Weather Resilience in Grazing Beef Heifers with Differing Residual Feed Intake

by

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Abstract

The beef industry seeks sustainability through improved feed efficiency, yet the interaction between residual feed intake (RFI) and weather resilience in grazing beef heifers remains underexplored. This thesis evaluated rumen temperature (RT), blood parameters, growth performance, and activity behavior of beef heifers with varying RFI during summer and winter seasons in Western Canada. Forty-one crossbred beef heifers [351 ± 40 kg body weight (BW); 14 mo of age] were classified as more (LOW-RFI; n = 21, -0.96 ± 0.70) or less (HIGH-RFI; n = 20, 1.4 ± 1.00) feed-efficient. Heifer behavior was monitored using accelerometer-based sensors and individual virtual fence collar technology. Rumen temperature and water access events frequency were monitored using a bolus device, and blood samples were collected to analyze parameters related to protein, lipid, and growth metabolism, as well as neurotransmitters and hormones associated with metabolic homeostasis. The neutrophil-to-lymphocyte ratio (N:LR) was also assessed as a marker of inflammation. Environmental conditions were estimated by calculating the Climate Comprehensive Index. In summer, LOW-RFI heifers tended to exhibit greater free triiodothyronine levels ($P = 0.08$) and gamma-aminobutyric acid (GABA; $P = 0.01$) when weather was classified to impose moderate stress. LOW-RFI heifers also tended to produce more heat shock protein 70 ($P = 0.10$), while HIGH-RFI heifers had greater haptoglobin ($P = 0.02$) and leptin ($P = 0.04$) concentrations. The RT was greater in HIGH-RFI heifers during specific hours of the day ($P = 0.002$). For activity behavior, HIGH-RFI heifers had a greater number of steps on certain days, sought shade and water spots during hotter days, and had more daily transitions. In winter, greater leptin concentrations were found in HIGH-RFI heifers ($P = 0.04$) while GABA tended to decrease in HIGH-RFI ($P = 0.08$). The LOW-RFI heifers had higher RT on certain days ($P = 0.009$), more standing activity during moderate cold stress, and higher N:LR on the coldest days.

The HIGH-RFI heifers stood more on extreme cold days but had fewer steps on mild cold days and more steps on severe cold days. LOW-RFI heifers had fewer daily transitions. Blood parameters were significantly affected by day, RFI and RFI × day interaction in both seasons ($P < 0.001$), but there was no significant difference in growth performance between LOW and HIGH-RFI heifers ($P > 0.24$). Feed-efficient beef heifers in Western Canada exhibited greater resilience to extreme weather conditions, showing variations in blood parameters and rumen temperature (RT) without affecting growth performance. This study identified distinct behavioral and physiological responses between LOW-RFI and HIGH-RFI heifers. LOW-RFI heifers displayed enhanced thermoregulatory activity during both summer and winter, although chronic cold exposure may have potentially negative consequences. These findings explain the potential of selecting LOW-RFI heifers, leading to environmental resilience and sustainable beef production. However, further research is necessary to understand the long-term impacts of chronic cold stress on LOW-RFI heifers.

Preface

This thesis is an original work done by Maria Camila Londono-Mendez at the University of Alberta in the Department of Agricultural, Food & Nutrition Science (AFNS) under the supervision of Dr. Gleise Medeiros da Silva. This thesis was written according to the guidelines provided by the Faculty of Agricultural, Life & Environmental Sciences (ALES). The research studies of this project involved the use of beef heifers with a previous ethics approval by the University of Alberta Animal Care and Use Committees under the AUP# 00004004.

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List of Abbreviations

ADG	Average daily gain
BHBA	β-Hydroxybutyric acid
BUN	Blood urea nitrogen
BW	Body weight
CBC	Complete blood cell count
CCI	Comprehensive climate index
CH_4	Methane
CP	Crude protein
CS	Cold stress
DNA	Deoxyribonucleic acid
DM	Dry matter
DMI	Dry matter intake
DSR	Direct solar radiation
fT3	Free triiodothyronine
FUFAT	Final ultrasound rib fat thickness
GABA	Gamma amino butyric acid
GH	Growth hormone
GHG	Greenhouse gas
HIGH-RFI	High residual feed intake
HP	Haptoglobin
HPA	Hypothalamic-pituitary-adrenal
HS	Heat stress

HSP70	Heat shock protein 70
IGF-1	Insulin-like growth factor type 1
LEP	Leptin
LOW-RFI	Low residual feed intake
MDWT	Midpoint weight
MJ	Megajoule
mRNA	Messenger ribonucleic acid
MWT	Metabolic bodyweight
NEFA	Non-esterified fatty acid
N:LR	Neutrophil to lymphocyte ratio
RAD	Solar radiation
RFI	Residual feed intake
RFIf	Residual feed intake corrected for final ultrasound rib fat thickness
RH	Relative humidity
RT	Rumen temperature
SAA	Serum amyloid A
STR	Surface temperature
Ta	Air temperature
T cells	T Lymphocytes
Tc cells	Cytotoxic T cells
TDN	Total digestible nutrients
Th cells	Helper T Cells
THI	Temperature humidity index

TMR	Total mixed ration
TNZ	Thermoneutral zone
T3	Triiodothyronine
T4	Thyroxine
WBC	White blood cell count
WS	Wind speed
5-HT	Serotonin

Chapter 1. Introduction and objectives

In recent years, certain areas of Canada have experienced a significant increase in environmental variability. The summer of 2022 was one of the warmest on record (Lin et al., 2022), with temperatures exceeding 49.6°C in British Columbia during the summer of 2021 (WMO, 2021). Projections suggest that average temperatures may rise by +1.5 to 2°C, leading to hotter conditions and longer drought periods (Madakumbura et al., 2019). These weather changes could exacerbate environmental conditions for cattle in various systems, including feedlots and grazing environments.

Simultaneously, due to global warming, the Arctic region has experienced significant melting processes (Zhang et al., 2020), resulting in increased occurrences of cold spells and heavy snowfalls in regions geographically close to the Arctic, such as Canada. As Arctic waters warm, melting increases and reduces sea surface salinity, facilitating stagnation and freezing in the Nordic seas. This phenomenon contributes to decreased airflow and spreads lower temperatures (Zhao et al., 2019). Consequently, there has been a rise in extreme winter conditions, colloquially called the "warm-Arctic/cold-continents pattern" (Cohen et al., 2018), that means the cold spread is expected to move from the arctic to the south part of the continent. It is crucial to note that extreme weather events, namely heat waves or cold snaps, have the potential to significantly impact cattle physiology and well-being, ultimately affecting animal productivity. The concept of weather resilience, which has gained prominence in scientific discourse, was originally defined by Holling et al. (1973) as the capacity of systems to persist and adapt in the face of change and disturbances while maintaining their essential structure and function (Macmillan et al., 2022). This concept is closely related to the ability to maintain homeostasis under extreme environmental conditions.

Heat stress (HS) occurs when the body temperature rises beyond a comfortable range, triggering the need to dissipate heat to maintain homeostasis, which can affect customary grazing behavior (Lefcourt and Schmidtmann, 1989). Conversely, cold stress (CS) arises when external environmental conditions are extremely cold, and the animal's body cannot generate enough heat to maintain normal body temperature (Roland et al., 2016), causing body temperature to drop. In cattle, thermoneutral zone (TNZ) denotes the ability to maintain constant body temperature and physiological conditions under varying environmental temperatures (Kadzere et al., 2002). In other words, it is the temperature range in which an animal can maintain a constant internal body temperature without activating mechanisms to generate or dissipate heat. During HS, anticipated responses in cattle include elevated respiratory rate (Gebremedhin et al., 2008), increased heart rate (Beatty et al., 2006), and vasodilation (Sammad et al., 2020). Conversely, CS triggers vasoconstriction and shivering (Wang et al., 2023), accompanied by an increase in dry matter intake to meet the heightened energy demands of 2.89 kJ/kg for every Celsius degree below the TNZ (He et al., 2022). It is widely acknowledged that cattle in extreme weather conditions require more energy for thermoregulation response (Wang et al., 2023). In this context, significant physiological changes ensue, which include adaptive modifications in metabolism, hormonal responses, growth performance, behavior, feed intake (Nardone et al., 2006), and tissue metabolic rate (NASEM, 2016). These alterations constitute crucial adaptations to cope with challenging environmental conditions, allowing organisms to maintain vital physiological functions and sustain homeostasis.

Cattle in both grazing and feedlot settings encounter varying environmental conditions that influence their heat gain and loss through seasonal changes in factors such as air temperature, wind speed, humidity, and solar radiation (Mader et al., 2010). The air temperature and solar radiation contribute to heat gain and retention (Pontiggia et al., 2024), intensifying summer conditions, but providing warmth during colder days. Meanwhile, wind speed may help in heat dissipation through convection (Shephard et al., 2023), cooling the body during the summer but increasing wind chill in winter. However, humidity may worsen both hot and cold sensations, making it difficult to pant and sweat during the summer (Gebremedhin et al., 2008; Brody et al., 1952) and exacerbating the sensation of cold during winter. Windy days can further increase the extreme winter sensation and create a more significant wind chill effect (Olson et al., 2000).

Cattle employ various mechanisms for heat regulation, including convection, where wind speed aids in lowering body temperature, and conduction, which transfers heat to or from cold surfaces (Bastian et al., 2003; Godyń et al., 2019). Additionally, conduction from hot surfaces plus solar radiation may increase the heat gain (Mader et al., 2010). The mechanism for self-body temperature relief includes panting (Islam et al., 2023), sweating (Gebremedhin et al., 2008) and more saliva production (Toledo et al., 2022) for evaporative cooling and vasodilation over the skin for heat dissipation **(Figure 1.1).**

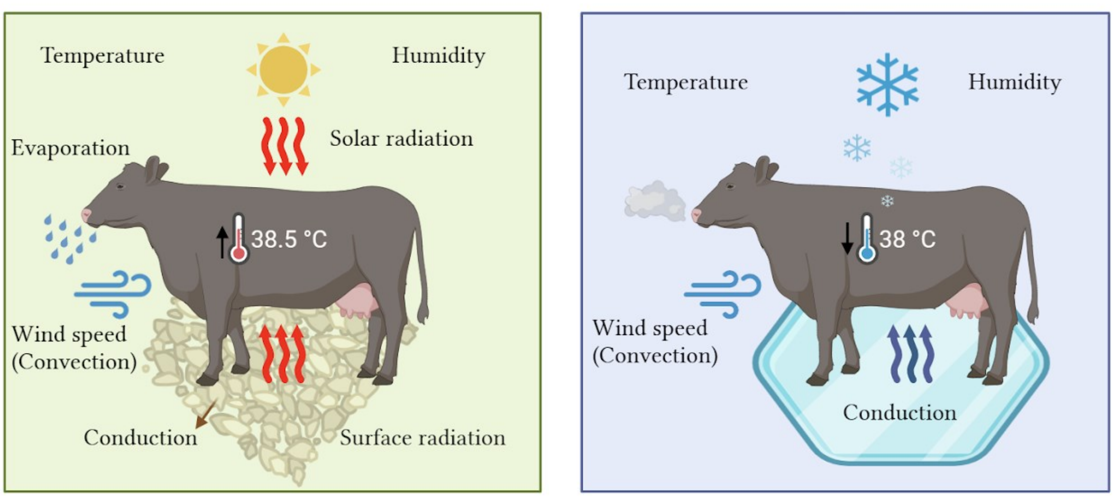

Figure 1.1. Cattle exposed to environmental conditions typical of grazing settings experience varying challenges during both summer and winter seasons. Various environmental variables,

such as air temperature, wind speed, humidity, and solar radiation, are shown influencing heat gain and loss. Mechanisms such as convection and conduction help in the thermoregulation process. Often, heat gain (A) is found in summer with more solar radiation and greater air temperature but heat loss (B) occurs in the winter due to decreased air temperature and icy surface which by conduction may reduce body temperature.

Adapted from Mader et al., 2010 and Most et al., 2021.

Understanding the several physiological changes that occur in extreme weather conditions is crucial to effectively manage the health and productivity of beef cattle. From a metabolic perspective, environmental stress can further impact metabolism in a manner that decreases the concentration of thyroid hormones alongside high concentrations of β-Hydroxybutyric acid ([BHBA] Nardone et al., 1997). Thyroid hormones are in charge to regulate metabolism (Trenkle, 1978) and may be negatively associated with an increase in body temperature (Yousef et al., 1968), while BHBA levels are directly related to fat mobilization during fasting (Schäff et al., 2013). In hot environments, accelerated protein catabolism in muscle tissues provides additional energy substrates for thermoregulation (Baumgard & Rhoads, 2013). However, this increased breakdown metabolism is compounded by a reduction in dry matter intake (De Rensis et al., 2003), leading to impaired growth performance in beef heifers (Nonaka et al., 2007). Blood urea nitrogen (BUN) levels are also affected, reflecting alterations in protein metabolism and amino acid use efficiency (Qin et al, 2022). Another physiological change observed during HS involves a reduction in insulin-like growth factor 1 (IGF-1) production (Bernabucci et al., 2010). This hormone is pivotal in the control of cell cycle and apoptosis, and its production is closely related to growth hormone levels (Le Roith et al., 2001), which is a predictor for growth rate. Greater IGF-1 concentrations are associated with increased feed intake and protein anabolism in muscles (Hill et al., 1999). Moreover, alterations in energy demands triggered by HS can also impact the production of leptin (LEP), an important indicator of energy reserves and body condition score, which also has the function of signaling to the central nervous system to regulate feed intake (Leó et al., 2004; Morrison et al., 2001).

Due to the physiological adaptations prompted by challenging environmental conditions, several other blood components can serve as biomarkers indicating stress due to environmental variability. For instance, haptoglobin (Hp), an acute-phase protein, has been linked to inflammatory processes, cell disruption, and stress with a reference value of < 0.1 mg/mL for healthy cows (Huzzey et al, 2009). Conversely, heat shock protein 70 (HSP70) functions as a marker for CS (Hu et al., 2019) and acute HS (Kim et al., 2024). Gamma aminobutyric acid (GABA) is associated with feed intake, due to its relationship with insulin secretion and glucagon response (Xu et al., 2006). Furthermore, GABA is the primary inhibitory neurotransmitter in the central nervous system and has the function of relieving the pressure of stress (Dai et al., 2012). Serotonin (5-HT) is crucial for thermoregulation and has vasoactive effects (Slominski et al., 2005), enhancing intestinal absorption by promoting mucosal 5-HT production and stimulating the maturation of the enteric nervous system, which supports nutrient absorption and intestinal transit (De Vadder et al., 2018; Lucki, 1989). This influence becomes particularly pronounced during periods of energy exhaustion resulting from the heightened energy demands of thermoregulation under environmental stress. Additionally, Alsemgeest et al. (1994) investigated the importance of serum amyloid A (SAA), an acute phase protein, as an indicator to evaluate inflammatory diseases in cattle. The SAA concentrations were identified as diagnostic tools for tracking inflammatory conditions in HS (Abeyta et al., 2023) and CS (Griffin et al., 2021). The hormones, proteins, and neurotransmissions mentioned are orchestrated by the organ-stimulus response and mainly by the neuroendocrine system **(Figure 1.2).**

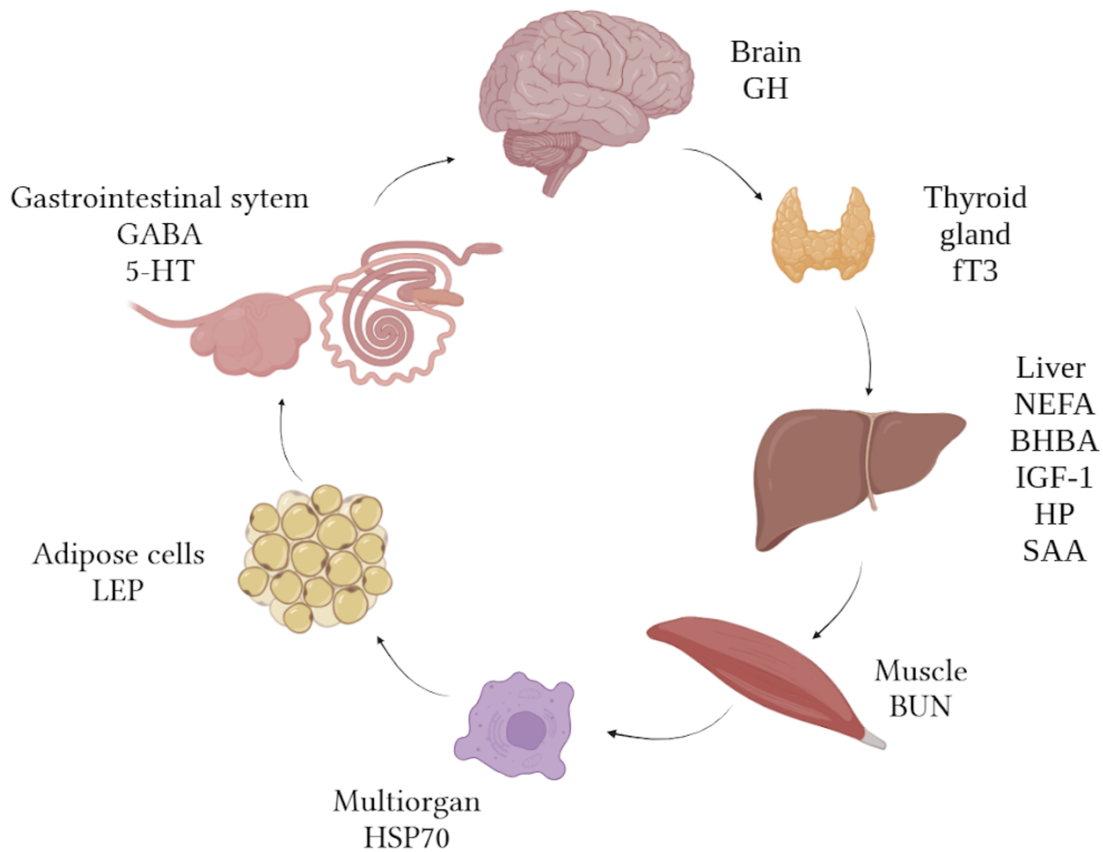

Figure 1.2. Mammals have a complex physiological response involving various organs such as the brain, thyroid gland, liver, muscles, adipose cells, and gastrointestinal system. These organs work together to produce hormones, proteins, and neurotransmitters.

Currently, many equations are available to assess the combined effects of environment on cattle, such as the temperature humidity index (THI; Gaughan et al., 2008), which aims to predict when environmental conditions might negatively impact cattle physiology. However, due to the lack of solar radiation and wind speed in the THI model, Mader et al. (2010) proposed the comprehensive climate index (CCI) as an alternative indicator of thermal stress in grazing beef herds or species produced under the influence of direct solar radiation. The calculated CCI fits into categories as posing no risk of stress to mild, moderate, severe, extreme, and extreme danger stress risk using adjustments for temperature, solar radiation, wind speed, and relative humidity. Additionally, the CCI includes the surface where the animals are placed as a variable, making it a valuable metric to provide evidence of animal well-being.

Lastly, from the perspective of sustainable beef production, genetic selection presents a promising alternative to mitigate the adverse impact of extreme weather conditions on grazing beef herds during summer and winter. For instance, the residual feed intake (RFI) is intended to capture variations in efficiency. Animals displaying a negative RFI value have actual feed intake levels lower than the expected group intake, thereby indicating enhanced efficiency (Koch et al., 1963) and greater energy utilization (Marín et al., 2024). Such animals have consumed a reduced amount of feed to achieve a specific production level, thereby exhibiting enhanced biological or cellular efficiency (Richardson et al., 2004; Herd et al., 2009). In this regard, selection for more efficient beef heifers based on RFI has gained prominence over the years.

Five major physiological processes are likely to contribute to variation in RFI, which includes feed intake and digestion, metabolism (anabolism and catabolism), physical activity, and thermoregulation; studies on Angus steers selected with divergent RFI estimated that heat production from metabolic processes and physical activity explained 73% of the variation in RFI (Herd and Arthur, 2009). Richardson et al. (2001) reported a positive correlation between RFI and daily activity, where less efficient cattle had approximately 5% more feed energy intake cost due to their higher level of activity compared with more efficient cows. These studies demonstrated a greater energy demand associated with activities in less efficient animals. Differences in heat production also play a role in feed efficiency. Animals with greater body temperature, all else being equal (such as feed intake), are expected to direct a greater proportion of feed energy into metabolic heat production than into productivity, which reduces their production efficiency (Hill and Wall, 2017). More feed-efficient cows might be less susceptible to thermal stress (stresses associated with high or low temperatures) than less efficient cows because of better thermoregulatory abilities in the former. For example, recently Sprinkle et al. (2019) demonstrated that on a cooler day (max of 23°C), inefficient cows grazed 1.5 h longer than efficient cows, however, on a hot day (max of 30°C) they grazed 2 h less than efficient cows. Authors argue that less efficient cows would be expected to have greater appetite than efficient ones to compensate for increased energy requirements and should increase daily grazing time when conditions are favorable. Yet, greater appetites are accompanied by larger gastrointestinal tracts (Sprinkle et al., 2000), increasing metabolic heat load and reducing heat tolerance. On the contrary, in non-ruminants, Schmitt et al. (2021) reported that newborn piglets classified as less feed-efficient had more difficulties to maintain body temperature by displaying lower ear tip temperatures than more feed-efficient cohorts. This indicates that more efficient piglets can better adjust body temperature when required during stressful conditions.

Although various studies have evaluated the relationship among RFI, metabolism, fertility, and carcass traits, there is still a lack of information on the association of the immune and stress responses to divergence in RFI in cattle (Naddafi, 2021). A recent study performed by Toghiani et al. (2020), have highlighted the significant effects of extreme weather conditions on cattle, particularly regarding their body weight and weaning weight under cod stress. These findings emphasize the need to consider environmental factors when assessing cattle growth and development, as extreme temperatures can influence key performance indicators.

1.1 Research objectives

The increased energy requirements for beef cattle associated with thermoregulation during cold and heat extremes suggest that environmental conditions are worthy of consideration when evaluating RFI (Thompson et al., 2018). There is still a lack of research focused on the evaluation of the interaction between cattle feed-efficiency in forage-based systems and the environment, specifically during the winter season. Such information is necessary to understand the impacts of environment on animal health and production to support more sustainable beef production. This thesis will address this knowledge gap by revealing the physiology and behavioral (activity budget) changes employed by heifers with divergent RFI during natural fluctuations in weather conditions (i.e., summer vs. winter) in Western Canada.

The specific objectives of this study are to reveal the relationship between beef heifer weather resilience and feed efficiency by assessing heifer physiological status (rumen temperature, IGF-1, free triiodothyronine, BUN, non-esterified fatty acids, LEP, Hp, SAA, HSP70, BHBA, GABA, 5-HT and, complete blood cell count), growth performance, and behavioral responses (lying, standing, transition times) during summer of 2022 (June to August) and winter of 2023 (January to March). With the results of this project, we will provide information on the physiology and behavior responses employed by divergent feed-efficient beef heifers under natural fluctuations in weather conditions and reveal the impact of those responses on heifer performance.

1.2 General thesis hypotheses

We hypothesized that more feed-efficient beef heifers are more weather resilient because of their greater efficiency in energy utilization, resulting in maintenance of adequate behavior, body weight, and physiological and immune status compared to less efficient heifers.

1.3 Conclusion

In conclusion, the increasing environmental variability in Canada, with extreme heat in summer and intense cold in winter, presents significant challenges for beef cattle management. The anticipated temperature rise, prolonged droughts, and the "warm-Arctic/cold-continents pattern" causing more frequent cold spells further stress cattle. Heat and CS disrupt cattle's homeostasis, impacting their physiology and productivity. Understanding these THI and CCI for better stress management. Genetic selection for feed efficiency, especially focusing on RFI, shows promise in enhancing cattle resilience to extreme weather.

Chapter 2. Literature Review

2.1 Beef cattle production in Canada

In Canada, the province of Alberta is the largest beef cattle producer, with 4.612 million heads, which includes beef cows and feeder cattle (Statistics Canada, 2024). In Alberta, it is common to grow cattle in three different operating systems as mentioned by Sheppard et al. (2015):

1) Cow-Calf: Cow-calf production involves raising cattle specifically for breeding and producing calves. In this system, most producers, about 91%, focus on marketing weaned calves. This means that their primary business is to breed cows and raise the calves until they are weaned, which typically occurs when the calves are around 6 to 8 months old. Once weaned, these calves are sold to other operations, such as feedlots or stocker operations (backgrounding), where they are further grown and fattened for beef production. This specialization allows producers to concentrate on the breeding, health, and early growth stages of the calves, ensuring they are well-prepared for the next phase in the beef production process.
2) Backgrounding: involves feeding weaned calves until they are ready for the finishing phase. About 38% of beef producers specialize in backgrounding, which focuses on growing and

preparing the calves after weaning. These producers feed and manage the calves to achieve optimal growth and health, typically for several months. During this time, calves are fed a diet designed to promote steady weight gain and muscle development, preparing them for the finishing phase where they are further fattened for slaughter. This specialization allows backgrounding producers to bridge the gap between weaning and finishing, ensuring calves are in prime condition for the next stage of beef production.

3) Finishing: This phase involves raising steers, cows, and heifers until they reach market weight. Approximately 13% of beef producers specialize in finishing, dedicating their efforts to bringing the cattle to their optimal weight for slaughter. These producers provide high-energy diets, often consisting of grains and other concentrates, to promote rapid weight gain and fattening. The goal is to produce well-marbled beef that meets market standards for quality and yield. By concentrating on this final stage of beef production, finishing producers ensure that the cattle achieve the desired size and body composition required for the meat market.

In Canada, the management of nutritional resources to feed animals in growing conditions often involves the grazing system, distinct from feedlots that are used for finishing purposes. Within these grazing systems, the rotational grazing method is commonly employed. This approach entails moving cattle between different pastures or paddocks during the growing season, allowing vegetation time to rest and produce seeds. Although implementing rotational grazing requires additional labor, time, and careful planning, it significantly benefits pasture health by promoting the regeneration of specific areas and enhancing overall pasture productivity (Rothwell, 2005).

In addition to grazing management, Canada is actively working to reduce greenhouse gas emissions from beef production, with a particular focus on minimizing enteric methane (CH4) emissions from mature beef cows, which account for about 84% of enteric CH_4 emissions within the cow-calf herd. Research by Beauchemin et al. (2010), utilizing the Holos empirical model, estimated greenhouse gas emissions by considering CH4, nitrous oxide, and carbon dioxide emissions and removals on the farm, including those from manure. Within the beef production cycle, the cow-calf system accounted for about 80% of total GHG emissions and the feedlot system for only 20%. Notable, 84% of enteric CH_4 was from the cow-calf herd, mostly from mature cows. Therefore, mitigation practices to reduce GHG emissions from beef production should focus on reducing enteric CH_4 production from mature beef cows. Nevertheless, it is observed that today's Canadian beef cattle production has a lower environmental footprint and higher efficiency compared to 30 years ago. This is evident as cattle emit lower greenhouse gases per kilogram of beef produced and generally yield more beef per animal with a relatively lower input requirement (Legesse et al., 2016).

The above-mentioned observations present a significant opportunity to maintain the selection criteria for more efficient animals in Canada. Studies centered on residual feed intake (RFI) classification have indicated a comparatively reduced environmental impact in animals that exhibit greater efficiency (Haugen-Kozyra, 2021). Cattle classified as more efficient achieve the energy requirements for physiological processes more effectively, converting less feed into more body weight and obtaining more nutrients from the same quantity of feed. (Castro bulle et al., 2007). Moreover, the more efficient animals have lower energy requirements for maintenance (Herd et al. 2003) and produce less metabolic heat as consequence of less energy waste coming from digestion (Sainz et al., 2016). Although efforts in genetic selection based on RFI have a significant impact on fat deposition, with efficient animals being leaner than less efficient animals (Robinson and Oddy, 2004), favoring efficient energy utilization (Carstens and Kerley, 2009), differing results have been found in reproductive traits. For instance, some studies have found no differences in average calving dates (Basarab et al., 2011), while others have reported delays at first calving in efficient animals (Crowley et al., 2011). Efforts to understand differences between the more efficient and less efficient cattle based on feed-efficiency was described before as complex, given that RFI is a trait influenced by various factors, including genetic (Durunna et al., 2011).

However, there is a limited number of studies that explore the impact of weather variations on the physiological and metabolic responses of cattle that were previously tested for RFI, specifically concerning metabolism, activity behavior, and immunity response. Such studies could provide valuable insights into the mechanisms underlying the health and well-being of these animals. It is therefore imperative that further research is conducted in this area to enhance our understanding of the complex interactions between weather and cattle physiology.

2.2 Feed efficiency and residual feed intake in beef cattle

Residual feed intake is a crucial metric in the cattle industry, serving as a key indicator of feed efficiency that producers use to evaluate and select animals for optimal production efficiency (Koch et al., 1963). This metric is calculated by subtracting the expected feed intake required for livestock growth and maintenance from the actual feed intake (Arthur et al., 2001; Basarab et al., 2003), which provides insights into how efficiently an animal utilizes the feed it consumes.Studies by Hoque et al. (2009) and Seabury et al. (2017) have shown that RFI's genetic potential has a heritability ranging from moderate (0.21) to high (0.60). This indicates that genetic factors significantly influence an animal's feed efficiency, making RFI a valuable tool for cattle producers in breeding genetically feed-efficient offspring (Herd et al., 1997).

