The Pennsylvania State University

The J. Jeffrey and Ann Marie Fox Graduate School

INFLUENCE OF BREED COMPOSITION AND NOVEL TOOLS ON LONGEVITY AND BEHAVIOR OF DAIRY COWS

A Dissertation in

Animal Science

by

Muratori Tanya

Submitted in Partial Fulfillment

of the Requirements

for the Degree of

Doctor of Philosophy

December 2024

The dissertation of Muratori Tanya was reviewed and approved by the following:

Chad Dechow
Associate Professor of Dairy Cattle Genetics
Dissertation Advisor
Chair of Committee

Wansheng Liu
Professor of Genomics

Kathy Soder
Research Animal Scientist
USDA-ARS-Pasture Systems and Watershed Management Research Unit

Idan Shalev
Associate Professor of Biobehavioral Health

Ramesh Ramachandran
Professor of Reproductive Biology
Director of Graduate Studies

ABSTRACT

My dissertation presents an overarching objective to integrate novel tools and grazing behavior in genetic selection programs to enhance longevity and performance in dairy cows. Two specific objectives were pursued to address this overarching aim. The first objective was developed to test the hypothesis that telomere length (TL) is favorably associated with the longevity of dairy cows and that integrating TL as a parameter within the genetic selection index for dairy cows will result in the production of animals with longer telomeres, leading to enhanced overall health, prolonged productive lifespans, and reduced healthcare requirements.

The second objective was to investigate and analyze the impact of different cow breeds composition on the behavior of cattle in pasture settings, with the aim of providing insights into how breed composition-specific characteristics influence grazing behavior and overall management practices. The hypothesis behind this objective was that the breed composition of cows is a significant factor influencing behavior while on pasture.

Chapter 2 aimed to evaluate the heritability of TL and its relationship with longevity and productivity traits in dairy cattle using both single and multi-trait models. TL was determined via qPCR on 402 samples from 336 cows and 413 samples from 330 heifers. Genomic data from 998 animals involving 74,973 effective SNPs, were used for genetic analyses. Heritability estimates for TL from the single-trait analysis were 0.40 ± 0.17 in cows and 0.21 ± 0.02 in heifers. A seven-trait model, incorporating TL, milk yield, fat, protein, somatic cell score (SCS), and milk urea nitrogen (MUN), was also

used to investigate genetic correlations. Genomic estimated breeding values (gEBV) for TL with an accuracy ≥ 40% were correlated with sire genomic predicted transmitting ability (gPTA) for cow livability (COWLIV), heifer livability (HEIFLIV), and productive life. Approximate genetic correlations between cow gEBV for TL and COWLIV gPTA were 0.44 ($P<0.01$) and 0.60 ($P<0.001$) for HEIFLIV. Heifer TL from the multi-trait model was also significantly correlated with HEIFLIV (0.65, $P<0.01$). However, gPTA for productive life was not significantly correlated with TL in either model. These findings indicate that TL is favorably correlated with livability traits in dairy cattle, supporting its potential use in selective breeding programs aimed at improving longevity and health.

Chapter 3 focuses on investigating TL in purebred Holstein cows and two rotational crossbreeds, GrazeCROSS and ProCross. Blood samples were collected from 101 cows at the West Central Research and Outreach Center, University of Minnesota, and TL was quantified using a multiplex qPCR procedure. The cows, aged between 31 and 128 months, were divided into Holstein (n = 17), GrazeCROSS (n = 29), and ProCross (n = 55) breed composition groups, and further categorized into first (n = 28), second (n = 30), and third-plus (n = 43) lactation groups. All cows were sired by Holstein (n = 32), Jersey (n = 15), Montbéliarde (n = 17), Normande (n = 4), or Viking Red (n = 33) bulls. Statistical analyses were performed using mixed-effects models in SAS (v9.4) to assess the effects of breed composition group, sire breed, and cow age on TL. Results indicated that sire breed (P = 0.03) and age (P = 0.003) had significant effects on TL, while breed composition group did not (P = 0.20). Tl decreased with increasing cow age,

and Montbéliarde-sired cows exhibited significantly longer telomeres ($P < 0.05$) compared to cows sired by other breeds. These findings suggest that TL may serve as a useful biomarker for evaluating the influence of breed composition and age in dairy cows, highlighting the potential for breed selection strategies aimed at enhancing production efficiency and longevity.

In Chapter 4, we elucidate the diurnal grazing and ruminating behavior patterns of grazing dairy cows across different breed composition groups and sire breeds, with a focus on estimating feed efficiency through novel tools such as potentially degradable neutral detergent fiber (pdNDF) and thermal heat loss. The study included 13 cows (two Holstein, six GrazeCross, and five ProCross) from the West Central Research and Outreach Center, fitted with RumiWatch devices to track rumination, grazing, and other activities between June 15-21, 2021. In addition to behavior data, body condition score (BCS), fly density levels, thermal imaging, milk yield, fat, protein, SCS, MUN, and fecal samples were collected to assess breed composition differences in performance and efficiency. Statistical analyses using mixed-effects models in SAS (v9.4) revealed distinct hourly grazing and rumination patterns, with grazing peaking in the evening around 8 pm and morning around 7 am, and nearly ceasing by 8 am. Rumination peaked at 11 pm and remained elevated into the early morning, following a consistent circadian rhythm across breeds. While no significant differences in hourly behavior were found between breed compositions after applying Tukey's adjustments, GrazeCross cows spent significantly more time grazing daily (604.1 minutes/day) compared to ProCross and Holsteins ($P<0.05$). Fecal analysis indicated higher pdNDF levels in Normande cows (14.56)

compared to other sire breeds (9.79-12.92), suggesting less complete digestion in Normande cows. Thermal imaging showed significant breed-specific differences in leg temperatures, with Viking Red cows exhibiting cooler legs (29.16°C) than Normande cows (32.49°C) (P=0.03), reflecting potential differences in metabolic efficiency and heat dissipation. Fly resistance scores ranged from 0.94 in Montbéliarde to 1.86 in Holstein (P=0.02), while body condition scores (BCS) were highest in Montbéliarde cows (3.53) and lowest in Holsteins (3.19) (P=0.05). These findings highlight the potential of adjusting farm routines such as milking and feeding times to better coincide with the animals' natural peaks in grazing and rumination behaviors. This adjustment could enhance cows' productivity, nutrient utilization, and digestion. Additionally, tracking novel tools such as pdNDF, thermal heat loss, and fly resistance in pasture-based systems could help monitor cow health and performance, providing opportunities for enhancing production efficiency in pasture management.

In Chapter 5, we investigate the origin and accuracy of immune cell genotypes in neonatal calves, focusing on potential contamination from maternally derived cells or colostrum. Blood samples from a one-day-old calf were compared to its own hair, dam hair, and consumed colostrum. Polymorphonuclear cells (PMN) and peripheral blood mononuclear cells (PBMC) were separated, with PBMC further divided into T-cells, B-cells, and monocytes via flow cytometry. All samples were genotyped for 139,376 single nucleotide polymorphisms (SNPs). Using the calf’s hair as the reference genotype, chi-squared tests assessed the consistency of blood sample genotypes. Call rates were high for hair and PMN (98.6%) and slightly lower for T-cells (96.2%). Most cell lineages

showed strong concordance with the hair genotype, with mismatches in 2, 37, 56, and 656 loci in PMN, B-cells, monocytes, and T-cells, respectively. T-cells displayed higher heterozygosity (33.9%) and differed significantly from the hair genotype ($P<0.05$). Colostrum genotyping had a low call rate (78.3%) and high heterozygosity (65.4%), reflecting DNA contamination resulting from the pooling of samples from multiple cows. Blood-derived genotypes from neonatal calves were generally reliable, with some interference from colostrum cells relating to the T-cell genotype.

The results from this dissertation address a gap in the literature by demonstrating the genetic component of telomere length (TL) and its association with longevity traits in dairy cows. It also identifies distinct grazing and ruminating behavior patterns across breeds, emphasizing the importance of aligning farm routines with cows' natural circadian rhythms. Furthermore, the dissertation introduces novel tools like fly resistance, pdNDF, and heat dissipation, highlighting their potential to improve cow health and performance in pasture-based systems. By integrating these traits into genetic selection, this research provides a framework for improving health and productivity in dairy herds.

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ACKNOWLEDGEMENTS

I still can't believe this moment is finally here! Grad school has been an incredible journey, full of beautiful and challenging moments that allowed me to grow and become the scientist I've always wanted to be. I don't know how to express how deeply grateful I am for all the support and friendship I received over the years at Penn State.

First, I would like to thank my committee members, Dr. Wansheng Liu, Dr. Kathy Soder, and Dr. Idan Shalev, for accepting this position and always offering a kind word of support. I would also like to thank my advisor, Dr. Chad Dechow, for the amazing opportunity to be part of his team, for constantly pushing me to become a better scientist, and, more importantly, for teaching me how to write a paper properly. Thank you for never losing faith in me, even when I made you struggle, and for creating an environment that made me feel at home 4,300 miles away from home.

Thank you to Dr. Jud Heinrichs for bringing his expertise in heifer nutrition to my workplace in Italy and changing my life. If he had not seen something in me, I would never have met my advisor, and this moment would still be a dream. I am also grateful to Dr. Kevin Harvatine for allowing me to run all my qPCR in his lab and to the other faculty members in the Department of Animal Science, including Dale Olver, Dr. Ramesh Ramachandran, Dr. Joy Pate, Dr. Erika Ganda, and Dr. Adele Turzillo, for their support, wisdom, and guidance.

To my past and current lab mates Dr. Han Longfei, Dr. Isaac Haagen, Wasim Yousaf, and Hannah Kelley, thank you for the laughs, the discussions, and the exchange of ideas. These memories will stay with me forever and made my time in the Dechow lab an incredible experience. A heartfelt thank you goes especially to Dr. Lydia Hardie, who has been a fantastic friend in both my professional and personal life. Thank you for helping me believe in myself during the lowest points, for teaching me new things, reviewing my papers, and for all the time we spent drinking tea, always with your kind smile.

Thank you to my incredible undergraduate helpers, George Demers, Sydney Jewell, and Elizabeth Gilpatrick, for allowing me to teach you and for learning so well how to run qPCR that I could step away from hands-on activities and focus on learning new skills myself.

To my friends Dynisty, Emily, Victoria, Chiara, Federico, and Ellie, thank you for making my time in State College full of beautiful moments. To Martina, Silvia, Eleonora, Elisa, and Alessia, thank you for showing me that distance means nothing and that we'll always pick up exactly where we left off, no matter how much time passes.

To Mikhail, thank you for all the incredible support you've shown me over the years. To Dylan, thank you for your constant guidance and support; you've helped me navigate every challenge life and the dissertation have thrown at me. To Valentina, thank you for being my partner in crime and always answering the phone, even at 2 a.m., to keep me awake while working. I would be lost without you!

To my boyfriend Ethan, thank you for taking such good care of me through all the sleepless nights. Thank you for always having coffee, popcorn, and ice cream ready at a moment's notice and for ensuring I would end each day with a smile. You never stop amazing me.

Thank you to my wonderful family, without whom I wouldn't be the person I am today. Since I was born, there has never been a moment when I didn't feel your love, support, and presence. Thank you for standing by my side when I decided to move across the world to follow my dream and for supporting me every day since, even if it meant being absent for countless birthdays, Christmases, and celebrations. Thank you for always being my biggest fans and for laughing, crying, or celebrating every event in my life with me. I can't wait to hug you and move forward to the next milestone of my life, hopefully with far fewer miles between us!

Last but not least, I want to thank myself. I would never have survived the past six intense years if not for my determination and persistence. I know I proved everyone right regarding my capabilities, but I also exceeded my own expectations as a first-gen student earning a Ph.D. overseas and in my second language. I want to thank myself for never losing sight of the bigger picture—the dream that led me to start this adventure in the first place—even in the face of all the obstacles life threw at me. I've proven once again that there's nothing I can't accomplish if I set my mind to it.

This work is supported by USDA-NIFA-OREI competitive grant no. 2016-51300-25862 and by the US-Israel Binational Agricultural Research and Development Fund # US-5000-1 to the last author. The authors have not presented any conflict of interest. Any

opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the funding agencies.

And now, I can finally say:

Veni, Vidi, Vici

Chapter 1

Literature review

Section 1

TELOMERES

Genetic selection programs have increasingly emphasized nonproduction traits that include disease resistance. In their revision, VanRaden et al. (2021) presented how traits related to dairy cows' health and reproductive performance, including new traits such as early first calving, heifer livability, and feed saved, have been included in the most recent Net Merit Index. Cow health and well-being are influenced by environment and genotype by environment interactions. Hardie et al. (2023) suggested that genetics play a role in determining cow health, highlighting the heritability of total lactational health costs (HCOST) and nulliparous health costs. This genetic influence implies that there can be variation in how cows respond to environmental factors. Telomeres may be a biomarker that facilitates selection for healthier cows and provides an indication of adaptability to varying environments.

Definition and structure

Telomeres are conserved non-coding tandem repeats identified as physical ends of eukaryotic chromosomes (Pfeiffer and Lingner, 2013) (Figure 1-1). They protect the end of the chromosome from damaging events like deterioration or fusion with surrounding chromosomes.

Telomeres have an essential role in maintaining genome integrity. Telomeres are dynamic structures that differ in length and sequence in distinct species, ranging from 20-70 repeats in Tetrahymena thermophila, all the way up to 150 kb in Mus Musculus (Blackburn et al., 2006; Shay and Wright, 2019). The sequence in question consists of repetitive units that are iterated up to thousands of times. The telomere repeat sequence in humans and vertebrates, including mice, birds, reptiles, and fish is TTAGGG (Blackburn and Gall, 1978). Telomeres were identified for the first time in 1938 when Herman Muller discovered that chromosome ends were the only part not affected by fusion or rearrangement events, even when the rest of the structure was broken (Blackburn et al., 2009).

With each round of DNA replication, telomeres undergo progressive shortening due to the inherent limitations of DNA polymerases in fully replicating the chromosome ends (Blackburn and Gall, 1978). Due to the elaborate components in their structure, maintaining genomic integrity relies heavily on telomeres. Their shortening process impacts cellular senescence and aging speed (Harley et al., 1990).

Telomeres are connected to the rest of the chromosome through two structures: interstitial sections and subtelomeres. Looking deeper into the telomeric structure, there is a difference between the proximal and distal regions. While the latter is a single-

stranded guanine-rich G-tail, the former presents itself as double-stranded (Baird, 2018). The connection between the two regions is formed through a t-loop, which occurs when the long single-stranded G-tail folds back on itself and invades the double-stranded region of the telomere. During this invasion, the G-tail pairs with the complementary strand, displacing the original strand and creating a loop structure known as a d-loop within the duplex DNA at the invasion site. The connection between the two regions is created through a t-loop generated when the long single strained G-tail undergoes a folding mechanism on itself, invading the double-stranded region of the telomere and pairing with the complementary strand by displacing the other strand. This displacement creates a loop structure (d-loop) within the duplex DNA at the point of invasion (Nandakumar and Cech, 2013). Other identifiable structures include different protein complexes, like the shelterin complex proteins, which function to stabilize the unstable fragile sites along the telomeric sequence (Baird, 2018). The G-tail can also fold into a G-quadruplex structure in regions of DNA rich in guanine bases. These regions comprise stacked planar guanine-rich tetrads and are stabilized by metal ions such as potassium and sodium (Rhodes and Lipps, 2015). G-quadruplex structures play a pivotal role in the maintenance of telomeres and are intricately involved in the pathogenesis of telomere-associated diseases, including cancer. G-quadruplexes formation in telomeric DNA has been shown to inhibit and effectively impede the activity of telomerase, an enzyme primarily responsible for maintaining and, in some cases, extending the length of telomeres.

As a consequence, telomeres undergo a gradual process of reduction in length and the onset of cellular senescence. This process is considered one of the critical

mechanisms by which telomeres act as an aging clock. Additionally, G-quadruplexes in telomeres have been shown to promote chromosomal instability, which is associated with cancer development.

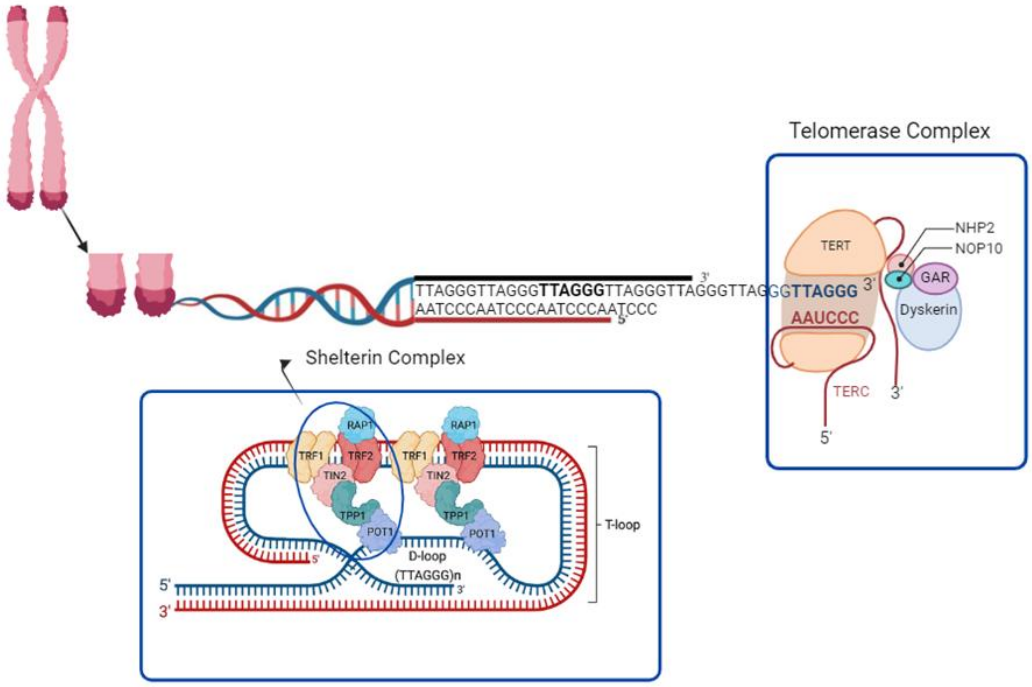

Figure 1-1: Telomere sequence, structure, and location of associated protein complexes.

