescue are beneficial to the plant but detrimental to cattle, as they induce different negative responses that are commonly referred to as fescue toxicosis. The exposure to heat stress during the summer aggravates the symptoms of this disease, and this interaction significantly reduces animal performance and productivity.

The results presented by these studies provide new insights about the complex interaction between fescue toxicosis and heat stress and their impact on the cardiopulmonary system of cattle, and highlight the need for an extensive research framework that can generate improved selection and management strategies to improve cattle performance. As discussed, a relationship between the vasoconstrictive effects of fescue toxicosis and the pulmonary artery is deemed unlikely, which may be associated with the interaction between ergot alkaloid molecules and the required presence of specific receptors in vascular tissue. Further, a novel interaction between heat stress exposure and changes in the pulmonary arterial pressure (PAP) is highlighted, as both treatment groups reported an increase in PAP as thermal stress decreased. Considering that PAP is a moderately heritable trait widely used to identify cattle with increased resistance to high-altitude disease, future studies should incorporate heat stress into their analyses to enhance the comprehension of how this environmental stressor influences physiological changes in future progeny.

Based on the various methodologies to assess efficiency in a cow-calf operation and considering that the provision of feed may account for approximately three quarters of its total direct costs, the incorporation of feed efficiency metrics such as RFI and RADG may represent an opportunity to decrease financial outputs and increase the profitability of these operations. However, the results presented herein reinstate that more research is needed to improve the determination of nutritional efficiency in cattle exposed to fescue toxicosis and heat stress. Among the multiple negative effects caused by these stressors, decreased feed intake and increased water intake impose additional challenges for the development of uniform and comprehensive methodologies for efficiency classification. In fact, the lack of significant differences observed in the RFI and RADG evaluations of the heifers that consumed an endophyte-infected ration suggest an impact of ergot alkaloid exposure on the efficiency assessment. Nonetheless, a correlation between water intake and feed efficiency was observed in the endophyte-infected group, as the low-RFI heifers consumed less water than their high-RFI pairs. Consequently, selection of animals with increased feed efficiency may also contribute to improve water efficiency.

The reported changes in efficiency rankings when comparing RFI results between years may have been impacted by the differences in diet composition, including the use of endophyte-infected tall fescue seed in one of the treatment diets, the exposure to different levels of heat stress, as well as the different physiological stages that were evaluated. Future investigations that implement a longitudinal approach are needed to better understand the impact that ergot alkaloid consumption has on metabolic and digestive processes that influence nutritional efficiency determination, as a significant number of cow-calf operations are established in the Southeastern USA, where these stressors are commonly found.

An additional topic that requires further investigation is the relationship between fescue toxicosis and heat stress exposure on feed efficiency classifications of cattle grazing on endophyte-infected pastures. Most research efforts have focused on evaluating RFI classifications of young, growing cattle, and these trials and results are limited to the assessment of animal performance in a confined environment due to the methods and technologies used for data collection. Further, the diets utilized in these trials are typically high in digestibility and energy content, which differs from the forage quality consumed by these animals in pasture-based operations. Recent studies conducted at different locations have evaluated the grazing performance of cattle with different RFI classifications, and their findings may offer resources and methodologies that could be adapted to the regions dominated by endophyte-infected fescue. Additionally, incorporating other technological applications, such as infrared thermography, accelerometers, and portable GPS and temperature data loggers, may improve the quantity and quality of data collected and enhance the analysis of results to develop more comprehensive management strategies.

These results also demonstrate the complexity of FT and its negative effects on health and performance parameters of beef cattle, particularly during periods of thermal stress. As changes in global climate have a greater impact on agricultural and livestock production, species with more robust traits that support resiliency, such as endophyte-infected tall fescue, will have advantages in expanding their coverage area. Therefore, it is critical to gain deeper insights into the impact that fescue toxicosis and heat stress exposure have on the physiological mechanisms and nutritional efficiency in beef cattle, to develop improved animal selection strategies that can generate more resilient, productive and efficient cattle herds.

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# APPENDIX

**Appendix A:** Experimental unit and design justification for Chapter 2.

Figure (Suppl. A1): Aerial image of the Upper Piedmont Research Station Research Barn containing the Vytelle Sense System. The barn structure runs North/South with Vytelle Sense System pens on the west side of the barn. The adjacent pens comprise of a shaded area under roof (125 m2 per pen) and an exercise lot per pen (1,175 m2).