The RFI term hardly explored by Basarab et al. (2003) in Canada, has garnered widespread acceptance in animal breeding and research. By accurately assessing feed efficiency, RFI empowers producers to make informed decisions that enhance overall production efficiency and contribute to sustainable livestock farming practices. Thus, RFI's significance extends well beyond its role in genetic selection.

Estimating RFI in beef cattle involves several detailed steps. Firstly, the daily feed intake is determined by dividing the total feed intake recorded over a specific test period, typically obtained from a system like GrowSafe, by the number of days in the test period. This involves subtracting the expected feed intake, which is calculated based on regression covariates, from the actual feed intake. The daily feed intake for each female or male beef cattle is then calculated using the dry matter (DM) content of the diet and the daily feed intake. To standardize the dry matter intake (DMI) and make comparisons more meaningful, it is adjusted to a standard energy content, often expressed as 10 MJ per kg of DM based on the diet's energy content for heifers. Next, the average daily gain (ADG) of each animal is calculated based on the plotted weights over time, typically using linear regression to determine growth rates. The midpoint weight (MDWT) of each animal is then calculated, which involves adding the product of ADG multiplied by half of the days on the test to their initial weight in kilograms. Metabolic bodyweight (MWT) is derived from the MDWT raised to the power of 0.75.

Next, a linear regression model is employed to generate regression coefficients that predict the expected DMI of an animal based on its body weight, growth rate (ADG), and potentially other factors such as final ultrasound rib fat thickness (FUFAT). The regression model takes the form:

$$Y_i = \beta_0 + \beta_1 ADG_i + \beta_2 MWT_j + \beta_3 FUFAT_k + e_{ijk}$$

Where Y_i represents the standardized daily DMI, β_0 is the intercept, β_1, β_2, and β_3 are the partial linear regression coefficients and e_{ijk} represents the residual error.

The RFIf, or RFI corrected for FUFAT, is then computed for each individual by calculating the difference between their standardized daily DMI and the expected DMI predicted using the regression model's intercept and coefficients. This adjustment allows animals to be categorized as either high or low RFI, offering valuable insights into their feed efficiency and metabolic performance. A high RFI indicates less efficient animals, while low RFI values mean more efficient animals because they eat less to maintain all the physiological requirements for maintenance and growth. Incorporating the RFIf into the animal's evaluation enhances accuracy by accounting for the variations in body composition, particularly concerning fat and protein variances in energy demand.

Following the categorization of cattle based on RFI, it is crucial to consider that selection for feed efficiency can potentially affect the size of visceral organs, such as the liver (Zhang et al., 2017). Studies have indicated that the more efficient group of cattle may exhibit relatively lower liver weights compared to less efficient counterparts (Basarab et al., 2003). Additionally, it is known that liver may be 1C° degree warmer than other core tissues (Sessler, 2005). This finding can be associated with a better thermoregulation process under extremely hot environmental conditions favoring more efficient animals.

2.3 Factors affecting feed efficiency (genetics, nutrition, and management)

Feed efficiency in cattle is influenced by several key factors, including genetics, nutrition, and management **(Figure 2.1)**. Genetics play a crucial role, as RFI is influenced by numerous small-effect deoxyribonucleic acid (DNA) variants, in contrast to DMI, ADG, and

MWT, which are influenced by a mix of large and small-effect variants (Zhang et al., 2020), with a moderate heritability in cattle (Foroutan et al., 2021). In the context of determining an animal's RFI, genetic factors play a significant role in the potential interaction with the environment, where animals that excel in one environment may not perform as well in another (Lahart et al., 2020). This becomes particularly significant when aiming to improve feed efficiency through selective breeding. The concern arises because the ranking of animals based on their feed efficiency could change depending on the type of the diet cattle receive (Lawrence et al., 2012). This variability in diet is especially evident in different beef production systems, which adds complexity to efforts to consistently enhance feed efficiency across various environments.

Nutrition also plays a critical role in feed efficiency. Hafla et al. (2013) revealed a positive correlation between RFI and the increase in back fat depth in growing Brangus heifers fed a forage-based diet. In experiments performed on a forage-based diet, lower feed intake is expected due to the slower rate of passage through the rumen (Forbes et al., 2005). This variation can impact RFI compared to studies with high-concentrate diets, which alter rumen metabolism and microbial communities. These dietary shifts alter gene expression related to energy metabolism and nutrient use, affecting feed utilization efficiency in cattle (Ramos et al., 2021). Furthermore, diets high in starch, as described by Ezequiel et al. (2021), may lead to altered insulin responsiveness in yearling bulls categorized as less efficient based on RFI, necessitating higher insulin levels for glucose uptake by peripheral tissues compared to their more efficient counterparts. The re-ranking of RFI has attracted significant attention within the scientific community and will be further discussed after the management impact on RFI.

Lastly, management practices, such as developing strategies to reduce cortisol levels or metabolites resulting from cell disruptions, may help cattle express their genetic potential more effectively, including adequate prenatal nutrition, which may impact reproductive progeny capacity (Foroutan et al., 2021). Llonch et al. (2016) assessed cortisol levels following stress-inducing events, such as transportation, and found notable differences in cortisol responses among cattle with varying RFI levels. These findings suggest that cattle with more efficient metabolism may have improved stress tolerance, which could positively impact their overall feed efficiency.

Genetic, nutrition, and management factors collectively influence the repeatability of the RFI index across different time points and production stages, as highlighted by Kenny et al. (2018). This previous assumption underscores the potential utility of RFI, which required further investigation to assess feed efficiency consistently in different environments.

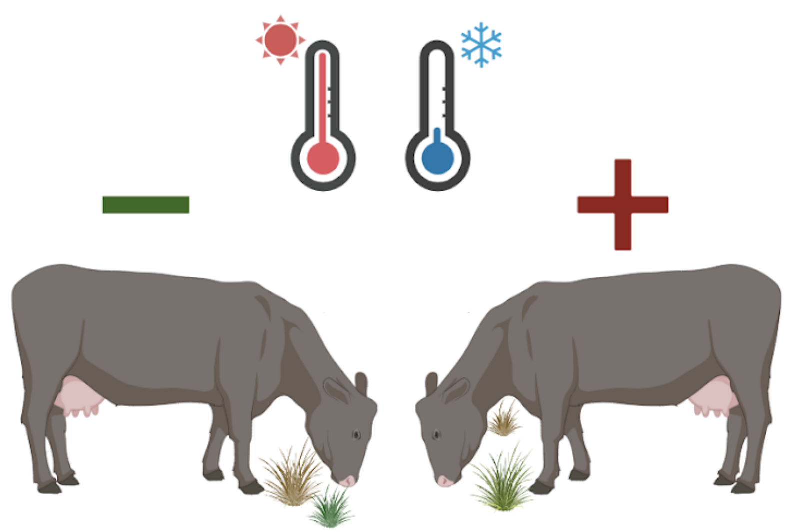

Figure 2.1 Residual feed intake classification is influenced by DNA variants, where factors like dry matter intake (DMI), average daily gain (ADG), and metabolic bodyweight (MWT) play roles with moderate heritability in cattle. External factors such as nutrition, particularly forage diets,

may reduce feed intake, while high-concentrate diets can alter rumen metabolism, thereby impacting RFI evaluation.

2.4 Environmental conditions in Canada

Western Canada faces a myriad of challenges during extreme summers, as highlighted by extensive research. Geissinger et al. (2024), identified a troubling tendency of increasing extreme heat events, surpassing historical norms, and leading to frequent heat waves in Canada. Concurrently, these have increased the risks of droughts due to climate shifts, resulting in diminished soil moisture and water scarcity, representing new challenges faced in agriculture (Hale et al., 2024; Lai et al., 2024). This combination of high temperatures and drought conditions has direct implications for the increased occurrence of wildfires, as demonstrated by research from Flannigan et al. (2009), posing significant threats to ecosystems, communities, and air quality. Additionally, extreme heat events have serious repercussions for both human health and livestock. Wolski et al. (2019) discuss how such events lead to heat-related illnesses, increased mortality rates, and diminished efficiency in livestock, with cattle being particularly affected.

The Climate Change Adaptation Platform (CCAP, 2021) underlines the economic and social repercussions of extreme summers on agriculture, forestry, tourism, and public health services. This underscored the urgent need for implementing adaptive strategies and resilience measures to mitigate these impacts. For example, during the summer of 2022 temperatures ranged between 31.1°C and 6°C, with an average of 19.1°C at Kinsella, in the province of Alberta, Canada (ACIS, https://www.alberta.ca/acis-find-current-weather-data#jumplinks-1, 2024), illustrating the extent of temperature variability experienced in the region.

On the other extreme, extreme cold winters in Canada bring about exceptionally low temperatures and heavy snowfall, creating challenging conditions that profoundly affect both human activities and natural ecosystems (Smith et al., 2020). These harsh winter phenomena are especially prominent in regions like the northern territories and provinces, encompassing Alberta, Saskatchewan, Manitoba, Ontario, Quebec, and the Atlantic provinces (Stewart et al., 2019 and Ward Jones et al., 2019). Treacherous road conditions due to ice and snow, coupled with reduced visibility during snowstorms, contribute to disruptions in travel and raise safety concerns among residents. For instance, during the winter of 2023 at Kinsella, Alberta, temperatures as low as -34 °C were reported, with a maximum temperature of 5.7 °C and an average of -14.7°C (ACIS, https://www.alberta.ca/acis-find-current-weather-data#jumplinks-1)

The impact of extreme cold extends beyond human endeavors to encompass wildlife and ecosystems. Species such as caribou, moose, and various birds must adapt to these harsh conditions by seeking shelter, conserving energy, and coping with limited food resources (Tomchuk, 2019). For livestock systems, extreme cold winters in Canada can have significant impacts on beef cattle, affecting their health, productivity, and overall well-being. During these periods, temperatures can plummet to extreme lows, posing challenges for cattle management and welfare. The CS in cattle can lead to several adverse effects. For instance, extreme cold temperatures can increase the animals' energy requirements as they need more energy to maintain their body temperature and stay warm (Young, 1983). This increased energy demand can result in higher feed intake to meet their nutritional needs, potentially leading to increased production costs for farmers (Baile and Forbes 1974). Extreme cold weather can disrupt water availability for cattle due to frozen water sources, leading to dehydration and further health issues (Young, 1983 and Petersen et al., 2016). Furthermore, the harsh conditions of extreme cold winters can also impact cattle behavior and grazing patterns. Cattle may seek shelter in barns or windbreaks to escape the cold winds and snow, altering their usual grazing routines (Collier and Collier, 2012). This behavior change can affect their nutrient intake and overall productivity. Extreme cold winters in Canada can significantly impact beef cattle by increasing their energy requirements, compromising immune function, disrupting water availability, and altering grazing behavior, all of which can have implications for their health and productivity.

2.5 Environmental conditions impacting cattle

Extreme weather conditions have been shown to significantly impact feed and water intake of beef cattle, resulting in heightened stress levels (NRC, 1981). Specifically, periods of extreme weather characterized by higher temperatures can impose a greater heat load on cattle, leading to what is commonly known as heat stress (HS). Heat stress can trigger a decrease in feed intake, affecting cattle overall nutritional status and productivity. The reduction in feed consumption is a critical concern as it directly impacts the energy and nutrient intake necessary for optimal growth and performance. Furthermore, HS can also influence the grazing behavior of cattle.

Researchers such as Lee et al. (2019) have discussed how cattle may adjust their grazing patterns in response to HS. This adjustment often involves shifting grazing activities to cooler parts of the day, such as early morning or late evening, to avoid the peak heat experienced during midday hours. This behavioral adaptation underscores the significant impact that extreme weather conditions can have on the daily routines and nutritional habits of beef cattle, highlighting the need for effective management strategies to mitigate these challenges. Another study by Wang et al. (2020) examined how energy, protein, carbohydrate, and lipid metabolism contribute to consistent energy production for better thermoregulation process and underscoring the importance of adequate water consumption and electrolyte balance under dehydration. This is crucial, as dehydration and ion imbalances can compromise cattle physiology due to mineral losses associated with HS. Similarly, Colditz and Kellaway (1972) found that HS has been shown to reduce DMI in heifers due to various factors, such as heightened respiration rates, resulting in increased water loss, subsequently diminishing the animal's appetite. Moreover, HS induces discomfort and disrupts metabolic processes, leading to a decreased inclination to consume feed. Lastly, Colditz and Kellaway (1972) highlight that hormonal shifts associated with HS also play a role in appetite regulation and contribute to the observed reduction in DMI among heat-stressed heifers. Under HS, cattle reduce feed intake to reduce metabolic heat from the digestion process and then, the body temperature will not increase (Collier et al., 2012).

Conversely, extreme winter conditions are expected to have the opposite feeding behavior to allow more energy for thermoregulation. A study developed by Wang et al. (2023) highlights the detrimental effects of long-term CS in cattle, negatively impacting growth performance and reducing activity behavior associated with feeding patterns. Additionally, variations in blood parameters, such as greater cortisol, non-esterified fatty acids (NEFA), and time spent standing after exposure to extreme CS (Kim et al., 2023). Additionally, increased thyroid hormones levels, and diminished insulin sensitivity were also observed in response to alterations in energy metabolism and nutrient utilization in beef cattle (Wang et al., 2023). Lower insulin sensitivity impairs glucose utilization, while increased thyroid function accelerates cell metabolism, resulting in higher heat production from the tricarboxylic acid cycle.

2.6 Thermotolerance, heat and cold stress in beef cattle

Thermotolerance in beef cattle can be defined as "the ability of cattle to withstand and adapt to elevated environmental temperatures while maintaining normal physiological functions and minimizing heat-induced stress responses" (Nienaber and Hahn, 2007). This includes efficient heat dissipation mechanisms, such as increased sweating, panting, and vasodilation, as well as behavioral adaptations like seeking shade and adjusting grazing patterns to avoid peak heat periods. Conversely, in extreme cold, thermotolerance can be defined as "the capacity of cattle to cope with low environmental temperatures, maintain body temperature within a tolerable range, and minimize CS-induced physiological disturbances" (Soren, 2012). This involves physiological adaptations such as increased metabolic activity for heat production, shivering, seeking shelter, and modifying grazing behavior to conserve energy and minimize heat loss.

When extreme environmental conditions have been imposed, production may have a negative correlation with feed efficiency traits because more energy and nutrient utilization for maintenance and alleviating HS are required (Britt et al, 2003). Regarding size, the smaller individuals are more thermotolerant when compared with bigger mature cows (Wang et al., 2020). However, studies on feed efficiency have found that less efficient lambs often have small pancreas and spleen compared to their more efficient counterparts, but enzymes function of this organs was not different between groups (Meyer et al., 2015). These observations highlight the importance of understanding the relationship between feed efficiency and thermotolerance in cattle.

Under prolonged periods of extreme cold, growth performance may be negatively impacted with lower weight gain and decreased feed efficiency when compared to cattle in proper environmental conditions. Further, changes related to behavior, such as greater restlessness, less physical activity, greater heart rate, and lower respiratory rate are also observed. Consequently, energy allocation for growth and production will be shifted towards thermoregulation (Wang et al, 2023). Alternatives like providing heated water can potentially enhance cattle performance and welfare. Therefore, optimizing management practices for beef cattle in cold weather conditions, particularly regarding their energy needs and water consumption patterns in this extreme weather, is crucial (He et al, 2022). During CS, cattle can also exhibit changes in respiratory rates, which can be observed by a decrease in respiratory rate as they conserve energy and reduce metabolic heat production (Habibu et al., 2019). This decrease in respiratory rate helps minimize heat loss through respiration and maintain body temperature. However, prolonged exposure to cold temperatures can lead to respiratory issues, such as pneumonia, which may cause an increase in respiratory rate as the animals try to cope with respiratory infections (Dennis, 1986).

Conversely, HS in cattle can lead to an increase in respiratory rate as they attempt to dissipate excess heat and maintain their body temperature within a normal range (Gaughan et al., 2000). During periods of extreme heat, cattle may pant more frequently, which is a physiological response to regulate their body temperature by evaporative cooling through the respiratory system (Idris et al., 2021). Increased respiratory rate helps in heat dissipation but can also result in respiratory alkalosis due to excessive loss of carbon dioxide through panting (Robertshaw et al., 2006).

During HS, cattle can also experience vasodilation as part of their thermoregulatory response to dissipate excess heat and cool down (Collier et al, 2019). Vasodilation allows for increased blood flow to the skin, facilitating heat transfer to the environment through convection, radiation, and evaporation (dos Santos et al., 2021). This mechanism helps reduce body temperature during periods of elevated heat, preventing heat-related illnesses such as heat stroke. In contrast, CS triggers vasoconstriction rather than vasodilation in cattle. When exposed to cold temperatures, blood vessels in the skin constrict to reduce blood flow and minimize heat loss from the body (Maraia et al., 2010). This vasoconstrictive response helps conserve body heat and maintain core temperature, ensuring that cattle can withstand cold weather conditions without experiencing hypothermia.

Under HS, cattle may also experience a decrease in heart rate as part of their thermoregulatory mechanisms to cope with elevated temperatures (Ferrazza et al., 2017). However, the increased heart rate helps in redistributing blood flow to the skin for heat dissipation through vasodilation and enhanced evaporative cooling, thereby aiding in maintaining body temperature within a normal range (Charkoudian., 2023). This physiological response is crucial for preventing heat-related illnesses and maintaining overall homeostasis during periods of HS. However, during CS the decreased heart rate is part of a broader strategy to minimize heat loss and maintain core body temperature, primarily through vasoconstriction and behavioral adaptations such as seeking shelter (Sejian et al., 2018). Yet, prolonged exposure to extreme cold conditions can also lead to increased heart rates due to stress and increased energy demands for thermogenesis.

2.7 Genetic, environment, and physiological factors influencing thermotolerance

Body size plays a significant role in determining thermotolerance in cattle. Calves and heifers, due to their smaller body mass relative to body surface area, produce less metabolic heat. This characteristic allows them to effectively dissipate body heat, making them more heat tolerant than mature cattle. This phenomenon is well-documented in studies such as West (2003), which highlight the efficiency of heat dissipation in younger cattle with greater body surface area-to-mass ratios.

Moreover, Branton et al. (1966) observed that cattle with a lower metabolic rate tend to exhibit greater thermotolerance. A low metabolic rate means that the animal produces less internal heat during metabolic processes. As a result, these cattle have a reduced heat load and are better equipped to withstand high ambient temperatures without experiencing HS.

Fat storage and insulation processes also contribute to thermotolerance. In *Bos Indicus* breeds, the hump muscle mass located above the withers on the back of the thoracic region contains a significant amount of fat tissue, which acts as an insulating layer. This fat layer helps in reducing heat transfer from the external environment to the body, thus providing a degree of thermal insulation (Berman, 2004). Such insulation is particularly beneficial during hot weather conditions, as it helps prevent excessive heat absorption by the body. *Bos indicus* cattle, which often have cervicothoracic humps, are well-adapted to arid and hot climates. The hump's characteristics, such as fat storage and heat dissipation properties, are evolutionary adaptations that enable these cattle to thrive in environments with high temperatures and limited water resources (Gaughan, 2012). Studies have shown that Zebuine cattle with cervicothoracic humps experience less HS compared to cattle breeds without such humps (Hansen, 2004). This indicates that the hump plays a crucial role in enhancing thermotolerance and reducing the negative impacts of HS in Zebuine cattle.

Hair coat characteristics are also closely linked to thermotolerance. In Brazil, Bertipaglia et al. (2005), measured the hair length of cattle, which ranged from 1.5 to 5.3 mm. They observed that cattle with longer hair, within this specific range, exhibited better heat conductance compared with those with shorter hair. This finding supports the idea that hair coat characteristics, such as length and density, play a significant role in determining the heat conductance and thermotolerance of cattle, especially in regions with high temperatures as cattle with sleek hair demonstrate the ability to maintain lower rectal temperatures, particularly under HS, with variations ranging from 0.18 to 0.61°C (Olson et al., 2003). The slick gene is a mutation in the prolactin receptor gene (Huson et al., 2014). This observation highlights the potential benefits of sleek hair in managing thermal stress in cattle. This trait, often associated with the "slick hair coat gene", proves especially advantageous in regions with elevated temperatures or during periods of HS. Furthermore, cattle hair with more color density retains more direct solar radiation compared to color-lighter hair coat (Hutchinson et al., 1969).

In contrast to periods of HS, during CS, the hair coat of cattle provides insulation against cold temperatures by trapping air close to the skin, creating a layer of warmth (Mader et al., 2009). Cattle with thicker and denser hair coats have enhanced insulation, which reduces heat loss and helps maintain body temperature in cold environments. To conserve heat, cattle can regulate blood flow to their extremities through vasoconstriction, reducing blood flow to peripheral areas like the skin surface and extremities (Suttle, 2010). This physiological response minimizes heat loss through the skin and maintains core body temperature. Additionally, when exposed to cold, cattle increase metabolic heat production through processes such as shivering and increased muscle activity (Carroll et al., 2012). This heat generation helps offset heat loss and contributes to maintaining body temperature. Behavioral adaptations in response to CS include seeking shelter, huddling together with other cattle for warmth, and altering grazing patterns to conserve energy (Eicher, 2012). These behaviors help reduce heat loss and improve thermoregulation. Adequate nutrition is also essential for supporting these cold dissipation mechanisms, as sufficient energy and nutrients in the diet enables cattle to maintain the metabolic processes required for heat production and thermoregulation (Johnson et al., 2014).

Heterosis, or hybrid vigor, also plays a significant role in thermotolerance. This phenomenon happens when breeding two genetically different parents results in offspring that outperform both parents in traits such as growth rate and overall performance (Shull, 1914). In mice, Lynch et al. (2009) found that the influence of heterosis on thermoregulation response is associated to metabolic efficiency in mice. Similarly, in beef cattle heterosis was also associated with thermoregulation under extreme hot conditions (Hammond et al.,1996). These findings suggests that environmental factors such as temperature, humidity and solar radiation may influence the extent of heterosis in crossbred cattle. However, several factors as environmental conditions and forage availability can contribute to the better expression of heterosis as:

- Improved nutrient availability: Warm seasons are often associated with abundant forage growth and enhanced nutrient availability, which can support the growth and development of crossbred offspring, leading to enhanced heterosis (Fitzhugh et al., 1975).
- Reduced stress: In the tropics, the most significant heterosis occurs in crosses of breeds adapted to different environments, allowing for the combination of complementary traits to increase profitability (Bunning et al., 2019).
- Efficient metabolic processes: Warmer temperatures can promote more efficient metabolic processes in cattle, leading to better utilization of nutrients and higher productivity in crossbred animals (Meyer et al., 2008).

2.8 Rumen temperature and body temperature responses to environmental stress

Understanding the relationship between rumen temperature (RT) and body temperature in beef cattle is crucial for evaluating their thermoregulatory mechanisms and health status. Research by Godyn et al. (2019) and Rose-Dye et al. (2011) has shown that RT correlates with body temperature and can indicate HS and metabolic activity in cattle. Therefore, monitoring both RT and body temperature provides valuable insights into the physiological responses of cattle to environmental conditions, particularly during HS, helping assess their thermoregulation and overall health.

A study by Boehmer et al. (2015) revealed that RT was impacted by hot environmental conditions, with a positive correlation between RT and rectal temperature observed using the temperature-humidity index (THI) as a measure of environmental comfort in dairy cows. Additionally, during early and late lactation stages, when metabolic demands are higher, vaginal temperature was found to be higher in comparison to dry cows under consistent climatic conditions as reported by Araki et al. (1984). Srikandakumar and Johnson (2004) noted that Holstein cows exhibited significantly higher rectal temperatures compared to Jersey or Australian Milking Zebu cows when breeds were compared. Additionally, the study by Mader et al. (2002) found that beef cattle with black hair coats exhibited higher tympanic temperatures compared to those with light color, suggesting that hair coat color is an important characteristic to consider when selecting individuals. Howard et al. (2014) explored the impact of genetic factors on body temperature regulation and thermotolerance in beef cattle exposed to climatic stress. Their study found that core temperature had high heritability in summer and moderate heritability in winter, underscoring the role of genetic selection in breeding programs aimed at enhancing cattle adaptability to extreme environmental conditions.

2.9 Physiology and blood parameters responses to environmental stress

Temperature regulation in cattle involves intricate coordination between the autonomic nervous system and the neuroendocrine system, leading to changes in blood biochemical parameters in response to environmental variations. Environmental stressors, such as extreme weather, can significantly alter thyroid hormone levels in cattle. For instance, during HS, feed consumption decreases (Nardone et al., 2006), a phenomenon also observed in other farm animals, which has been linked to reduced thyroid secretion (Garg et al., 2001). Conversely, during CS, a study conducted by Li et al. (2015), concluded that exposure to cold temperatures led to notable increase in the concentrations of triiodothyronine (T3), thyroxine (T4), and adrenocorticotropic hormone in the bloodstream. Moreover, raised circulating norepinephrine and epinephrine hormones has been reported under HS conditions (Starkie et al., 2005). These hormonal changes in HS, result in reduced levels of T3 and T4 in the bloodstream, leading to a lower basal metabolic rate and consequently, reduced heat production (Gaughan et al., 2012). The decrease in T3 and T4 is an immediate adaptive reaction, which, combined with decreased plasma growth hormone (GH), synergistically contributes to lowering heat production (Yousef et al., 1966). Furthermore, Hu et al. (2018) documented notable physiological alterations under HS, including greater GH, lactate, prolactin, superoxide dismutase, alongside lower blood urea nitrogen, C-reactive protein, lactate dehydrogenase, lipid peroxide, norepinephrine and erythrocyte potassium. Conversely, during CS, they reported lower levels of dopamine, GH, lactate, prolactin, superoxide dismutase and adrenocorticotrophic hormone, but higher levels of cortisol, corticosterone and erythrocyte potassium among Chinese Holstein cows following exposure to hot and cold environmental conditions.

Stressful conditions, such as those encountered during transportation, have been shown to downregulate the hypothalamic-pituitary-adrenal (HPA) axis in Limousine cattle (Fazio et al., 2023). During HS, cattle may also experience changes in plasma serotonin (5-HT) levels. Heat stress actuvates the HPA axis and the sympathetic nervous system, leading to increased cortisol levels and catecholamine release (Borell et al., 2001). These hormonal changes can influence 5-HT production, increasing its levels in the plasma while decreasing its output in the brain to help alleviate HS (Shen et al., 2012). High ambient temperatures can also directly affect 5-HT metabolism, with HS potentially altering tryptophan metabolism, a precursor for 5-HT synthesis, thus potentially impacting plasma 5-HT levels (Li et al., 2022). The CS can also impact 5-HT synthesis and metabolism with a significant influence in metabolic rate (Lin et al., 1978).

Serotonin plays a crucial role in brain functions such as mood regulation and cognitive processes (Homberg, 2012), as well as in various physiological processes in peripheral tissues, particularly in gastrointestinal systemic function with greater motility (Srinivas, 2022). Due to its distribution throughout the body, with less than 2% of 5-HT being in the brain, the vast majority of 5-HT found in the peripheral blood is sourced from the gastrointestinal tract. This phenomenon underscores the significant role of the gastrointestinal system in 5-HT production and regulation within the body (Llambías et al., 2003).

During HS, recent research by Arneson (2021) has highlighted the impact of insulin and glucagon secretion within the endocrine pancreas, sparking interest in its potential role in altered glycemic states. Furthermore, elevated levels of gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter, have been observed to influence the heat-regulating center, potentially aiding in core body temperature regulation by facilitating heat dissipation (Guo et al, 2018). Gamma-aminobutyric acid plays a role in gastrointestinal function, where it interacts with excitatory or inhibitory GABA receptors and glutamate receptors to modulate gastric motility and mucosal function (Tsai et al., 1993). However, after HS, gastrointestinal motility, punctually gut motility, is reduced (Calamari et al., 2018). Arneson et al. (2024) found that GABA levels also tended to be lower in cattle following HS, suggesting a potential impact on gut motility and overall gastrointestinal health. Gamma-aminobutyric acid is a neurotransmitter produced by specific probiotic bacteria, which play a crucial role in maintaining gut health and overall well-being. Among these beneficial bacteria are *Lactobacillus brevis* and *Bifidobacterium dentium*, which have been identified as significant producers of GABA. These probiotic bacteria reside in the gastrointestinal tract and contribute to the synthesis of GABA through enzymatic processes (Mazzoli et al., 2016). The production of GABA by these bacteria supports digestive functions and has potential implications for neurological health, mood regulation, and immune system modulation (Barrett et al., 2012). Thus, the presence of GABA-producing probiotics underscores the intricate connection between gut microbiota and physiology, highlighting the importance of a balanced gut microbiome for optimal health outcomes. The GABA shows gastrointestinal function where neurons may target excitatory or inhibitory GABA receptors and glutamate receptors, which in turn modulate gastric motility and mucosal function as reported by Tsai et al. (1993). After HS, gastrointestinal motility, punctually gut motility is reduced (Calamari et al., 2018) and was found GABA with a tendency to be lower in cattle after this environmental stimulus (Arneson et al., 2024).

Recent molecular biology research has identified that HSP70 gene as a highly promising candidate to detect CS for further validation across diverse cattle populations and a particular interest exists due to its potential role in enhancing CS resilience in cattle, a critical aspect of their adaptation to varying environmental conditions. Among a pool of 193 candidate genes, the expression of HSP70, which encodes for heat-shock protein 70, exhibited a statistically significant increase following cold exposure (Xu et al., 2017). This finding suggests that HSP70 may play a crucial role in the cellular response to CS and warrants additional functional studies to elucidate its exact mechanisms and potential applications in improving cattle welfare and productivity under challenging climatic conditions. Additionally, a study developed by Bharati et al (2017) suggests that HSP70 plays a role in helping cattle adapt to HS. The gene's two-phase expression pattern might provide extra protection for cattle during extended periods of HS, indicating its significance in managing thermal stress and supporting overall adaptation strategies.