Associated proteins

There are key proteins that stand out as pivotal players in the dynamic and complex world of telomere maintenance. These proteins (Figure 1-1) include the shelterin complex, which serves as a protective shield for telomeres, nucleosomes that organize and modulate chromatin structure, RecQ family helicases responsible for unwinding telomeric DNA, and telomerase, the enzyme tasked with the delicate

balance of extending telomeric DNA while maintaining genomic stability. The intricate functions and interactions of these proteins are central to understanding the mechanisms underlying telomere biology, aging, disease and the potential therapeutic avenues they may open in the realm of cellular and molecular biology.

The shelterin protein complex assumes a critical function in safeguarding and preserving telomeres' structural and functional stability. The dysregulation of shelterin proteins can lead to dysfunction of both telomere and genome (Palm and de Lange, 2008), and its interaction with other telomere-interacting complexes, such as CST, is crucial for coordinating the maintenance of telomere structure and function (Sexton et al., 2014).

The shelterin protein complex comprises a set of six main proteins (Telomeric Repeat-binding Factor 1 and 2 (TRF1 and TRF2), Repressor/Activator Protein 1 (RAP1), Protection of Telomeres 1 (POT1), TRF1-interacting Nuclear factor 2 (TIN2), and Tripeptidyl Peptidase 1 (TPP1). The first two subunits recognize and bind to the TTAGGG repeats in telomeric DNA, forming a protective cap that prevents the DNA damage response pathways from being activated. The third subunit is a repressor/activator protein that binds to TRF2 and helps to regulate the shelterin complex by recruiting additional proteins and preventing inappropriate DNA repair pathways from being activated. The fourth subunit binds directly to the single-stranded telomere and helps avoid degradation and repair pathways. TPP1 binds to both POT1 and TIN2. It helps to regulate telomerase, the enzyme responsible for adding telomeric repeats to chromosome ends in stem and germ cells. TPP1 interacts with POT1 and TIN2 and acts in the process of telomerase regulation. TIN2 assists in the coordination

of the subunits functions, as well as the assembly and stability of the shelterin complex (Palm and de Lange, 2008, Vulliamy and Dokal, 2008).

The shelterin protein complex also exhibits regulatory functions regarding gene expression and chromatin organization at telomeres. TRF1 protein has been demonstrated to govern the expression of genes, specifically at telomeres, through its ability to recruit chromatin-modifying enzymes to these regions (Koering et al., 2002).

Telomeres are maintained by shelterin subunit interactions with proteins (TIN2, TPP1, POT1, Rap1) and DNA, which with the subunits TRF1 and TRF2 binding to the telomeric repeat sequences (Sfeir and de Lange, 2012). In higher eukaryotes, telomeres are typically found in heterochromatic regions, where they are enriched with specific histone modifications like H3K9me3, H4K20me3, and HP isoforms (Blasco, 2007; Zhang et al., 2013). SUV39H1 and SUV39H2 play a key role in promoting the methylation of H3K9 residues, which in turn connects to HP1 proteins that are essential for compacting chromatin and maintaining telomeric structural integrity (Takai et al., 2010; Tennen et al., 2011). However, the depletion of heterochromatic marks can lead to problems like the open structure of the chromatin and its instability, as well as faulty telomeres or anomalous telomere length (TL) (García -Cao et al., 2003). A critical aspect of histone-mediated telomere regulation is the establishment of heterochromatic structures that transcriptionally silence nearby genes, a phenomenon known as the telomere position effect. This is mediated by the shelterin protein RAP1 and the histone deacetylase SIRT6. RAP1 recruits SIRT6 to telomeres, where SIRT6 deacetylates histones, leading to repressive chromatin marks and a closed chromatin structure,

preventing transcription of telomere-proximal genes (Blasco, 2007; O'Sullivan and Karlseder, 2010; Tennen et al., 2011).

In addition to shelterin, a complex of CTC1-STN1-TEN1 (CST) participates in TL regulation (Miyake et al., 2009; Wang et al., 2012; Sexton et al., 2014) and facilitates replication by unfolding G-quadruplex structures and localizing with Polα at DNA damage sites. The STN1-TEN1 subunit of the CST complex also resolves replication forks during replication stress and regulates telomerase-mediated extension of the 3' G-overhang (Miyake et al., 2009; Wang et al., 2012).

RecQ-family DNA helicases, such as Werner (WRN) and Bloom (BLM), are also recruited to telomeres through TRF1 and TRF2 and play a crucial role in unwinding G-quadruplex structures and DNA replication initiation (Mohaghegh et al., 2001; Opresko et al., 2004). Moreover, proteins such as excision repair cross-complementing associated with xeroderma pigmentosum group F (ERCC/XPF), RAD51, and RTEL1 are associated with telomeres and participate in the replication, recombination, and repair of telomeric DNA (Ray et al., 2009; Zhang et al., 2015). ERCC/XPF is also involved in the DNA damage response mechanism (Schröder et al., 2008).

Telomerase is a DNA polymerase that facilitates the elongation of chromosome ends with telomeric repeats using RNA as a template (Blackburn, 2001). Telomerase plays a pivotal role in the preservation and potential extension of TL through the addition of extra telomere repeats at the chromosomal termini (Greider and Blackburn, 1985). Telomerase exhibits activity in specific cell lineages, including stem cells and

germ cells (Greider and Blackburn, 1985), while typically remaining dormant in the majority of somatic cells (Kim et al., 1994).

The telomerase complex consists of two primary constituents: telomerase reverse transcriptase (TERT), which acts as the catalytic subunit, and telomerase RNA component (TERC), serving as a template for the elongation of telomeric nucleotide repeats (Vulliamy et al., 2001; Tomlinson et al., 2006; Nandakumar et al., 2013). The intricate processes of telomerase biogenesis, its subcellular localization, and its functional regulation are influenced by various accessory molecules, such as Dyskerin (DKC1), Glycine-Arginine Rich Protein 1 (GAR1), Nucleolar Protein Family A Member 2 (NHP2), Nucleolar Protein 10 (NOP10) (Vulliamy et al., 2001; Tomlinson et al., 2006). The 3' end of the telomerase RNA component presents a H/ACA domain remarkably conserved. This domain binds components that work in concert to form a stable complex where the dyskerin acts as a scaffold for the assembly, while NOP10 and GAR1 associate with dyskerin, and NHP2 directly interacts with the RNA moiety (Tycowski et al., 2009; Nandakumar et al., 2013). A fully developed telomerase complex, in collaboration with telomerase cajal body protein 1 (TCAB1), can recognize the Cajal body (CAB) box. During the S-phase of the cell cycle, Cajal bodies play a crucial role in facilitating the recruitment of the mature telomerase complex to the telomeres, as also observed by Jády et al. (2006) and Nandakumar et al. (2013). Vulliamy et al. (2001) and Tomlinson et al. (2006) described how ATPases reptin and pontin interact with TERT and dyskerin and play a crucial role in facilitating the assembly and stabilization of the telomerase complex.

Replication process

The replication of telomeres is an intricate process that involves telomerase and the shelterin complex (Blackburn, 2001). Shelterin prevents the recognition of telomeres as double-stranded breaks by effectively shielding telomeres from engaging the DNA damage response (DDR) machinery, thereby preserving genomic stability and mitigating potential cellular repercussions (de Lange, 2005). During DNA replication, the replication machinery encounters difficulties replicating telomeres because of the inability to synthesize the 3' overhang (Blackburn, 2001). Therefore, telomerase is required to elongate the telomeric DNA.

Despite the existence of telomerase and shelterin, the replication of telomeres can still undergo processes that lead to their shortening or lengthening, influenced by diverse factors.

An important factor to consider is the oxidative damage caused by reactive oxygen species (ROS), (chemically reactive molecules containing oxygen, which are natural by-products of cellular metabolism), which impacts DNA integrity (Passos et al., 2007). The oxidative damage inflicted on DNA leads to breaks in the single-stranded DNA, which subsequently triggers the recruitment of the DDR machinery. This recruitment ultimately activates a control site for DNA damage, impairing telomeres (d'Adda di Fagagna et al., 2003). In addition, telomere replication can be hindered by telomere-binding proteins competing with shelterin to bind telomeric DNA, leading to damage to telomeres (Zhong et al., 2012). Furthermore, the replication process can result in telomeric fusions, triggering chromosomal aberrations (Capper et al., 2007).

Disturbances in the regulation of TL and telomerase activity have been implicated in various diseases, including cancer (Counter et al., 1992), cardiovascular disease (Samani and van der Harst., 2008), and neurodegenerative disorders (Rodríguez-Fernández et al., 2022). Unraveling the intricacies of TL and function control constitutes a significant focal point of research in biology and medicine (Blackburn et al., 2015).

Telomerase reactivation

Telomerase reactivation is a prospective approach to fighting aging and respective diseases by restoring TL and preventing cellular senescence. However, telomerase reactivation also poses several challenges and potential problems, including the risk of cancer, immune-related problems, and potential effects on stem cell function and differentiation (Blasco, 2005; Jaskelioff et al., 2011; Bernardes de Jesus et al., 2012).

Over the years, strategies like telomerase activators, telomerase modulators, and gene therapy are a few of the approaches formulated to reactivate telomerase (Harley et al., 2011; Bernardes de Jesus et al., 2012; Liu et al., 2016). The gene therapy approach is based on inserting external telomerase genes that, once introduced in the stem cells, will rehabilitate telomerase activity. Harley et al. (2011), Jaskelioff et al. (2011), and Bernardes de Jesus et al. (2012) communicate that several studies have proven the practicality and viability of this innovative approach through successful in vitro and in vivo experimentation. Doubts are still present regarding its safety, as telomerase reactivation could lead to uncontrolled cell growth and the development of cancer

(Blasco, 2005). Small molecule-based strategies involve using compounds that target telomerase or its regulators to increase telomerase activity. Several small molecules, such as Imetelstat, a telomerase activity inhibitor, have shown promise in preclinical studies (Marian et al., 2010), but their clinical development has been hampered by toxicity and other side effects (Liu et al., 2016). In the study by Frink et al. (2016), the effectiveness of imetelstat treatment appeared to be influenced by TL, with cancer cells with longer telomeres showing greater susceptibility to the drug.

Telomerase activators indirectly enhance telomerase activity by affecting signaling pathways involved in telomerase regulation. For example, resveratrol is a natural polyphenol that is prominent in the skin of grapes and shines. This compound activates telomerase through SIRT1. This pathway is intricately involved in governing cellular senescence and promoting longevity, thereby facilitating the activation of telomerase (Baur et al., 2006; Ju et al., 2007).

The transplantation of telomerase-positive stem cells has shown potential, but their safety and efficacy are still being evaluated (Bernardes de Jesus et al., 2012).

Telomerase reactivation presents several challenges and potential problems. One primary concern is the risk of cancer, as telomerase reactivation could lead to uncontrolled cell growth and the development of tumors. Several studies provided evidence indicating that the reactivation of telomerase can impact primary human cells by facilitating their conversion, and it may increase the risk of cancer in animal models (Hahn et al., 1999; Blasco, 2005). Another potential problem is immune-related problems, as telomerase is a common target of the immune system, and telomerase reactivation could lead to autoimmunity or immune system suppression (Blasco, 2005).

Finally, telomerase reactivation could also affect stem cell function, supporting the self-renewal and proliferation process, and differentiation by either influencing the stem cells' ability to maintain genomic stability by committing to specific cell lineages or by influencing epigenetic changes and thereby affecting the differentiation potential of stem cells. Earlier studies have provided evidence indicating that the reactivation of telomerase can induce the proliferation of stem cells. In most somatic cells and tissues, telomerase is downregulated (Blackburn et al., 2015). Telomere shortening can lead the cells to death through multiple pathways, from DNA damage to cellular senescence and eventually apoptosis (Campisi, 2013; Blackburn et al., 2015). However, this rule has some exceptions, as some cells have evolved systems that preserve telomeres even when there is no telomerase activity (O'Connor et al., 2004).

Bernardes de Jesus et al. (2012) described how telomerase reactivation could be the right approach for age-related tissue degeneration or cancer. This process also presents significant challenges, particularly concerning the potential for inducing cancer (Chen et al., 2019). Cancer cells often exhibit high telomerase activity levels, allowing them to bypass the senescence checkpoint and continue dividing indefinitely (Shay and Wright, 2019). As such, telomerase reactivation in non-cancerous cells could promote pre-cancerous cell growth or even induce new tumors (Bernardes de Jesus et al., 2012; Chen et al., 2019).

Genetics of telomere length

Recent investigations have revealed that genetic and environmental factors both exert an influence on TL and their shortening process in humans (Zhan and Hagg, 2020). Twin studies have indicated that TL is highly heritable, with several specific loci identified as related to TL (Hjelmborg et al., 2015). Additionally, there is evidence linking genetic mutations correlated with shortened TL to a range of disorders called telomere syndrome, which occurs due to accelerated cellular aging across a range of tissues in the same organism (Armanios and Blackburn, 2012).

Multiple studies have genetically predicted TLs. These studies have shown proof of a consistent association between genetic potential for longer telomeres and an elevated risk of various types of cancers. (Mendelian Randomization Collaboration 2017). On the other hand, several conditions characterized by the deterioration of various bodily functions over time, including cardiovascular disorders, facial aging, and Alzheimer's disease, are observed to be more prevalent in individuals with genetically shorter telomeres (Zhan and Hagg, 2020).

Despite telomere "reprogramming" during embryonic development, parental germ cells' influence on TL is still a notable factor, as elucidated by Kalmbach et al. (2014). The heritability of TL can arise from two possible factors: inherited variations in regions not located within telomeres and differences in TL observed in gametes responsible for zygote formation (Delgado et al., 2019). Compelling evidence has emerged regarding this correlation. The offspring's TL tends to increase with the advancing of the father's age, due to the observation that telomerase activity in spermatogonial stem cells, and consequently sperm TL, increases with the father's age.

However, the advancing age of the mother has been linked to shorter TL in offspring potentially because oocytes do not undergo the same level of telomerase activity and cellular renewal as sperm, which may contribute to the lack of elongation and potential shortening of telomeres with increased maternal age (Eisenberg and Kuzawa, 2018; Eisenberg et al., 2019).

Moreover, environmental and lifestyle factors throughout the life course, from adverse events in utero or the early stages of life to TL at birth, have been shown to affect life-course TL dynamics (Belsky and Shalev, 2016; Entringer et al., 2018; Ridout et al., 2018; Beijers et al., 2020; Gorenjak et al., 2020; Habibi et al., 2020). Adverse events at the neonatal stages of life may impact both the subject affected and its offspring (Vaiserman et al., 2017). Negative physical or psychological events in the adult stages of life leave long-term effects on the length of telomeres.

Telomere length and disease

In recent years, there has been growing interest in the role of telomere biology in governing the trajectory of aging and the potential for longevity during early life. (Vaiserman 2018). Numerous epidemiological studies have consistently shown that TL plays a pivotal role as an influential factor in determining the risk of age-associated chronic events.

Vaiserman (2018) proved that intrauterine growth restriction (IUGR) is a potential cause of reduced neonatal body weight and increases the risk for dysregulation of metabolic processes in later stages of life. The IUGR leads to lasting changes in metabolism, raising the likelihood of insulin resistance, type 2 diabetes, obesity, and

cardiovascular diseases. These effects are due to epigenetic alterations and developmental changes in key organs during fetal growth. Entringer et al. (2018) demonstrated a correlation between the rate of TL changes and events during the early stages of life. Additional research supporting such a correlation was proposed by Belsky and Shalev (2016) and Shalev and Belsky (2016). These papers describe how the aging process can be expedited by events during the fetal and adult stages of life. Aviv and Shay (2018) similarly stated that the threat of ailments associated with elevated rates of cellular propagation could be intensified by extended TL. In contrast, truncated telomeres can be associated with limited cellular propagation and premature deterioration of tissues.

Numerous studies indicate an elevated susceptibility to heart complications linked to atherosclerosis with reduced telomerase activity and consequent shorter TL (Zhan and Hagg, 2019; Herrmann and Herrmann, 2020). An important link was observed between high blood pressure and the accelerated erosion of telomeres with age (Liu et al., 2019), as well as obesity and metabolic diseases (Mundstock et al., 2015).