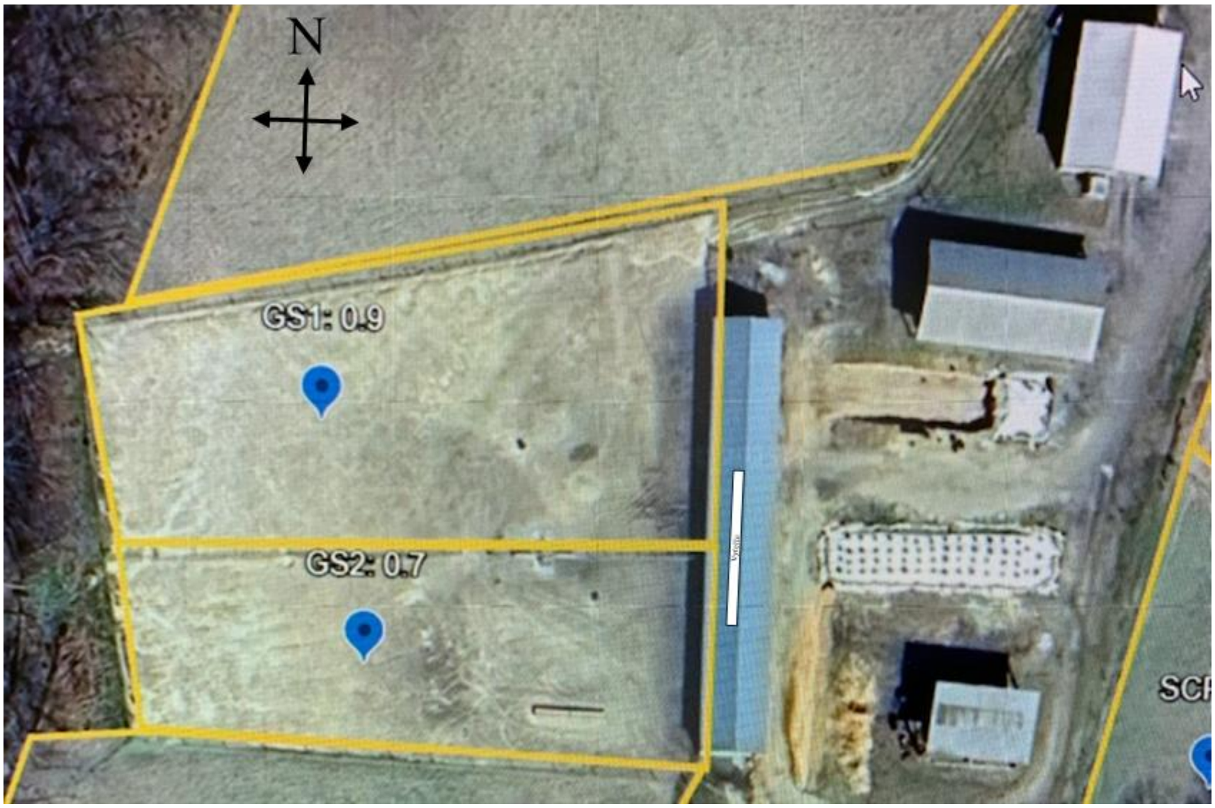

Figure (Suppl. 2): Environmental temperatures (ºC) collected at the Vytelle feed bunk for treatment groups over time: There was no difference in environmental temperature between pens (P = 0.9086) throughout the study, thus ensuring that heifers experienced equal environmental conditions within each pen.

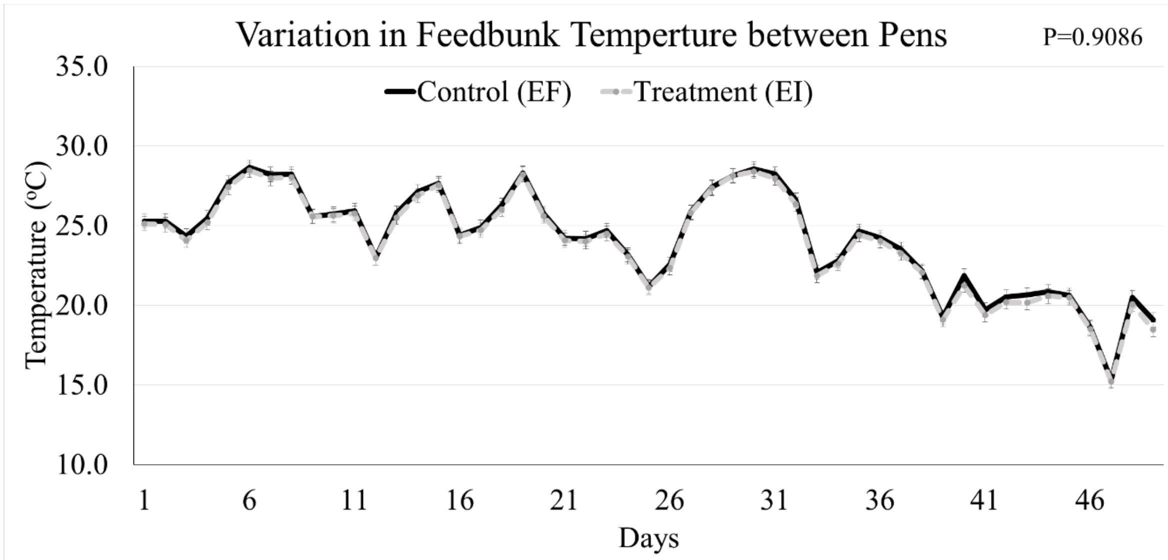


Figure (Suppl. 3): Water temperatures (ºC) collected within the Vytelle water system for treatment groups over time: There was no difference in water temperature between pens (P = 0.9531) throughout the study.