Haptoglobin (Hp), an acute-phase protein, has been utilized as a reliable physiological marker to assess the welfare status of animals, as highlighted by Arthington et al. (2003). This indicates that increased Hp levels can indicate compromised welfare, offering valuable insights into the health and stress status of animals in various management contexts. For instance, after exposure to HS is expected greater concentrations of Hp. However, in a study involving cooling interventions for Holstein cows, Hp levels decreased, which was attributed to the improved environmental comfort provided by the cooling measures (Cheng et al., 2018). Haptoglobin acts as a marker of inflammation in cattle (Moriel et al., 2018) under certain conditions, reflecting a different aspect of the physiological response to stress and environmental factors in livestock. Under moderate CS, calves with a satisfactory diet did not show greater levels of Hp (Nonnecke et al., 2009). Cooke and Bohnert (2011) observed that cortisol, a hormone linked to stress response, is more susceptible to variations induced by physiological stress and management practices compared to Hp. Tadich et al. (2013) established a benchmark of 0.1 mg/ml or 100 ng/ml for healthy cows, with a baseline Hp level of 0.06 mg/ml in non-lame cows. Additionally, Heegaard et al. (2000) found a strong correlation between the extent and duration of the Hp response and the severity of clinical symptoms. Another acute phase protein important in cattle is the serum amyloid A (SAA), which is a more sensitive indicator of acute disease when compared with Hp (Alsemgeest et al., 1994).

The assessment of growth metabolism can be performed using the insulin-like growth factor type 1 (IGF-1) hormone, an essential regulator of metabolism and growth. During periods of HS or chronic CS, fluctuations in IGF-1 levels can occur, exerting an influence on various aspects of cattle health and performance. Research by Baumgard and Rhoads (2013) highlights the dynamic response of IGF-1 to thermal stress in cattle, noting that HS can lead to a reduction in circulating IGF-1 levels, affecting growth rates and nutrient utilization in beef cattle. Additionally, CS may also impact IGF-1 concentrations, although the specific responses can vary depending on factors such as duration and severity of cold exposure, as discussed by Kadzere et al. (2002).

Insulin-like growth factor 1's regulation is closely tied to GH, which primarily affects the liver and stimulates the release of about 80% of plasma IGF-1. Then, IGF-1 mediates the indirect effects of GH on different body tissues (Le Roith et al., 2001). Rhoads et al. (2009) delve into the mechanisms underlying the modulation of IGF-1 during extreme weather conditions. These mechanisms include alterations in hormone secretion, metabolic pathways, and cellular signaling pathways, all of which contribute to the overall thermoregulatory and metabolic responses of beef cattle to environmental stressors. During HS, DMI is reduced and less IGF-1 concentrations are expected (Bernabucci et al., 2010). Cortisol, which generally promotes catabolic processes and stress responses (Rhoads et al., 2010), contrasts with IGF-1, which supports anabolic activities and growth. In the other hand, IGF-1 was greater during the stimulus of CS in grazing sheep that are thermotolerant (Zhang et al., 2020), due to more IGF-1 receptors in the brown adipose tissue (Boucher et al., 2012) that is regulated by IGF-1 production (Desautels and Ram, 1996).

Another important physiological path to understand during environmental stress is protein metabolism. Blood urea nitrogen (BUN) is primarily originating from the rumen, where ammonia nitrogen is produced through the breakdown of dietary crude protein (CP) and the deamination of amino acids. This ammonia is absorbed through the rumen wall and then converted into urea in the liver, which is subsequently released into the bloodstream (Huntington et al., 1999). The process involves a series of biochemical reactions in the rumen and liver, highlighting the dynamic interplay between dietary protein degradation, nitrogen metabolism, and urea synthesis in ruminant animals (Qin et al., 2022). An imbalance in the profile of adsorbed amino acids, characterized by an unequal distribution or inadequate proportions of amino acids, leads to an increase in the concentration of BUN. Increased BUN concentrations in calves, for example, have been used as an indicator of dehydration (Lee et al., 2020). Elevated BUN levels in serum have also been associated with improved protein absorption, as demonstrated by research conducted by Huang et al. (2015) and Liu et al. (2023). This implies that an increase in BUN may signify enhanced utilization of dietary CP, highlighting a positive relationship between BUN levels and efficient feed CP utilization. However, a study conducted by Cabral da Silva et al. (2020) on crossbred Holstein x Gyr heifers failed to establish a direct link between elevated BUN levels and improved nitrogen balance, particularly in groups classified as more or less feed-efficient. Additionally, recent findings from de Assis Lage et al. (2019) suggest that animals with low RFI consume less protein but retain similar or more amounts of protein, indicating the potential for improved nitrogen use efficiency (i.e., nitrogen retention relative to nitrogen intake). Moreover, a microbiome study by Zhou et al. (2023) found that more feed-efficient animals had a greater abundance of *Prevotella* in the rumen when compared with the less efficient animals. *Prevotella* is known for generating polypeptides from the consumed diet (Walker et al., 2003), highlighting the potential impact of rumen microbiota on protein metabolism and efficiency.

The liver is pivotal in fat management, especially during negative energy balance when energy intake is less than expenditure. Under such conditions, circulating NEFA becomes abundant and exceeds the liver's capacity for complete oxidation. This surplus of NEFAs undergoes a process known as ketogenesis, where they are transformed into ketone bodies such as acetoacetate, β-hydroxybutyrate (BHBA), or acetone. This metabolic shift may lead to a condition called ketosis (Crociati et al., 2017). Furthermore, NEFA has alternative fates within the liver. They can be either reconfigured into triglycerides through re-esterification, forming lipid droplets stored within hepatocytes' cytoplasm or be exported from the liver as very low-density lipoproteins, contributing to the transport of fats throughout the body (Barletta et al., 2017). In ruminants, factors such as feed restriction and body fat mobilization are common to increase NEFA and BHBA concentrations (Orquera-Arguero et al., 2023). Interestingly, under chronic HS, NEFA concentrations in blood did not change (O'Brien et al., 2010). Similarly, during exposure to cold temperatures no differences were observed on the levels of NEFA circulating in the bloodstream, indicating that the animals' metabolic responses to CS did not involve changes in NEFA levels (Kang et al., 2020). However, periods of reduced feed intake, often due to adverse environmental conditions, can lead to increased concentrations of BHBA and NEFA in the blood (Do Amaral et al., 2009; Lu et al., 2007).

Leptin, a hormone associated with adipocyte numbers, often shows higher concentrations in peripheral blood when fat stores increase (Minton et al., 1998; Florant et al., 2004). The correlation between blood LEP levels and carcass composition metrics suggests that LEP concentrations could serve as an additional marker for assessing fat content in feedlot cattle (Geary et al., 2003). However, in terms of RFI, Perkins et al. (2014) suggest that animals with LOW-RFI in warm conditions show increased expression of orexigenic neuropeptide genes, regulating appetite and feeding behavior, independently of adipose tissue-derived LEP expression. Leptin levels were found to be positively associated with DMI and RFI, suggesting that LEP plays a role in regulating both how much the animals eat and how efficiently they convert that feed into body mass (Foote et al., 2015). Higher LEP concentration was found in the LOW-RFI group, which deposited more fat (Foote et al., 2016). However, low LEP concentrations may be expected after HS as it could reduce feed intake. Nevertheless, in pigs tested for efficiency and evaluated for thermoregulatory status, no differences in LEP levels were found after HS (Campos et al., 2014).

2.10 Blood cell count and residual feed intake

Cell counts in cattle refer to the measurement of somatic cells, primarily white blood cells (WBC) in blood samples. Variations in WBC can indicate an immune response or inflammation, which may be indicative of health issues in cattle. Studies by Roland et al. (2014) and O'Loughlin et al. (2014) emphasize the significance of cell counts in blood samples as a marker of systemic inflammation or infection in cattle. Elevated WBC counts can indicate an immune response to pathogens or other stressors, providing valuable insights into the health status of the animal. The complete blood cell count (CBC; **Figure 2.2**) in beef cattle could be used as a diagnostic tool for evaluating various health conditions, such as infections, anemia, and inflammatory diseases. The CBC helps to assess the red blood cell count, WBC, hemoglobin levels, hematocrit, and platelet count, providing insights into the animal's health status and potential health issues. The author Roland et al. (2014) mentioned a physiological leukocytosis in response to various stressors such as stress, excitement, fear, physical exercise, or parturition **(Figure 2.3)**. A typical stress leukogram involves an increase in neutrophils (neutrophilia), a decrease in lymphocytes (lymphocytopenia), a reduction in eosinophils (eosinopenia), and occasionally an increase in monocytes (monocytosis), as described by Roland et al., (2014).

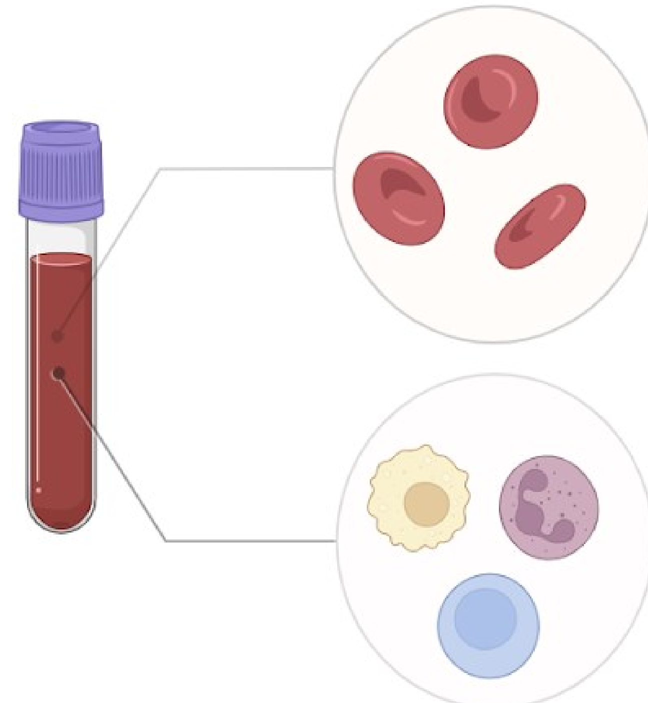

Figure 2.2. Hematological evaluations as the complete blood cell count have been widely utilized by researchers to evaluate the health status of cattle and to accurately diagnose diseases. The evaluation of the health status of cattle by measuring various parameters such as red blood cell count, white blood cell count (WBC), hemoglobin levels, hematocrit, and platelet count provides vital insights into hematopoietic cell disorders, while WBC counts, encompass neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Both evaluations are crucial for diagnosing specific diseases and monitoring immune system.

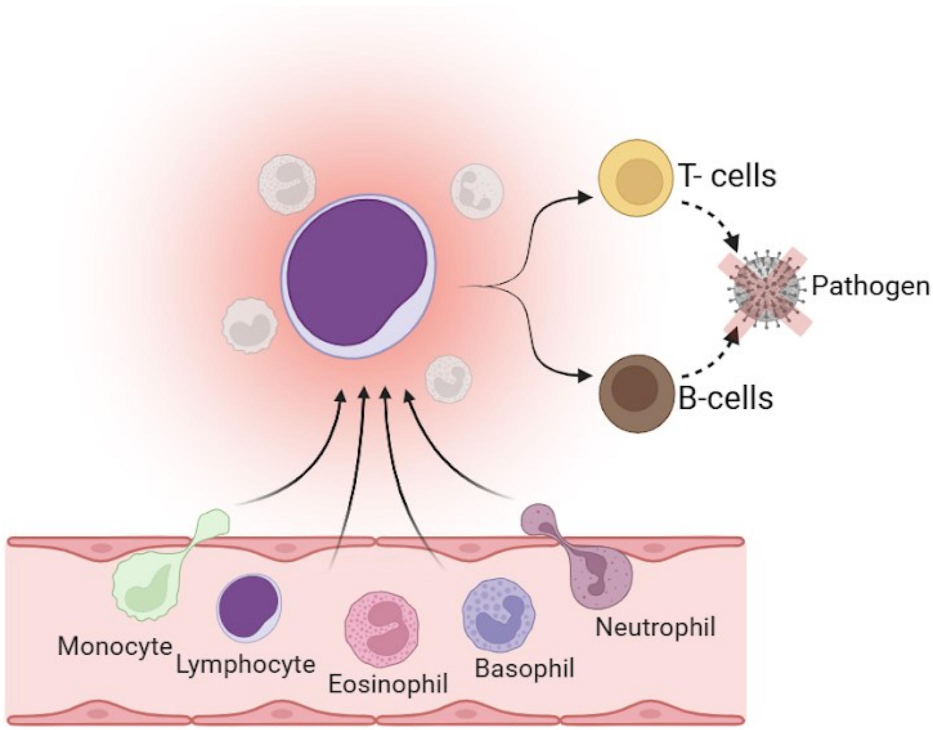

Figure 2.3. White blood cells have leukocytes as part of the cell pool and are essential for fighting infections. The two main types are T cells (T lymphocytes) and B cells (B lymphocytes). T cells include helper T cells (Th cells) and cytotoxic T cells (Tc cells). B cells produce antibodies. Lymphocytes recognize germs and help the body remember how to fight them. Additionally, T cells also help regulate immune responses (Crotty et al., 2018).

Stressful management practices can elevate plasma cortisol levels, which impact immune function, as catecholamines released during stress stimulate cortisol secretion, affecting immune cell activity (Carroll et al., 2007). The influence of cortisol on immune responses is well-documented (Roth et al., 1985; Caroprese et al., 2010). Extreme environmental conditions, such as HS, disrupt the hormonal responses of the HPA axis, leading to increased cortisol levels and suppressed production of immune signaling molecules called cytokines (Bagath et al., 2019).

In a study made by Taiwo et al. (2024) in beef cattle that were previously tested as more efficient displayed a notable increase in messenger ribonucleic acid (mRNA) expression of genes associated with immune cell functions, this was observed in both whole blood and liver tissues of these animals' recognizing pathogens and regulating processes like phagocytosis, highlighting the crucial role of RFI in immune system modulation. Moreover, Arias et al. (2016) found under CS a significant impact of alpaca monocytes by increasing the expression of Interleukin-1 beta cytokines, these cytokines play crucial roles in immune responses, inflammation, and pulmonary defense mechanisms, particularly in combating adverse weather conditions and infections caused by pathogens.

2.11 Activity behavior responses in extreme environments and their relationship with residual feed intake

Elevated ambient temperatures can alter grazing behavior of cattle, causing cattle to shift their grazing activities to either early morning or late evening periods (Dwyer, 1960). An Australian study on beef cattle behavior activity emphasised that grazing activity is the predominant behavior, accounting for an average of 51% of observation scans and corresponding to approximately 6.1 hours of grazing time per day per herd with a maximum of 7.3 hours (Kilgour et al., 2012). Moreover, it describes the diurnal rhythm of grazing, with peaks in the early morning and late afternoon in four of six beef herds. While grazing receives significant attention in the literature, other behaviors such as standing resting, lying resting, and walking are less explored (Kilgour et al., 2012). In an observational study of six herds of beef steers under commercial conditions in Australia, Kilgour et al. (2012) found that cattle spend approximately 95% of their time grazing, resting/ruminating, and walking. Additionally, more steps are related with better reproductive performance, associated with greater estrogens and greater walking activity (López-Gatius et al., 2005) that plays an important role when the prediction of ovulation is required (Roelofs et al., 2005).

Generally, dairy cattle are observed to lie down for an average of 11 hours per day, consistent with findings from similar studies in Switzerland (Wechsler et al., 2000). Beef cattle have been reported to spend approximately 10.3 hours per day in a lying position (Aharoni et al., 2009). In a study conducted in Ireland, visual observation detected grazing activity at a median of 40.5 minutes per hour, while an automated sensor system recorded a median of 47 minutes per hour for grazing time. In terms of walking, visual observation showed a median of 1 minute per hour, ranging from 0 to 18 minutes per hour, whereas the automated system recorded a median of 2 minutes per hour, with a range of 0 to 17 minutes per hour (Werner et al., 2018). In general, the normal average lying time is 11 hours per day, which aligns with previous studies made in Switzerland in dairy cattle (Wechsler et al., 2000). Similarly, research on beef cattle indicates they spend approximately 10.3 hours per day in a lying position (Aharoni et al., 2009).

As environmental temperatures rise, cattle exhibit a notable behavior of reducing their lying time by approximately 30%, adjustments that allows them to increase their body surface area, facilitating heat dissipation, as discussed in studies by Cook et al. (2007) and Schütz et al. (2011). This adaptive response is crucial for alleviating HS. Furthermore, the prolonged standing time that accompanies increased ambient temperature can have significant implications for the cows' energy balance and nutrient utilization. Extended periods of standing can elevate energy expenditure and lead to alterations in nutrient metabolism, potentially increasing the cows' maintenance requirements. This phenomenon has been highlighted in research by West (2003) and underscores the complex interplay between environmental factors, behavior, and metabolic demands in cattle exposed to HS. Additionally, the process of rumination, which is essential for proper digestion and metabolic activities in cattle, primarily takes place during lying down. Therefore, when cows have shorter lying times, it can negatively impact their metabolic processes and digestive efficiency (Chaplin et al., 2000).

In ambient temperatures where cows are comfortable, they typically eat around 12 to 15 meals throughout the day. However, during periods of HS, their eating frequency reduces to about 3 to 5 meals per day. This change is coupled with larger meal sizes, which may have implications for the health of their digestive system (Kadzere et al., 2002). Conversely, CS leads to increased lying and feeding time (Wang et al., 2023), though cattle often spend more time standing in extreme cold to maximize heat absorption from solar radiation (Olson and Wallander, 2011).

Behavioral patterns and their relationship to feed efficiency also vary. Hafla et al. (2013) found that first-parity females exhibited significantly higher daily step counts and more lying-bout frequencies compared with those in second parity. However, these metrics did not differ based on RFI classification, suggesting that while parity influences physical activity, feed efficiency classification does not have a clear impact on these behaviors. In grazing systems, more feed-efficient heifers spent less time in a standing position and more time lying than less efficient heifers (Lawrence et al., 2012). Recently, Sprinkle et al. (2019) demonstrated that on a cooler day (max of 23°C), inefficient cows grazed 1.5 h longer than efficient cows, however, on a hot day (max of 30°C) they grazed 2 h less than efficient cows. Authors argue that less efficient cows would be expected to have greater appetite than efficient ones to compensate for increased energy requirements and should increase daily grazing time when conditions are favorable. Yet, greater appetites are accompanied by larger gastrointestinal tracts (Sprinkle et al., 2000), increasing metabolic heat load and reducing heat tolerance. Efficient cows, however, displayed compensatory behavior during extended periods of high temperatures by adapting their grazing patterns and utilizing more challenging terrain to manage heat stress. However, this literature review revealed a notable gap in scientific information regarding the activity behavior of cattle previously tested for feed efficiency based on RFI. This indicates a need for further research to better understand the relationship between feed efficiency and activity behavior in cattle, which could have significant implications for livestock management and production practices.

2.12 Conclusion

In conclusion, Alberta is a foundation of Canadian beef cattle production, hosting most of the nation's cattle inventory. The industry's operations—from cow-calf production to backgrounding and finishing—are finely tuned to maximize efficiency at each growth stage. Environmental stewardship practices, such as rotational grazing, are pivotal in promoting sustainable beef production across Canada. Efforts to mitigate greenhouse gas emissions, particularly enteric methane from mature beef cows, underscore Canada's commitment to environmental sustainability in beef production. Residual feed intake has emerged as a crucial metric for measuring feed efficiency, influenced by genetics, nutrition, and management practices. The moderate heritability and repeatability across production stages highlight the RFI utility in optimizing feed utilization and, consequently, enhancing cattle health and productivity. However, there is a need for more research to understand how weather variations affect the physiological and metabolic responses of RFI-tested cattle, which could refine management strategies. Extreme weather conditions, such as HS and CS, significantly impact beef cattle, altering their feeding behavior, metabolic processes, and overall health. Heat stress reduces feed intake and disrupts thermoregulation, whereas CS increases energy demands and alters feeding patterns. Behavioral and physiological adaptations, including body size, fat storage, and hair coat characteristics, are critical for cattle to cope with these extremes. Effective management strategies are essential to mitigate these challenges and ensure optimal cattle well-being and productivity amid evolving climatic conditions. Furthermore, immune system monitoring through cell counts, mainly WBC counts, provides valuable insights into cattle health. Elevated WBC counts indicate immune responses to pathogens or stressors, emphasizing the importance of regular health assessments. Stressful conditions, like HS, can suppress immune functions, underscoring the vulnerability of cattle under environmental stress. Conversely, cattle selected for higher feed efficiency exhibit enhanced immune-related gene expression, linking metabolic efficiency with immune modulation and overall health resilience.

Ongoing research and innovative management practices are crucial to sustainably enhance beef cattle production in the face of climate change impacts. Integrating scientific advancements in genetics, nutrition, and environmental management will ensure resilient and productive beef cattle populations across Canada's diverse agricultural landscapes.

Chapter 3. Metabolism and growth performance of grazing beef heifers with divergent residual feed intake under hot and cold environments

3.1 Abstract

The beef industry is increasingly focused on improving feed efficiency to advance sustainability in beef production. Nevertheless, a notable knowledge gap remains concerning the interplay between residual feed intake (RFI) and challenging environmental conditions for grazing beef heifers. This study evaluated blood parameters, rumen temperature (RT), and growth performance of beef heifers with divergent RFI during the summer and winter seasons in Western Canada. Forty-one crossbred beef heifers (351 ± 40 kg of body weight [BW] and 14 ± 1 months of age) previously characterized as more (n = 21; LOW-RFI = -0.96 ± 0.70) or less feed-efficient (n = 20; HIGH-RFI = 1.4 ± 1.00) in a drylot setting were used. Rumen temperatures were automatically recorded every 10 min throughout the study using an automatically logging rumen bolus. Plasma was collected every 18 ± 5 d for 42 d (summer) and 54 d (winter) to determine plasma concentrations of blood urea nitrogen (BUN), non-esterified fatty acids (NEFA), insulin-like growth factor 1 (IGF-1), β-Hydroxybutyric acid (BHBA), leptin (LEP), free triiodothyronine (fT3), haptoglobin (HP), heat shock protein 70 (HSP70), gamma-aminobutyric acid (GABA) and serotonin (5-HT). Environmental conditions were assessed by calculating the Climate Comprehensive Index using temperature, wind speed, solar radiation, and humidity data from a meteorological station. Daily weather conditions were considered to impose non-stress, mild, moderate, severe, extreme, and extreme danger risk in the summer (10, 19, 50, 19, 2, and 0% of the days, respectively) and winter season (0, 5, 27, 35, 24, 9% of the days, respectively). Plasma, RT, and performance were analyzed using GLIMMIX procedure of SAS (SAS 9.4). The fT_3 tended to be greater in LOW-RFI when compared with HIGH-RFI (*P* = 0.08; 8.6 vs. 8.0 ± 1.032 pmol/L) during the summer season. A tendency for an RFI × day interaction was detected for BUN with lower concentrations in HIGH-RFI vs. LOW-RFI at d 28 in the summer (P = 0.08; 34 vs. 43 ± 3.52 mg/dL). When a moderate risk of environmental stress occurred during d 14 of the summer sampling, GABA was greater in LOW-RFI vs. HIGH-RFI (P = 0.01; 8.8 vs. 7.1 ± 1.081 ng/ml). Additionally, GABA tended to decrease when environmental conditions reached extreme risk of cold stress in HIGH-RFI when compared with LOW-RFI (P = 0.08; 3.9 and 5.8 ± 1.117 ng/ml). The LOW-RFI group tended to produce more HSP70 in the summer (P = 0.10; 3.20 vs 2.99 ± 0.092 ng/ml), while the HIGH-RFI had greater concentrations of Hp (P = 0.02; 2185 vs. 1274 ± 25.61 ng/ml). Greater LEP concentrations were found in HIGH- vs. LOW-RFI in the winter (P = 0.04; 5.21 vs. 4.56 ± 0.122 ug/L). An RFI × hour interaction was detected for RT where HIGH-RFI heifers had greater RT by hour from 1:00 to 6:00 am, 10:00 am to midday, and 8:00 to 22:00 (P = 0.002) compared with LOW-RFI. During winter, an RFI × day interaction was found (P = 0.009) with higher RT in the LOW-RFI vs. HIGH-RFI group at d 224 and 205 (39.0 vs. 38.8 and 39.0 vs. 38.8 ± 0.005 °C, respectively). Except for GABA, there was an effect of day for all the blood parameters in the summer, and for fT_3 and HSP70 in the winter (P < 0.001). Growth performance did not differ between LOW and HIGH-RFI in both summer and winter seasons (P > 0.24). In summary, summer and winter weather conditions changed blood parameters and RT of replacement beef heifers with divergent RFI without affecting growth performance. Feed-efficient beef heifers were shown to be more resilient to varying extreme weather conditions.

Keywords: cattle, cold stress, environmental stress, heat stress, weather resilience.

3.2 Introduction

Since the onset of the 21st century, there has been a notable increase in extreme environmental conditions globally (Parr et al., 2003; Trapp et al., 2007). This includes a significant rise in global temperatures and heightened variability in severe cold air during the winter months (Cohen et al, 2013). Western Canada has undergone a marked increase in environmental variability, notably with the summer of 2021 being one of the warmest on record (Lin et al, 2022). Environmental data collected between 2007 and 2023 at Kinsella, Alberta, Western Canada, shows that summer temperatures increased by 1.4°C, while winter temperatures decreased by 4.6°C. The highest summer temperatures were 31.3, 32.3, and 32.7°C although the lowest winter temperatures were -37.7, -36.9, and -41.6°C, recorded between 2007 to 2012, 2013 to 2018, and 2019 to 2023, respectively (Alberta Climate Information Service [https://acis.alberta.ca/acis/]). During winter, greater variability might result in increased precipitation and higher relative humidity (RH; Zhang et al, 2000); which suggests a potential worsening of adverse weather conditions. Cow longevity in production systems can be as high as 15 years (Turner et al., 2013) and 4 to 6 years until culling in cow- calf operations (Naazie et al., 1999).Therefore, understanding the influence of environmental extremes on weather resiliency is particularly important for cow-calf producers, as these cows remain in the herd for many years and are directly exposed to a range of extreme conditions.

Elevated environmental temperatures affecting beef heifers are considered an external hazard known to be detrimental when heat abatement is not available for animals (Silva et al., 2023). When body temperature increases beyond 38.5°C, the absence of a proper equilibrium in heat dissipation leads to detrimental physical responses (Mishra et al., 2021) that can cause heat stress (HS). These responses include elevated respiratory (Gebremedhin et al., 2008) and heart rates

(Beatty et al., 2006), and vasodilatation (Sammad et al., 2020), and reduced nutrient intake, immune status, performance, reproductive efficiency, and abnormal behavioral patterns (Lefcourt and Schmidtmann, 1989; St-Pierre et al., 2003; Farooq et al., 2010; Sexson et al., 2012; Carroll et al., 2013; Chauhan et al., 2021). During cold stress (CS), the primary observed responses include vasoconstriction, shivering (Wang et al., 2023), and elevated dry matter intake. Those responses are expected to increase requirements and heightened energetic demands, reaching 2.89 kJ/kg for every Celsius below the thermoneutral zone (He et al., 2022; Britt et al., 2003; Wang et al., 2023). Additionally, adaptive changes in physiology may occur, including variations in lipid, energy, and protein metabolism. These changes can lead to catabolism, hormonal responses, and behavioral differences (Geary et al., 2003; Nardone et al., 2006).

A practical solution to address environmental extremes might be genetic selection through feed efficiency. This approach has enhanced breeding programs for more than 60 years (Warwick et al., 1958; Koch, 1963). In the last years, selection for more feed-efficient beef females based on residual feed intake (RFI) has increased (Herd et al., 2003). Five major physiological processes are likely to contribute to variation in RFI, which include feed intake and digestion, metabolism, physical activity, and thermoregulation (Herd and Arthur, 2009). DiGiacomo et al. (2014), showed that more feed-efficient cattle tend to lose less body weight in colder temperatures (Hoelscher, 2001), while Silva et al. (2023) showed that replacement beef heifers previously classified as thermotolerant based on multiple vaginal temperature measurements collected throughout the summer had decreased when measured in the fall, indicating a potential relationship between heat tolerance and feed efficiency. However, the association between RFI and thermotolerance has not been thoroughly explored (Silva et al., 2022).

3.3 Hypothesis

Therefore, it was hypothesized that beef heifers exhibiting higher feed efficiency are more resilient to weather-related stressors, attributed to their superior energy utilization and thermotolerance capacity.

3.4 Objective

This study evaluated blood parameters, growth performance, and rumen temperature of grazing beef heifers classified based on RFI during both summer and winter seasons in Western Canada. By examining the relationship between feed efficiency classification and physiologic response in varying seasonal conditions, this research aims to enhance animal well-being and productivity by identifying if more efficient grazing beef heifers thrive in a high-risk environment.

3.5 Materials and Methods

3.5.1 Feed efficiency test (Phase I)

This study was conducted at the Roy Berg Kinsella Research Station, University of Alberta, Kinsella, Alberta, Canada, during the summer and winter seasons of 2022 and 2023, respectively. The animal protocol was approved by the Institutional Animal Care and Use Committee (protocol #AUP00004004).