TL in cancer cells can be influenced by several factors, including telomere lengthening and reduction, the associated proteins, and the age of the patient (Shammas et al., 2011). Although most tumor cells have controlled telomeres due to telomerase activation, recent evidence suggests that some tumor-derived and immortalized cell lines exhibit clonal heterogeneity in telomerase activity and TL (Kim et al., 1994; Shay and Bacchetti, 1997; Savre-Train et al., 2000). Moreover, certain tumor types have longer telomeres than expected, possibly due to recent telomerase activation (Albanell

et al., 1999). TL can also vary during cancer progression, with shorter telomeres observed in the late stage of chronic myeloid leukemia compared to its early stage (Counter et al., 1995). Similarly, TL in colorectal carcinoma is shorter than in normal controls, and there is no significant correlation between TL and telomerase activity (Kim et al., 2002). Telomerase activity is reportedly expressed only in the early phase of gastric carcinogenesis (Maruyama et al., 1997).

Numerous epidemiological studies have demonstrated that a longer TL is associated with an increased risk of various types of cancer, such as melanoma, basal cell carcinoma, glioma, lung cancer, tumors of the urogenital system, soft tissue sarcoma, and lymphoma (McGrath et al., 2007; Svenson et al., 2011; Wentzensen et al., 2011; Blackburn et al., 2015; Xu et al., 2020). This relationship is supported by genetic evidence from Mendelian randomization studies, which have shown that various polymorphisms that affect TL are also associated with different cancer risks (Svenson et al., 2011; Wentzensen et al., 2011; Machiela et al., 2016).

Telomeropathies, which arise from genetic mutations resulting in extremely short telomeres, are linked with poorer patient survival in multiple cancers (Holohan et al., 2014; Zheng 2021). Some studies have reported that a shorter TL is associated with a heightened susceptibility to specific types of cancers, such as pancreatic cancer (Wentzensen et al., 2011). Furthermore, the impact of TL on cancer risk can be modified by carcinogenic substances. (Srinivas et al., 2019). In a research study concerning carcinoma in basal cells, a dose-dependent synergistic increase in cancer risk was observed in individuals with both arsenic exposure and short telomeres (Srinivas et al., 2019).

Overall, while TL plays a significant role in cancer development, the relationship is complex and varies depending on the specific context (O'Sullivan and Karlseder, 2010; Armanios and Blackburn, 2012; Graham and Meeker, 2017; Martínez and Blasco, 2017; Cleal et al., 2018; Wang et al., 2018; Shay and Wright, 2019; Fan et al., 2021; DeBoy et al., 2023; Holesova et al., 2023). Further research is necessary to understand the underlying mechanisms better and develop effective strategies for targeting telomeres in cancer treatment.

Telomere length variation

TL varies between individuals and within the same cell, and some chromosomes exhibit consistently short telomeres across individuals (Londono-Vallejo et al., 2001). For example, telomeres in germ cells exhibit greater length compared to somatic cells due to higher expression of telomerase, and there is substantial variation in the average TL among CD34+ human hematopoietic progenitor cells from diverse sources. (Engelhardt et al., 1997; Lansdorp 2022). Germ cells and early embryonic cells maintain long telomeres to ensure genomic stability and proper functioning. (Engelhardt et al., 1997; Lansdorp 2022). Additionally, without a working telomerase, cells lose TL in each successive cell division, resulting in the onset of replicative senescence (Zhang et al., 1999). Replicative senescence is induced in a specific subset of chromosomes by the presence of the shortest telomeres (Zou et al., 2004). During the crisis phase, cells undergo a state in which telomeres are shorter than in the initial phase (Halvorsen et al., 1999).

Integrating leukocyte telomere as a regular parameter in clinical practice has been suggested for monitoring the process of biological aging and assessing the risk for related diseases in human medicine. However, many unresolved issues persist in this field's pre-analytical and analytical aspects, currently hampering its widespread use. In particular, the substantial variation between individuals in terms of leukocyte TLs and the variability of TL across different tissue types are major challenges that must be addressed (Semeraro et al., 2020).

Several molecular epidemiological studies have proven the substantial variation of leukocyte TLs across different populations and age groups. This variability can pose challenges when interpreting data on one specific subject (Bodelon et al., 2014). Factor-Litvak et al. (2016) defined the range of leukocyte TL in newborns and their parents. These findings highlight the need for population-specific reference values and the consideration of age and gender effects when interpreting leukocyte TL data.

Another critical issue is the substantial variability of TL amongst tissues. Semeraro et al. (2020) pointed out that TL can exhibit variation within an organ, depending on the specific site of sampling. Conversely, recent studies have shown that TLs may exhibit correlation across organs despite these variations. For instance, Hiam et al. (2020) demonstrated this correlation in normal subjects of different ages by comparing leukocytes and muscles. The observed association between the length of telomeres in white blood cells and various bodily tissues within a subject may be attributable to the considerable inter-individual variation in TL, which is evident from birth (Factor-Litvak et al., 2016). This variation has been found to be approximately three times greater than the TL variation within an individual's somatic tissues (Aviv

and Levy, 2019). Nonetheless, differences in TLs among somatic tissues mainly arise from the replicative history of stem and progenitor cells in these tissues.

TL is most commonly determined in peripheral blood leukocyte samples due to the non-invasive nature of this procedure (Blackburn et al., 2015). However, it is uncertain to what extent the results from leukocyte studies are reproducible using different subsets. Several studies have reported a strong correlation between TL in leukocytes and other somatic cell types, such as skin, skeletal muscle, and subcutaneous fat (Daniali et al., 2013). These observations suggest that TL in circulating leukocytes could serve as a reasonable proxy marker for TL in various other tissues. However, evidence suggests this may not be true for all tissues (Mather et al., 2011; Thomas et al., 2008). Therefore, caution should be exercised when generalizing TL findings in leukocytes to other tissues. In summary, telomere length varies among different cell types due to differences in telomerase activity, cell division rates, exposure to oxidative stress, DNA repair capacity, and epigenetic regulation. These factors collectively contribute to the dynamic nature of telomere maintenance and the functional diversity of cells within an organism.

The cellular composition of blood leukocytes is characterized by significant heterogeneity. The makeup of leukocytes can vary significantly, even in healthy individuals, depending on diverse stress exposures (Semeraro et al., 2020). Various stressors can induce the migration of leukocytes from immune reservoirs to both the bloodstream and specific peripheral tissues (Dhabhar et al., 2012). Thus, TLs can differ among distinct subtypes of leukocytes obtained from the same donors possessing a variety of cell types, such as lymphocytes, monocytes, and granulocytes. B cells have

been found to have the highest level of telomerase activity and longest TLs, while CD4+ T cells had slightly higher telomerase activity and similar TLs to CD8+CD28+ T cells (Lin et al., 2010). Moreover, transient telomerase expression can occur due to antigen-induced lymphocyte stimulation (Huang et al., 2017). Therefore, the leukocyte population's composition should be considered when interpreting TL data.

In calves, the development of the immune system progresses from conception to about 6 months after birth when they reach maturity (Chase et al., 2008). Maternal immunoglobulins, immune cells, and cytokines passively acquired through the colostrum from the mother grant the calf protection. These cellular components interact with the development of local immunity and modulate active immunization of the neonatal intestine. T lymphocytes are the most active component, with their ability to transfer immune functions and secrete cytokines (Chase et al., 2008).

Telomere length in different blood cell types

TL varies among different blood cell types. For example, Alter et al. (2007) have shown that TL is shorter in granulocytes (neutrophils, eosinophils, and basophils) compared to)lymphocytes (white blood cells). Granulocytes undergo less cell divisions than erythrocytes and lymphocytes. (Rufer et al. 1999)

Immune cells are a complex mixture of many different cell types, each with its own unique developmental pathways and functional characteristics. T cells and B cells are two of the main types of immune cells that play important roles in the immune system. T cells are responsible for cell-mediated immunity, while B cells are responsible for humoral immunity. Lin et al. (2016) aimed to investigate the changes in

TL in subsets of lymphocytes in response to different experimental conditions. They reported that TL changes in CD4+, CD8+CD28+, and CD8+CD28− T cells and B cells from the same participant were correlated, suggesting systematic responses to biochemical factors that impact TL. However, the rates of TL change differed among immune cell types, indicating cell type-specific responses. This finding suggests that while certain factors may generally impact TL in immune cells, the specific impact can vary depending on the cell type.

Significant discrepancies are observed in the pace of telomere erosion associated with aging among various subsets. The reduction in TL in lymphocytes as a result of aging was significantly more prominent than in granulocytes (Aubert et al., 2012). Thus, caution should be exercised when comparing TL data from blood.

It is worth noting that leukocyte TL is a dynamic parameter that reflects temporary variations in the immune system for each unique individual. More leukocytes could be circulating during inflammatory conditions, leading to increased TL in response to oxidative stress (Epel et al., 2004). Therefore, the influence of inflammation and stress on TL measurements should be considered in TL studies.

Tools to measure telomere length

There are various methods to measure the length of telomeric sequences. Harley et al. (1990) described the first method developed as the telomere restriction factor, which bases TL determination on a southern blot. Since scalability limitations have been established, its use has been restricted.

Two fluorescent in situ hybridization methods can be applied to analyze TL. One is a quantitative method (Q-fish), while the other is based on a flow cytometer (Flow-fish) (Baerlocher et al., 2006). Q-fish utilizes a conventional fish methodology as its foundation, employing a peptide nucleic acid probe to scrutinize cells affixed to a slide surface. Data is read through digital image software, and the fluorescence signals need to be sufficiently quantified because TL will be defined as directly proportional to the intensity of the acquired fluorescence. A negative aspect of Q-fish is that regions other than telomeres may also be included, such as subtelomeres, convoluting the results.

Flow-fish uses fish technology as well. The peptide nucleic acid probe analyzes the hybridized cells in a flow cytometer, estimating the average TL based on the average telomere amount in individual cells. Data are then expressed as mean fluorescence intensity. The flow-fish technology is very efficient in measuring the number of telomeres in specific cell subsets, but to do so, it requires two laser flow cytometers and regular calibrations. The beads need to maintain sensitivity, and the emission channel must be able to collect fluorescence data. For these reasons, flow-fish is an expensive technology. Another problem is that the peptide nucleic acid probe is not specific and may be bound to unwanted cytoplasmic items (Baerlocher et al., 2006).

A widely used method to determine TL is qPCR (Cawthon, 2002). This is the most economical technology for this analysis. It requires a small amount of DNA sample (nanograms compared to micrograms required by TRF analysis). The qPCR also has the benefit of being able to be prepared and conducted in different ways. We can determine TL using the SybrGreen approach, where a fluorophore interacts with

double-stranded DNA, emitting a fluorescent signal that is then measured. Otherwise, we could use the TaqMan approach, which includes probes with a fluorophore attached. Upon the completion of target DNA sequence amplification, the fluorophores undergo liberation, resulting in the emission of a detectable signal for subsequent quantification.

One of the main issues with qPCR technology is that the quality and yield of the DNA sample used directly impact the efficiency of the process. Even the results can be presented differently. They are represented as absolute values when a standard curve is used as a comparison measure against the results of the PCR. Alternatively, the results may be presented as a relative value when we used a reference gene as a comparison term for the target gene signals' average.

Another possible method is the single TL analysis (STELA) (Baird et al., 2003). The STELA technology targets TL on a single chromosome blending PCR and Southern blot methodologies. This method can uncover possible genomic instability events by measuring telomeres short enough to suffer fusion. It can be beneficial for clinical information. The main limitation is the specificity of proximal primers. This parameter is a requirement but could only be applied to a small subgroup of chromosomal ends. Thus, studies on chromosomal comparison for TL shortening rates and consequent cellular senescence are restricted.

The outcomes achieved through a methodology may exhibit significant disparities when compared to those achieved through alternative approaches. This discrepancy poses challenges in the utilization of these methods in epidemiological and clinical studies. Standardizing measurement techniques, using reference standards, validating methods through inter-laboratory studies, and combining different

methodologies could improve the accuracy and comparability of telomere length measurements in epidemiological and clinical studies.

DNA contamination: effect on genotypes

A complication for the accuracy of TL assessment and genotyping in young calves is potential contamination of their DNA with cells absorbed from colostrum. Single nucleotide polymorphism (SNP) chips are widely used in genetic studies and breeding applications (Laoun et al. 2020) for livestock, poultry, and plants. Genotype call rates are regularly used to measure data quality and as a screening tool for genomic studies and evaluations of dairy cattle (Khatkar et al., 2012).

DNA contamination will result in genotyping errors and reduced power for association testing. Flickinger et al. (2015) examined the effects of DNA sample contamination on genotype calling in sequencing data. The authors demonstrate that contamination results in systematic genotype misclassification with a bias towards heterozygotes, leading to reduced power for association testing.

In 2019, Zajac et al. aimed to identify and estimate the sources of DNA contamination in genotyped samples from multiple studies and found that contamination occurred during sample collection, processing, and storage. They also identified specific sources of contamination, such as reagents and equipment, and identified ways to reduce contamination in future studies.

The possible uses of telomere length as a biomarker of aging

TL has been a topic of significant interest in gerontological research since its discovery as a potential biomarker of aging (Blackburn et al., 2015). Despite its theoretical potential, the empirical evidence regarding TL's relationship with aging is conflicting (Mather et al., 2011). Biomarkers associated with aging have the potential to evolve throughout an individual's lifespan, and it is important to recognize that a solitary biomarker might not accurately represent aging across all biological systems (Blackburn et al., 2015). As a result, TL's use as a routine clinical diagnostic tool is limited.

Many epidemiological and clinical studies have examined the weak relationship that telomeres have with physical parameters for well-being (Mather et al., 2011). More robust associations have been found between TL and cognitive performance measures (Mather et al., 2011). Nonetheless, these conclusions suggest that TL does not serve as a universal marker of biological aging. Small sample sizes and narrow age ranges in these studies could explain contradictory findings (Der et al., 2012).

The pace at which aging occurs has been assessed using TL as a sole determinant in numerous studies. A prospective study in a Scottish population by Der et al. (2012) compared age and health associations with two metrics, one including and one excluding TL. Indexes, including TL, were better predictors for overall health outcomes than chronological age. TL was significantly associated with age and physical and cognitive functioning measures related to normal aging. However, due to the variability of TL and the influence of external factors and age-related diseases, TL alone was not sufficient to accurately predict age.

Hastings et al. (2019) conducted a study to quantify composite measures of biological aging that included TL. The study compared TL from leukocytes to three different combined measures of biological age that have been determined using algorithms. The first algorithm, the Klemera-Doubal method (KDM), was divided into Biological Age and Homeostatic Dysregulation; while both referred to a reference population, the former was based on a combination of biological markers for the well-being of different organs, and the latter referred to the Mahalanobis distance of the markers in use. This distance measurement quantifies the distance between a point and a distribution of points in a multi-dimensional space. The last algorithm, the Levine-method (LM) Biological Age, was composed using markers for the prediction of the risk of death.

All measures correlated with chronological age. However, while the three composite biomarkers were correlated with each other, none showed a correlation with TL. The authors concluded that TL measures different aspects of the aging process, compared to the than patient-level physiological aspects captured by the biomarker composites. Furthermore, effect sizes tended to be larger for these measures than for TL. However, marginal increases in effect sizes were observed when TL was integrated into these biomarker composites compared to indices constructed without TL.

It is still unclear if TL is a valid predictor of aging, when used individually. Due to the complexity of the aging process, composite biomarker measures may be more precise (Blackburn et al., 2015). Thus, future research should continue exploring composite biomarker measures to understand the complex aging process more thoroughly.

Telomere length as a potential biomarker in animal production

TL measurements hold significant potential as a metric for assessing cow health and welfare. Based on the human TL observations that have been described above, it can be hypothesized that TL may serve as a biomarker for cellular aging and could reflect cumulative exposure to environmental and management stressors, providing insights into an animal's overall health and activity levels. Monitoring this parameter in dairy cows could offer early detection of suboptimal health conditions, enabling timely interventions and improved welfare. Additionally, it may serve as an indicator of genetic fitness, aiding selective breeding for longevity and resilience.

While the study of TL in dairy cows has not reached the same extent as in humans, significant progress has been made in this area. Brown et al. (2012) and Meesters et al (2023) found a significant association between TL and chronological age in lactating Holsteins. Average TL was shorter for older cows, mainly due to a reduction in the number of older cows with long telomeres when compared to younger animals. This suggested that TL could serve as a potential biomarker for aging in this population. Cows with short telomeres were more likely to be culled in the subsequent year after considering the age of the cow (Brown et al., 2012).

Since understanding the relationship between telomeres and important traits such as milk yield, reproductive performance, and overall health could have practical implications for management and breeding strategies in the dairy industry, studies have also been conducted to identify tissue-specific differences. Specifically, adipose tissue had longer telomeres than liver and muscle tissues (Laubenthal et al., 2016). Researchers observed that TLs were shorter in peripheral blood and mammary gland

during late lactation compared to early lactation, suggesting a potential association between TL and the physiological demands of milk yield and metabolic stress (Laubenthal et al., 2016).

Another critical parameter in dairy management is feeding management. A study conducted by Seibt et al. (2022) aimed to contribute to the scientific knowledge regarding the effects of feeding levels on cellular aging markers and mitochondrial function in dairy heifer calves. The researchers used TL and Mitochondrial DNA Copy Number (mtDNAcn) as markers in the study. While telomeres assess cellular aging in dairy heifer calves by reflecting the highest telomere lengths observed at birth and their subsequent decrease with age, mtDNAcn reflects the abundance of mitochondria in a cell. Since mitochondria play a crucial role in energy production and are the primary producers of reactive oxygen species, changes in mtDNAcn can indicate alterations in mitochondrial function, which can affect cellular health. The findings suggested that the feeding levels investigated in this study did not induce noticeable alterations in cellular aging markers or overall health in dairy heifer calves during the preweaning phase.

Genetic differences in TL among individual cows or breed compositions may exist but the investigation of TL in a cow population has primarily focused on the Holstein Friesian breed. This has made it challenging to assess the relationship between TL and aging, primarily due to the Holstein Friesian breed's early culling practices (Iannuzzi et al. 2022). In their study, Iannuzzi et al. (2022) used milk and blood samples to determine TL in an Agerolese population. The researchers discovered that these animals have longer telomeres compared to Holsteins Friesian at the same stage in life.

Another interesting study was conducted on Italian local beef breeds by Tilesi et al. (2010). The researchers aimed to assess the length of telomeres in between Chianina and Maremmana, breeds known for their extended lifespan and extensive range breeding practices. The results indicated that the median TL in Chianina was significantly shorter compared to Maremmana. Additionally, there was minimal variation in TL among individuals within the Chianina breed, whereas the Maremmana breed exhibited a relatively higher degree of variation. In summary, these studies align with the idea that TL shortens with age, and the intensive genetic selection of Holstein over the years may have negatively impacted the TL compared to breed compositions that are not as intensively selected.

Section 2

BREED COMPOSITION EFFECTS ON GRAZING BEHAVIOR

Grazing Behavior

Grazing constitutes a foundational behavior among cows in pasture-based systems, encompassing the selection, intake, and processing of forage plants (Pauler et al., 2020). In this process, cows rely on a combination of sensory cues, including visual, olfactory, and tactile inputs, to identify and choose desirable forages. Furthermore, cows use flavor-feedback associations and learning from past experiences to guide their food choices, associating the flavors of foods with their post-ingestive consequences. This learning process begins in utero and continues throughout their lives, allowing cows to adjust their diet dynamically based on the nutritional content of foods and their physiological needs (Provenza et al., 2015).

This discerning approach to grazing involves a selective preference for certain plants based on characteristics such as taste, texture, or nutrient content (Soder et al., 2009). Grazing occupies a substantial portion of cows' daily activities on pasture and is indispensable for fulfilling their nutritional needs and maintaining an optimal dry matter intake (Bargo et al., 2003). Understanding cattle feeding patterns, biting rates, forage preferences, avoidance behaviors, rumination, and the crucial aspect of meal timing (Boland, 2011) is essential in optimizing cattle management and optimal production to promote farm profitability.

The impact of restricted pasture access on milk yield

From an economic standpoint, grazing behaviors influence feed efficiency and milk yield, which are crucial economic factors in dairy farming (Iqbal et al., 2023). Grazing dairy cows with access to high-quality pasture forage can produce more milk compared to cows grazing on lower-quality pasture or with inconsistent pasture quality, while also reducing the need for expensive supplemental feeds (Soriano et al., 2001; Tozer et al., 2003; Baudracco et al., 2010; Macdonald et al., 2017; Molle et al., 2022). Although total milk yields from grazing systems may sometimes be lower than those from total mixed ration (TMR) systems, high-quality pasture can optimize milk production relative to poorer grazing systems (Soriano et al., 2001; Tozer et al., 2003; Baudracco et al., 2010; Macdonald et al., 2017; Molle et al., 2022). To avoid potential economic losses, understanding bovine grazing behavior can help farmers anticipate and manage risks associated with forage availability, weather conditions, and disease outbreaks. For example, in an automatic milking system, Kismul et al. (2019) aimed to assess whether providing cows nighttime access to pasture with restricted indoor silage feeding during the day yielded better production outcomes compared to an exercise paddock system with ad libitum indoor silage feeding. Milk production yield was lower for cows with pasture access, but there was no significant change in fat and protein yield. Regardless of treatment, all cows were motivated to access pasture during the early evening hours more so than at night. Due to the observed animal's strong preference for evening grazing, the authors recommended that cows have access to pasture in the late afternoon and early evening, peak grazing hours, to maximize grazing intake to improve cow welfare while optimizing milk yield.

Studies by Pérez-Ramírez et al. (2009) and Kennedy et al. (2009) found that for lactating dairy cows, the availability of pasture significantly influences the effect of restricted grazing time on pasture intake and milk yield. Pérez-Ramírez et al. (2009) explored how restricting daily pasture access impacts milk yield, pasture dry matter intake (DMI), and grazing behavior, with a focus on varying pasture allowances. In their study, cows received no forage or concentrate supplements during confinement periods. They found that as pasture allowance increased from 13 to 24 kg of DM/day per cow, pasture intake rose by 1.6 kg/day, and milk yield increased by 1.8 kg/day. Therefore, management decisions should consider both the economic benefits of higher milk production and the potential stress or nutritional deficiencies that could arise from limited grazing time; restricting pasture access time can also affect milk component concentrations. Kennedy et al. (2009) observed that while restricting pasture access time did not significantly reduce overall milk yield, it did lower milk protein concentration by 4.8% and total DMI by 10.1% compared to cows with full-time access. Cows with restricted access compensated by increasing their DMI per minute of grazing by 45.2% and DMI per bite by 46.8%, resulting in greater grazing efficiency as they spent more of their grazing time actively feeding.

Learned behavior and food preferences in cows

Several studies have shown that dairy cows use past experiences to select the forages to ingest while eating. Villalba et al. (2009) found that herbivores, including dairy cows, develop learned food preferences through associative learning, from peers and personal experiences, where sensory cues like taste and smell are linked to the post

ingestive effects of foods. This connection allows them to their nutritional needs while avoiding harmful plant secondary metabolites (Villalba et al., 2009). Furthermore, Provenza and Launchbaugh (1999) discovered that herbivores animals, including cows, adapt to new environments through trial-and-error learning and behavioral flexibility, the ability of adjusting the food intake based on nutrient and toxin levels in plants available in the area. The study from Provenza and Launchbaugh (1999), also showed better flexibility in young animals especially when exposed to varied foods in early life.

RumiWatch System

Grazing intensity (forage consumption in a specific area over time) and duration (time spent actively grazing) are vital factors for sustainable pasture management. Monitoring these factors helps prevent overgrazing, ensures adequate pasture regrowth, and optimizes forage utilization. For this reason, Williams et al. (2016) aimed to develop a robust model for classifying the behavior of grazing dairy cattle using Global Positioning System (GPS) data because understanding individual cattle behavior is crucial for improving productivity and overall health. The final model demonstrated high accuracy on an independent dataset, even in uncontrolled grazing conditions, proving that small, low-cost GPS receivers on neck collars have the potential to be a practical and versatile tool for monitoring cattle behavior and aid in early disease identification by tracking temporal changes in cattle behavior.

The RumiWatch system (Itin and Hoch GmbH, Liestal, Switzerland) utilizes advanced sensors to collect high-resolution data on feeding behaviors in dairy cows (Li et al., 2021; Norbu et al., 2021). These sensors, integrated into the halter, accurately

quantify ingestive behaviors such as jaw motion and chewing patterns. The system can also incorporate additional sensors to monitor environmental factors like temperature and humidity, providing context to the behavioral data. Data are collected continuously, with the option to download and view data at intervals that can be customized to every 1 second, 1 minute, or 1 hour, allowing for flexible and precise monitoring.

Recent studies have evaluated the RumiWatch system's accuracy in monitoring cow behavior, particularly grazing. Norbu et al. (2021) and Pereira et al. (2020) assessed the system's performance in both grazing and stall-fed conditions, focusing on prehension bites and mastication chews. Norbu et al. (2021) found that the system was more accurate in grazing conditions for measuring prehension bites and total jaw movements ($P < 0.01$), though it struggled with accurately quantifying mastication chews in both environments.

Pereira et al. (2020) validated the RumiWatch system, which can include a halter and a pedometer, for monitoring feeding and locomotive behaviors in grazing dairy cattle by comparing it to direct visual observations. The system accurately monitored rumination ($r = 0.93$, $P < 0.001$), eating ($r = 0.94$, $P < 0.001$), and standing and lying behaviors ($r = 0.20$, $P < 0.05$), though it faced challenges in accurately detecting drinking and walking behaviors. Similarly, Kajava et al. (2014) validated the RumiWatch pedometer for measuring lying, standing, and walking times against video-based continuous behavior recordings. While walking time measurements needed improvement to enhance accuracy, the pedometer reliably measured lying and standing times.

Steinmetz et al. (2020) studied the accuracy of algorithms in the RumiWatch Converter (RWC) version V0.7.4.5 for classifying behavioral characteristics in dairy cows. The system showed high accuracy in classifying rumination ($r = 0.91$, $P < 0.001$) and ruminating chews per bolus, with solid agreement between the system's results and direct observations. The study also compared classification results from 1-minute and 1-hour time summaries, finding consistent accuracy in classifying rumination behavior across both. However, eating behaviors were sometimes misclassified as rumination, leading to overestimations of rumination time ($P < 0.05$), and drinking behavior was particularly challenging to detect accurately.

Zehner et al. (2012) also demonstrated the RumiWatch system's high accuracy in measuring rumination ($r = 0.95$, $P < 0.001$) and feed intake compared to direct observations and video recordings. Additionally, Pereira et al. (2021) highlighted the system's capability in monitoring the locomotion behaviors of grazing dairy cows, accurately identifying normal gait patterns and assessing cows' mobility and comfort. Despite these strengths, the technology still requires improvements in differentiating between mastication chews and prehension bites while eating (Rombach et al., 2018).

Li et al. (2021) validated the RumiWatch sensor for monitoring grazing behaviors in lactating dairy cows within a pasture-based automatic milking system. Their research found a moderate correlation between eating chews ($r = 0.55$, $P < 0.01$), mastication chews ($r = 0.61$, $P < 0.01$), and daily milk yield, highlighting the potential of these behavioral indicators to predict and assess milk production. The system's ability to monitor feeding behavior enables the assessment of crucial health indicators, such as feeding patterns and rumination duration, and the identification of abnormal

behaviors. This functionality supports early detection of potential health issues, evaluation of management interventions, and optimization of feeding strategies to enhance cow welfare and productivity.

Precise measurements provided by the RumiWatch system allow researchers to explore the effects of various diets, forage availability, nutritional interventions, and breed differences on cows' feeding patterns and performance. The data collected may facilitate the development of personalized feeding regimens, optimization of nutrient intake, and assessment of the impact of dietary modifications on rumen health and microbial activity. Additionally, Pereira et al. (2021) demonstrated the system's capability to detect signs of lameness or discomfort, offering support for early detection and timely intervention in potential health issues.

In conclusion, in pasture-based dairy systems, grazing behavior is critical for optimizing nutritional intake, feed efficiency, and milk production. Cows utilize sensory cues and learned experiences to select and process forages, directly impacting dry matter intake and overall productivity. Effective grazing management, including monitoring grazing intensity and duration, is essential for sustainable pasture use and maximizing milk yield. The RumiWatch system provides a precise and reliable method for quantifying grazing behavior, rumination, and locomotion in dairy cows, offering valuable data for enhancing herd management, improving animal welfare, and optimizing feeding strategies.

Investigating Breed Composition Differences in Grazing and Ruminating Behavior Using Novel Tools

There is a notable gap in the literature regarding the genetic differences among breed composition in pasture grazing behavior and the use of novel tools to investigate these breed composition differences. Few studies have specifically addressed how animal behavior on pasture, including grazing, varies across breed composition. Grodkowski et al. (2023) investigated the differences between European Holstein (HO) and Brown Swiss (BS) for grazing behavior and pasture adaptability, finding that both groups increased activity around sunrise and sunset. HO spent 8.24% less time grazing than BS and approximately 8.94% more time ruminating. Breed composition differences for grazing and ruminating behaviors were also analyzed by Koczura et al. (2019), using HO, Montbéliarde (MO), and Valdostana Red Pied (VA) on different types of pastures. The authors concluded that MO showed an optimal compromise between time spent grazing (about 5% more than HO but less than VA) and ruminating.

Understanding breed composition differences could impact genetic and breeding strategies aimed at optimizing grazing efficiency. This dissertation aims to fill this gap by investigating novel tools such as TL, fly resistance, pdNDF and thermal images and investigate the breed composition differences and whether these differences are a significant factor influencing grazing and ruminating behavior.

CONCLUSIONS AND OBJECTIVES

Telomeres, repetitive DNA sequences at chromosome ends, play a critical role in genome integrity by protecting against damage and fusion. Their gradual shortening with DNA replication is linked to cellular senescence and aging acceleration. Telomeres consist of specialized DNA sequences and are safeguarded by the Shelterin protein complex. Dysregulation can lead to genomic instability. Genetic and environmental factors influence TL. Genetic mutations and non-telomeric variations are associated with TL, which can be inherited. Life events, from early adversity to adult stress, also affect telomeres. Short telomeres are associated with various diseases, while longer telomeres have complex associations with specific cancers.

Introducing TL into the genetic selection index for dairy cows holds promise in several key areas. By considering TL in the selection process, dairy farmers could benefit from enhanced longevity and overall health in their herds, as TL correlates with cellular aging. This can contribute to more productive and long-lived cows, which is advantageous for the dairy industry. Selecting cows based on TL may also result in more stress-resilient animals, promoting their welfare and reducing stress-related health issues. Overall, integrating TL into genetic selection indices has the potential to enhance herd health and reduce premature culling, contributing to the sustainability and prosperity of dairy farming. However, the practical implementation of this approach should be rigorously validated through further research.

Grazing behavior is a pivotal aspect of cows' daily activities, driven by sensory cues and preferences. Access to pasture positively impacts cow welfare, reducing resource competition and fostering motivated behaviors. Monitoring grazing intensity

and duration is essential for sustainable pasture management. Overall, understanding cows daily grazing and rumination behavior and how to implement farm management to exploit the natural peak can improve the animal feed efficiency by allowing a more complete utilization of nutrients and consequently generate economic growth by incrementing animal's milk yield. However, further research is required to validate this approach.

Hypothesis and Objectives

The overarching objective of this dissertation is to integrate novel tools and grazing behavior in genetic selection programs to enhance longevity and performance in dairy cows.

There will be two central hypotheses tested. The first is that TL is favorably associated with the longevity of dairy cows, and that integrating TL as a parameter within the genetic selection index for dairy cows will lead to enhanced overall health, prolonged productive lifespans, and reduced healthcare requirements. This dissertation will test this hypothesis by assessing the variation in TL among a diverse population of dairy cows and investigating its heritability, breed composition differences, and associations with longevity.

The second hypothesis addressed is that the breed of a cow is a significant factor influencing grazing and ruminating behavior. This will be tested by analyzing the impact of crossbreeding and sire breed on the grazing behavior of cattle in pasture settings.

Chapter 2

Genomic analysis of telomere length in Holsteins and associations with other routinely recorded traits

ABSTRACT

This study aimed to evaluate if telomere length (TL) was heritable and to elucidate the relationship of TL with longevity and productivity traits in dairy cattle. Animals included belong to the Penn State University dairy herd and a nearby commercial herd. qPCR for TL determination was conducted on 402 samples from 336 cows and 413 samples from 330 heifers, and TL records were merged with 3,446 production records. Genomic analyses were conducted including genotypes from 998 animals, involving 74,973 effective SNPs. Of the 998 genotyped animals, 253 had cow TL records, 276 had a heifer TL record, and 816 had milk yield records. Single trait analyses of cow TL and heifer TL included contemporary group effects plus the random effects of permanent environment, animal, and error. A seven-trait model included TL for cows and heifers, milk yield, fat (kg), protein (kg), somatic cell score (SCS), and milk urea nitrogen (MUN). Genomic estimated breeding value (gEBV) for TL with accuracy ≥ 40% were correlated to sire genomic predicted transmitting ability (gPTA) for cow livability (COWLIV), heifer livability (HEIFLIV), and productive life,

and cow gPTA for the same traits (n=388 animals from Penn State herd) to generate approximated genetic correlations. TL exhibited a heritability of 0.40 ± 0.17 in cows in the single trait analysis and was 0.21 ± 0.02 in heifers. Cow EBV for TL were positively correlated with sire gPTA for livability, with approximate genetic correlations with COWLIV of 0.44 (P<0.01) and 0.60 (P<0.001) for HEIFLIV from the single trait model. Heifer TL from the multiple trait model was significantly correlated with HEIFLIV (approximate genetic correlation = 0.65, P<0.01). gPTA for productive life was not significantly correlated with TL. These results indicate that TL is a favorably correlated with livability in dairy cattle, supporting its potential use in selective breeding programs to enhance longevity and health.