Forty-one Kinsella Composite black-hair coat heifers were obtained from a single university research herd for a feed efficiency test and divided into two pens based on BW (351 ± 40 kg of body weight [BW] and 14 ± 1 month of age) for 80 d (from d - 90 to d - 10 relative to the start of the summer sampling period) that included a 21-d adaptation period used to acclimatize cattle to the GrowSafe system for individual daily feed intake measurements (GrowSafe System, Ltd., Airdrie, Alberta, Canada). Heifers were fed a single total mixed ration composed of barley silage and oats that was fed ad libitum (14.6% crude protein [CP], 44% neutral detergent fiber, 32.5% acid detergent fiber, 0.97% calcium [Ca], 0.36% phosphorus [P], and 62.6% total digestible nutrients [TDN]). Individual BW was double-recorded at the beginning and end of the experiment while single BW measurements were obtained every 28-d. At the end of the RFI test, individual back fat (mm) was measured between the 12–13th rib using an Aloka 500 V diagnostic real-time ultrasound (Aloka, Wallingford, CT) with a 17 cm 3.5 M Hz linear array transducer. The RFI was calculated from actual feed intake minus expected feed intake considered the regression covariates. An individual growth curve for each animal was modeled by linear regression of observed BW against days on the test to estimate the animal's average daily gain (ADG) and initial BW on the test. Initial BW and ADG were used to calculate mid-test BW and mid-test metabolic BW (MIDMBW). Linear regression of observed average daily standardized dry matter intake (DMI) on ADG, MIDMBW, and end-test back fat (BFEND) were used to calculate the fat-adjusted RFI (RFIf; Manafiazar et al., 2021). After the completion of the test (Phase I), heifers were moved to a single pasture, in rotational grazing, for the remainder of phases II and III. Residual feed intake was calculated and heifers with RFI < 0, were ranked as more efficient (n = 21; LOW-RFI = -0.963 ± 0.70), while RFI > than 0, as less feed-efficient (n = 20; HIGH-RFI = 1.40 ± 1.0).

3.5.2 Sampling periods (Phases II and III)

In phase II, environmental data, plasma, RT, BW, rump, and rib fat deposition were collected during the summer season (July to August 2022) on d 0, 14, 28, and 42 of the study. Subsequently, during winter (January to March), phase III involved a second round of sampling on d 185, 204, 227, and 239, relative to the start of the summer sampling at the same location. The fall season was excluded for a sampling period due to the absence of anticipated extreme environmental conditions.

The pasture grazed during the experimental period was mostly composed of *Poa pratensis*, *Bromus inermis Leyss*, and *Hesperostipa curtiseta.* From July to August two bulls were added to the group for natural mating. During winter, heifers were offered free choice grass-alfalfa hay bale twice a day in the same location. Forage and hay samples were collected on d 0, 14, 28, 42, 185, 204, 227, and 239 for chemical composition analysis performed by a commercial laboratory (Down To Earth Labs, Lethbridge, Canada). Herbage mass (DM/kg) was estimated (d 0, 14, 28, 42) with randomization within each pasture, and stratification based on topography. A quadrat ($0.25m^2$) was positioned over a randomly chosen sample area, ensuring that only biomass rooted within this quadrat square was clipped 2 cm above ground. Following this, the samples underwent drying at a temperature of 79°C for a week and were immediately weighed after extraction from the dryers.

3.5.3 Environmental data

The comprehensive climate index (CCI) developed by Mader et al. (2010) was used as the environmental stress indicator and animal comfort in grazing beef cattle. The CCI was calculated using air temperature (Ta), RH, wind speed (WS), and solar radiation (SR) obtained from a weather station within 1 km where the heifers were placed and were corrected according to Mader et al., (2010):

$$Ta + RH\ corrected + WS\ corrected + RAD\ corrected$$

RH correction formula:

$$e^{(0.00182 \times RH + 1.8 \times 10^{-5} \times Ta\ \times RH) \times (0.000054 \times Ta^2 + 0.00192\ \times Ta - 0.0246) \times (RH - 30)}$$

WS correction formula:

$$\frac{-6.56}{e^{\frac{1}{2.26 \times WS + 0.23}} 0.45 \times (2.9 + 1.14 \times 10^{-6} \times WS^{2.5} - (2.26 \times WS + 0.33)^2} - 0.00566\ \times WS^2 + 3.33$$

RAD correction formula:

$$0.0076 \times RAD - 0.00002 \times RAD \times Ta + 0.00005 \times Ta^2 \times \sqrt{RAD} + 0.1 \times Ta - 2$$

Additionally, an adjustment to the Ta was applied using the solar direct radiation (DSR) formula:

DSR correction formula:

$$0.0057 \times RAD - 0.00002 \times RAD \times Ta + 0.00005\, Ta^2 \times \sqrt{RAD}$$

To know the total radiation influence on heifers in the grazing system, the variable RAD was used to estimate the effects of surface temperature (STR). A correction for surface temperature was utilized:

STR correction formula:

$$0.1 \times (Ta + 0.019 \times RAD) - 2$$

Lastly, the final CCI equation was:

$$CCI = DSR + STR$$

For summer and winter seasons, the calculated CCI classifies the environmental conditions based one of the thresholds: non-stress, mild, moderate, severe, extreme, and extreme danger (< 25, 25 to 30, > 30 to 35, > 35 to 40, > 40 to 45 and, > 45 vs. > 0, 0 to -10, < -10 to -20, < -20 to -30, < -30 to -40 and, < -40, respectively for each season). The mean, minimum, and maximum of the environmental variables between each sampling period (d 0-14, 14-28, 28-42, 185-204, 204-227, and 227-239) and CCI's are reported in Table 2.

3.5.4 Growth performance

Body weight (Gallagher Smart TSi Gallagher Australia Pty, Ltd.) was double-recorded at the beginning and at the end of the 21-d adaptation period. During the summer season, the initial BW was calculated as the average of full BW on d -1 and 0 and on d 184 and 185 for the winter, while the final BW was the average of d 42 and 43 for the summer and only recorded at d 238 for winter. Additionally, during phases II and III, BW was collected every 18 ± 5 d for a total of 42 d (summer; on d 0, 14, 28, 42,) and 18 ± 8 d for 54 d (winter; on d 185, 204, 227, and 239, relative to the start of summer trial) to assess ADG between sampling days (d 0-14, 14-28, 28-42, 0-42 for summer season and d 185-204, 204-227, 227-239 and 185-239 for winter season). Fat deposition was estimated using rib and rump fat thickness on the BW sampling days.

3.5.5 Blood parameters and rumen temperature

Blood samples were collected through jugular venipuncture during the summer and winter seasons (d 0, 14, 28, and 42 for summer and d 185, 204, 227, and 239 for winter) into heparin tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) and placed on ice until it was centrifuged at 3200 rpm for 15 minutes at 4°C. The plasma was then transferred to polypropylene vials (12 × 75 mm; Fisherbrand; Thermo Fisher Scientific Inc., Waltham, MA) and stored at -80°C until further analysis.

Commercial ELISA kits were used to determine free triiodothyronine (fT_3; inter- and intra-assay coefficients of variation were 5.37 and 5.70, respectively; Cat. No. CSB-EQF027510BO Cusabio Technology llc, Houston, TX, USA), heat shock protein 70 (HSP70; inter- and intra-assay coefficients of variation were 6.76 and 6.0, respectively; Cat. No. CSB-E13452B Cusabio Technology llc, Houston, TX, USA), bovine β-Hydroxybutyric acid (BHBA; inter- and intra-assay coefficients of variation were 7.38 and 7.91, respectively; Cat. No. CSB-E10056b Cusabio Technology llc, Houston, TX, USA), serum amyloid A (SAA; inter- and intra-assay coefficients of variation were 7.84 and 3.28, respectively; Cat. No. CSB-E08592b Cusabio Technology llc, Houston, TX, USA), blood urea nitrogen (BUN; inter- and intra-assay coefficients of variation were 3.93 and 2.20, respectively; cat. No. EIABUN Invitrogen, Carlsbad, CA, USA), Haptoglobin (Hp; inter- and intra-assay coefficients of variation were 4.05 and 4.34, respectively; Cat. No. E-

10HPT ICL, Newberg, OR, USA), GABA (inter- and intra-assay coefficients of variation were 8.55 and 5.79, respectively; Cat. No. BOEB1223 Assay Genie, Dublin, Ireland) and Serotonin (5-HT; inter- and intra-assay coefficients of variation were 9.43 and 6.55, respectively; Cat. No. BOEB1217 Assay Genie, Dublin, Ireland), insulin-like growth factor type-1 (IGF-1) previously validated for cattle (Moriel et al., 2012; inter- and intra-assay coefficients of variation were 7.15 and 9.61, respectively; Cat. No. SG100B R&D Systems, Minneapolis, MN, USA), non-esterified fatty acids (NEFA; inter- and intra-assay coefficients of variation were 2.08 and 5.30, respectively; Cat. No. 999-34691, 995- 34791, 991-34891, 993-35191, 276-76491 Fujifilm Wako Diagnostics, Mountain View, CA, USA) and leptin (LEP; inter- and intra-assay coefficients of variation were 9.46 and 9.74, respectively; Cat. No. EK760144, AFG bioscience, Northbrook, IL, USA) concentration in plasma.

On d 14, an automated recording system (Smart Rumen Bolus by Moonsyst, Hungary) was orally inserted using a bolus gun device to obtain individual RT with a 10 min logging interval.

Altogether, the summer season was represented by d 14 to 42, and the winter, from d 185 to 239.

3.6 Statistical analyses

Except for BW and ADG, data were analyzed as linear mixed models under assumption of a completely randomized design with repeated measurements using the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC, USA, version 9.4). The RT was evaluated by hour and by day of the study.

Individual heifer was the experimental unit for all analyses and were included as a random effect nested within treatment, and RFI classification obtained in Phase I was used as a fixed effect for all the analyses (i.e., BW, ADG, and fat thickness). For RT and plasma measurements, data were analyzed as repeated measures and tested for fixed effects of RFI, day, and RFI × day interaction (or hour for RT only). For RT by the hour, day of study was included as a random effect. The drops in RT associated with water/snow intake (below 36.5°C), were removed manually from the raw data before starting the analysis from each season.

Residuals and variables were tested for normality using the Kolmogorov-Smirnov test $P <$ 0.05. Moreover, abnormal data were transformed to have normal distribution using the box-cox transformations. All blood variables except for BUN in the summer season, were transformed. Means were back-transformed for data reporting and separated through the Tukey-Kramer test. The lowest Akaike Information Criterion was used to select the best covariance structures. Significance was set at $P < 0.05$, and tendencies were declared when $0.05 \geq P \leq 0.10$.

3.7 Results and Discussion

During this study, cattle were exposed to weather conditions ranging from no stress to extreme stress during the summer months. During the winter period, animals were exposed to a range of mild to extreme danger stress (**Figure 3.1**). Both seasons represented challenges for the beef cattle industry in Western Canada. In Kinsella, Alberta, Canada, summer temperatures have risen by 1.4°C, while winter temperatures have decreased by 4.6°C over the past 16 years (2007 to 2023). Currently, equations exist to estimate the impact of environmental variables on cattle stress (Buffington et al., 1981; Gaughan et al., 2008, Dikmen and Hansen et al., 2009; Mader et al., 2010). Mader et al. (2010) introduced the CCI as an alternative indicator of thermal stress in grazing cattle which includes SR and WS. Adjusting for those variables is crucial because SR and WS significantly influence how animals dissipate or retain heat (da Silva et al., 2009). Based on the average calculated CCI, this research found the summer of 2022 to pose less risks of stress (mild to moderate) than the winter season (severe; **Table 3.2**). Yet, the environmental extremes that we observed during our experiment can significantly affect cattle's ability to maintain stable body temperature and physiological processes (Kadzere et al., 2002).

Although monitoring body temperature continuously is troublesome, several technologies have been developed to better monitor animal body temperature (Kaufman et al., 2018, Vitali et al., 2024). For ruminants, RT can be used as a proxy for body temperature with a moderate correlation (Lees et al., 2019).

In HS, as the body and RT increase, adaptation patterns reflect the extreme weather's impact on daily routines and nutritional habits. These adaptations include shifting activities to cooler times, such as early morning or late evening (Lee et al., 2019). Additionally, HS reduces dry matter intake in heifers due to hormonal shifts affecting appetite regulation (Colditz and Kellaway., 1972). Heifers with higher body temperatures are anticipated to allocate more feed energy towards the regulation of body temperature rather than productivity. This allocation reduces their overall production efficiency (Hill and Wall, 2016). Research has shown that steers with high RFI had higher heat production when compared to their more efficient cohorts. A potential explanation for this could be in response to the differences in metabolic efficiency (Nkrumah et al., 2006). Conversely, in the winter season, a decrease in body temperature induces opposite feeding behaviors (i.e., increased feed intake) which is needed for thermogenesis.

An effect of RFI was detected for RT when analyzed by day of the study during the summer season ($P < 0.0001$; **Figure 3.2**). Also, an RFI × hour interaction was detected for RT during summer ($P = 0.002$; **Figure 3.3**), where HIGH-RFI had greater RT from 1:00 to 6:00 am, 10:00 to 12:00 am, and 8:00 to 10:00 pm. These results were similar to those reported by DiGiacomo et al. (2014), which found better thermoregulation capacities in more efficient dairy cows compared to less efficient ones based on RFI, Nevertheless, this author evaluated body temperature through infrared camera. A study conducted on pigs, revealed that those classified as more feed-efficient exhibited better thermoregulation capabilities (Campos et al., 2014). In poultry, Tixier-Boichard et al. (2002) identified heat production as one of the potential physiological mechanisms by which variation in RFI may occur, which agrees with Herd et al. (2004). The reduced thermoregulation ability observed in HIGH-RFI cattle could possibly be explained by their expected increase in forage consumption, which is associated with lower feed efficiency. This increased forage intake leads to higher heat increment, potentially affecting body temperature and making it more challenging for these cattle to cope with hotter environments. However, forage consumption was not measured in this study. In summer, RT was higher for HIGH-RFI from 10:00 am to midday when environmental temperatures started to rise signaling lowered thermoregulation ability during the heat exposure ($P = 0.002$; **Figure 3.3**).

During winter, an RFI × day interaction was observed ($P = 0.0086$; **Figure 3.4**), showing higher RT in the LOW-RFI compared to the HIGH-RFI group on d 205 and 224 (39.0 vs. 38.8°C and 39.0 vs. 38.8°C, respectively) when CCI classified the environmental conditions to impose extreme danger risk and extreme risk, respectively. In non-ruminants, Schmitt et al. (2021) reported that new-born piglets classified as less feed-efficient had more difficulties to maintain body temperature by displaying lower ear tip temperatures than more feed efficient cohorts. It was concluded that more efficient piglets can better adjust body temperature when required during stressful conditions. Our results demonstrate that LOW-RFI heifers exhibited better thermoregulation abilities. They were able to maintain higher temperatures during winter and lower temperatures during summer, effectively coping with extreme seasonal conditions.

Heat stress in cattle leads to several physiological alterations, including low circulating concentrations of thyroid hormones. This decrease in thyroid hormone levels is part of the adaptive response to reduce metabolic heat production and cope with elevated environmental temperatures. Those hormones are positively correlated to weight gain and increased basal metabolic rate (Magdub et al., 1982). Under thermal stress conditions, Limousine cattle exhibited a decrease in T3 hormone concentration to 76% of the levels observed under thermoneutral conditions (Pereira et al., 2007). A tendency to have more concentration of fT3 was found in the LOW-RFI ($P = 0.08$; **Figure 3.5**) when compared with the HIGH-RFI heifers during the summer. These results could suggest that LOW-RFI heifers are more resilient to hot-stress environments. The benefits of greater fT3 includes the ability to permeate target cells more effectively. This permeability is crucial for several physiological functions, such as basic maintenance, muscle growth, and the onset of early puberty in heifers (Hornick et al., 2000; Cassar-Malek et al., 2001; Huszenicza et al., 2006). According to Ferlazzo et al. (2018), fT3 concentrations are more sensitive and prone to variations caused by stressors due to the negative feedback effect of cortisol on the hypothalamic-pituitary-adrenal (HPA) axis. This sensitivity leads to significant changes in fT3 concentrations during stress exposure, as observed in Limousin bulls during prolonged transport (Fazio et al., 2005). High cortisol levels may reduce the HPA function as corticosteroids reduce the activity of the 5-deiodinase enzyme, which converts thyroxine to fT3 (Charmandari et al., 2005). Heifers exposed to HS will decrease feed intake which may also be paired with reduced thyroid secretion (Garg et al., 2001). In our study, the HIGH-RFI group and their lowered fT3 concentrations could potentially indicate a lowered feed consumption in the found adverse summer conditions. However, future research should validate these results in extensive systems, as feed intake was not estimated during phases II and III of our trial. In this study, greater fT3 levels and lower RT were observed in the LOW-RFI group in the summer. Ultimately, our results suggest that feed-efficient animals exhibit superior thermoregulatory ability. No differences in fT3 were observed during the winter (P = 0.53; **Figure 3.5**).

Environmental stress can impact blood concentrations of BHBA and NEFA (Nardone et al., 1997; Garner et al., 2017). Higher concentrations of BHBA and NEFA are associated with prolonged low feed intake and lipid reserve mobilization (Leduc et al., 2021), which could occur during stressful conditions. Heat-stressed cows, despite eating less, show improved glucose clearance and higher insulin levels while mobilizing less fat. This may be because the β-oxidation of NEFA produces more metabolic heat than carbohydrate oxidation (Wheelock et al., 2010; Baumgard and Rhoads, 2007). Other studies (Calamari et al., 2013 and Rhoads et al., 2013) did not find an increase in NEFA for heat-stressed cattle, which are also supported by our results (**Table 3.4**). Our study found an effect of day ($P < 0.0001$; **Table 3.4**) during summer and winter for NEFA, but not an effect of RFI or interaction of RFI × day ($P \geq 0.71$). In winter, NEFA concentrations were greater in the extremely dangerous vs. extreme cold day (0.619 vs. 0.230 mEq/L). During the coldest conditions, the expected increase in feed intake may not have been sufficient to reduce fat mobilization, which likely explains the elevated NEFA levels seen at d 227 ($P < 0.0001$). Similar increases in NEFA during negative energy balance in response to CS have been reported before (Kim et al., 2023).

During the summer, heifers exhibited the highest NEFA concentration (0.279 mEq/L) on d 0, indicating potential detrimental effects on body condition due to tissue mobilization (Cappellozza et al., 2014). Interestingly, BHBA levels as reflective of ketone bodies from liver acetyl-CoA oxidation, did not significantly increase during summer in our study, contradicting the NEFA findings which suggested lipolysis.

Blood urea nitrogen is related to protein metabolism and amino acid use efficiency (Qin et al., 2022) and is reported to increase 4–8 h after cattle are fed (Butler et al.,1998). A tendency for an RFI × day interaction ($P = 0.08$; **Figure 3.6**) in the summer season was observed for BUN. The LOW-RFI heifers had greater BUN than HIGH-RFI heifers (43 vs. 34 mg/dL, respectively) during a mild environmental risk (d 28). A recent study found greater counts of *Prevotella* in the rumen of more feed-efficient beef bulls (Zhou et al., 2023), which helps explain the greater protein metabolism function founded in this study. While BUN concentrations were elevated in both LOW-RFI and HIGH-RFI groups compared to findings in other studies (Clemmons et al., 2023), it is important to note that our animals were in an extensive system with early morning access to feed prior to blood sampling. This access, along with the higher CP content in their diet (**Table 3.1**), likely explains the elevated BUN levels observed in our study. High BUN levels in heat-stressed cattle indicate metabolic shifts, which are partly explained by expected changes in microbial fermentation leading to reduced use of rumen ammonia for microbial crude protein synthesis (Cowley et al., 2015). High levels of BUN can result from the ineffective assimilation of rumen ammonia into microbial protein and the liver's process of deaminating amino acids released from skeletal muscle (Wheelock et al., 2010). Future research should investigate N metabolism in more feed-efficient cattle in response to environmental extremes. Low BUN concentrations are typically observed when cattle decrease feed intake to mitigate additional heat production associated with digestion (Beatty et al., 2008). Accelerated protein catabolism in the muscles provides more energy substrate for thermoregulation in a hot environment (Baumgard & Rhoads, 2013) which may result in lower growth performance in heifers (Nonaka et al., 2007). However, no differences in BW, ADG, and fat deposition ($P \geq 0.24$; **Table 3.3**) were found in this experiment. Along with this, the low BUN concentration findings on the extremely dangerous coldest day (d 227) in the winter trial (4.77 mg/dL; **Table 3.4**) could be attributed to the lower CP of the hay provided.

Insulin-like growth factor type 1 plays a significant role in regulating the cell cycle and apoptosis, and it serves as a predictor of growth rate (Le Roith et al., 2001). Higher concentrations of IGF-1 were found during the winter when compared with the summer season (**Table 3.4**) with an observed effect of day ($P < 0.0001$). Greater IGF-1 concentrations are related to an increase in feed intake and anabolism of proteins in muscles (Hill et al., 1999). The lower IGF-1 concentrations in the summer at d 49 could be associated with HS (Bernabucci et al., 2010), specifically with chronic HS based on CCI records (**Table 3.2**). Nonetheless, the connection of lower IGF-1 concentrations in the more efficient group as documented by Moore et al., (2005), were not found in this study. During the winter season concomitant with a late gestation period, the IGF-1 decreased while NEFA was high (**Table 3.4).** Another study shows that an extended period of CS resulted in a 10% reduction in feed efficiency and ADG among feedlot cattle (Hoelscher et al., 2001).

An important indicator of energy reserves and body condition score, as well as the function of transmitting to the central nervous system and regulating feed intake is LEP (Morrison et al., 2001; Leó et al., 2004). Leptin acts in many areas of the brain and in doing so, it alters metabolism and energy expenditure (Morrison, 2008). Our study found greater LEP concentrations in the HIGH-RFI heifers when compared with LOW-RFI ($P = 0.04$; **Figure 3.7**; 5.2 vs. 4.6 ug/L, respectively) in the winter season. Research involving mice has indicated that LEP induces thermogenesis and helps maintain body temperature, partly through its influence on the thyroid hormone axis (Deem et al., 2018). Therefore, the elevated LEP levels in HIGH-RFI animals in our study may suggest the activation of additional mechanisms necessary for less-feed-efficient heifers to cope with CS. Additionally, LEP could increase the retention of heat through vasoconstriction (Fisher et al., 2016) which signals a potential physiological adaptation response on less feed-efficient animals.

Haptoglobin is a protein released by the liver during an acute-phase response, a part of the body's immediate reaction to inflammation processes, cell disruption, and stress (Moriel and Arthington, 2013). The Hp reference value below to 0.1 mg/mL for healthy cows was reported previously (Tadich et al, 2013). Concentrations 100-fold under (0.1 mg/mL of Hp) were found for HIGH-RFI vs. LOW-RFI heifers in the summer ($P = 0.02$; 2185 vs. 1274 ng/mL; **Figure 3.8**). On d 227 with an extremely dangerous coldest environment, a tendency for RFI × day was observed ($P = 0.06$; **Figure 3.9**) with greater concentrations in the LOW-RFI group (1043 ng/ml). However, Hp concentrations in both groups and seasons were lower than those with health issues. Similar to our results, LOW-RFI pigs had lower Hp concentrations when compared with HIGH-RFI (Mani et al., 2013). This might indicate a lower inflammatory process in the efficient group.

Heat shock protein 70 is known to play a significant role during CS (Hu et al, 2019) and in the present study, it was greater in average during the winter than in summer (6.48 vs. 3.10 ng/ml). The LOW-RFI tended to produce more HSP70 when compared with the HIGH-RFI in the summer ($P = 0.10$; **Figure 3.10**; 3.20 vs. 2.99 ng/ml). However, this study did not find effects of RFI, day, or RFI × day in the winter season ($P \geq 0.59$, **Figure 3.10**). During the summer season, an effect of day was observed ($P < 0.0001$, **Table 3.4**). The HSP70 concentration was the highest at d 42 after extreme risk environmental exposure at d 41, while it was at its lowest at d 28 after twelve hours of severe risk on d 27 (40 vs. 38 CCI [Data non-showed] and 3.26 vs. 2.88 ng/ml, respectively; $P < 0.0001$; **Table 3.4**). Signaling non-cellular damage during extreme summer conditions in both groups. Our results were aligned with others showing higher HSP70 concentrations in LOW-RFI pigs which is explained by a greater metabolic capacity in the liver of feed efficient animals (Grubbs et al., 2013).

Serotonin serves as an immunomodulatory biogenic amine, acting both as a neurotransmitter and a mediator in stress responses, with heat-stressed dairy calves producing more 5-HT (Marrero et al., 2021). An effect of day was detected for serotonin in the summer and winter seasons ($P < 0.0001$ and $P = 0.0008$, respectively; **Table 3.4**). In the present study, serotonin levels increased during summer; however, they lowered under extreme and extremely dangerous cold conditions. Serotonin is involved in thermoregulation processes (Natarajan et al., 2015), with studies showing increased concentrations in the peripheral circulation of rodents following acute HS exposure (Sharma et al., 1992). However, chronic stress disturbs the modulatory neurotransmitter system, affecting 5-HT levels (Lapiz-Bluhm et al., 2009) with the hypothalamus of chronically stressed rats producing less 5-HT (Van riel et al., 2003). Therefore, 5-HT concentration were heightened during summer, however, reduced under extreme and extremely dangerous cold conditions.

Similarly, GABA decreases body temperature and regulates stress responses (Bongianni et al, 2006) and is expected to decrease after hyperthermia in rabbits (Sharma et al., 2005). An RFI × day interaction was found when the summer was moderately hot on d 14, with the lowest concentration for the HIGH-RFI group ($P = 0.01$; 7.1 ng/ml; **Figure 3.11**). The HIGH-RFI heifers had lower GABA concentrations than LOW-RFI heifers ($P = 0.01$; 7.1 vs. 8.8 ng/ml) during the summer and tended to have lower concentrations in the winter ($P = 0.08$; 3.9 vs. 5.8 ng/ml; **Figure 3.11**). Daily and seasonal variations were found with the lowest concentration of GABA at d 239 with extreme cold weather in the less efficient heifers ($P = 0.08$; 3.9 vs. 5.8 ng/ml; **Figure 3.11**) in the winter, indicating low neurotransmitter concentrations after long-term cold exposure.

Studies have shown that individuals with low plasma GABA levels are more vulnerable to stress-related disorders (Vaiva et al., 2004). It is possible that high-RFI individuals may also be more susceptible to the negative effects of stress. However, no significant differences were observed in growth performance between cattle categorized as LOW-RFI and HIGH-RFI during both summer and winter seasons ($P > 0.24$; **Table 3.3**).

Heifers generate less metabolic heat and have a higher body surface area-to-body mass ratio compared to mature animals. This makes them more efficient at dissipating body heat and more tolerant to HS (West et al., 2003). Our findings suggest that LOW-RFI heifers demonstrate greater thermotolerance capacities. They can maintain physiological homeostasis despite extreme temperature fluctuations in both seasons, indicating an adaptation to thermal environments without significantly impacting their metabolism.

3.8 Conclusion

Our study highlights the significant environmental challenges faced by beef cattle in Western Canada, ranging from non-stressful to extremely stressful conditions in summer and winter seasons. The LOW-RFI heifers exhibited physiological parameters (RT, fT3, GABA and LEP) that indicate a better thermoregulation and greater resilience to heat stress and winter environments. Overall, our findings emphasize the importance of understanding the complex interactions between environmental stress, the physiology of feed efficiency, and physiological responses to improve management practices and animal welfare in challenging environments.

Table 3.1. Herbage mass and chemical composition of pasture and hay consumed by beef heifers with divergent residual feed intake during summer (July to August) and winter (January to March).

	Summer (pasture)				Winter (hay)			
Item [1]	0	14	28	42	185	204	227	239
HM, kg/ha	1374	1000	996	1793	-	-	-	-
			%					
CP	12.0	22.3	16.1	9.1	14.3	7.9	8.1	8.7
ADF	30.9	25.8	27.9	33.3	29.5	45.1	31.8	45.3
NDF	58.2	46.9	55.4	59.5	39.5	58.2	50.1	54.0
TDN	62.8	65.1	64.1	61.7	59.5	50.6	57.7	49.0
			ppm					
Copper	3.4	7.6	3.8	1.9	6.4	5.3	3.2	4.7
Manganese	45.1	44.6	35.6	41.9	61.9	29.9	10.8	10.3
Zinc	21.3	29.8	24.4	16.7	22.4	17.2	11.5	9.7
Iron	76.4	92.7	87.6	101.1	75.8	55.1	14.8	15.6
			%					
Sulfur	0.1	0.2	0.2	0.2	0.2	0.1	0.1	0.1
Sodium	BDL[2]	BDL[2]	BDL[2]	BDL[2]	0.1	0.1	0.1	0.1
Potassium	1.7	3.0	1.7	1.4	2.5	1.3	1.8	1.6
Phosphorous	0.3	0.4	0.3	0.3	0.2	0.1	0.1	0.1
Calcium	0.3	0.3	0.3	0.4	0.9	0.8	0.5	0.9
Magnesium	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2

[1]HM: Herbage mass, CP: Crude protein, ADF: Acid detergent fiber, NDF: Neutral detergent fiber, TDN: Total digestible nutrients.
[2]BDL: Below the detection limit.

Table 3.2. Environmental descriptive statistics with average, maximum, and minimum values for each variable during the summer and winter seasons (July to August 2022 and January to March 2023; respectively).