INTRODUCTION

The pursuit of optimizing dairy cattle productivity has long been intertwined with the challenge of ensuring animal health and longevity. Telomeres, the protective non-coding tandem repeat sequences that safeguard chromosome ends, have emerged as a possible selection tool to facilitate selection for longevity. These DNA sequences, conserved across eukaryotic species, are vital in preventing chromosomal deterioration and fusion, thereby serving as guardians of genomic stability (Pfeiffer and Lingner, 2013).

The length of telomeres (TL), which decreases with cell division and under oxidative stress, has been correlated with an organism's life span and propensity for age-related diseases. In humans, the interplay between genetics and environment in determining TL is well-documented (Zhan and Hagg, 2020). Twin studies have underscored that TL is heritable, revealing that specific genetic loci are associated with its variation (Hjelmborg et al., 2015).

The environment, too, exerts a significant impact on telomeres, beginning from in-utero development to adverse events in early life that can affect telomere dynamics throughout an individual's lifespan (Belsky and Shalev, 2016; Entringer et al., 2018; Gorenjak et al., 2020; Habib et al., 2020; Ridout et al., 2018; Beijers et al., 2020). Stressors experienced during adulthood, whether physical or psychological, have been shown to leave an imprint on TL (Vaiserman et al., 2017).

The association between TL and age in dairy cows has been investigated for the potential impact on traits such as milk production, reproductive performance, and

overall health (Brown et al., 2012; Laubenthal et al., 2016). A study from Laubenthal et al. (2016) looked at the TL variation across organs suggesting that different tissues aging rates could impact overall health and productivity, with relative telomere quantity (qT) in the mammary gland decreasing by 16% ($P = 0.02$) from early to late lactation. Furthermore, Brown et al. (2012) suggested that cows with shorter telomeres were more likely to be culled within the following year and that the TL shortened with age. Shortened telomeres could potentially impact a cow's productive lifespan and stayability in the herd (Seeker et al., 2018).

In a study conducted on dairy heifer calves, Seibt et al. (2022) investigated how environmental factors, particularly nutrition and feeding management, influence TL and mitochondrial DNA copy number (mtDNAcn). During a 14-week preweaning phase, TL showed a length reduction with age while mtDNAcn increased during the early weeks of life, plateaued around weaning, and then decreased again by the first year of life.

Livability measures the cow's ability to stay alive while in a milking herd. This trait only accounts for the death of the animal and does not include culling (Norman et al., 2016). HEIFLIV represents the ability of the animal to stay alive on the farm until 18 months of age (Neupane et al., 2021). In 2016, the CDCB introduced COWLIV as an evaluation trait in dairy cows (Council on Dairy Cattle Breeding, 2016). The initial study by Wright and VanRaden (2016) demonstrated a correlation of 0.70 between livability and productive life and estimated the trait heritability at 1.3%.

Given the relationship between TL and physiological traits that could translate to variation in survival, this study aims to estimate the heritability of TL, evaluate the

correlation between TL and various production parameters including milk yield, milk composition (fat, protein), somatic cell score, and milk urea nitrogen, and to determine relationships of TL with routinely recorded longevity traits in US national evaluations.

MATERIALS AND METHODS

Data

All cows and heifers were born between May 2011 and July 2018. The trial ran from November 2017 through November 2019, and cows ranged between 21 and 87 months of age while heifers ranged between 1 day of age and 24 months. Blood samples were obtained from the tail vein of animals from the Penn State University dairy herd and a nearby commercial herd and DNA was extracted using a phenol-chloroform isolation with ethanol precipitation and resuspension in nuclease-free water (Gautam, 2022). Samples quality and DNA concentration were assessed using a Nanodrop spectrophotometer (Thermo Fisher Scientific) and those with a 260/280 ratio of 1.6 to 2.0 were retained. Following DNA extraction and quality control, the total number of observations was 402 from 336 cows (278 cows had 1 observation, 50 cows had 2 observations and 8 cows had 3 observations), and 413 from 330 heifers (259 heifers had 1 observation, 60 heifers had 2 observations, 10 heifers had 3 observations and 1 heifer had 4 observations) (Table 2-1). Thirty-two of the animals had observations both as heifers and cows, for a total of 35 observations.

Telomere length determination

TL was determined using a multiplex quantitative polymerase chain reaction (qPCR) method based on the primers and thermal cycle settings described by Brown et al. (2012) with β-globin used as the standard reference gene and samples run in triplicate on a given plate. To facilitate across-plate standardization, some samples were run on multiple plates with44% of the samples run on a single plate, 33% on two plates, and 23% on three or more plates. Cycle thresholds (Ct) were recorded at the machine default ΔRN (relative fluorescence) value of 0.262. The determination of amplification efficiency was based on the slope during the amplification phase, which was assumed to occur from 3 cycles prior to the cycle threshold (ΔRN = 0.033) to a half cycle after (ΔRN = 0.39). Amplification efficiencies were calculated separately for each plate as 10^{slope} with slope the regression coefficient for cycle number with average efficiencies of 1.86 for TL and 1.84 for β-globin. TL was calculated using the approach described by Hastings et al. (2021) with the formula $TL = ln\left(\frac{eTL^{ctTL}}{eBG^{ctBG}}\right)$, where ln represents the natural logarithm to normalize TL. eTL and eBG represent the plate efficiencies for telomere and β-globin, respectively. ctTL and ctBG denote individual sample cycle thresholds for the telomere and β-globin.

To reduce variability, samples with a coefficient of variation (CV) greater than 5% were excluded from the analysis which was 1.9% of samples. As samples were evaluated on multiple plates, a single TL value per sample was derived with an across-plate mixed model with TL = sample + random plate effect. The sample effect represented the final TL value used for subsequent analysis.

Genomic evaluation

A single-step genomic analysis was conducted with pedigree and genomic relationships blended to create the relationship matrix. This methodology was defined and executed through a series of programs from the BLUPF90 suite (Misztal et al., 2018), with variance components estimated with REMLF90 or AIREMLF90, EBV estimated with BLUPF90, and DNA marker effects estimated with POSTGSF90. For the construction of the genomic relationship, a total of 74,973 SNPs remained for the analysis after quality control. Of the initial 79,294 SNPs processed, the quality control process removed 174 monomorphic SNPs, those with a call rate < 0.90 (n=2) and SNPs with minor allele frequencies < 0.05 (n =4,145). Out of the 1000 genotypes, 2 were eliminated due to a low call rate, leaving 998 effective genotyped animals with 529 having TL records, 816 having production records, and 109 were relatives without records.

Single trait analyses of cow TL and heifer TL were conducted with the model:

$TL_{ijk} = LG_i + HYS_j + A_k + PE_k + \varepsilon_{ijk}$, where TL denotes TL; LG_i represents the fixed effect of lactation group i (1, 2, ≥3); HYS_j represents the fixed effect of herd year season j; A_k represents the random additive genetic effect for cow k; PE_k = the permanent environmental effect of cow k; ε_{ijk} denotes the random error term. For cows, HYS was the herd-year-season of collection and there were no LG 0 animals, whereas HYS was the herd-year-season of birth and LG was not included for heifers.

A 7-trait model was fit that included TL for cows, TL for heifers, 305-day milk yield, fat, and protein yields, somatic cell score (SCS), and milk urea nitrogen (MUN).

The data regarding production traits were retrieved from herd records from all animals born during the same period as those with TL. There were 3,446 observations from 1,372 cows for all traits except MUN (1725 records from 721 cows) which was only recorded in the Penn State herd. The final model was as follows:

$Y_{ijk} = LG_i + CG_j + CG_j + CG_j + A_k + PE_k + \varepsilon_{ijk}$, where Y_{ij} include TL for cows, TL for heifers, milk yield, fat yield, protein yield, SCS, and MUN. LG_i was lactation group as described above and not included for heifers. CG_j represents the contemporary group (HYS of calving, HYS of sample collection, and HYS of birth for cows; HYS of sample collection and HYS of birth for heifers); A_k is the random additive genetic effect; PE_k is the permanent environmental effect; ε_{ijk} defines the random error term.

The GWAS was executed with POSTGSF90 to assess SNP associations with TL. gEBV were first derived in BLUPf90 and subsequently decomposed into SNP effects. A Bonferroni adjustment of 0.05 / number of SNPs was implemented to determine genome-wide significance. Genomic regions of interest were identified using the Bos taurus ARS-UCD2.0 as the genome reference (Rosen et al., 2017).

Heritability was calculated as: $h^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_E^2 + \sigma_{PE}^2}$ where σ_A^2 is the additive genetic variance; σ_E^2 is the error variance assumed to represent temporary environment; σ_{PE}^2 is the permanent environmental variance. Genetic correlations from the 7-trait model were derived as $r_{gAB} = \frac{\sigma_{gAB}}{\sqrt{\sigma_{gAA}^2 * \sigma_{gBB}^2}}$ where σ_{gAB} represents the covariance between the genetic effects of traits A and B; and σ_{gAA}^2 and σ_{gBB}^2 are the genetic variances of traits

A and B, respectively. The phenotypic correlations were derived as $r_{pAB} = \frac{\sigma_{pAB}}{\sqrt{\sigma^2_{pAA} * \sigma^2_{pBB}}}$

where σ_{pAB} represents the phenotypic covariance between traits A and B; and σ^2_{pAA} and σ^2_{pBB} are the phenotypic variances for traits A and B, respectively.

Correlations of TL EBV with nationally recorded traits

We retrieved genomic Predicted Transmitting Abilities (gPTA) from the Council of Dairy Cattle Breeding December 2023 evaluation for bulls and Penn State cows. Traits considered included productive life (PL), COWLIV, and HEIFLIV. These were correlated with genomic EBV (gEBV) for TL from animals with at least 40% accuracy for one or more of the following gEBV: cow TL from the single trait model, cow TL from the multi-trait model, heifer TL from the single trait model, and heifer TL from the multi-trait model.

For bull evaluations, correlations were derived from 216 sires of which 163 had cow TL from the multi-trait model; 165 had cow TL from the single-trait model; 82 had heifer TL from the multi-trait model; and 28 had heifer TL from the single-trait model.

For correlations based on gPTA from 388 Penn State cows, 327 had cow TL from the multi-trait model; 176 had cow TL from the single-trait model; 174 had heifer TL from the multi-trait model; and 106 had heifer TL from the single-trait model.

Approximated genetic correlations were obtained by adjusting the correlation results for the reliability of the specific traits following the model:

$\sim rg = \frac{r_{tl,x}}{\sqrt{rel_{TL} * rel_x}}$ where $r_{tl,x}$ represent the Pearson correlation between the sire or cow gPTA for the trait of interest (COWLIV, PL and HEIFLIV) and the gEBV for TL; rel_x indicates the reliability for the trait of interest; and rel_{TL} indicates the reliability for TL gEBV (Calo et al., 1973).

RESULTS AND DISCUSSION

For cows, the estimated heritability of TL was 0.40 ± 0.17, from the single trait model, indicating a moderate genetic contribution to TL variation within this age group. This result is in alignment with Zhang et al. (2023), whose study defined TL heritability in cattle as ranging between 0.36 and 0.46 when evaluated through qPCR. A study from Chik et al. (2022) compared meta-analysis of >100 heritability estimates derived from over 403 studies covering 18 vertebrate species and concluded that while the overall heritability of TL is 44.9%, these estimates are affected by the laboratory methods and statistical approaches used. Conversely, heifers in our study exhibited a lower heritability estimate at 0.21 ± 0.02.

The heritability, genetic and phenotypic correlations from the 7-trait model are reported in Table 2-1. TL had a somewhat lower heritability in both cows (0.22 ± 0.17) and heifers (0.18 ± 0.15) than observed from the single trait models. In general, the moderate heritability of TL determined in our study aligns with a study from Seeker et al. (2018) where the heritability of TL from leukocytes were estimated between 0.32 and 0.47 in Holstein cows, and Ilska-Warner et al (2019), where TL heritability was defined as moderate to high going from birth to first lactation (0.36 birth; 0.46 first

lactation). While there is variation among studies regarding the heritability of TL, it seems clear that there is sufficient genetic variation to consider TL as a potentially useful trait for selective breeding programs aiming to improve cow longevity (Seeker et al., 2018).

Heritability for production traits, such as milk yield (0.24 ± 0.04), protein yield (0.25 ± 0.037), fat yield (0.39 ± 0.039), and somatic cell score (0.21 ± 0.034) were similarly moderate, aligning with common estimates for these traits in dairy cattle (Atashi and Hostens, 2021).

Genetic correlations of production in kg of milk with fat (0.42 ± 0.08) and protein (0.78 ± 0.05) were in line with expectations. The genetic correlation of TL between cows and heifers was 0, but with a very large standard error (± 0.65). Genetic correlations require a sample size of at least several hundred animals to be estimated with precision (Lynch and Walsh., 1998) which was not achieved in our study. Likewise, genetic and phenotypic correlations of TL with production traits had sizable standard errors.

Manhattan plots of the SNP P-values from single-trait and multi-traits analysis are reported in Figures from 2-1 to 2-6. Figure 2-1 demonstrates the significance level of DNA marker effects for fat yield from the multiple trait analysis. This was performed to verify the GWAS procedures because there is a well-known QTL from variation in the DGAT1 gene (diacylglycerol acyltransferase isoform 1) on chromosome 14 (Yang et al., 2022).

The significance of DNA markers effect for TL in lactating cows from the single-trait analysis is presented in Figure 2-2. Even though no genome-wide significant

region was identified, peaks on chromosome 10 and the X chromosome were discernable. The significance of the DNA markers for TL cows was investigated from the multi-trait analysis (Figure 2-3) with the aim that the multi-trait analysis would be able to capture more genetic variance through genetic covariances among the traits. However, only the strong influence of DNA markers associated with fat yield (DGAT1 gene on chromosome 14) were amplified in the multiple-trait analysis, complicating the identification of potentially significant regions across the genome. Therefore, we considered the single-trait GWAS results to be more reliable.

We investigated the percentage of additive genetic variance explained by regions of 1Mb (Figure 2-4) which also highlighted regions on chromosome 10. The most significant markers on chromosome 10 were from two regions (between 0Mb and 2Mb and between 10Mb and 11Mb). While our analysis is not designed to identify candidate genes with precision, there are genes with plausible connections to variation in TL. A list of genes mapped to the first genomic region of interest (between 0M and 2M) is presented in Table 2-2 but did not have obvious connections to TL. In the second genomic region of interest (Table 2-3), one potential gene of interest was identified. Thrombospondin 4 (THBS4) has been implicated in various aspects of cancer biology, particularly in the context of the endoplasmic reticulum (ER) and cancer progression. For example, Brody et al. (2016) reported that THBS4 plays a critical intracellular role in regulating the adaptive ER stress pathway through activating transcription factor 6α (Atf6α). Endoplasmic reticulum (ER) stress has been shown to have a significant impact on telomerase activity and regulation. Zhou et al. (2013) also demonstrated that ER stress can activate telomerase. Furthermore, Cheng et

al. (2017) discovered that upregulating human telomerase reverse transcriptase (hTERT) induces cell apoptosis and ER stress, indicating a complex interplay between telomerase and ER stress in cellular processes.

Significance of DNA marker effects for TL in heifers from the single-trait (Figure 2-5) and multiple trait (Figure 2-6) analyses are also reported. Non consistent regions were identified with DGAT masking effects in the multiple-trait analysis.

Correlations of gEBV for TL with gPTA for PL, COWLIV, and HEIFLIV are reported in Table 2-4 for sires with at least 40% accuracy for TL gEBV and in Table 2-5 for Penn State cows that reached that level of accuracy. gPTA of sire COWLIV was correlated with higher gEBV for TL in cows with approximate genetic correlations of 0.37 (P <0.05) and 0.44 (P <0.01) when gEBV were derived from the multiple trait or single trait model, respectively. The approximate correlations of sire gPTA for HEIFLIV with heifer TL gEBV from the multiple trait model was 0.65 (P <0.01), and with cow EBV for TL in single trait model was 0.60 (P <0.001).

In Table 2-5, COWLIV gPTA had an approximate genetic correlation of 0.36 with cow TL gEBV from the multi-trait model (P <0.01). HEIFLIV gPTA was correlated with heifer TL gEBV from the multi-trait model (approximate genetic correlation = 0.42; P <0.05) supporting the idea that heifers with longer telomeres had higher probabilities of remaining alive until 18 months of age. HEIFLIV gPTA was also correlated with cow TL gEBV from the single trait model at 0.34 (P <0.05).

TL in heifers and cows against age is plotted in Figure 2-7, which indicated a correlation of -0.08 (P = 0.04). However, it was apparent that there was no association between TL and age in heifers (correlation = 0.03; P = 0.56), but older cows did have

shorter TL than younger cows (correlation = -0.10; $P = 0.04$). Seeker (2021) also reported that the relationship between TL and age in dairy cattle is not linear, but in a different direction. Their study showed TL shortening during the first year of life, but with older animals exhibiting less telomere depletion. A study from Kochan et al. (2023) reported a substantial reduction of approximately 9,225 telomeric copies between first parity (123,197 ± 4,426) and second parity (113,972.5 ± 3,973) cows which aligns with the general result shown in Figure 2-7. The authors also reported that TL in leukocytes is a significant predictor of longevity and survival in cattle, with shorter TL associated with higher mortality rates and reduced lifespan.

CONCLUSIONS

The investigation of the relationship between TL and key traits in dairy cattle found that TL has moderate heritability, with estimates ranging from 0.21 (± 0.02) for heifers to 0.40 (± 0.17) for cows in single-trait analysis. Both cow and heifer livability were correlated to both cow TL and heifer TL. The findings suggest TL could serve as a biomarker for breeding programs aimed at enhancing cattle health and longevity, but further research is needed to uncover the genetic mechanisms by which TL influences these traits.

Table 2-1: Heritability ± se (diagonal) of telomere length (TL) in cows and heifers, milk, fat, and protein yield, SCS, and MUN with genetic correlations ± se (above diagonal) and phenotypic correlations ± se (below diagonal)

	TL Cows	TL Heifers	Milk (kg)	Fat (kg)	Protein (kg)	SCS	MUN
TL Cows	0.22	-0.00	-0.28	0.059	-0.29	-0.08	0.29
	0.17	0.65	0.51	0.34	0.50	0.67	0.59
TL Heifers	-0.00	0.18	0.29	-0.05	0.18	-0.14	-0.02
	0.20	0.15	0.45	0.50	0.40	0.54	0.44
Milk (kg)	-0.05	0.21	0.24	0.42	0.78	-0.17	0.13
	0.06	0.31	0.04	0.08	0.04	0.12	0.13
Fat (kg)	0.08	-0.04	0.64	0.39	0.72	-0.31	0.44
	0.06	0.25	0.02	0.04	0.05	0.10	0.10
Protein (kg)	0.07	0.14	0.90	0.74	0.25	-0.26	0.33
	0.06	0.30	0.00	0.01	0.04	0.12	0.12
SCS	0.08	-0.11	-0.15	-0.16	-0.11	0.21	-0.29
	0.06	0.37	0.00	0.02	0.02	0.03	0.13
MUN	0.17	-0.02	0.12	0.33	0.20	-0.14	0.45
	0.09	0.35	0.03	0.03	0.03	0.03	0.06

Table 2-2: List of genes mapped to the first genomic region of interest (between 0M and 2M) on chromosome 10 in cattle

Gene symbol	Name	Start	Stop
LOC787243	coiled-coil domain-containing protein 3-like	141605	259227
LOC132346318	liprin-alpha-1-like	297375	326956
LOC132342136	uncharacterized LOC132342136	394909	411650
OR5W28P	olfactory receptor family 5 subfamily W member 28, pseudogene	409757	410722
OR5W56P	olfactory receptor family 5 subfamily W member 56, pseudogene	416109	417287
MCC	MCC regulator of WNT signaling pathway	451668	985178
LOC132346319	transmembrane protein 126A-like	607881	651772
LOC112448351	uncharacterized LOC112448351	905651	914086
LOC132346320	uncharacterized LOC132346320	981769	983012
DCP2	decapping mRNA 2	1030712	1043567
REEP5	receptor accessory protein 5	1094713	1145331
SRP19	signal recognition particle 19	1153147	1161237
LOC132346321	uncharacterized LOC132346321	1161498	1167261
APC	adenomatous polyposis coli	1167671	1299376
EPB41L4A	erythrocyte membrane protein band 4.1 like 4A	1518392	1814679
LOC112448611	small nucleolar RNA SNORA13	1816633	1816769
LOC132346322	uncharacterized LOC132346322	2058774	2124887

Table 2-3: List of genes mapped to the second genomic region of interest (between 10M and 11M) on chromosome 10 in cattle

Gene symbol	Name	Start Position	Stop Position
JMY	junction mediating and regulatory protein, p53 cofactor	10305748	10387185
TRNAC-GCA	transfer RNA cysteine (anticodon GCA)	10343370	10343441
LOC132346326	uncharacterized LOC132346326	10387020	10396450
HOMER1	homer scaffold protein 1	10407010	10546778
TENT2	terminal nucleotidyltransferase 2	10611812	10671414
CMYA5	cardiomyopathy associated 5	10675335	10773418
LOC112448606	small nucleolar RNA SNORA72	10713653	10713780
THBS4	thrombospondin 4	10826387	11060139
MTX3	metaxin 3	10950682	10961442
SERINC5	serine incorporator 5	11108319	11216774

Table 2-4: Pearson correlations and approximated genetic correlations (~rg) of sire gPTA[2] for livability and productive life with daughter EBV[1] for telomere length

		EBVTL_CMT	EBVTL_HMT	EBV_CST	EBV_HST
PTAPL	Correlation	0.03	0.04	0.09	-0.06
	~rg	0.06	0.09	0.18	-0.12
PTACOWLIV	Correlation	0.18*	0.19	0.22**	0.12
	~rg	0.37	0.40	0.44	0.27
HEIFLIV	Correlation	0.03	0.30**	0.29***	0.08
	~rg	0.06	0.65	0.60	0.18

[1]EBVTL_CMT = Cow telomere length from the multi-trait model; EBVTL_HMT = Heifer telomere length from the multi-trait model; EBV_CST = Cow telomere length from the single-trait model; EBV_HST = Heifer telomere length from the single-trait model

[2]PTAPL = Productive Life; PTACOWLIV = Cow livability; HEIFLIV = Heifer livability.

*P <0.05; **P <0.01; ***P <0.001.

Table 2-5: Pearson correlations and approximated genetic correlations (~rg) between cow and heifer gPTA[2] for productive life and livability with EBV[1] for telomere length.

		EBVTL_CMT	EBVTL_HMT	EBV_CST	EBV_HST
PTAPL	Correlation	0.03	-0.07	-0.07	-0.09
	~rg	0.08	-0.17	-0.13	-0.21
PTACOWLIV	Correlation	0.15**	-0.01	-0.03	0.06
	~rg	0.36	-0.03	-0.05	0.14
HEIFLIV	Correlation	0.01	0.16*	0.15*	-0.02
	~rg	0.01	0.42	0.34	-0.06

[1]EBVTL_CMT = Cow telomere length from the multi-trait model; EBVTL_HMT = Heifer telomere length from the multi-trait model; EBV_CST = Cow telomere length from the single-trait model; EBV_HST = Heifer telomere length from the single-trait model

[2]PTAPL = Productive Life; PTACOWLIV = Cow livability; HEIFLIV = Heifer livability.

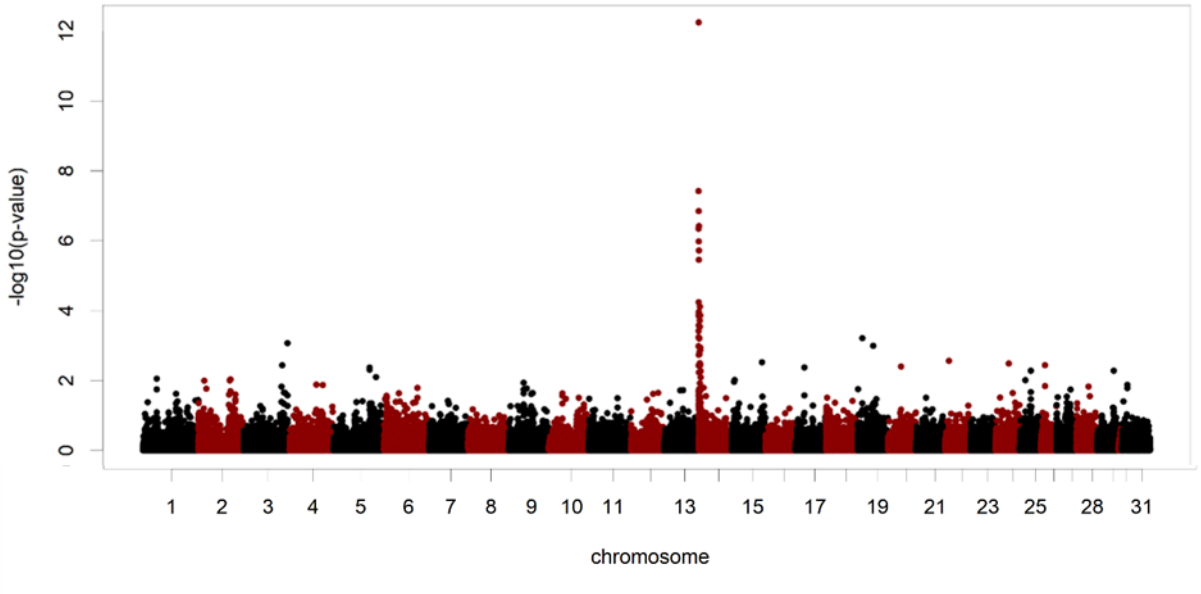

Figure 2-1: Significance of DNA marker effects for fat yield in cows from a multi-trait analysis by chromosome[1].

[1]30 = unplaced genetic markers; 31 = X chromosome.

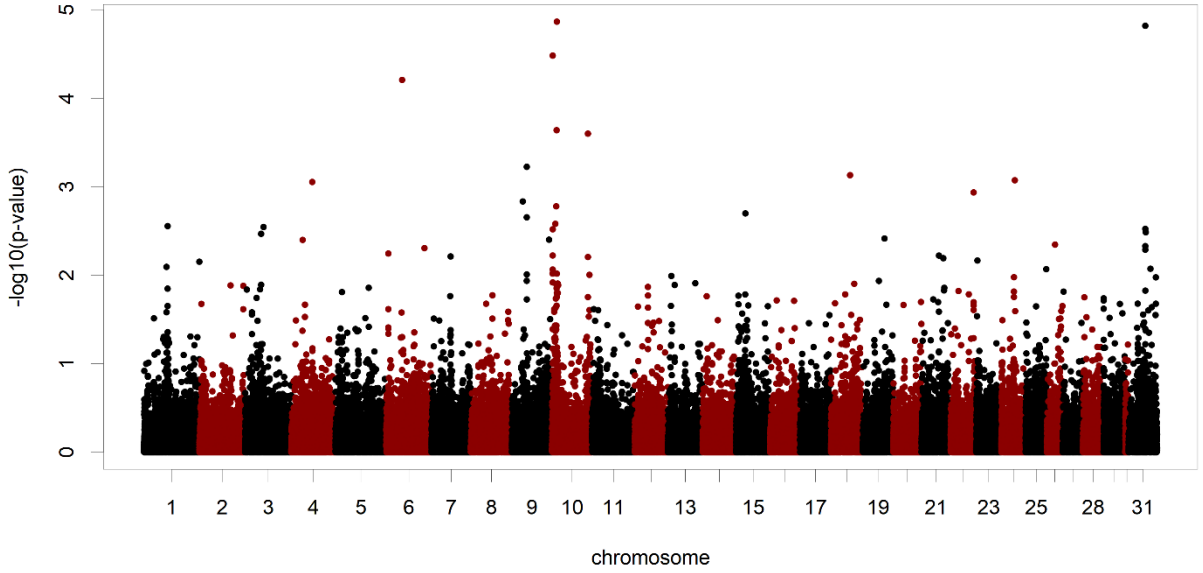

Figure 2-2: Significance of DNA marker effects for telomere length in cows from a single-trait analysis by chromosome[1].

[1]30 = unplaced genetic markers; 31 = X chromosome.

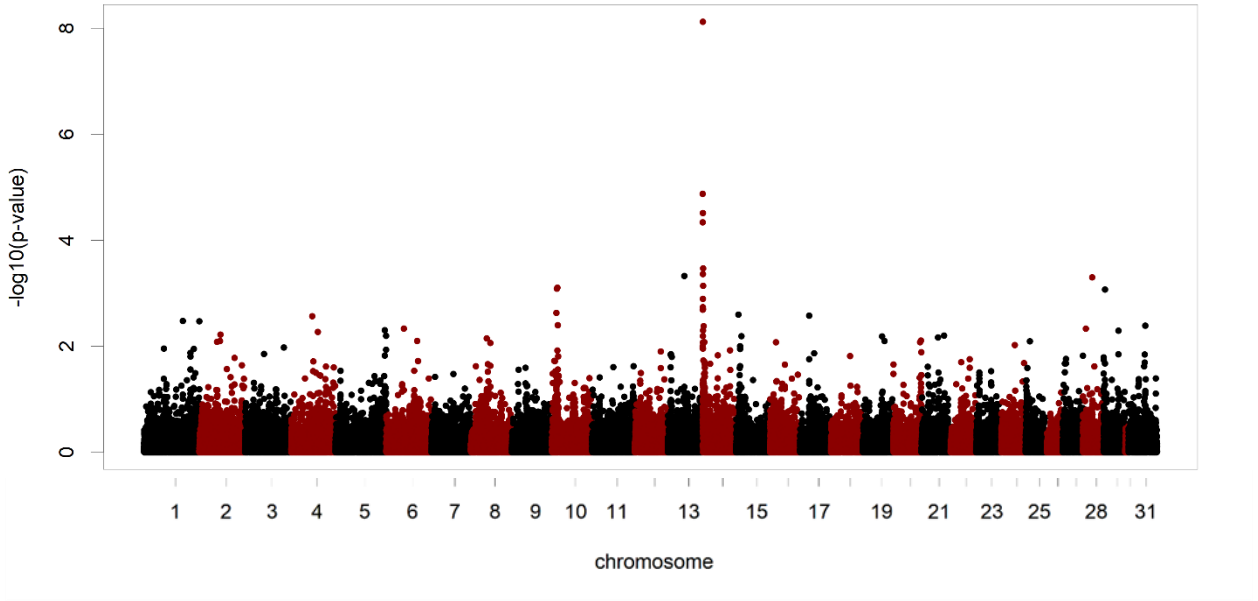

Figure 2-3: Significance of DNA marker effects for telomere length in cows from a multi-trait analysis by chromosome[1].

[1]30 = unplaced genetic markers; 31 = X chromosome.

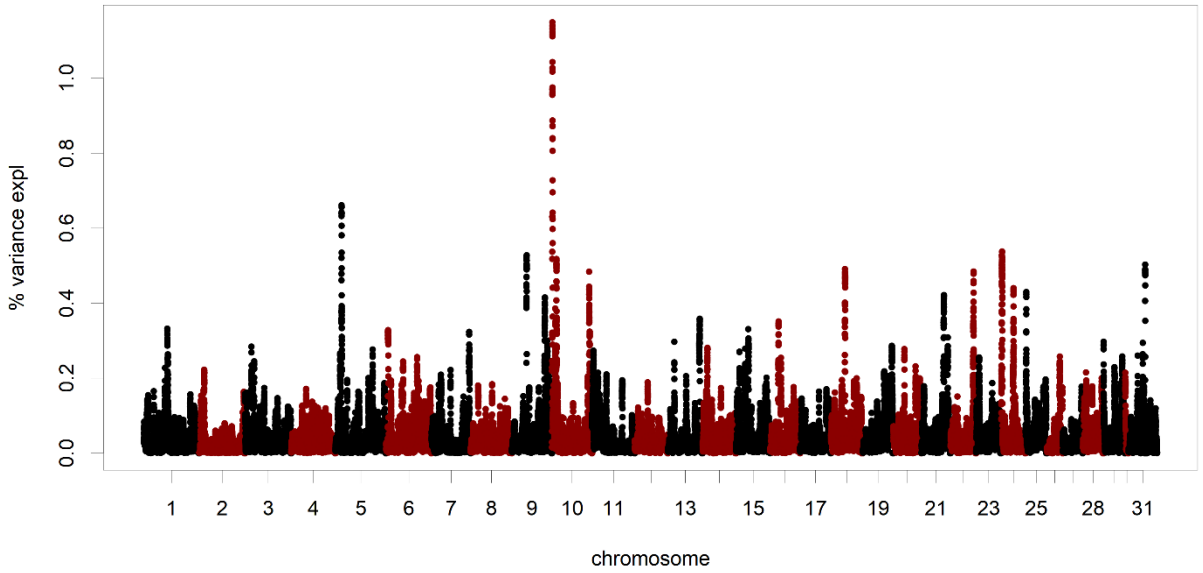

Figure 2-4: Variance for telomere length explained by DNA markers over 1 MB windows in cows from a single-trait analysis by chromosome[1].

[1]30 = unplaced genetic markers; 31 = X chromosome.

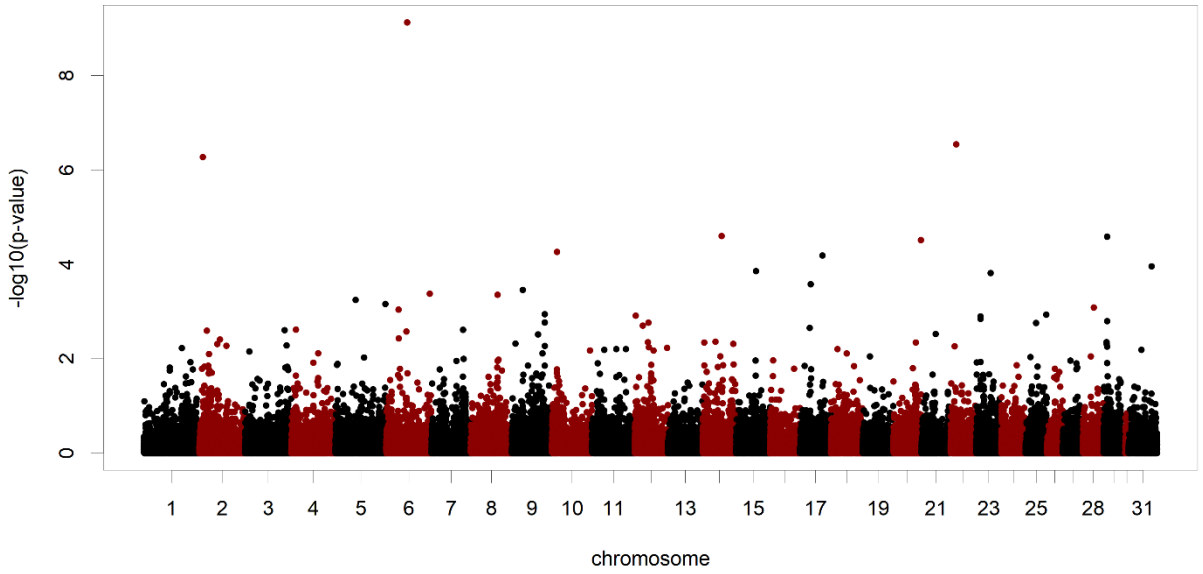

Figure 2-5: Significance of DNA marker effects for telomere length in heifers from a single-trait analysis by chromosome[1].