	Day of the experiment relative to the start of the summer sampling					
Environmental variable[1]	0-14	14-28	28-42	185-204	204-227	227-239
Air temperature (°C)	18.3	17.2	19.1	-8.2	-9.0	-14.7
Max	29.6	28.4	31.1	4.5	5.7	3.0
Min	7.2	5.8	6.0	-25.6	-32.6	-34.1
Relative humidity (%)	74.3	79.5	72.9	88.7	77.2	77.7
Max	100	100	100	100	98	95
Min	34.8	35.1	27.1	66.7	48.2	49.8
Solar radiation (w/m^2)	297.6	256.1	265.8	50.7	88.5	135.6
Max	905	853	838	367	493	604
Min	0.2	0.3	0.2	0	0	0
Wind speed (km/h)	8.9	9.8	7.1	9.4	10.0	10.9
Max	22.1	28.7	16.8	37.0	35.8	22.4
Min	1.6	1.6	1.2	0.3	1.6	1.7
CCI [2]	32.4	28.7	32.9	-24.3	-22.8	-29.3
CCI Index	Moderate	Mild	Moderate	Severe	Severe	Severe

[1]Values from meteorological station placed within approximately 1 km from the experimental site.
[2]CCI = Comprehensive Climate Index as described by Mader et al., 2010.

Table 3.3. Growth performance during the summer and winter season of beef heifers previously classified as more (LOW) or less (HIGH) feed efficient based on residual feed intake.

	RFI			
Item[1]	LOW	HIGH	SEM[3]	*P*-value[4]
n	21	20	-	-
RFI Classification	-0.96	1.40	0.193	< 0.0001
RFI test				
BW, kg				
Initial	230	235	3.57	3.45
Final	376	377	4.77	0.85
Summer				
ADG, kg				
0 to 14 d	0.75	0.93	0.105	0.25
14 to 28 d	1.01	1.07	0.270	0.88
28 to 42 d	0.80	0.64	0.287	0.71
0 to 42 d	0.35	0.37	0.030	0.70
BW, kg				
Initial	359	357	4.71	0.83
Final	394	394	4.86	0.95
Fat deposition, mm				
Initial Rib	2.87	2.57	0.196	0.28
Final Rib	2.39	2.48	0.244	0.80
Initial Rump	4.65	4.42	0.347	0.64
Final Rump	3.87	3.76	0.317	0.81
Winter				
ADG, kg				
185 to 204 d	0.5	0.1	0.278	0.37
204 to 227 d	-0.4	-0.2	0.097	0.24
227 to 239 d	2.3	2.4	0.234	0.86
185 to 239 d	0.5	0.5	0.095	0.75
BW, kg				
Initial	433	440	7.62	0.56
Final	462	466	6.98	0.69
Fat deposition, mm				
Initial Rib	3.1	2.8	0.234	0.31
Final Rib	-	-	-	-
Initial Rump	5.3	4.8	0.365	0.40
Final Rump	-	-	-	-

[1]RFI = Residual Feed Intake, BW = body weight, ADG = average daily gain
[2]Pregnancy weight was corrected as described by Gionbelli et al., 2015.
[3]SEM = Standard error of the mean.

[4] Statistical significances at $P < 0.05$ and tendency between $P > 0.05$ and $P < 0.10$.

Table 3.4. Blood parameters during summer and winter of beef heifers previously classified as more (LOW) or less (HIGH) feed efficient based on residual feed intake.

Item	Day of the experiment relative to the start of the summer sampling									
	0	14	28	42	185	204	227	239	SEM[12]	*P*-value[13]
Summer										
fT_3, pmol/L[1]	6.9^{b}	7.2^{b}	9.1^{a}	10.6^{a}	-	-	-	-	1.040	< 0.0001
IGF-1, ng/ml[2]	11.2^{a}	11.7^{a}	9.8^{a}	7.0^{b}	-	-	-	-	1.088	< 0.0001
BHBA, nmol/L[3]	237.8^{ba}	199.3^{c}	218.5^{bc}	253.7^{a}	-	-	-	-	1.040	< 0.0001
NEFA, mEq/L[4]	0.279^{a}	0.150^{c}	0.213^{b}	0.205^{b}	-	-	-	-	0.004	< 0.0001
LEP, ug/L[5]	4.1^{c}	8.4^{b}	13.4^{a}	8.0^{b}	-	-	-	-	1.075	< 0.0001
HSP70, ng/ml[6]	3.2^{b}	3.0^{ba}	2.9^{c}	3.3^{a}	-	-	-	-	0.857	< 0.0001
Hp, ng/ml[7]	1939^{ba}	1029^{b}	2760^{a}	1408^{b}	-	-	-	-	1.222	0.0005
5-HT, ng/ml[8]	25.4^{b}	49.6^{a}	42.6^{a}	59.6^{a}	-	-	-	-	0.185	< 0.0001
Winter										
IGF-1, ng/ml[2]	-	-	-	-	38.7^{a}	33.8^{a}	26.1^{b}	19.6^{c}	0.040	<0.0001
β-OHB, nmol/L[3]	-	-	-	-	269.0^{a}	272.1^{a}	128.2^{b}	233.4^{ba}	30.277	<0.0001
NEFA, mEq/L[4]	-	-	-	-	0.230^{c}	0.453^{b}	0.619^{a}	0.497^{ba}	0.001	<0.0001
BUN, mg/dL[9]	-	-	-	-	7.0^{b}	16.8^{a}	4.8^{b}	6.6^{b}	0.001	<0.0001
LEP, ug/L[5]	-	-	-	-	3.7^{b}	7.5^{a}	4.7^{b}	4.6^{b}	0.025	<0.0001
Hp, ng/ml[7]	-	-	-	-	834^{a}	591^{b}	989^{a}	539^{b}	1.086	<0.0001
GABA, ng/ml[10]	-	-	-	-	7.1^{a}	7.2^{a}	4.4^{b}	4.7^{b}	1.082	<0.0001
5-HT, ng/ml[8]	-	-	-	-	56.7^{a}	22.8^{b}	31.9^{b}	37.1^{ba}	1.167	0.0008
SAA, ug/ml[11]	-	-	-	-	12.4^{a}	8.6^{b}	3.3^{c}	1.3^{c}	1.267	<0.0001

[1]fT_3: Free triiodothyronine, [2]IGF-1: Insulin-like growth factor type 1, [3]BHBA: Bovine β-Hydroxybutyric acid, [4]NEFA: Non-esterified fatty acids, [5]LEP: Leptin, [6]HSP70: Heat shock protein 70, [7]Hp: Haptoglobin in ng/ml, [8]5-HT: Serotonin in ng/ml, [9]BUN: Blood urea nitrogen, [10]GABA: Gamma-aminobutyric acid, [11]SAA: Serum amyloid A.

[12]SEM: Standard error of the mean.

[13]Within a row, means with different superscripts differ, $P \leq 0.05$.

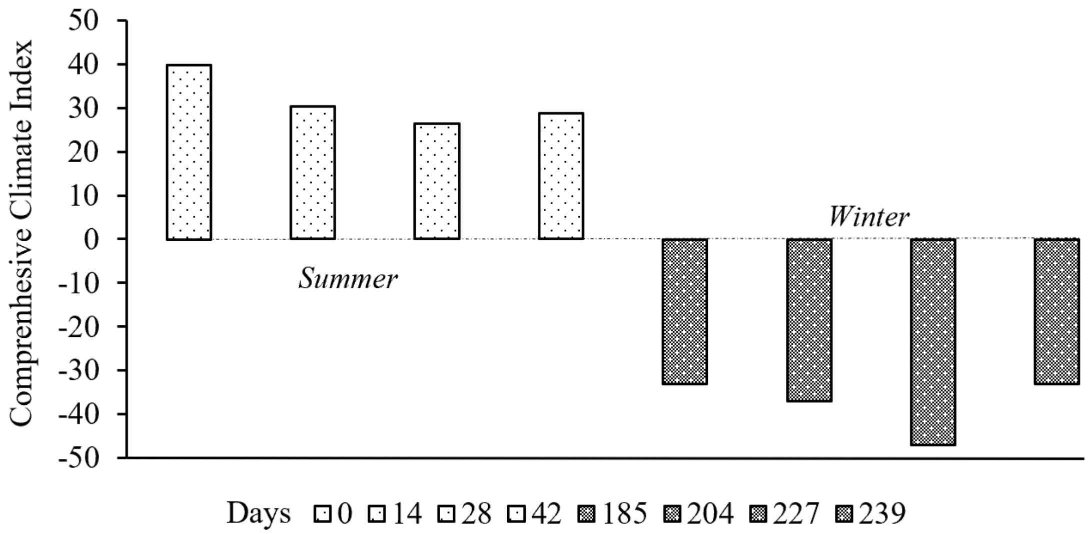

Figure 3.1. Environmental conditions during the sampling days based on the Comprehensive Climate Index (Mader et al., 2010). During summer, environmental conditions were classified to impose risk of severe to mild stress during the summer (July to August) and extreme to extreme dangerous in the winter season (January to March).

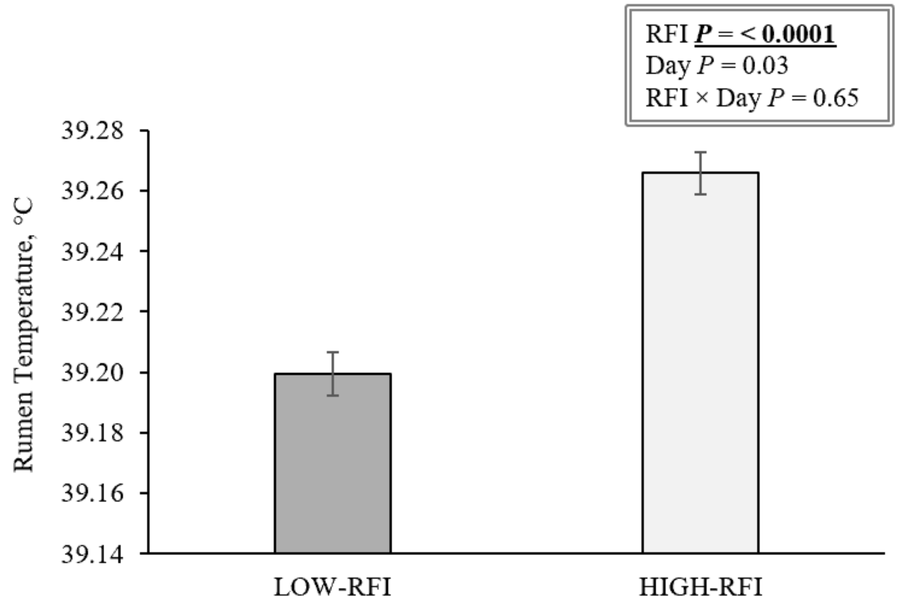

Figure 3.2. Rumen temperature, recorded during the summer, of beef heifers previously classified as more (LOW-RFI) or less feed-efficient (HIGH-RFI). Effect of residual feed intake on rumen temperature when analyzed by day ($P < 0.0001$; 39.20 vs. 39.27 ± 0.007°C).

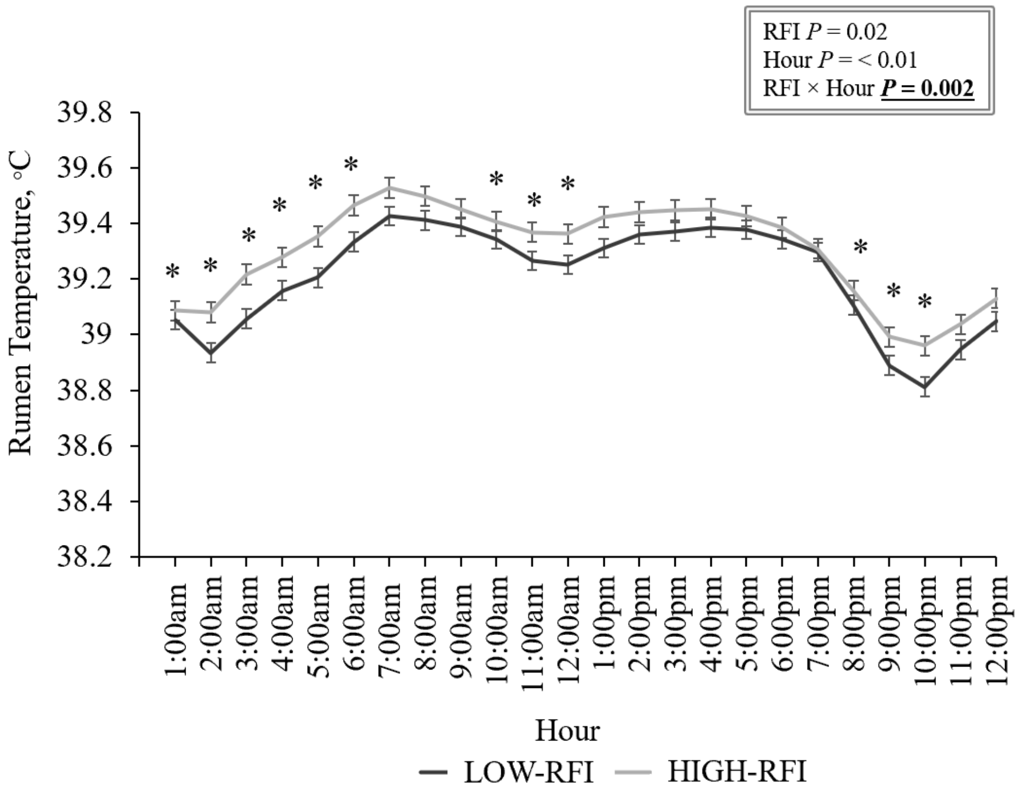

Figure 3.3. Rumen temperature recorded during the summer of beef heifers previously classified as more (LOW-RFI) or less feed-efficient (HIGH-RFI). An RFI × hour interaction was detected (P = 0.002; SEM ± 0.028), with HIGH-RFI having greater RT from 1:00 to 6:00 am, 10:00 to 12:00 am, and 8:00 to 10:00 pm compared with LOW-RFI. Data were recorded every 10 min and averaged by hour. *Within hour, means with an asterisk are different (P < 0.05).

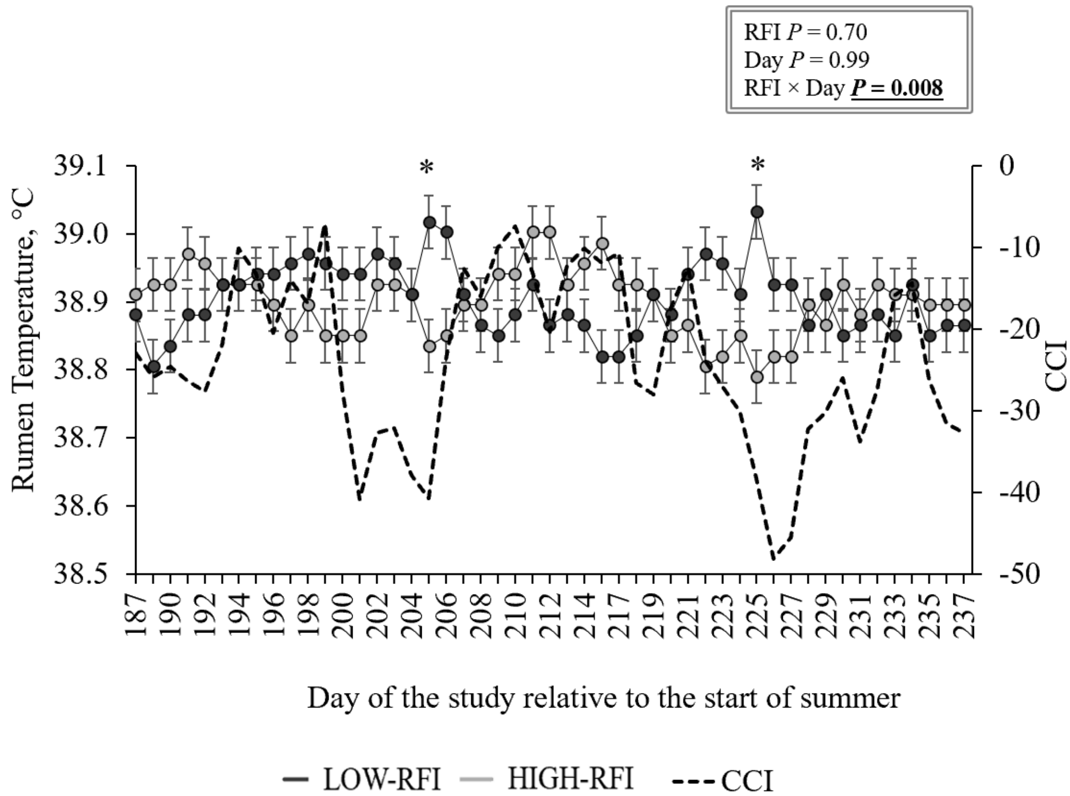

Figure 3.4. Rumen temperature recorded during the winter of beef heifers previously classified as more (LOW-RFI) or less feed-efficient (HIGH-RFI). An RFI × day interaction was detected (P = 0.0086; SEM ± 0.038), with HIGH-RFI having lower RT at days 205 and 224 with extreme danger and extreme cold stress.

*Within day, means with an asterisk are different (P < 0.05)

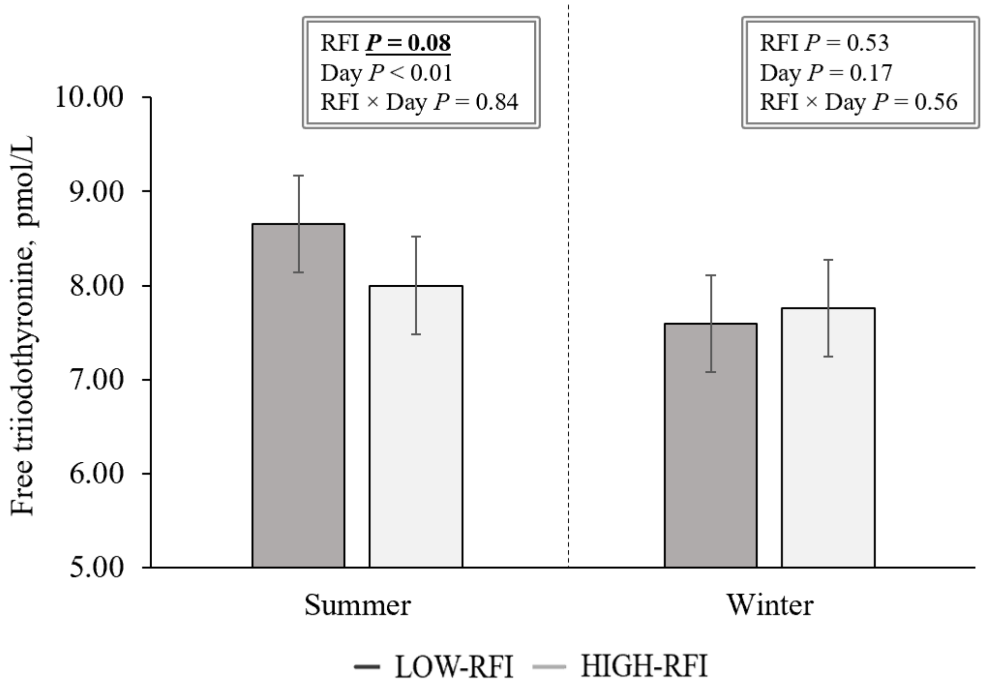

Figure 3.5. Plasma free triiodothyronine (fT3) concentration, during summer and winter, of beef heifers previously classified as more (LOW-RFI) or less feed-efficient (HIGH-RFI). Free T3 tended to be greater in the LOW-RFI group when compared with HIGH RFI (P = 0.08; 8.65 vs. 8 pmol/L SEM ± 1.032 vs. 1.032) during the summer season. However, during the winter season, no significant differences were found (P = 0.53).

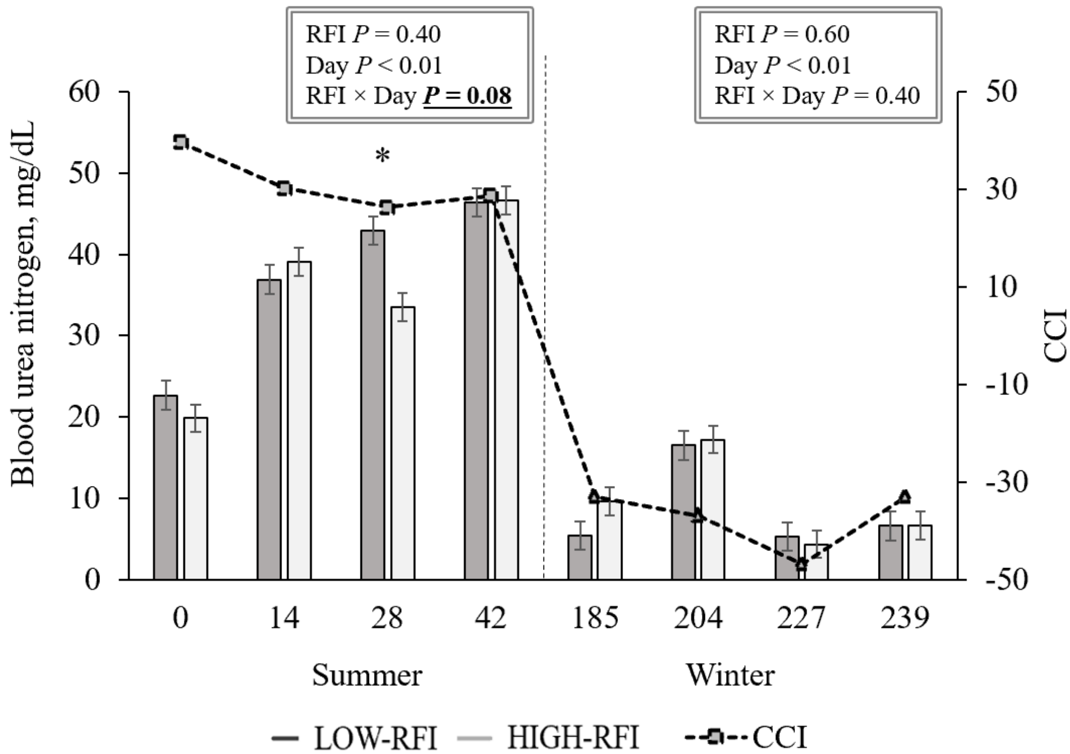

Figure 3.6. Plasma blood urea nitrogen concentrations of beef heifers previously classified as more (LOW-RFI) or less feed-efficient (HIGH-RFI). The comprehensive climate index (CCI) was calculated as described by Mader et al. (2010). A tendency for an RFI × day interaction was observed during the summer (P = 0.08) The LOW-RFI tended to have greater BUN compared with HIGH-RFI heifers on d 28 (43 vs. 34 ± 3.52 mg/dL). In the winter, an RFI × day interaction was not observed (P = 0.40).

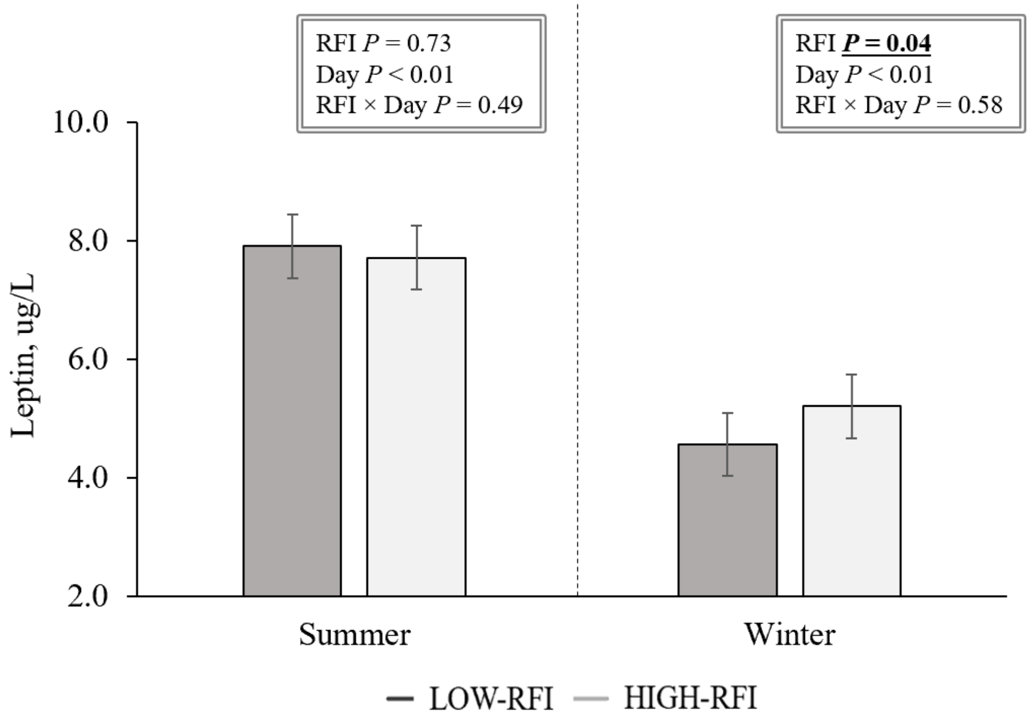

Figure 3.7. Plasma leptin (LEP) concentrations of beef heifers previously classified as more (LOW-RFI) or less feed-efficient (HIGH-RFI) during the summer and winter season. An effect of RFI was found in the energy demand by satiety pathway with greater LEP concentrations in the HIGH-RFI heifers when compared with LOW-RFI during the winter ($P = 0.04$; 5.2 vs. 4.6 ug/L, SEM ± 0.107 vs. 0.122, respectively).

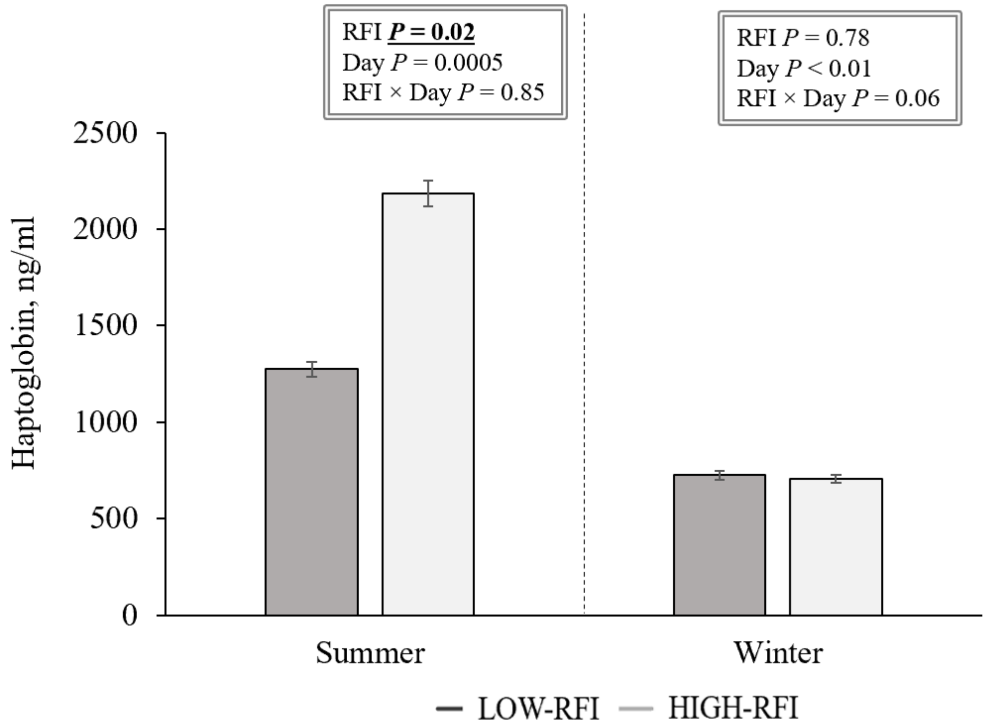

Figure 3.8. Plasma haptoglobin (Hp) concentrations of beef heifers previously classified as more (LOW-RFI) or less feed-efficient (HIGH-RFI) during the summer and winter season. An effect of RFI was found with greater HP in the HIGH-RFI when compared with the LOW-RFI in the summer season (P = 0.02; 2185 vs. 1274 SEM ± 25.61 vs 14.89 ng/ml, respectively).