[1]30 = unplaced genetic markers; 31 = X chromosome.

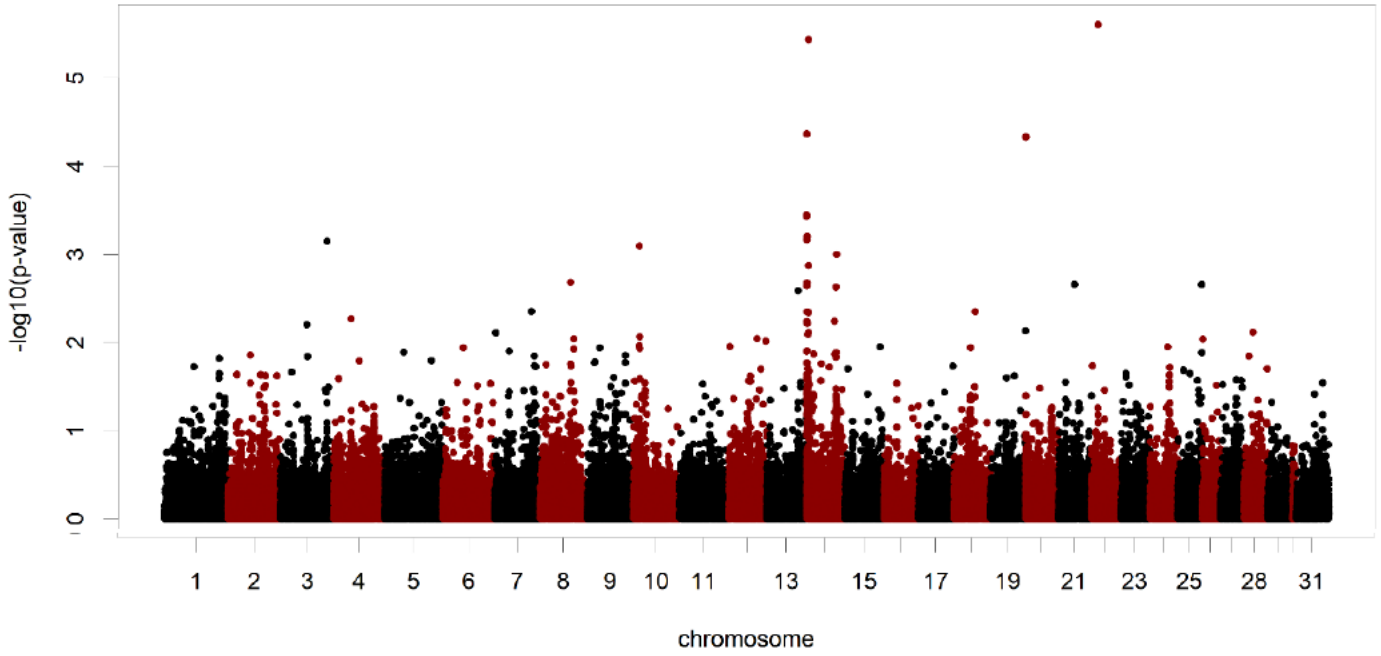

Figure 2-6: Significance of DNA marker effects for telomere length in heifers from a multi-trait analysis by chromosome[1].

[1]30 = unplaced genetic markers; 31 = X chromosome.

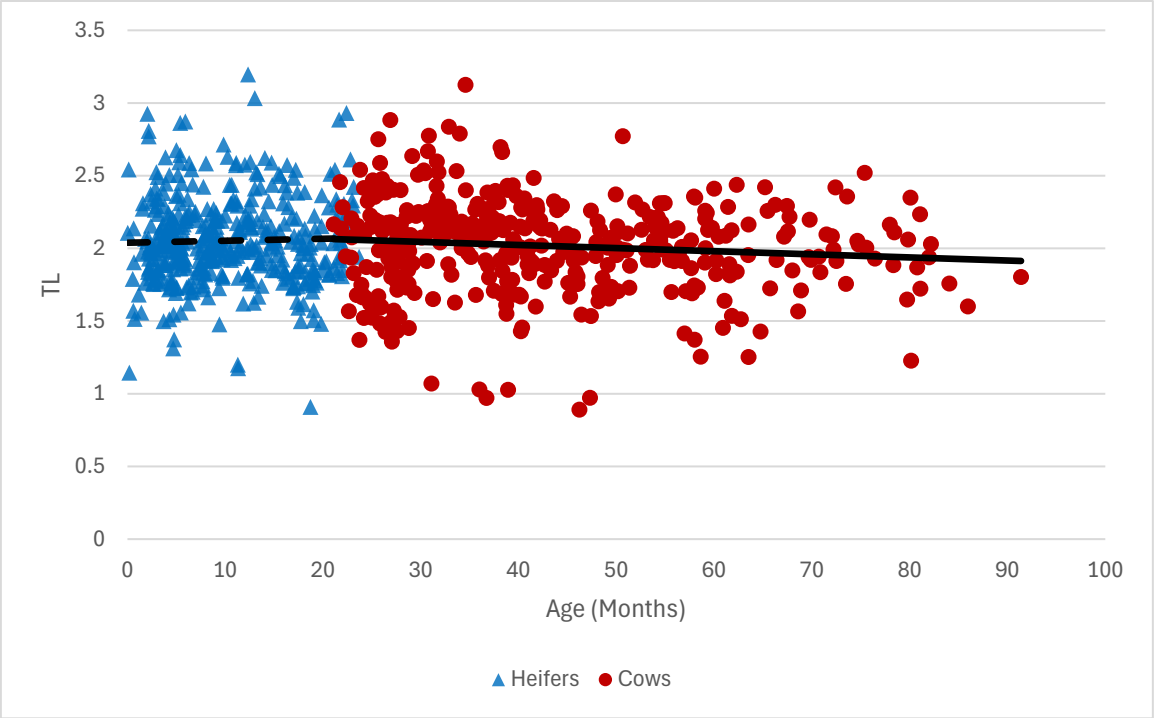

Figure 2-7: Association of telomere length with age in months for cows and heifers

Chapter 3

Sire breed differences of telomere length in a grazing herd

ABSTRACT

This study investigates the telomere length (TL) in purebred Holstein and two rotational crossbreeds, GrazeCROSS and ProCross. Blood samples from 101 dairy cows were collected at the West Central Research and Outreach Center, University of Minnesota, with TL quantified using a multiplex qPCR procedure. The cows, aged 31-128 months, were divided into Holstein (n = 17), GrazeCROSS (n = 29), and ProCross (n = 55) breed composition groups, and into first (n = 28), second (n = 30), and third plus (n = 43) lactation groups. All cows had a sire belonging to Holstein (n = 32), Jersey (n = 15), Montbéliarde (n = 17), Normande (n = 4), or Viking Red (n = 33). Statistical analysis was conducted using SAS (v9.4) with mixed-effects models to assess breed composition group or sire breed, and cow age on TL. Sire breed ($P = 0.03$) and age ($P = 0.003$) were significantly associated with TL, whereas breed composition group was not ($P = 0.20$). TL declined as age increased. Montbéliarde-sired cows had longer telomeres ($P < 0.05$) than cows sired by the other breeds. TL may be a useful biomarker to investigate the effects of breed composition and age on dairy cows, highlighting potential breed selection strategies for enhancing production efficiency and longevity.

INTRODUCTION

Telomeres, the terminal non-coding tandem repeat sequences of chromosomes, serve as protective caps for eukaryotic chromosomes and play a critical role in cellular aging and integrity (Pfeiffer and Lingner, 2013). The measurement of telomere length (TL) holds considerable promise as an index for evaluating health, welfare, and the potential for genetic selection in dairy cattle. For instance, Seeker et al. (2018) revealed a favorable association between relative leukocyte telomere length (RLTL) and the productive lifespan of Holstein Friesian dairy. The study suggested a potential association between TL and longevity since cows with longer RLTL had lower mortality and consequently had the tendency to live longer.

Crossbred cows have demonstrated improved survival rates compared to their Holstein herd mates. For example, Montbéliarde x Holstein (MO × HO) crossbreds were reported to have comparable component yield to HO, but superior fertility (45.1% conception rate vs. 26.9% for pure HO), reduced mortality (5.1% vs. 17.7% for pure HO), and increased survival (81.4% survival to second calving compared to 68.1% for pure HO) (Hazel et al., 2014).

In a similar study, Hazel et al. (2020) compared pure HO to MO × HO and Viking Red × Holstein (VR × HO) crossbreds. Both crossbred groups outperformed pure HO in survival, with higher percentages calving beyond the first lactation. MO × HO crossbreds showed an 11% increase in survival from the first to fourth calving compared to HO, rising to 19% in a three-breed composition crossbred (HO, VR, and MO) (Hazel et al., 2020). Additionally, Clasen et al. (2019) found that crossbreds outperformed purebreds in fertility (93% of the crossbred cows had successful fertility, versus 87% of the purebred

HO), stillbirth (7% of calves born to crossbred cows died within the first 24 hours vs. 9% for purebred HO), and survival (97% of crossbred cows survived from their first to their second calving, compared to 95% of purebred HO), regardless of herd production levels. Whether higher survival in crossbreds is associated with differences in TL is not clear.

ProCROSS and GrazeCross are rotational crossbred systems that have been researched in the US (Jaafar et al., 2022). ProCROSS stem from the crossing of VR, HO, and MO and are reported to have high milk solids production with enhanced health and fertility. GrazeCross was developed to focus on efficient feed conversion in grazing cows and incorporates the VR, Normande (NO), and Jersey (JE) breeds.

This study aims to investigate whether TL differs in purebred HO relative to ProCROSS, GrazeCross, and breed of sire in an experimental grazing herd.

MATERIALS AND METHODS

Data

Blood samples were collected from the tail vein of 101 cows at the West Central Research and Outreach Center (WCROC) facility of the University of Minnesota, on July 14th, 2022. Animals were divided into three breed composition groups as HO (n = 17), GrazeCROSS (n = 29) and ProCross (n = 55). The cows were from first lactation (n= 28), second lactation (n = 30), and third plus lactation (n = 43). The animals' ages ranged between 31 and 128 months of age. All animals were sired by a bull belonging to one of five breeds (Table 3-1): HO (n = 32), JE (n = 15), MO (n = 17), NO (n = 4) and VR (n = 33).

DNA was extracted with a phenol-chloroform extraction followed by precipitation of DNA using ethanol and samples with a 260/280 ratio between 1.6 and 2.0 were retained for analysis. TL was then quantified employing a multiplex qPCR procedure, as described in chapter 2.

Statistical analysis

Data analysis was conducted in the SAS (SAS, v9.4, Cary, NC) with the following mixed-effects model:

$TL_i = \beta_0 + SB_i + \beta_1 * Age + \varepsilon_i$, where TL_i denotes the TL for cow from sire breed i; $\beta 0$ is the model intercept; SB_i represents the effect of sire breed i; β_1 represents the regression coefficient on age of the cow in months and ε_i denotes the random error term. Sire breed was replaced with breed composition group (HO, ProCross, or GrazeCross) in some analyses.

Correlation coefficients were computed to assess the linear relationships between TL and production metrics (milk, fat, protein, and SCS) using the CORR procedure of SAS. Production data records were calculated as the average between two consecutive test dates.

RESULTS AND DISCUSSION

Breed composition group had no significant effect on TL (P = 0.59), while the breed of the sire (P = 0.04) was a significant determinant of TL (Table 3-1; Figure 3-1). MO had significantly higher TL compared to HO (P = 0.02), NO (P = 0.04), JE (P = 0.01), and VR (P = 0.01). Iannuzzi et al. (2022) presented results in support of the idea

that TL may be associated with longevity among dairy breed composition with TL differences between HO and Agerolese cows, as the HO had a shorter productive life and shorter telomeres. MO are reported to have high longevity in modern production systems, which could be related to the longer TL reported here.

Hazel et al. (2014) found that MO crossbreds are more robust than pure HO. MO × HO and MO × JH crossbreds showed significantly higher survival rates to subsequent calvings compared to pure HO, with 13% to 25% greater survival rates to the second, third, fourth, and fifth lactations, respectively. Mortality rates were lower for MO × HO (5%) and MO × JH (12%) compared to 18% for pure HO. A significant survival advantage for MO crossbreds was also confirmed by Heins, Hansen, and Vries (2012). In their study, MO × HO crossbreds had significantly higher survival rates compared to pure HO and were comparable to NO × HO and Scandinavian Red (SR) × HO crossbreds. During the first 305 days of the first lactation, the mortality rate for MO × HO cows was 2.0%, much lower than the 5.3% for pure HO. NO × HO and SR × HO had similarly low mortality rates at 1.2% and 1.6%, respectively. MO × HO crossbreds also had higher survival rates to subsequent calvings (86% to second, 76% to third, 55% to fourth) compared to pure HO (75%, 51%, 29%). On average, MO × HO crossbreds stayed in the herd 412 days longer than pure Holsteins, with NO × HO and SR × HO crossbreds also showing increased longevity (317 and 360 days longer, respectively). While crosses of many breeds have been reported to have higher survival than HO, only MO sired cows had longer TL in this study.

The age of the cow ($P = 0.003$) was also a determinant of TL. For every one-month increase in the age of the cow, TL declined by -0.003 ± 0.001 (Figure 3-1). A

significant negative correlation between chronological age and TL was also presented by Brown et al. (2012). Their findings also emphasized herd-specific TL variations, suggesting environmental factors influence TL. Cows with shorter telomeres exhibited a higher likelihood of being culled within the subsequent year, supporting the notion that TL may serve as a valuable biomarker for assessing age-related physiological changes and well-being in dairy cattle.

CONCLUSIONS

Sire breed and cow age were associated with TL, with younger cows and Montbéliarde-sired cows exhibiting the longest telomeres. Breed composition group was not significantly associated with TL; that observation coupled with the sire breed observations may indicate that TL will vary among crossbreds depending on which breed composition is most prevalent in a given generation. Given the longer survival associated with MO sired cattle relative to HO in other studies, the results suggest TL could be a useful biomarker to facilitate genetic improvement for longevity

.

Table 3-1: Telomere length (TL) least square means for each breed composition group[1] and sire breed group[2]

	# obs	TL
breed composition group[1]		
GC	29	1.96
HO	17	2.04
PC	55	2.03
sire breed group[2]		
HOS	32	2.00[a]
JES	15	1.95[a]
MOS	17	2.14[b]
NOS	4	1.91[a]
VRS	33	2.00[a]

[1]HO=Holstein, PC = Procross, GC = GrazeCross

[2]HOS=Holstein sired, MOS=Montbéliarde sired, NOS=Normande sired, VRS=Viking Red sired, JES=Jersey sired

Note: Different superscript letters (a, b) indicate significant differences between groups at $P < 0.05$. No significant differences were found between breed composition groups.

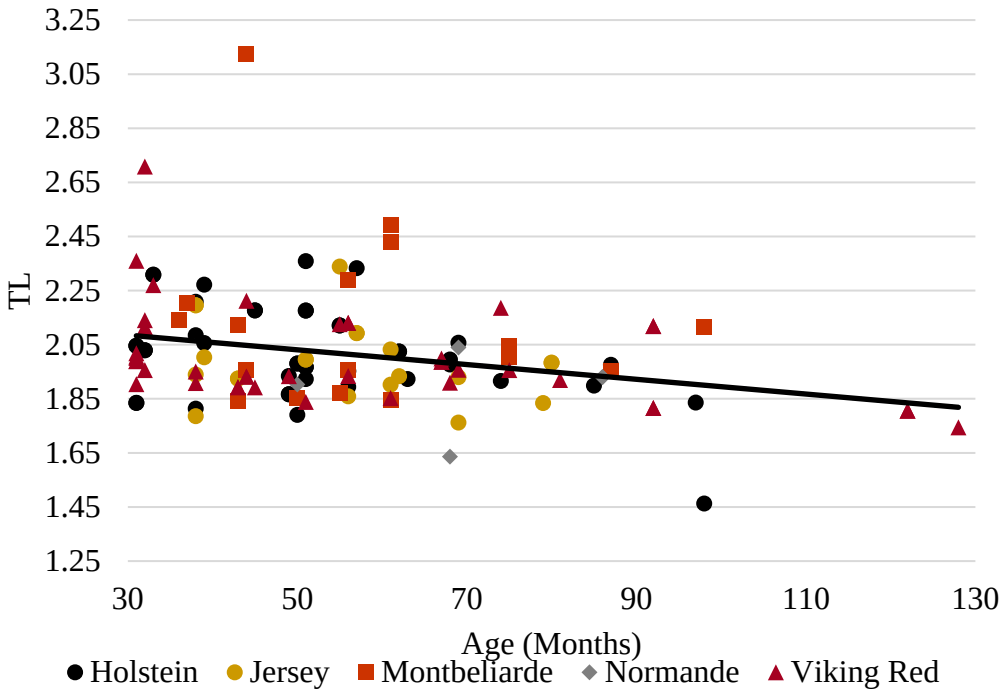

Figure 3-1: Regression of telomere length on age in months in sire breed groups (Holstein sired, Montbéliarde sired, Normande sired, Viking Red sired, Jersey sired)

Chapter 4

Association of breed composition and sire breed with diurnal grazing and rumination patterns.