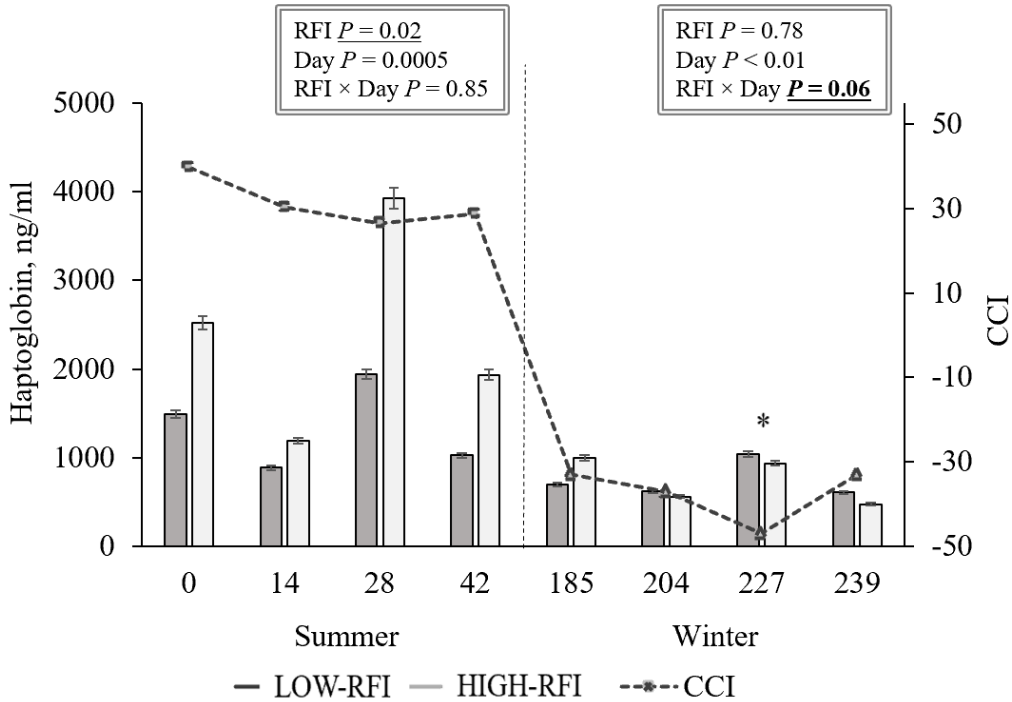

Figure 3.9. Plasma haptoglobin (HP) concentrations of beef heifers previously classified as more (LOW-RFI) or less feed-efficient (HIGH-RFI) during the summer and winter season. The comprehensive climate index (CCI) is the comprehensive climate index as described by Mader et al. (2010). A tendency to find interaction in the HP parameter was found ($P = 0.06$) in the winter season with an increase of this acute phase protein on the coldest sampling day (d 227; 1042 and 937 SEM ± 1.15 and 1.16 ng/ml).

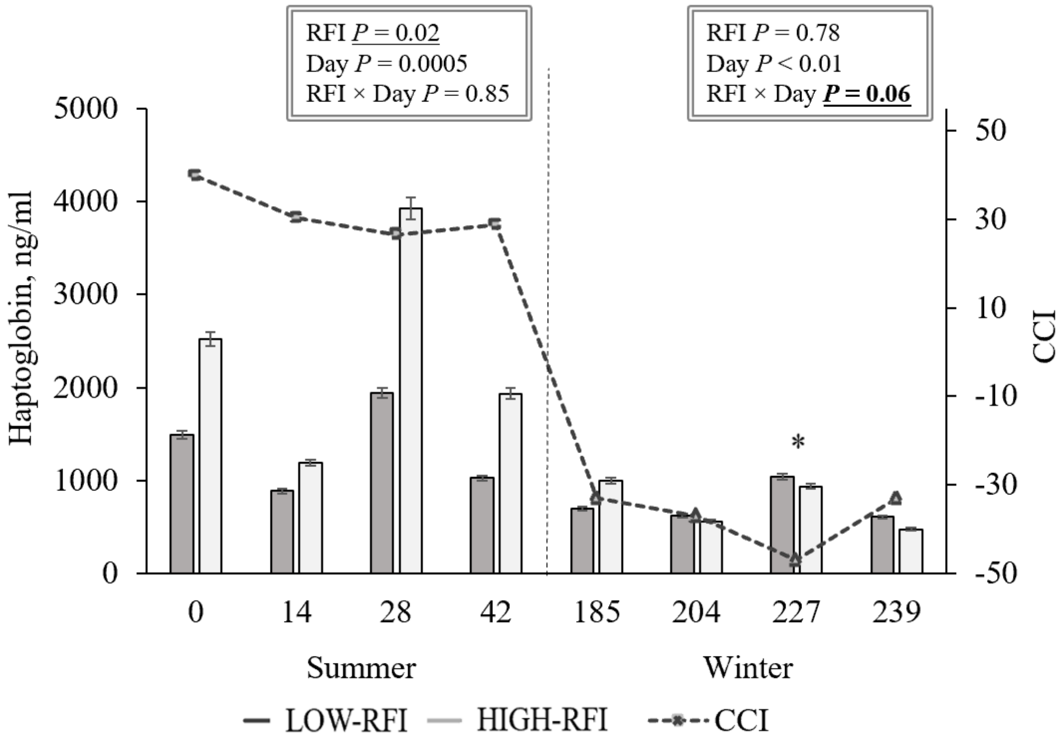

Figure 3.10. Plasma heat shock protein 70 (HSP70) concentrations of beef heifers previously classified as more (LOW-RFI) or less feed-efficient (HIGH-RFI) during the summer and winter season. A tendency was found for the LOW-RFI group to produce more HSP70 when compared with the HIGH-RFI in the summer ($P = 0.10$; 3.20 vs. 2.99 ng/ml SEM ± 0.092 vs. 0.091).

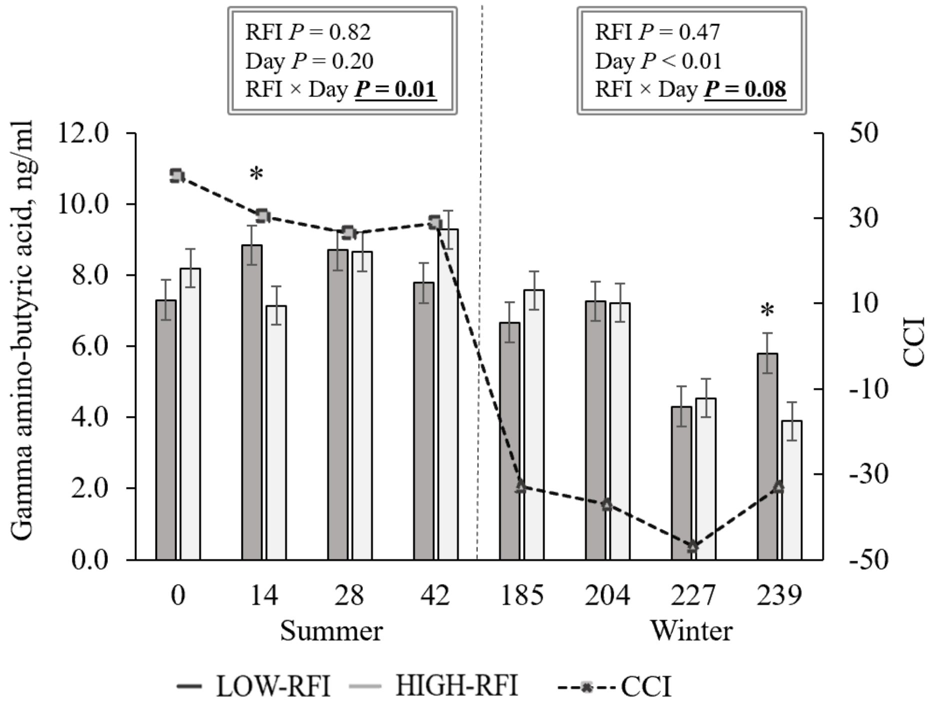

Figure 3.11. Plasma gamma amino-butyric acid (GABA) concentrations of beef heifers previously classified as more (LOW-RFI) or less feed-efficient (HIGH-RFI) during the summer and winter season. An RFI × day interaction was observed for GABA in LOW-RFI on d 14 with greater concentrations when compared with HIGH-RFI (P = 0.01; 8.8 vs. 7.1 ng/ml SEM ± 1.079 vs. 1.081, respectively) in the summer season. Moreover, GABA levels tended to decrease when environmental conditions reached extreme cold on d 239 in HIGH-RFI heifers vs. LOW-RFI (P = 0.08; 3.9 vs. 5.8 ng/ml SEM ± 1.117 vs. 1.117). CCI is the comprehensive climate index as described by Mader et al. (2010).

Chapter 4. Activity behavior and immunity of beef heifers with divergent residual feed intake under extreme summer and winter seasons in Canada

4.1 Abstract

Beef cattle have been selected for feed efficiency to reduce feeding costs and environmental impact. However, the potential associations on how selecting for feed efficiency influences the animal's ability to withstand environmental extremes remains to be studied. Therefore, this research assessed activity behavior, water access events (summer and winter), geolocation (summer only), and blood cell counts (winter only) of beef heifers with divergent RFI in Alberta, Canada. Forty-one crossbred beef heifers (351 ± 40 kg of body weight [BW]; and 14 mo of age) previously tested for RFI in drylot and classified as either more (n = 21; LOW-RFI = -0.96 ± 0.70) or less feed-efficient (n = 20; HIGH-RFI = 1.4 ± 1.00) were used in a completely randomized design in the summer (July to August) and winter season (January to March). Heifers were maintained in a single dormant pasture and received free-choice hay bales during winter and cultivated pastures in the summer. An accelerometer-based sensor was used to automatically assess activity behavior by recording steps, lying, standing, and transition times every 15 min. The geospatial location was evaluated through individual fence collar technology (®Nofence) in the summer. Water access events were recorded automatically through a bolus device (Moonsyst) every 10 min with a cut-off of < 36 °C of rumen temperature. Whole blood for cell counts were collected every 18 ± 8 d based on weather conditions and neutrophil to lymphocyte ratio (N:LR) was calculated to be used as an indicator for inflammation. Environmental conditions were assessed by calculating the Climate Comprehensive Index (CCI) using temperature, wind speed, solar radiation, and humidity from a weather station within 1 km of the pasture. Based on CCI, daily weather conditions were considered to impose mild, moderate, severe, extreme, and

extremely dangerous stress in both seasons. The standing activity was greater at 4:00 AM in the LOW-RFI compared to 8:00 and 10:00 AM in the HIGH-RFI ($P < 0.0001$) in the summer season. Conversely, time spent lying was lower in the LOW-RFI between 3:00 to 4:00 AM and 06:00 PM but greater at 11:00 AM ($P < 0.0001$) in the summer. During winter, at d 193, 213, and 214 (moderate CS) and at d 199 (severe CS) of the study relative to the start of the summer sampling, the LOW-RFI cows spent more time standing compared to the HIGH-RFI group ($P = 0.0107$). Conversely, on d 225, during extreme cold danger conditions, individuals in the HIGH-RFI spent more time standing when compared with LOW-RFI, but 24h later the tendency reversed, with the LOW-RFI group spending more time standing ($P = 0.0223$). An RFI × day interaction was detected for number of steps, indicating that on d 3, 5 to 12, 15, and 29 (summer), the HIGH-RFI group exhibited 6.6 to 11.5% more activity than LOW-RFI ($P = 0.0283$). During winter, an RFI × day interaction was found for steps taken at d 210, where the HIGH-RFI group showed 12.9% fewer steps under mild cold conditions, while at d 224 (during severe CS), the HIGH-RFI walked 11.4% more steps ($P = 0.0227$). The LOW-RFI had fewer transition times by day compared with HIGH-RFI heifers in the summer (3.8 vs. 4 / hr; $P = 0.0365$) and during winter (37 vs. 41 / d; $P = 0.0383$). On the hottest days of summer, the HIGH-RFI group kept clustered in a greater percentage close to the water spots, hills, and wooded areas (32.1 vs. 31.8, 45.7 vs. 26.7, 21.4 vs. 9, and 42.9 vs. 41.6%, for HIGH- and LOW-RFI respectively). At d 239 of winter, an RFI × day interaction ($P = 0.05$) was observed for relative lymphocytes. Additionally, the N:LR was greater in the LOW-RFI on the coldest day (d 224). In summary, results indicate differential behavior activity in the extreme summer plus cell count fluctuations between HIGH and LOW-RFI heifers under varying CS conditions. The HIGH-RFI group exhibited greater standing activity, step count, and transition times, while the LOW-RFI spent more time lying down, both in summer and winter. Moreover, a high N:LR was observed in the LOW-RFI group on the coldest winter day, suggesting that extremely dangerous cold temperatures may increase the inflammatory status in LOW-RFI.

Keywords: feed efficiency, immunity, thermotolerance, weather resilience, well-being.

4.2 Introduction

The beef cattle industry has implemented various strategies over the years to mitigate the adverse effects of extreme environmental conditions on cattle. The frequency and severity of environmental climatic abnormalities are on the rise globally, with North America experiencing prolonged and severe winters (Cohen et al., 2018) and extreme warming (Zhang et al., 2023) becoming a new reality. To overcome this, livestock producers need to monitor and identify animals with potential to withstand the expected climatic extremes more effectively.

Weather fluctuations can significantly influence the behavior of animals (Polsky et al., 2017). Alterations in behavior have been highlighted during heat stress (HS), including a decline in the number of steps taken by cattle (Chapinal et al., 2011). Similarly, Rushen et al. (2007) found that poor-quality surfaces negatively affect the time cattle spend lying down. Additionally, Mader et al. (2010) observed that ground surfaces accumulating solar radiation can hinder heat dissipation during extreme summer conditions, thereby increasing the discomfort for grazing cattle during rest periods. Greater transition periods between standing and lying have been linked to health issues or discomfort in cattle (Barraclough et al., 2020), which can also be related to HS. In hot environments, cattle reduce feed intake and increase water intake (Mallonee et al., 1985; O'Brien et al., 2010). In contrast, during the winter season, cattle tend to consume more feed to regulate body temperature, spend more time standing (Olson et al., 2002; He et al., 2022), and may resort to consuming snow when water sources are unavailable without increasing the energy requirement associated with thermoregulation (Degen and Young, 1990). Moreover, extreme environmental conditions have also been reported to decrease the immune response of cattle (Dahl et al., 2020), highlighting the negative effect of CS on blood T cell populations (Kang et al., 2016).

Identifying animals capable of better tolerating these extremes is crucial. Genetic selection is a viable option to alleviate the impact of extreme weather conditions that affect grazing beef herds throughout both summer and winter (Herd et al., 2003). For several years, cattle genetic selection has focused on residual feed intake (RFI; Koch, 1963). Cattle previously subject to RFI testing demonstrated heightened ruminal cellulose degradation and better nutrient acquisition (Auffret et al., 2020). Although several research studies have investigated RFI and its effects on economics, performance, and animal efficiency; to our knowledge, no one has tested RFI classification in beef cattle for overcoming extreme environmental conditions. Further, inherent environmental extremes in some production systems require planning for animals that are not only more feed efficient but also resilient to the system.

Therefore, this study aimed to identify how feed-efficient beef cattle differentially respond to extreme weather conditions and to monitor their potential impact on physiology and health. Specifically, this study aims to assess activity budget, number of water access events, and complete blood cell count of grazing beef heifers classified by RFI during the summer and winter seasons in Western Canada.

4.3 Hypothesis

We hypothesized that beef heifers with higher feed efficiency (LOW-RFI) exhibit greater resilience to weather-related stressors. This resilience is attributed to their superior metabolic mechanisms, which include more efficient energy conversion and enhanced thermotolerance. Additionally, efficient heifers may demonstrate improved behavioral responses to stress, better blood cell profiles, and fewer attempts to access water, enabling them to tolerate environmental extremes more effectively.

4.4 Objective

This study evaluated the activity behavior and health indicators in grazing beef heifers based on their RFI during summer and winter seasons in Western Canada. The research aimed to identify how grazing beef heifers with higher feed efficiency are capable of thriving in a high-risk environment, thereby enhancing animal well-being and productivity. The evaluation included steps taken, lying time, standing time, transition times, number of water access events, and blood cell count.

4.5 Materials and Methods

This experiment was conducted at the Roy Berg Kinsella Research Station, University of Alberta, located at Kinsella, Alberta, Canada, during the summer and winter seasons of 2022 and 2023 (July to August and January to March, respectively). The Institutional Animal Care and Use Committee officially conferred authorization for the animal protocol (AUP00004004).

4.5.1 Sampling Period – Phase I

Forty-one crossbred black hair coat beef heifers (351 ± 40 kg of body weight [BW]; and 14 mo of age) were initially classified for RFI utilizing automatic feed intake monitoring systems (GrowSafe System Ltd, Airdrie, Alberta, Canada) over 80 d (March to May 2022). To calculate RFI, individual back fat (mm) was measured between the 12–13th rib using an Aloka 500 V (Aloka, Wallingford, CT) diagnostic real-time ultrasound equipped with a 17 cm 3.5 MHz linear array transducer. Average daily gain (ADG) was computed through linear regression analysis to model observed BW against days of test duration. This facilitated the estimation of ADG and initial BW on the test for each animal. The RFI was calculated from actual feed intake minus expected feed intake considered the regression covariates and subsequently, initial BW and ADG were utilized to derive mid-test BW and mid-test metabolic BW (MIDMBW). The relationship between observed average daily standardized dry matter (DM) intake and ADG, MIDMBW, and end-test back fat was established through linear regression analysis to calculate fat-adjusted RFI, as per the methodology proposed by Manafiazar et al. (2021).

After the completion of the test (Phase I), heifers were moved to a single pasture, in rotational grazing, for the remainder of phases II and III. Residual feed intake was calculated and heifers with RFI < 0, were ranked as more efficient (n = 21; LOW-RFI = -0.963 ± 0.70), while RFI > than 0, as less feed-efficient (n = 20; HIGH-RFI = 1.40 ± 1.0) for the subsequent statistical analyses.

4.5.2 Sampling period – Phase II and III

During Phase II of the study, conducted from July to August 2022, environmental data, as well as information on lying, standing, steps, transition times, and water access events were recorded every 10 min throughout the study on d 0, 14, 28, and 42. In Phase III, which took place during the winter from January to March, a second round of sampling was conducted. This time, blood samples were also collected for a complete blood cell count (CBC) on days 185, 204, 227, and 239 relative to the start of the summer sampling at the same location. The fall season was excluded from sampling due to the lack of extreme environmental conditions.

The pasture primarily consisted of Kentucky bluegrass (*Poa pratensis*), smooth bromegrass (*Bromus inermis Leyss*), and needle-and-thread grass (*Hesperostipa curtiseta*). From July to August, two bulls were introduced to the group for natural mating. During the winter months, heifers were provided free-choice to grass-alfalfa hay twice daily at the same location. Forage and hay samples were collected on d 0, 14, 28, 42, 185, 204, 227, and 239 for chemical composition analysis, which was conducted by Down To Earth Labs in Lethbridge, Canada. Heifers and bulls were kept as a single group during Phase II and III.

4.5.3 Environmental analysis

The climate index developed by Mader et al. (2010), known as the comprehensive climate index (CCI), was utilized as the environmental stress indicator to assess the comfort of grazing beef cattle. The CCI was calculated using air temperature, relative humidity, wind speed, and solar radiation data from a weather station located within 1 km of the experimental station. For both summer and winter seasons, the CCI classified environmental conditions into one of six stress levels: non-stress, mild, moderate, severe, extreme, and extreme danger. The CCI thresholds for these classifications were defined as follows during summer: non-stress (< 25), mild (25 to 30), moderate (> 30 to 35), severe (> 35 to 40), extreme (> 40 to 45), and extreme danger (> 45). For winter, the thresholds were: non-stress (> 0), mild (0 to -10), moderate (< -10 to -20), severe (< -20 to -30), extreme (< -30 to -40), and extreme danger (< -40). Specific adjustments were made to variables such as solar radiation, wind speed, relative humidity, and environmental temperature based on the formulas described by Mader et al. in 2010. The results are present in Table 4.1.

4.5.4 Behavior Activity

The lying and standing patterns were summarized as activity budget, while steps and the number of behavioral transition times (changing from laying to standing and standing to laying) were reported by days of the study and hours of the day. Data were recorded every 15 min with the IceQube® accelerometer-based sensor (IceRobotics Ltd., Edinburgh, UK) attached to the left metatarsophalangeal joint using a velcro strap (IceRobotics Ltd., Edinburgh, UK) during both summer (July to August) and winter season (January to March) to track heifers' behavior. Data were directly exported from the CowAlert system (IceRobotics Ltd., Edinburgh, UK) and summarized per hour and by day using Microsoft Office Excel®, excluding days involving heifer handling (d 0, 14, 28, 42, 185, 204, 227, 239) from the data analysis. All variables were analyzed and converted from minutes into hours and further hours into days, resulting in two responsible variables (per hour and day) for each behavior.

4.5.5 Geolocation

All heifers in the experiment were collared, and their locations were assessed to determine whether they stayed close to the shade or spent the day without seeking shade during the summer season. The latitude and longitude data were recorded automatically using fence collar technology (®Nofence, AS, Batnfjordsøra Norway) every 10 ± 8 min. Data mapping to track heifers during the warmest days in the summer season (d 17, 26, 31, and 32) was created with Tableau software (v. 2021.3) using latitude and longitude information. Additionally, Google Earth and in-person characterization were used to classify areas such as wooded, water spots, and hills. The wooded areas were associated with more natural shade availability, the water spot with more hydration required, and hills with more wind speed utilization for thermoregulation purposes. Once areas were identified, locations were grouped by RFI and percentage of heifers present in wooded areas, water spots, and hills were described.

4.5.6 Water Access Events

Water access events in cattle was assessed using a Smart Rumen Bolus, an automated temperature monitoring system developed by Moonsyst (Cork, Republic of Ireland). The bolus was placed in the rumen using a copper gun (Agrimin Ltd., North Lincolnshire, UK) for bolus administration at the second handling (d 14) of the trial and recorded for a total of 28 d in the summer trial (d 15 to 42), and throughout the whole winter season (d 185 to 239) every 10 min. The cut-off for water access events were temperatures < 36°C in the rumen, then each drop in temperature were counted as times per day. Heifer's handling days (0, 15, 30, 43, 185, 204, 227, 239) were excluded from the analysis. The data collected was summarized by day as parametric values using Microsoft Office Excel®.

4.5.7 Blood Cell Count

A CBC analysis was evaluated using a semi-automated cell counting system, VetScan HM5 (Abaxis, Inc., Union City, CA, USA) in adherence to the manufacturer's recommended protocol. The VetScan HM5 reagent kit was utilized, along with the calibration controls, to ensure accurate results. Approximately 2 ml of blood was collected at d 185, 204, 227 and 239 through jugular venipuncture into vacuumed EDTA tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ), inverted 3 times, and immediately placed on ice until individual evaluation within first six hours after collection. Prior to analysis in the cell counter, tubes were inverted 15 times. The absolute and relative analysis comprised of white blood cells, lymphocytes, monocytes, neutrophils, eosinophils, basophils, red blood cells, hemoglobin, hematocrit, hemoglobin, means corpuscular volume, means corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width coefficient, red cell distribution width standard deviation, platelets, means platelet volume, plateletcrit, platelet distribution width coefficient, and platelet distribution width standard deviation. Additionally, the neutrophil lymphocyte ratio (N:LR) was calculated by dividing the relative count of neutrophils by the relative count of lymphocytes. This analysis was conducted only during the winter season due to delayed arrival of the required machine.

4.5.8 Statistical analyses

Data were analyzed as linear mixed models under assumption of a completely randomized design with repeated measures, either by hour or days, using the GLIMMIX procedure (SAS Institute Inc., Cary, NC, USA, version 9.4). For the behavior activity, data collected every 10 min were averaged by hour and day, and then analyzed as repeated measures, considering both hourly and daily intervals. Additionally, blood cell variable and water access events were also analyzed using the same model. The experimental unit for all analytical procedures was heifers, with RFI values treated as fixed effects. For blood cell variables and water events, day was included as a random effect while behavior activity, hour and day were selected as random. The inclusion of RFI in the model aimed to predict and assess the interaction between the environmental factors and the classification of heifers based on efficiency, serving as the independent variable. The significance level was set at $P < 0.05$, while tendencies were assumed within the range of $P \geq 0.05$ to < 0.1.

To validate the integrity of the data, an examination of normality was carried out for residuals and variables using the Kolmogorov-Smirnov test, with a predefined significance threshold set at $P < 0.05$. Additionally, a comprehensive assessment of normality was conducted for all variables. When normality was not met, data were transformed using the Box-Cox method, and the analysis proceeded with the normalized values. The covariance structure with the lowest Akaike Information was chosen as the best criterion for each variable. For multiple comparisons amongst means, the following adjustments were made: Tukey-Kramer was applied to CBC and water events in the statistical evaluation, while differences between groups (DIFFT) were applied in the experimental design of activity behavior results. Geospatial location was summarized and analyzed by day just in the hottest days of the summer (d 17, 26, 31, and 32) with a CCI imposed as severe (39, 35, 35 and 37, respectively) based on Mader et al., (2013). The percentage of heifers

(LOW-RFI or HIGH-RFI) in the wooded, lake, and hills, were calculated during the hottest hours of the day (11:00 to 4:00 pm) splitting each group for the analysis where the areas with more animal density were quantified using Image J software based on pixels by square area over the total dots in the area.

4.6. Results and Discussion

The welfare of cattle can be reliably assessed by tracking their behavioral activity (Vasseur et al., 2012). Huzzey et al. (2005) found that cows experiencing discomfort close to parturition increased the number of behavioral transition times, compared to the non-calving season. Similarly, Barraclough et al. (2020) demonstrated that multiparous dairy cows with more transition times exhibited signs of subclinical and clinical hypocalcemia, emphasizing the significance of monitoring behavioral patterns for assessing welfare and health status. Silva et al. (2021), showed that providing artificial shade, therefore mitigating direct solar radiation exposure, increased lying time in replacement beef heifers compared with heifers without access to shade. Our study found that the HIGH-RFI had more transition times than the LOW-RFI heifers (4.0 vs. 3.8 times by hour, respectively; **Figure 4.2**; $P = 0.037$) in the summer season. Also, during the winter, there was a difference in transition times in the HIGH-RFI group with more transitions than the LOW-RFI group (41 vs. 37 times by day; **Figure 4.3**; $P = 0.038$). This result might be attributed to discomfort when the environmental temperature variation was from non-stress to extreme risk and mild to extreme danger risk to cause thermal stress (CCI of 15 to 42 and -7 to -47 in the summer and winter, respectively; **Figure 4.1**). Additionally, the reduced transition times observed in low-RFI cattle across both seasons may be linked to their enhanced feed efficiency and greater energy availability, which is conserved due to lower physical activity levels. This characteristic, as noted by Herd and Arthur (2009), is typically expected in more efficient cattle, where less energy is expended on unnecessary movements, thereby contributing to their overall efficiency.

It is known that cattle require between 11 and 14 hours of lying time in a thermoneutral environment (Ito et al., 2010). Our analysis found that the HIGH-RFI spent 8.8 hr/d in a lying position on the coolest day of the winter trial (225 d) vs. the LOW-RFI group with 10.3 hr/d (**Figure 4.4**). This indicates that the HIGH-RFI group had 2.2 hours less rest than expected. Less rest in cattle may impact negatively feeding behavior (Gomez and Cook, 2010). Furthermore, another study found that the less efficient cattle exhibited greater feed intake and longer total duration of nonfeeding periods when compared to the LOW-RFI animals (Fitzsimons et al., 2014). Additionally, it was found that the CCI indicated an extremely dangerous cold day (< -45) for an accumulated period of more than 24 hours (days 225 and 226). In these environmental conditions, the LOW-RFI group spent more time in a lying position (**Figure 4.4**; $P = 0.022$) than the HIGH-RFI, with 10 vs. 9 hrs of lying. This suggests that the HIGH-RFI group exhibited more standing behavior during the first 24 hours of extreme danger cold day (d 225), possibly to increase feeding intake and alleviate adverse weather conditions, leading to more rumen fermentation and an increase in body temperature, as suggested by Taweel et al. (2004). Additionally, West (2003) described greater energy and nutrient utilization in a standing position, then, in our study finding at d 225 with greater standing time in HIGH-RFI address to more thermotolerance in LOW-RFI in the first 24 hours of continuous extremely dangerous winter conditions. However, after 24 hours of continuous extremely dangerous cold, the HIGH-RFI spent more time in the lying position (14.8 vs. 13.6 hours; **Figure 4.4**; $P = 0.011$) which may reduce body temperature due to conduction mechanism on icy surfaces (Bastian et al., 2003; Godyń et al., 2019). Furthermore, when winter was moderate to severe cold at d 193, 199, 213, and 214, the LOW-RFI spent more time in a standing position than the HIGH-RFI group (13.2 vs. 12.4, 14.1 vs. 13.3, 13.1 vs. 12.3 and 13.2 vs. 12.3 hours per day; **Figure 4.4**; $P = 0.011$), increasing the possibly of developing social interactions (Val-Laillet et al., 2009). In the summer season, the greatest difference in standing activity between LOW-RFI and HIGH-RFI heifers at 4:00, 8:00, and 10:00 AM (**Figure 4.5**; $P < 0.0001$) may suggest a preference for grazing at distinct times, where the LOW-RFI probably did more grazing at 4:00 am while, HIGH-RFI did at 8:00 and 10:00 AM. This may indicate a more dominant activity in the LOW-RFI group, as they initiate standing activity first (Arave et al., 1981) probably for grazing compared to the HIGH-RFI group. Additionally, the increased standing activity in the HIGH-RFI group as temperatures rise suggests a response to thermoregulation needs, as wind flow may alleviate HS through convection (Wang et al., 2018). The lying time was shorter in the LOW-RFI group between 3:00 to 4:00 AM and 06:00 PM when the temperatures were lower but increased at 11:00 AM in comparison to HIGH-RFI when summer temperatures started to rise (**Figure 4.5**; $P < 0.0001$). Simultaneously, at this time the HIGH-RFI started to seek shade or water access based on geospatial location (**Figure 4.8, 4.9, 4.10 and 4.11**). The greater lying time at 11:00 AM could potentially represent greater rumination and rest times (Schirmann et al., 2012), serving as a thermoregulatory strategy to reduce energy expenditure during the rising temperatures of the day.

A higher number of steps in a grazing system can be associated with better forage quality selection (Pauler et al., 2020) and greater female reproductive behavior (Kiddy, 1977). We found an RFI × day interaction with greater number of steps on d 3, 5, 6, 7, 8, 9, 10, 11, 12, 15 and 29 in the HIGH-RFI group when compared to LOW-RFI during the summer (**Figure 4.6**; $P = 0.028$). Specifically, HIGH-RFI showed increases of 7, 9, 11, 8, 15, 10, 6, 10, 7 and 9 % in the number of steps on each of those days. Heifers were exposed to bulls for natural mating until d 20 of the experiment, which might explain the observed increase in steps from d 3 to 15, leading to better reproductive behavior in the HIGH-RFI vs. LOW-RFI as increased number of steps is connected to higher levels of estrogen and increased walking activity in a reproductive season (López-Gatius et al., 2005). Additionally, on d 29, there was an increase in the number of steps by 11 % for the HIGH-RFI group after a blood sampling, which might indicate an increase in grazing or locomotion due to stress as previously described by Oesterheld et al. (1991). Conversely, during the winter, a RFI × day interaction was also found, indicating that the LOW-RFI group exhibited greater number of steps on d 210 under mild CS conditions when compared to the HIGH-RFI group but, lower steps on d 224 under extreme danger cold day (**Figure 4.7**; $P = 0.022$). This suggests that the more efficient group may not require increased activity to produce more energy and heat. This observation is further supported by the findings on d 224, where extreme danger CS prompted the HIGH-RFI to take more steps (3507 vs. 3106; **Figure 4.7**; $P = 0.023$), suggesting a behavioral thermoregulation response to produce body heat under extreme cold exposure. On d 17, the hottest summer day under severe thermal stress risk, the HIGH-RFI group was clustered together close to the hill and wooded area with more natural shade from midday to 3pm compared to the LOW-RFI group (32.1 vs. 31.8%; Figure 24). On d 26, 31, and 32 between midday and 3 pm, under the same environmental conditions, the HIGH-RFI group was again greatly clustered together on wooded areas with more shade when in comparison to LOW-RFI (45.7 vs. 26.7, 21.4 vs. 9 and 42.9 vs. 41.6%, respectively; **Figure 4.8, 4.9, 4.10 and 4.11**).

An increase in environmental temperature has a direct negative effect on the appetite center of the hypothalamus, leading to a decrease in feed intake (Ammer et al., 2018), which disrupts metabolism in cattle. This underscores the importance of adequate hydration for thermotolerance, particularly in hot climates where cattle do not cope with extreme hot weather. The amount of water an animal consumes directly impacts its capacity to withstand extreme conditions (Arias and Mader, 2010). In our study, we did not find differences in rumen temperature reduction, associated with water access events between HIGH-RFI or LOW-RFI. However, on summer days with moderate risk, changes in RT varied by day and were associated with increased drinking attempts (3 attempts per day; **Figure 4.12**; $P < 0.0001$). In the winter, greater water drinking attempts were found at d 214 with mild CS when compared with d 237 under extreme cold risk (3 vs. 1 attempt per day; **Figure 4.13**; $P < 0.0001$).

Endocrine disruptions, such as increased cortisol levels, can negatively affect the immunity of cattle. Under extreme winter conditions (d 239), significantly greater relative lymphocyte cells were found in the LOW-RFI group ($P = 0.052$; **Figure 4.14**), while a tendency towards fewer relative neutrophils ($P = 0.088$; **Figure 4.15**) was observed when compared to the HIGH-RFI group. The adaptive immunity involves the interaction between antigen-presenting cells and T and B lymphocytes and interaction targeting specific pathogens, creating immune memory, and maintaining the body's immune balance (Bonilla et al., 2010). In this study, a higher lymphocyte count was linked to an enhanced adaptive immune response, as was associated by Cooper et al. (2006). Kamwanja et al. (1994) reported that exposure of bovine lymphocytes to a temperature of 45°C for three hours decreased the number of viable cells. Additionally, lymphocytes are involved in cellular proliferation and actively secrete cytokines and antibodies, which are key energy-intensive functions of the immune system (Brand, 1985). However, further studies that characterize specific cell lines are needed to examine the immunity depletion of cattle under extreme winter. Moreover, platelet counts decreased from the coldest sampling day until the last date of the study (d 227 to 239). A similar decrease was also found in chickens during the winter (Majewski et al., 2005), although our values were in the normal range (160 – 650 uL; Wood et al.

(2010; **Table 4.2**; $P < 0.0001$). All the other blood cell variables were affected by day of the study (**Table 4.2**; $P < 0.049$).

Identifying novel prognostic inflammatory markers is critical in medical research. Recent studies have shown that the N:LR is a potentially reliable marker for predicting acute and chronic inflammation. These findings have significant implications for the diagnosis and treatment of physiological disturbances (Carpio-Orantes et al., 2020). A low N:LR of 0.68, which was below to the physiological range of 1 and 3 (Forget et al., 2017), was found in the LOW-RFI during the extreme danger cold at d 227. The low N:LR is associated with an inflammatory process (Li et al., 2014) and critical health status with poor prognosis after illness (Hwang et al., 2017) suggesting that the LOW-RFI may be experiencing a cell challenge under harmful weather conditions (Cartes et al., 2021).

4.7 Conclusion

Our study emphasizes the significant environmental challenges faced by beef cattle in Western Canada, which range from non-stressful to extremely stressful conditions during summer and winter seasons. We found that heifers with low residual feed intake demonstrate better thermoregulation and resilience to hot and CS compared to their less-efficient counterparts, particularly in terms of behavior activity and location. However, the neutrophil-to-lymphocyte ratio, as an indicator of inflammatory status, was higher in the more efficient group on extremely cold days. Nevertheless, our findings underscore the importance of comprehending the complex interactions between environmental stress, feed efficiency, activity behavior, and immune responses to enhance animal welfare in challenging environments, with special attention to extremely cold days for grazing heifers in Western Canada.

Table 4.1 Average, maximum and minimum values of environmental variables measured during the summer (July to August 2022) and winter (January to March 2023) seasons in Western Canada.

	Day of the experiment relative to the start of the summer sampling					
Environmental variable[1]	0-14	14-28	28-42	185-204	204-227	227-239
Air temperature (°C)	18.3	17.2	19.1	-8.2	-9.0	-14.7
Max	29.6	28.4	31.1	4.5	5.7	3.0
Min	7.2	5.8	6.0	-25.6	-32.6	-34.1
Relative humidity (%)	74.3	79.5	72.9	88.7	77.2	77.7
Max	100	100	100	100	98	95
Min	34.8	35.1	27.1	66.7	48.2	49.8
Solar radiation (w/m^2)	297.6	256.1	265.8	50.7	88.5	135.6
Max	905	853	838	367	493	604
Min	0.2	0.3	0.2	0	0	0
Wind speed (km/h)	8.9	9.8	7.1	9.4	10.0	10.9
Max	22.1	28.7	16.8	37.0	35.8	22.4
Min	1.6	1.6	1.2	0.3	1.6	1.7
CCI [2]	32.4	28.7	32.9	-24.3	-22.8	-29.3
CCI Index	Moderate	Mild	Moderate	Severe	Severe	Severe

[1]Values from meteorological station placed within approximately 1 km from heifers at Kinsella, AB.
[2]CCI = Comprehensive Climate Index as described by Mader et al. (2010).

Table 4.2 Complete blood cell counts of grazing beef heifers with divergent residual feed intake during winter months (January to March) in Western Canada.

Blood cells	Day of the study relative to the start of summer sampling				SEM[18]	*P*-value
	185	204	227	239		
LYM, 10^{9}/L[1]	5.25^{ba}	5.56^{a}	5.28^{ba}	5.07^{b}	0.1516	0.049
MON, 10^{9}/L[2]	0.21^{a}	0.11^{b}	0.13^{ba}	0.17^{ba}	0.0253	0.026
EOS, 10^{9}/L[3]	0.24^{a}	0.15^{b}	0.26^{a}	0.19^{ba}	0.0241	0.004
BAS, 10^{9}/L[4]	0.07^{a}	0.05^{b}	0.05^{b}	0.03^{b}	0.0052	< 0.0001
MON, %[5]	2.63^{a}	1.43^{b}	1.76^{ba}	2.24^{ba}	0.2529	0.008
EOS, %[6]	3.15^{a}	1.92^{b}	3.23^{a}	2.78^{ba}	0.3003	0.005
BAS, %[7]	0.97^{a}	0.59^{b}	0.61^{b}	0.44^{b}	0.0611	< 0.0001
RBC, 10^{12}/L[8]	8.39^{a}	8.11^{ba}	8.00^{b}	8.24^{ba}	0.1168	0.020
HGB, g/dl[9]	10.75^{a}	10.81^{a}	10.91^{a}	11.02^{a}	0.1298	0.050
HCT, %[10]	35.49^{b}	35.94^{b}	36.18^{ba}	37.55^{a}	0.5007	0.004
MCV, fl[11]	42.44^{c}	44.40^{b}	45.05^{ba}	45.79^{a}	0.4439	< 0.0001
MCH, pg[12]	12.80^{b}	13.34^{a}	13.60^{a}	13.43^{a}	0.1326	< 0.0001
MCHC[1], g/dl[13]	30.25^{a}	30.10^{a}	30.28^{a}	29.43^{a}	0.2542	0.043
RDWc, %[14]	27.39^{a}	27.84^{a}	26.31^{b}	26.13^{b}	0.2418	< 0.0001
RDWs, fl[15]	43.09^{b}	45.74^{a}	43.20^{b}	43.51^{b}	0.5025	< 0.0001
PLT, 10^{9}/L[16]	268.02^{a}	245.87^{a}	183.21^{b}	170.69^{b}	13.6327	< 0.0001
PDWc, %[17]	34.37^{a}	33.68^{b}	32.37^{c}	33.35^{b}	0.4707	0.015

[1]LYM: Lymphocytes, [2]MON: Monocytes, [3]EOS: Eosinophils, [4]BAS: Basophiles, [5]MON%: Monocyte percentage, [6]EOS: Eosinophils percentage, [7]BAS: Basophiles percentage, [8]RBC: Red Blood Cells, [9]HGB: Hemoglobin, [10]HCT: Hematocrit,[11]MCV: Mean Corpuscular Volume, [12]MCH: Mean Corpuscular Hematocrit, [13]MCHC: Mean Corpuscular Hemoglobin Concentration, [14]RDWc: Red Cell Distribution Width, [15]RDWs: Red Cell Distribution Width, [16]PLT: Platelet, [17]PDWc: Platelet Distribution Width (coefficient of variation); [18] Standard error of the mean.
[abc] Means within a row with different superscripts differ ($P < 0.05$).

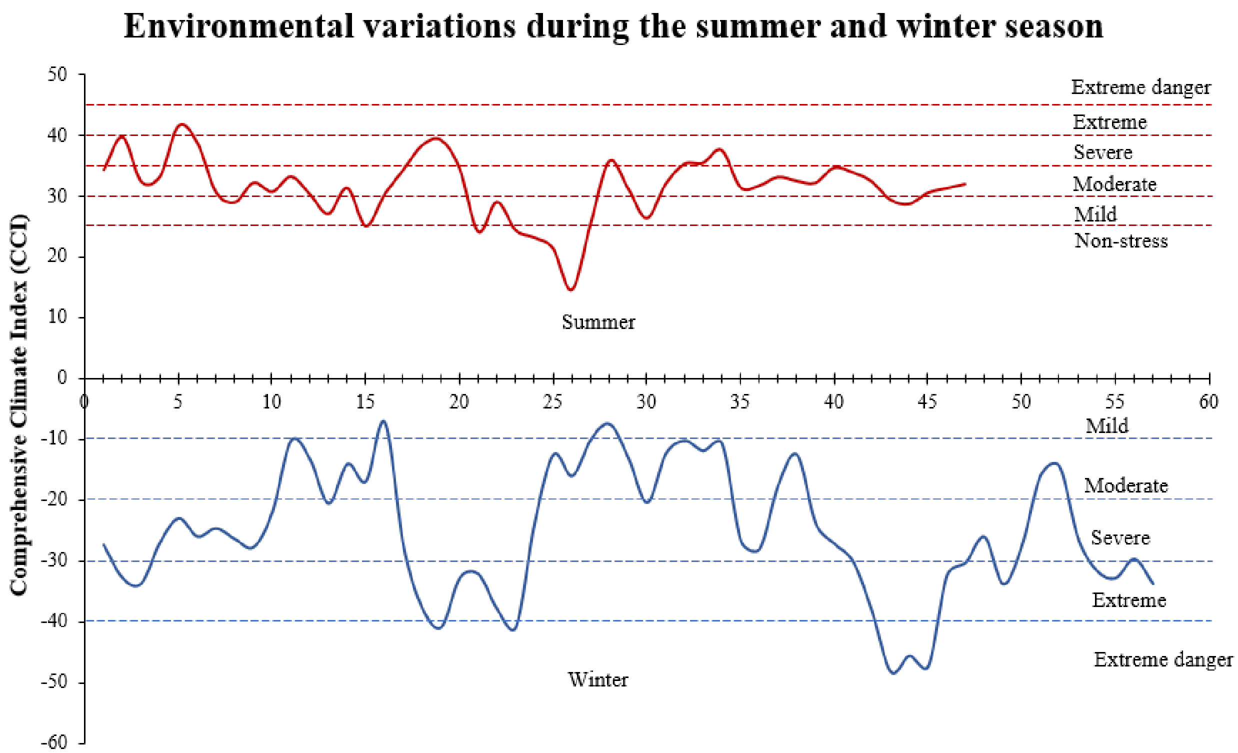

Figure 4.1. Environmental risk to generate stress from non-stress to extreme danger conditions in cattle in a grazing system based on Comprehensive Climate Index developed by Mader et al. (2010) during the summer and winter season in Kinsella, Alberta, Canada

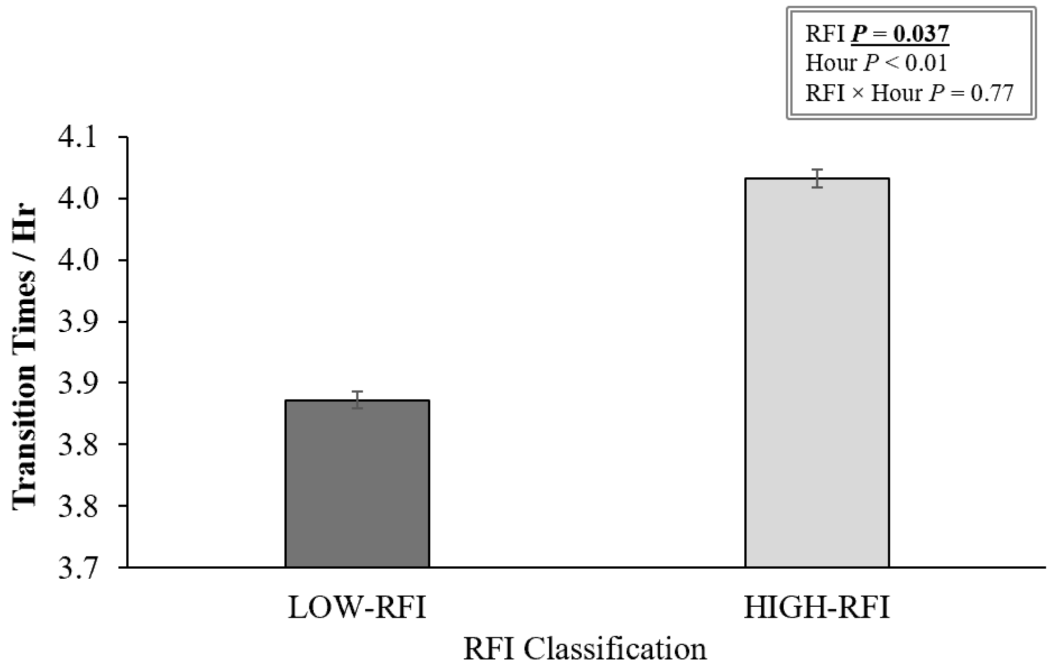

Figure 4.2. Transition times summarized by hour during the summer season. Differences between LOW-RFI and HIGH-RFI on behavioral transition times of grazing heifers in Western Canada during summer months (July to August) were found. The LOW-RFI show less transition times during summer months when compared with HIGH-RFI (3.8 vs. 4.0 times /hr ; $P = 0.037$).

LOW-RFI: More efficient group. HIGH-RFI: Less efficient group.

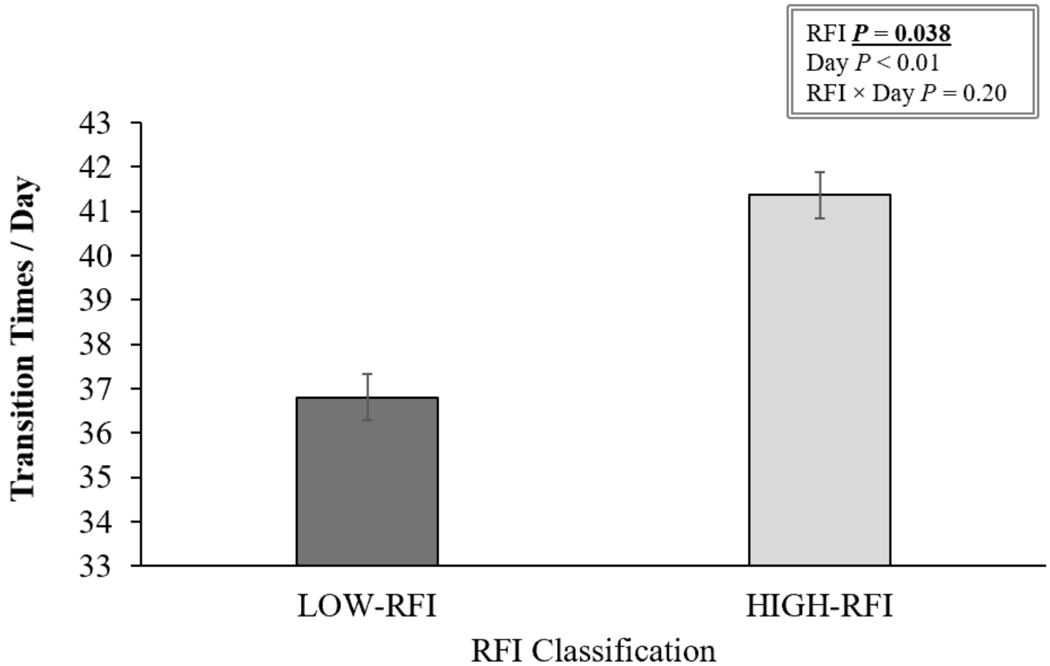

Figure 4.3. Transition times summarized by day during the winter season. Differences between residual feed intake on behavioral transition times of grazing heifers in Western Canada during winter months (January to March) were found. The LOW-RFI show less transition times during winter when compared with HIGH-RFI in a whole day (37 vs. 41 times / Day; $P = 0.038$).

LOW-RFI: More efficient group. HIGH-RFI: Less efficient group.

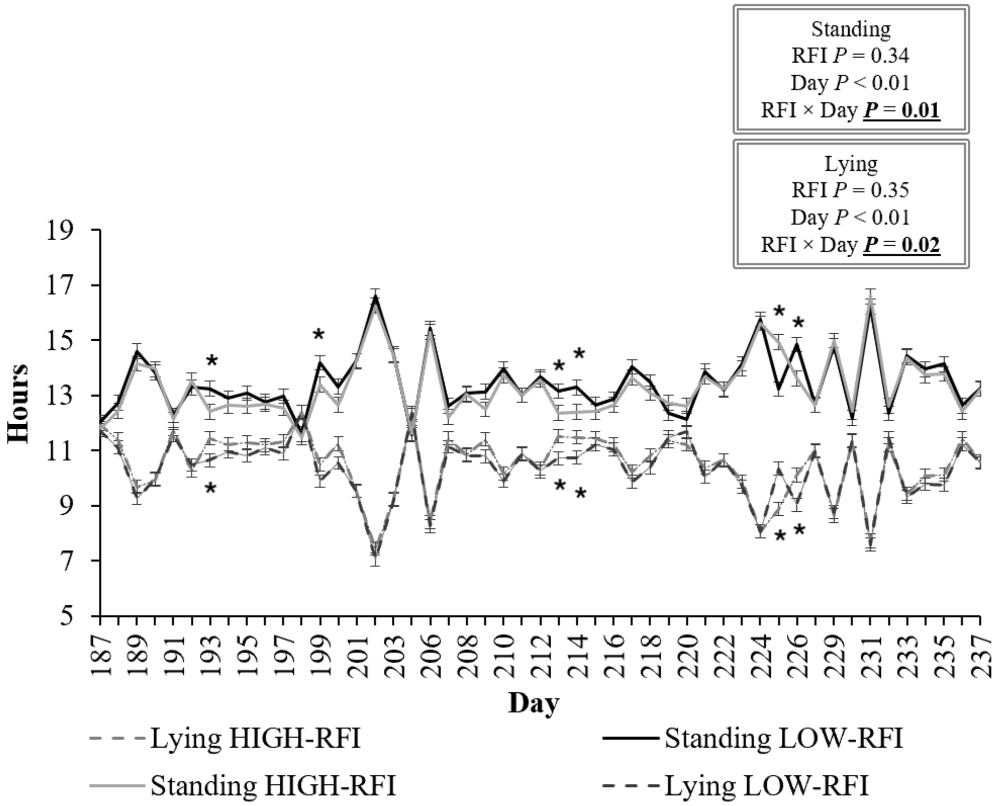

Figure 4.4. Behavior variations in lying and standing positions during the sampling days between the LOW-RFI and HIGH-RFI heifers in the winter season. The LOW-RFI heifers spent significantly more time standing on d 193, 213, and 214, under moderate cold stress (P = 0.011). Moreover, on d 199, under severe cold stress, LOW-RFI heifers also spent significantly more time standing (P = 0.011). Conversely, on d 225, during an extremely cold day (the coldest during the winter trial), HIGH-RFI heifers spent more time standing compared to LOW-RFI heifers. However, on d 226, following 24 hours of extreme cold, LOW-RFI heifers spent more time standing, with a notable reduction in lying time to 9 hours (P = 0.022).

LOW-RFI: More efficient group. HIGH-RFI: Less efficient group.

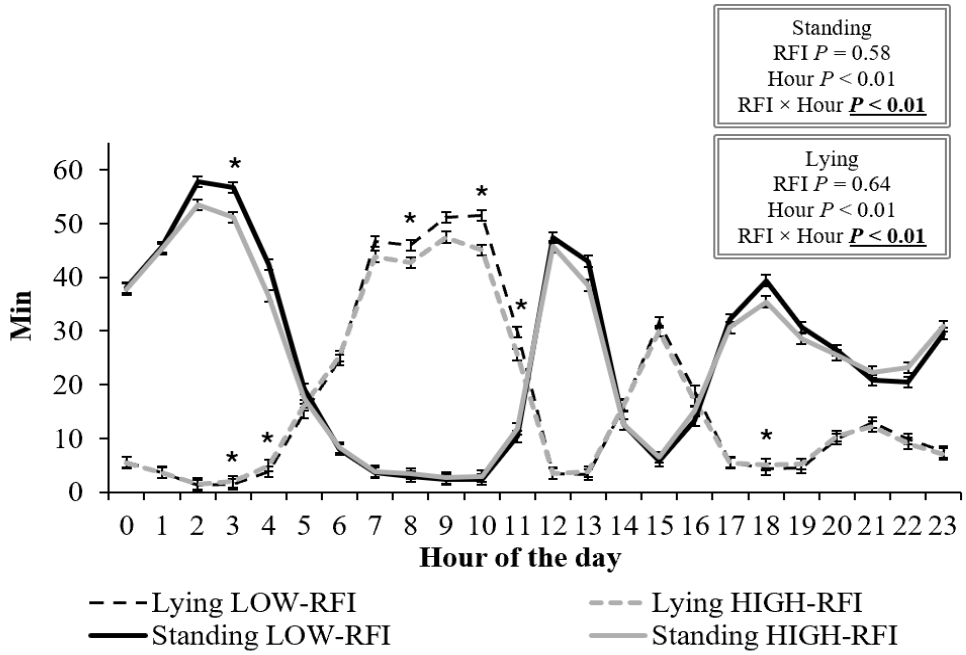

Figure 4.5. Activity budget by hour during the summer, with variations in lying and standing positions between the LOW-RFI and HIGH-RFI. The LOW-RFI heifers spent more minutes standing at 4:00 AM, while HIGH-RFI heifers showed higher standing activity at 8:00 AM and 10:00 AM. LOW-RFI heifers had lower lying time between 3:00 AM to 4:00 AM and at 6:00 PM, but they had greater lying time at 11:00 AM compared to HIGH-RFI heifers ($P < 0.0001$).

LOW-RFI: More efficient group. HIGH-RFI: Less efficient group.

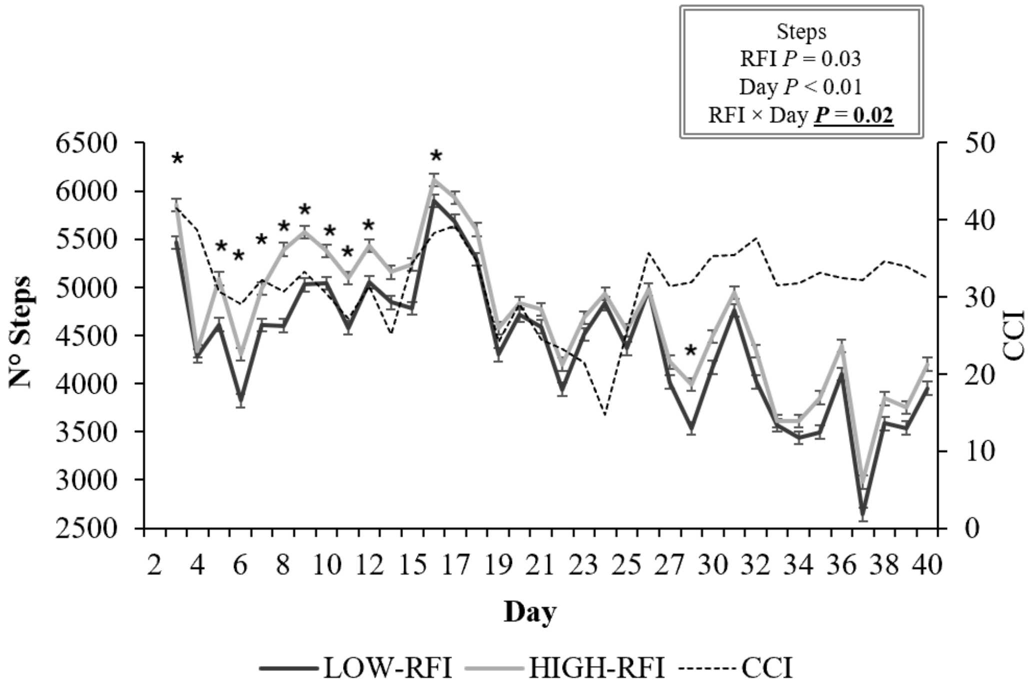

Figure 4.6. Number of steps recorded during the summer season in the LOW-RFI and HIGH-RFI by day. And RFI × day interaction showed that on d 3, 5, 6, 7, 8, 9, 10, 11, 12, 15, and 29 during the summer, HIGH-RFI heifers took more steps compared to LOW-RFI heifers ($P = 0.028$).

LOW-RFI: More efficient group. HIGH-RFI: Less efficient group. CCI: Comprehensive Climate Index.

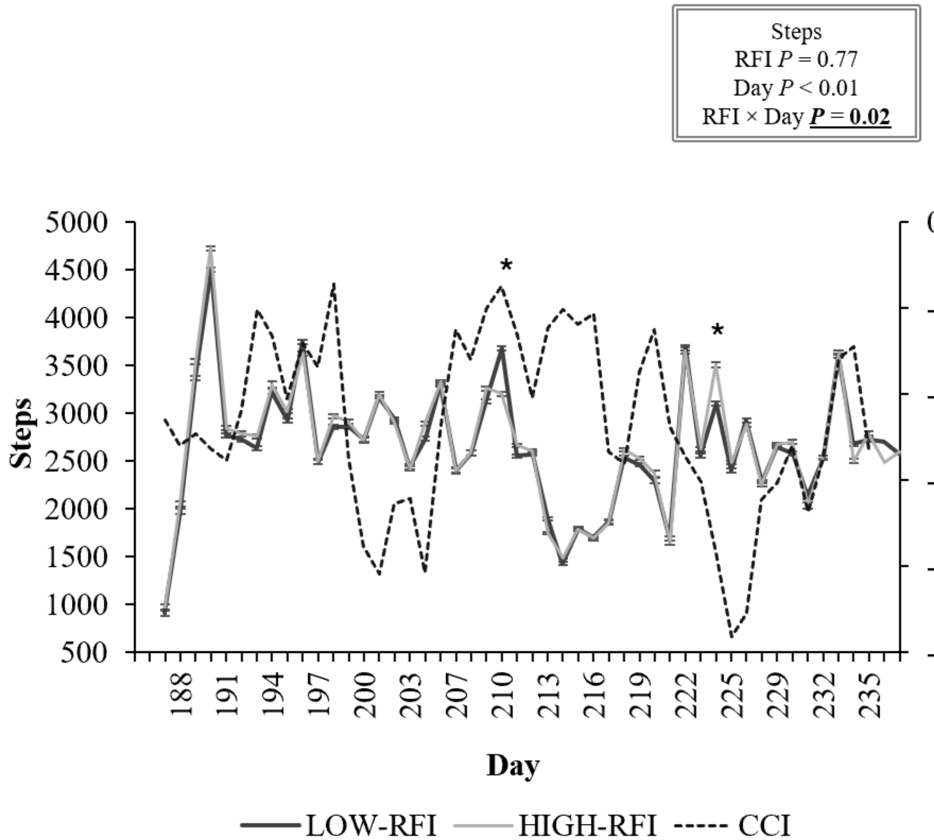

Figure 4.7. Number of steps by day recorded during the winter season in the LOW-RFI and HIGH-RFI. The RFI × day interaction showed that on d 210, classified as a mild cold day and one of the warmest during winter, HIGH-RFI heifers took fewer steps. However, on d 224, under severe cold stress, HIGH-RFI heifers took more steps ($P = 0.023$).

LOW-RFI: More efficient group. HIGH-RFI: Less efficient group. CCI: Comprehensive Climate Index.

Geospatial activity by groups with severe thermal stress risk

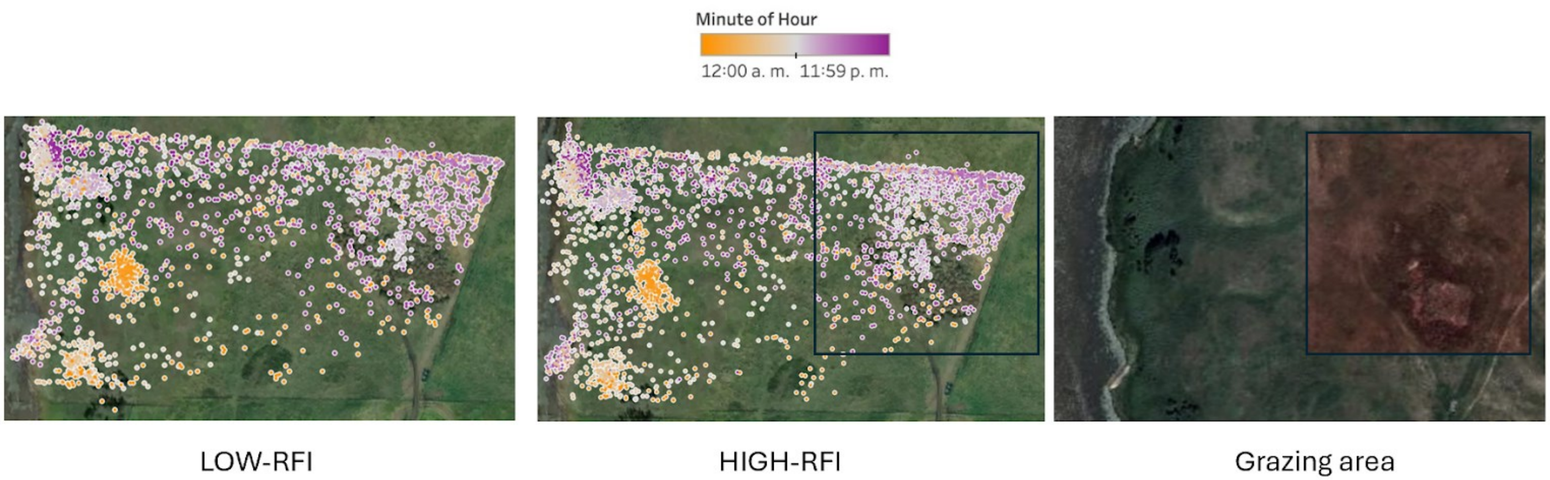

Figure 4.8. The GPS location of each group was recorded to understand behavior responses during the hottest hours of the day. On the hottest summer day (d 17; CCI: 39.2, indicating severe thermal stress risk), the HIGH-RFI group clustered together more than the LOW-RFI group (32.1% vs. 31.8%) near the hill and wooded area with more natural shade from midday to 3 PM.

LOW-RFI: More efficient group. HIGH-RFI: Less efficient group.

CCI: Comprehensive Climate Index.

Geospatial activity by groups with severe thermal stress risk

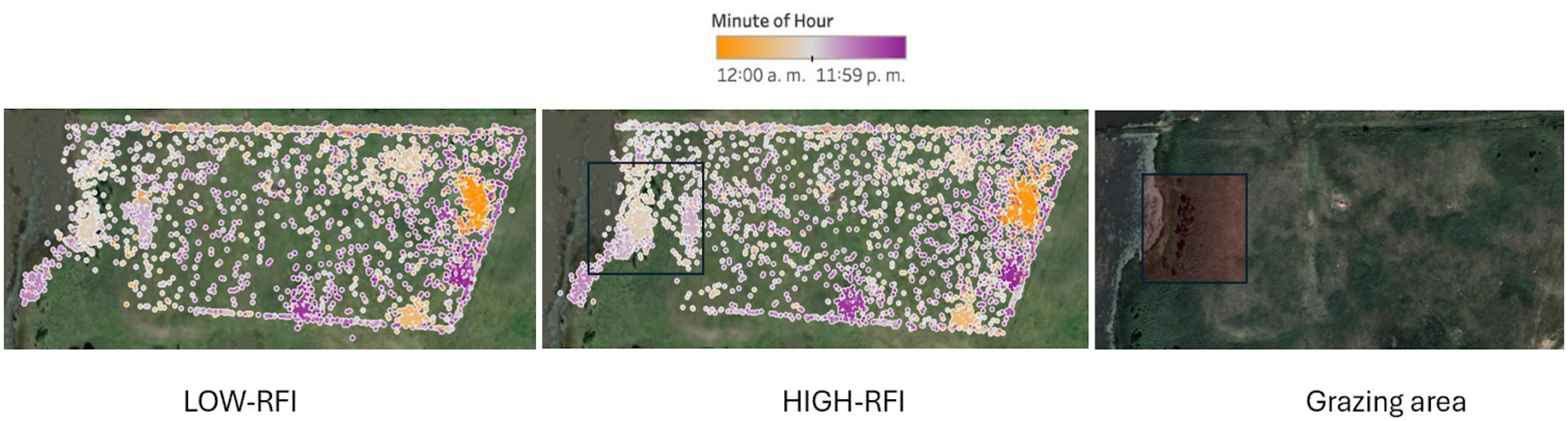

Figure 4.9. The GPS location of each group was recorded to understand behavior responses during the hottest hours of the day. On one of the warmest summer days (d 26; CCI: 35.7, indicating severe thermal stress risk), the HIGH-RFI group clustered together against LOW-RFI (45.7 vs. 26.7%) near the wooded area with more natural shade from midday to 3 PM.

LOW-RFI: More efficient group. HIGH-RFI: Less efficient group.

CCI: Comprehensive Climate Index.

Geospatial activity by groups with severe thermal stress risk

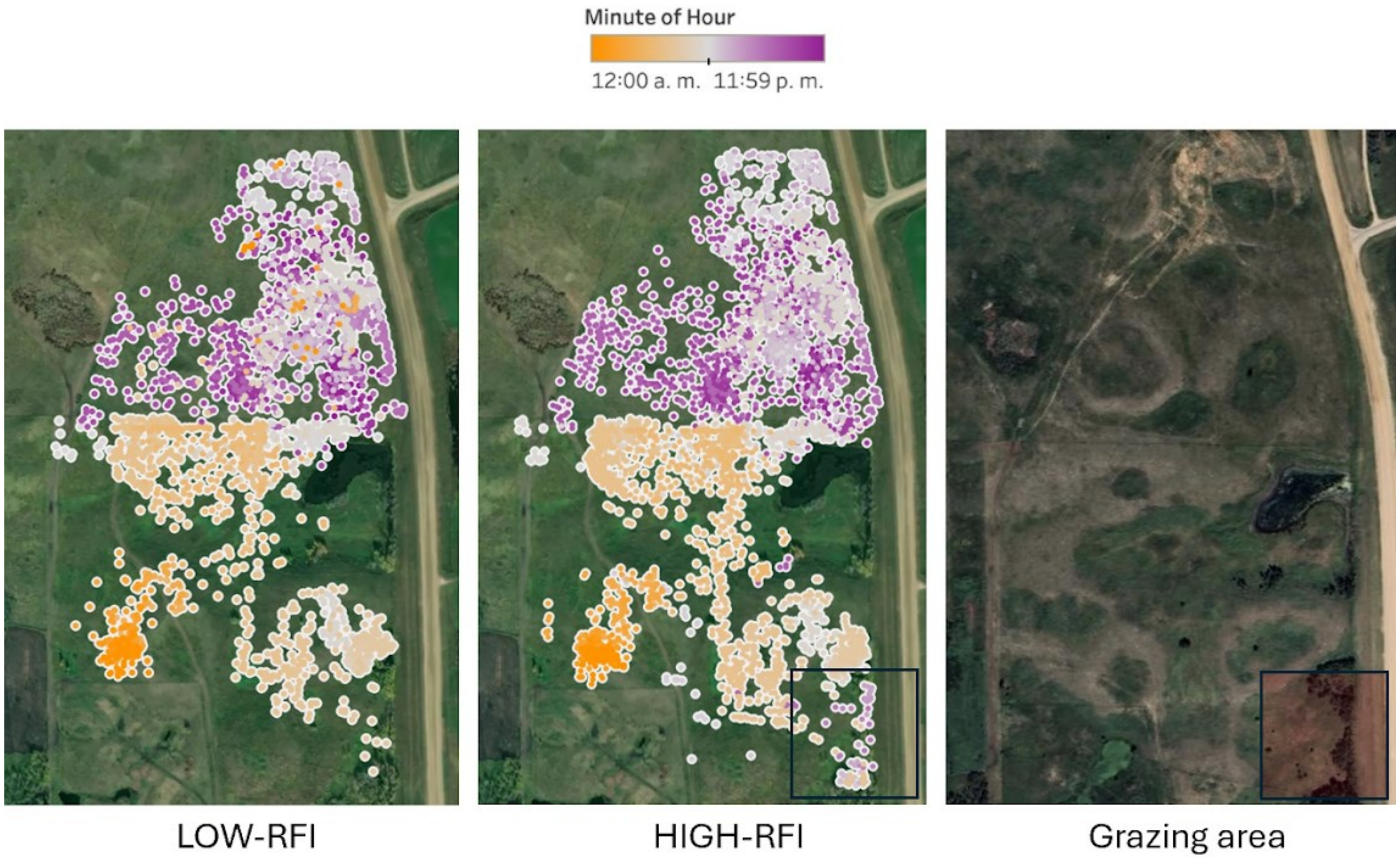

Figure 4.10. The GPS location of each group was recorded to understand behavior responses during the hottest hours of the day. On one of the warmest summer days (d 31; CCI: 35.4, indicating severe thermal stress risk), the HIGH-RFI group clustered against LOW-RFI (21.4 vs. 9%) near the wooded area with more natural shade from midday to 3 PM.

LOW-RFI: More efficient group. HIGH-RFI: Less efficient group.

CCI: Comprehensive Climate Index.

Geospatial activity by groups with severe thermal stress risk

Figure 4.11. The GPS location of each group was recorded to understand behavior responses during the hottest hours of the day. On one of the warmest summer days (d 32; CCI: 37.6, indicating severe thermal stress risk), the HIGH-RFI group clustered against LOW-RFI (42.9 vs. 41.6%) near the hill and wooded area with more natural shade from midday to 3 PM.

LOW-RFI: More efficient group. HIGH-RFI: Less efficient group.

CCI: Comprehensive Climate Index.

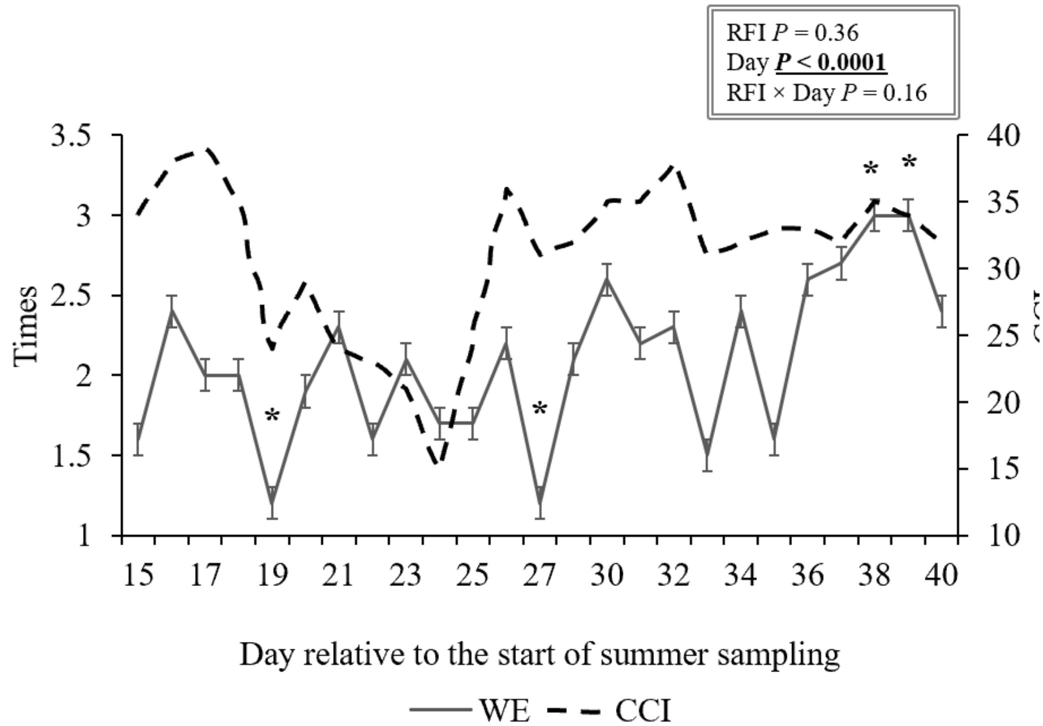

Figure 4.12. Water access events based on drops in the rumen temperature during the summer. An effect of day was found ($P < 0.0001$), on d 38 and 39, there was an increase in water access events, corresponding to moderate environmental risk based on CCI. Conversely, d 19, under no environmental stress, and d 27, with moderate heat risk stress, exhibited fewer water access events.

CCI: Comprehensive Climate Index, WE: Water access events.

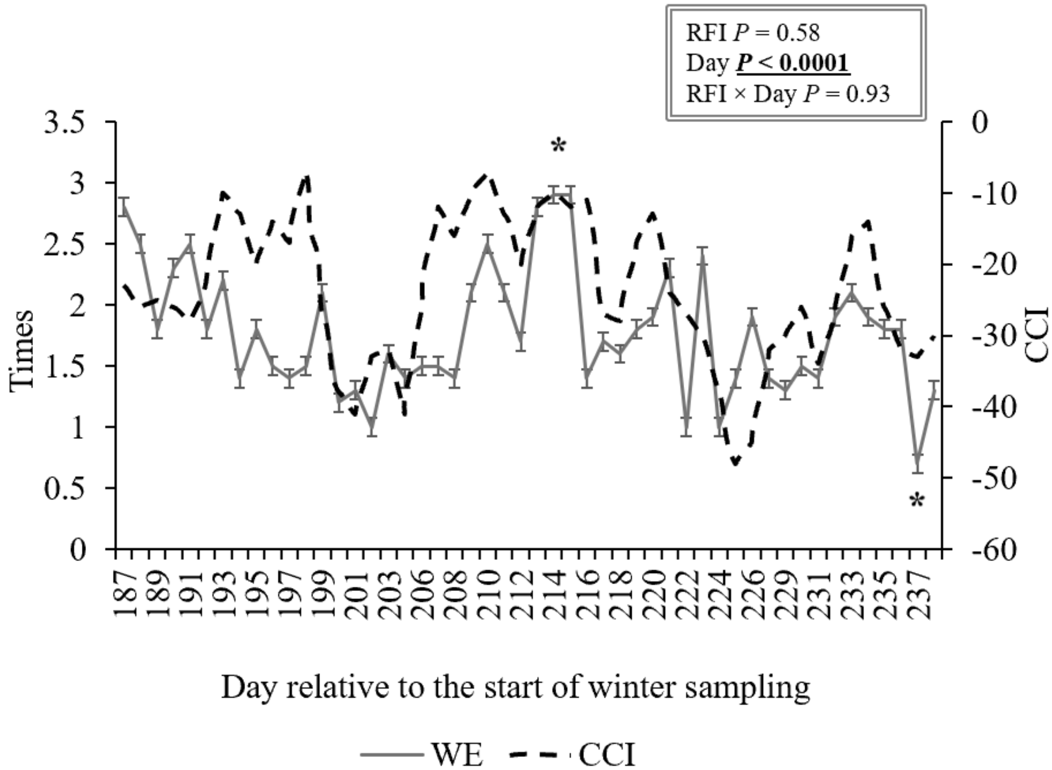

Figure 4.13. Water access events based on drops in the rumen temperature during the winter. An effect of day was found ($P < 0.0001$) on d 214, during mild cold stress, there was an increase in water access events. However, on d 237, water access events decreased.

CCI: Comprehensive Climate Index, WE: Water access events.

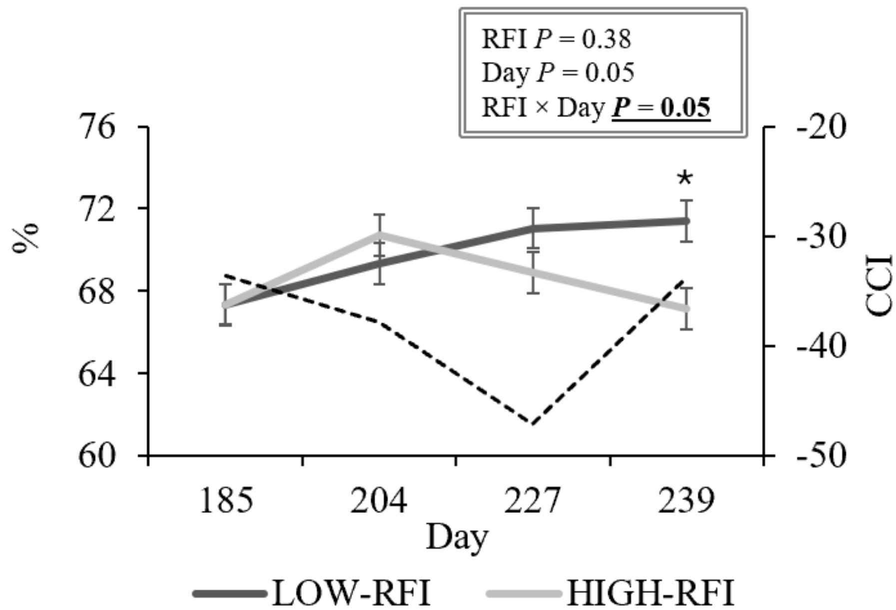

Figure 4.14. The relative lymphocytes in the complete blood cell count had an RFI × day interaction was detected for relative lymphocyte concentration with greater lymphocyte concentrations were observed in the LOW-RFI at d 239, when compared with HIGH-RFI group (71 vs. 67%; $P = 0.052$).

CCI: Comprehensive Climate Index.

LOW-RFI: More efficient group. HIGH-RFI: Less efficient group.

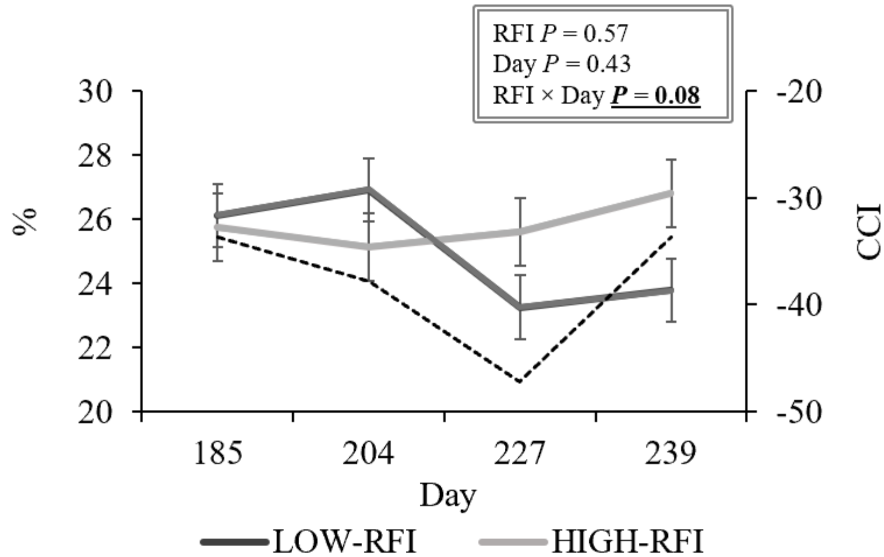

Figure 4.15. The relative neutrophile in the complete blood cell count had a tendency for an RFI × day interaction, without differences by day to highlight (P = 0.088).

CCI: Comprehensive Climate Index.

LOW-RFI: More efficient group. HIGH-RFI: Less efficient group.

Chapter 5. General Conclusions

This study offers a comprehensive analysis of the significant impact of environmental conditions on the physiology of forage-fed beef heifers in Western Canada, focusing on the seasonal contrasts between summer and winter and the influence of RFI classification. This research highlights the distinct responses observed between heifers characterized as more (LOW-RFI) and less feed efficient (HIGH-RFI).

During the summer of 2022, heifers were exposed to environmental conditions that could cause mild to moderate stress, whereas the winter of 2023 presented conditions capable of imposing severe to extreme stress. More feed-efficient beef heifers demonstrated superior thermoregulation, maintaining lower ruminal temperatures in the summer and higher temperatures in the winter. This may indicate a better adaptation to heat and could stress for LOW-RFI heifers. The LOW-RFI heifers displayed higher concentrations of fT3 during summer, possibly indicating superior metabolic efficiency and resilience to heat stress. Even though cortisol, an indicator of stress, was not measured in the present study, it is known that increased plasmatic corticoid concentrations may decrease the enzyme required for thyroxine to produce fT3. Therefore, having increased fT3 in the efficient heifers could signal lower plasmatic cortisol concentrations. Conversely, HIGH-RFI heifers exhibited elevated concentrations of LEP during the winter, which could contribute to increased fat deposition and further isolation from cold exposure. The HIGH-RFI group had greater haptoglobin concentration during summer and the LOW-RFI during winter, but not close enough to indicate inflammation during both seasons. LOW-RFI animals had greater GABA concentrations, which could indicate better nutrient absorption; conversely, decreased GABA for HIGH-RFI heifers could indicate stress.

Hormonal analyses revealed greater IGF-1 concentrations during winter which could indicate increased feed intake and protein anabolism, but also, a late gestation effect. LOW-RFI heifers had a neutrophil-to-lymphocyte ratio of 0.68 on a 1 to 3 normal scale, indicating that more efficient heifers may face challenges in maintaining physiological functions after exposure to extremely cold temperatures. However, further studies on immunity in LOW-RFI heifers under extreme winter conditions are needed. More transitions (lying to standing or standing to lying) and greater number of steps were found in the HIGH-RFI heifers in both seasons, indicating potential discomfort and increased grazing or time looking for shelter, respectively.

The study emphasizes the significance of comprehending cattle's physiological and metabolic adaptations to extreme weather conditions. More feed-efficient beef heifers showed potential for improved thermoregulation and metabolic efficiency, suggesting that selecting for feed efficiency can enhance cattle resilience to environmental stress, thereby increasing productivity and sustainability in beef production systems. Future research should investigate the molecular mechanisms underpinning these physiological adaptations, particularly concerning the immune system, and devise more effective management strategies to mitigate the impact of environment.

5.1 Limitations of the study

It is important to highlight some potential limitations of the study, such as the absence of a middle (or control) RFI group, limiting the ability to explore differences beyond the current extremes in RFI values. Furthermore, moving heifers from the drylot (test area) to a forage-based system could raise concerns about potential changes in RFI classification due to differences in the diet. However, to minimize this issue, the feed used during the testing period comprised mostly of silage, therefore, still a high-forage diet. Estimating forage intake to correlate with rumen temperature, along with quantifying grazing and rumination times, could provide additional valuable insights. Incorporating a regression or correlation analysis for blood parameters may help identify physiological differences within each specific RFI value (e.g., categorical vs. continuous RFI). However, to benefit from the use of this approach, more animals within similar RFI values would need to be used. Finally, during the summer, heifers were placed with bulls, while in the winter, they were in the last trimester of gestation. These different production stages (e.g., heat, pregnancy) could introduce some degree of variability in the data, potentially impacting the response variables.

5.2 Future directions

This thesis represents an examination of how environmental conditions affect physiological functions and thermoregulation of beef cattle in Western Canada. The study emphasizes the contrasting responses between cattle that are more feed-efficient (LOW-RFI) and those that are less feed-efficient (HIGH-RFI). Nonetheless, several areas of uncertainty identified during the study warrant additional investigation. These gaps in understanding present opportunities for future research. Specific aspects that emerged from the findings require closer examination and exploration to address these unresolved questions and enrich the scientific understanding of these processes.

In the summer of 2022, cattle experienced mild to moderate stress, while the winter of 2023 brought severe stress to extreme danger. The rising summer temperatures and colder winters have intensified these challenges. More feed-efficient beef heifers maintained better thermoregulation, with lower rumen temperatures in summer and higher ones in winter, compared to less-efficient heifers. However, it is essential to recognize that rumen temperature can be significantly influenced by dietary factors, particularly through the process of fermentation within the rumen.

Increased fermentation activity can lead to elevated rumen temperatures, as the microbial breakdown of feed generates heat as a waste product (Owens et al., 2016). This thermogenic effect of fermentation could potentially confound our understanding of RT as a measure of thermoregulation or body temperature in cattle. Therefore, incorporating detailed feed intake assessments and other measurements of body temperature into our analysis is crucial. By closely monitoring and evaluating the diet and feed consumption of cattle, we can better discern whether variations in RT are primarily due to differences in fermentation rates or if they are more directly related to the animals' overall body temperature regulation. This approach will enhance the accuracy and relevance of our findings, allowing for a more precise interpretation of the relationship between RT, feed efficiency, and thermoregulatory capabilities in both LOW-RFI and HIGH-RFI cattle.

The age of the herd is an important factor that could introduce variability into the results of this experiment. Hormonal fluctuations associated with puberty, such as changes in estrogen levels, can influence blood parameters, including those related to thyroid hormones, which may decrease as estrogen concentrations rise (Sawhney et al., 1978). Additionally, during puberty, the energy demands for growth, maintenance, and reproductive development increase, potentially diverting energy resources away from thermoregulation. This shift in energy allocation could impact the animals' ability to maintain stable body temperatures under varying environmental conditions.

Regarding the immune system, heifers classified as LOW-RFI exhibited a neutrophil-to-lymphocyte ratio of 0.68, in which the normal values range between 1 to 3. This relatively low ratio suggests that more feed-efficient heifers may encounter difficulties in sustaining their physiological functions when subjected to extremely cold temperatures. A low ratio is often associated with an immune system that may be under stress or struggling to mount an adequate response, potentially indicating a compromised ability to handle environmental challenges, such as severe cold. This finding raises important questions about the resilience of LOW-RFI heifers under extreme winter conditions, particularly regarding their immune competence. To thoroughly understand the implications, further research is needed to explore the immune function of these animals in harsh winter environments. Such studies should include techniques like flow cytometry, which would allow for the precise identification and quantification of specific immune cell populations. By analyzing which cell types are most affected during CS, researchers could uncover potential immunological imbalances or disorders in LOW-RFI heifers. This knowledge would be critical in developing strategies to enhance the immune resilience of these animals, ensuring their health and productivity in challenging environmental conditions.

In terms of activity behavior, increasing the size of the herd by adding more individuals could serve as a valuable approach to disrupting the established social structures and dominance hierarchies within smaller groups. When cattle are grouped in smaller herds, a clear social hierarchy often emerges, with dominant individuals exerting control over access to resources such as food and space. This can skew behavioral observations and make it difficult to accurately assess the true activity levels of individual animals, particularly when trying to evaluate them based on RFI classification. By transitioning from a small to a large herd, the social dynamics become more complex, potentially diluting the influence of dominant individuals and minimizing their control over the rest of the herd. This disruption in the established social structure allows for a more precise evaluation of each animal's behavior, as it reduces the impact of dominance-related activities such as aggressive encounters or monopolization of feeding areas. In a larger, more diverse group, the likelihood of any single animal asserting its dominance over others is diminished, leading to a more equitable distribution of resources and behaviors. This approach not only provides a clearer picture of each animal behavior status but also offers insights into how different individuals adapt to changes in social and environmental conditions. Observing cattle in a larger, more dynamic herd setting can reveal important behavioral patterns that might be masked in smaller groups, where social hierarchies play a more significant role (Grant et al., 2001), who suggested that altering herd composition and size could influence social behavior and reduce the dominance effects that often confound behavioral studies in livestock.

It is important to emphasize the need to select individuals with wide differences between negative and positive RFI values. Another statistical approach is to increase the number of individuals. However, adding more animals may not align with the 3Rs ethics principles for animal research protocols (replacement, reduction, and refinement). Therefore, focusing on increasing the extreme RFI values could be a more suitable option, as long as it does not compromise the power of the test.

Altogether, severe challenges posed by environmental extremes represent a significant danger to cattle. The potential reduction in adverse effects through selection of feed-efficient animals represents an empirical and sustainable solution to addressing some of these challenges. The metabolites and parameters examined herein represent baseline values and can serve as potential indices to evaluate environmental effects in the future and serve as a general basis for stress response in cattle. By focusing on the unique threats of winter, the study calls for targeted approaches to improve cattle resilience, ensuring that the beef industry can better withstand the growing challenges of climate variability. By assessing these parameters, these strategies are vital for protecting cattle health and sustaining beef production, offering a hopeful outlook for the industry's future in the face of increasingly severe winter weather.

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