ABSTRACT

This study aimed to elucidate the grazing and ruminating diurnal behavior patterns of grazing dairy cows across different breed composition groups and sire breeds and to estimate feed efficiency through indicators such as potentially degradable neutral detergent fiber (pdNDF) and thermal heat loss. Thirteen cows (two Holstein (HO), six GrazeCross, and five ProCross) at the West Central Research and Outreach Center, University of Minnesota, Morris, MN, were fitted with RumiWatch collars to track rumination, grazing, and other activities from June 15-21, 2021. Body condition score (BCS), fly density levels, thermal imaging, milk yield, fat, protein, somatic cell score (SCS), milk urea nitrogen (MUN), and fecal samples were also collected to determine breed composition differences for performance and efficiency. Statistical analyses were conducted using mixed-effects models SAS (v9.4) to evaluate diurnal behavior patterns, and correlations between average daily behavior and production metrics. Our study identified distinct hourly patterns in ruminating and grazing behaviors across different breed compositions and sire groups. Grazing peaked in the evening (around 20:00) and in

the morning (around 07:00), nearly ceasing by 08:00. Rumination peaked at 23:00 and remained elevated into the early morning, following a consistent circadian rhythm across breeds. No substantial breed composition differences were found for hourly behavior after conducting multiple comparisons using Tukey's adjustments. However, GrazeCross cows spent more time grazing each day (604.1 minutes/day) compared to ProCross and Holsteins (P <0.05). Fecal analysis showed higher pdNDF levels in Normande (NO) cows (14.56%) compared to other sire breeds (9.79-12.92%), indicating less complete digestion or selective grazing of less digestible grass varieties or plant parts. Thermal imaging revealed breed-specific differences in leg temperatures, with Viking Red (VR) cows exhibiting cooler legs (29.16°C) than NO cows (32.49°C) (P =0.03), suggesting differences in metabolic efficiency and heat dissipation among sire breeds. These preliminary results highlight breed composition differences in novel tools such as pdNDF (ranging from 9.79% in HO to 14.56% in NO), thermal heat loss (VR cows: 29.16°C; NO: 32.49°C), and fly resistance (fly scores ranging from 0.94 in Montbéliarde (MO) to 1.86 in HO). The results indicate that such traits could be useful for selecting cows or breed compositions to optimize pasture utilization. Further research is needed to explore the practical application of these traits and align farm management with cow behavioral patterns.

INTRODUCTION

Behavior exhibited by grazing dairy cows, such as more frequent walking, foraging, and relaxed postures, poses a lot of emphasis on the benefits of pasture access for cow health and performance, when high-quality forage and proper management to ensure cows meet their nutritional needs are assured (Popescu et al., 2013). The extent of pasture benefits on cow productivity, reproductivity, and feed efficiency can vary based on factors such as grazing time and pasture quality (Wagner et al., 2017), and challenges, such as maintaining proper body condition, can arise.

Pasture-based systems are often associated with lower body condition score (BCS), underscoring the need for effective pasture management to ensure adequate nutrient intake (Smid et al., 2020). Body condition score, a 1-5 scale developed by Wildman et al. (1982), is an accurate and practical indicator of the health and productivity of dairy cows by determining the cow degree of fitness independently from the animal frame size. Grazing behavior is correlated with sward characteristics, such as sward height, herbage mass, leaf and stem bulk density, and leaf tensile strength (Soder et al., 2022). Furthermore, the correlation between BCS and pasture characteristics highlights the significance of higher-quality pastures in directly contributing to improving nutrient intake, body condition, and overall health (Castelán-Ortega et al., 2018).

Consequently, effective pasture management and access are paramount for optimizing dairy cow health and performance. Bargo et al. (2003) and Boland (2011) outlined that optimizing the performance of pastured dairy cows while managing pasture allowance and supplementation requires understanding cattle feeding patterns,

biting rates, forage preferences, avoidance behaviors, rumination, and meal timing. Factors such as nutritional needs, social dynamics, topography, climate, and environmental conditions, including the availability of potable water, influence grazing patterns.

The selection of the right breed composition and crossbreeding system should also be matched to the herd management system in order to optimize performance and cow wellbeing (Barbano, 2017; Dechow and Hansen, 2017). Performance of Holsteins (HO), GrazeCross (GC; cross among Jersey, Normande, and Viking Red breeds), and ProCross (PC; cross among Holstein, Montbéliarde, and Viking Red genetics) has previously been compared in a grazing system to determine how genomic architecture was associated with high and low performance groups (Jaafar et al., 2022). Breed composition significantly affected animal performance. High-performing ProCross animals with higher MO genetics among their breed composition had increased fat and protein yields, while high-performing ProCROSS animals with higher HO genetics produced more milk yield. The study also found that MO ancestry was particularly influential in improving fat yield, and the combination of HO and MO contributed to higher protein yield. In GrazeCross, high-performing animals had 2% lower NO composition in their minimum range compared to low performers, suggesting that NO ancestry plays a significant role in production traits, but its influence on performance may be dependent on the optimal proportion of other breed contributions like VR (Jaafar et al., 2022).

Although breed composition differences in grazing behavior are not fully known, a few studies investigated the genetics of grazing systems. Washburn and

Mullen (2014) defined genetic selection as essential in grazing systems since specific breed composition and crossbreeding strategies offer distinct advantages. Washburn and Mullen (2014) showed that HO with high percentages of North American ancestry perform well in grazing systems with supplemental concentrates, while New Zealand Holstein-Friesian (NZH) and JE breeds excel in systems relying primarily on pasture. Washburn and Mullen (2014) also indicated that crossbreeding, such as HO × JE or including breeds like Norwegian Red (NR), MO, and NO, can enhance reproductive performance, longevity, and BCS which is critical for fertility in seasonal calving systems. For instance, NZH cows showed a 69% pregnancy rate within the first six weeks of breeding, outperforming North American HO at 54% in pasture-based systems. Economic indices like Net Merit $ (NM$) guide genetic selection by weighing traits such as milk production and fertility differently depending on the system. Some studies (Briske et al., 2008; Fetzel et al., 2017; Washburn and Mullen, 2014) suggest that creating specific indices for grazing systems may not be essential. Instead, genetic selection in these systems should prioritize reproductive efficiency and adaptability to optimize both production and economic returns (Washburn and Mullen, 2014).

In another study, Dillon et al. (2003) compared the performance of Dutch Holstein-Friesian (DHF), upgraded Irish Holstein-Friesian (CL), French Montbéliarde (MB), and French Normande (NR) breeds in a grass-based dairy system, revealing genetic differences in milk yield, milk composition, and body condition. The DHF cows produced the highest milk yield (5,994 kg) but had lower BCS (2.63) and greater BCS loss in early lactation (-0.46), indicating higher metabolic strain. In contrast, dual-purpose breeds MB and NR produced less milk (5,119 kg and 4,561 kg, respectively)

but with higher fat (40.0 g/kg for NR and 38.1 g/kg for MB) and protein (36.0 g/kg for NR and 34.9 g/kg for MB) content, showing better overall body condition and live weight stability.

Furthermore, Spaans et al. (2018) explored the genetic influence of JE and HO breeds on grazing system efficiency at different comparative stocking rates (CSR). The study found that HO cows, genetically selected for higher milk production, outperformed JE cows at a CSR of 80 kg BW/t DM, producing 17% more 4% fat-corrected milk (FCM) per cow. However, at a higher CSR of 100 kg BW/t DM, HO cows exhibited significant reductions in both pasture yield and milk production due to overgrazing, whereas JE cows, with their lower maintenance requirements and more efficient energy utilization, maintained better performance. This genetic advantage in energy efficiency made JE cows more suitable for higher stocking rates, particularly in restricted feed environments, highlighting the importance of breed composition-specific genetic traits in optimizing grazing systems.

While BCS is a well-developed and described tool to monitor animal performance (Montiel-Olguín et al., 2019), and behavior monitoring systems are improving, the development of additional tools to monitor cow health and performance on pasture would be beneficial. Very little is known regarding the application of novel tools such as potentially degradable neutral detergent fiber (pdNDF), thermal imaging, and fly scoring. pdNDF is a simple measure of feed digestion that could be useful as a tool to evaluate how well cows harvest nutrients in grazing systems. pdNDF can be derived as the difference between fecal neutral detergent fiber (NDF) and fecal indigestible NDF after 10-12 days of rumen digestion (Zanton, 2019). Thermal imaging

is a non-invasive method of performance that looks at body temperature variations, which correlate with energy expenditure and metabolic activity, as energy expenditure is found to be associated with metabolic activity in humans (Campbell, I. T. 2006).

Thermal heat loss has been linked to dry matter intake (DMI; Chang-Fung-Martel et al. 2021), and studies by Lahart et al. (2021) and Hardie (2016) have evaluated the relationship of thermal heat loss to feed efficiency. Lahart et al. (2021) found that using thermal imaging to measure thermal heat loss showed a tendency toward association with DMI, but it was not a robust predictor of feed efficiency in grazing dairy cows ($P < 0.25$). Hardie (2016) discovered that body surface temperature has moderate heritability (0.29 to 0.32) and a significant genetic correlation with residual feed intake (RFI). The study also showed that feed-efficient cows had lower surface temperature, especially in the leg region where each 0.25 kg/day increase in RFI corresponds to an increment of 1 standard deviation in temperature. Furthermore, Byrne et al. (2017) demonstrated the utility of detecting health issues like heat stress or individual health problems from thermal images. Fly scoring can also be used to evaluate fly infestation levels of grazed cows (Fraga et al., 2005). Fly infestation has an important economic consequence ($2.3 billion/year) for dairy cows in pasture-based systems due to decreased weight gain, compromised feed efficiency, the increased transmission of diseases such as mastitis, and reduced milk yield (Brewer et al., 2021). A study by Basiel et al. (2021) on pastured HO estimated that a heavy infestation of horn flies (>125 flies on one side of the cow) was associated with a loss in milk yield (2.2kg/d) compared to cows showing limited fly presence. Basiel et al. (2021) found that the heavy infestation was especially present on darker-coated Holsteins. The

infestation level of horn fly was assessed on the animals' right side from chine to loin and classified on a scale of 0 to 4 (Basiel et al., 2021).

The present study aimed to fill the gap in the literature by comparing diurnal grazing behavior across different cow breed compositions. Furthermore, breed composition differences were examined using novel predictors of pasture performance, specifically pdNDF, heat loss, and fly resistance to determine their future application in pasture-based systems.

MATERIALS AND METHODS

Data

Thirteen cows categorized as Pure Holstein (n=2), GrazeCross (n=6), and ProCross (n=5) at the West Central Research and Outreach Center (WCROC) organic herd at the University of Minnesota in Morris, MN were fitted with RumiWatch halters to evaluate grazing behavior activities; of these 2 of the GrazeCross were sired by a JE bull, 2 by a NO bull, and 2 by a VR bull, while for Procross 1 was sired by a HO bull, 2 by a VR bull, and 2 by a MO bull.

Data were collected from 06/15/2021 to 06/21/2021. Hourly RumiWatch estimates of rumination (min/h), grazing (graze bout duration min/h), and other activities (movements of the head that were not related to ruminating or grazing min/h) were retrieved for each date. Hourly data was summed to create daily totals for each cow for each activity (ruminating, grazing, and other). In addition to the 13 animals with the RumiWatch halters, performance and novel trait data were recorded from 36 herdmates. These records were collected from all breed composition groups (15

Grazecross 13 ProCross, and 5 HO) and sire breeds (8 HO, 4 JE, 6 MO, 7 NO, and 6 VR).

The herd was maintained on pasture at all-time except for milking, which served as the sole feed source for the animals. The pasture was composed by meadow bromegrass (*Bromus riparius* Rehamann), meadow fescue (*Schedonorus pratensis*), orchardgrass (*Dactylis glomerata* L.), red clover (*Trifolium pratense* L.) and white clover (*T. repens* L.). Cows were moved to a new pasture once every two days around 8am after the morning milking. Water was available at all times on pasture. Animals were milked twice daily between 07:30 – 09:00 and between 13:30 pm – 15:00. The behavior on pasture was measured using the RumiWatch system (Itin and Hoch GmbH, Liestal, Switzerland) which employs advanced sensors in halters designed to capture detailed jaw motion and chewing patterns (Weinert et al., 2020). The BCS (1=thin to 5=fat) (Wildman et al., 1982) and level of fly infestation (0 = no or few flies to 4 = heavy infestation) (Basiel et al., 2021) on the animal's right side from chine to loin were recorded daily by a trained operator. To collect thermal images, cows were isolated in a separate pasture area and images were captured focusing on three specific body regions: the lower part of the right rear leg, the right side of the rump (between the hip and pin bones), and the flank region (Hardie, 2016). A trained operator conducted the imaging using a Fluke Thermal Imaging Camera (Fluke Corporation, Everett, WA), ensuring reproducible measurements by maintaining a constant distance of 1.5 meters between the camera and the subjects. Analysis of the thermal images was performed using Fluke Smartview® software, with average temperatures determined for each region of the body.

Performance data was collected for each cow including days in milk, parity number, daily milk yield (kg), protein and fat (kg and %), somatic cell score, and milk urea nitrogen. These data were extracted directly from the PCDart farm management software (Raleigh, NC) for test dates prior to (05/25/2021) and subsequent to (06/30/2021) the data collection period. Records from the two dates were averaged and retained for 31 cows that had records on both dates.

Fecal samples were gathered twice per day from each animal (n = 33) on two separate occasions (6/17/2021 and 6/20/2021), totaling four collections per cow. Fecal samples were collected according to the methodology described by Lacey et al. (2020).

Briefly, fecal samples were collected during milking time (morning and evening) manually from the rectum of the cows using a plastic sleeve. Samples were dried for 72 hours at 55°C in a forced-air oven and combined per cow to obtain a total of two composite samples, morning and evening samples were composited into a single daily sample on date and dated per animal, which were analyzed by an external laboratory (Cumberland Valley Analytical Services; Waynesboro, PA) to determine the composition of fecal NDF from organic matter, and undigested NDF from organic matter (uNDF) after a 10-day in vitro digestion (Goering and Van Soest, 1970). Results were averaged per cow to investigate the total amount of potentially degradable NDF (pdNDF) which was derived as NDF – uNDF (Lacey et al. 2020).

Statistical Analysis

The MIXED procedure of SAS (v9.4, SAS Institute, Cary, NC) was used to evaluate diurnal behavior patterns. The initial model of 24-hour totals was:

$$y_{ijk} = \beta_0 + BG_i + D_j + C_k + \epsilon_{ijk},$$

where *y* = rumination time, grazing time, or other activities; β_0= the intercept; BG_i = the fixed effect of breed composition group i (HO, GrazeCross, ProCross), D_j = the fixed effect of date; observations were repeated across date for C_k= the random effect of cow k, and ϵ_{ijk} is random error. Least-squares-means (LSMeans) were calculated for breed composition group, and pairwise comparisons were performed with Tukey's adjustment. Significance was declared at $P<0.10$ for all analyses. An identical analysis was conducted with breed composition group replaced by sire breed; including maternal-grand-sire breed or breed composition group did not improve model fit so were not included.

A repeated-measures analysis was then conducted to evaluate hourly totals by breed composition or sire group as:

$$y_{ijklm} = \beta_0 + BG_i + H_j + D_k + BG_i * H_j + H_j * D_k + AM_l + C_m + \epsilon_{ijklm},$$

where *y* = rumination time, grazing time, or other activities; β_0= the intercept; BG_i = breed composition group (i=HO, ProCROSS, GrazeCross); H_j = hour of day j; D_k = day; AM_l = represents the fixed effect of age in months, C_m= the effect of cow m, and ϵ_{ijklm} is random error fit assumed to follow a normal distribution. Breed composition group was replaced with sire breed in a second series of analyses.

LSMeans were calculated for both sire breed and breed composition groups, and pairwise comparisons were performed with Tukey's adjustment.

Correlations of average daily rumination time, grazing time, and other activities with milk yield, fat and protein (kg and %), SCS, MUN, pdNDF, BCS, fly score, and thermal heat loss were derived with the CORR procedure of SAS (v9.4, SAS Institute, Cary, NC). Finally, LSMeans for milk yield, fat and protein (kg and %), SCS, MUN, pdNDF, BCS, fly score, and thermal heat loss were derived using the GLM procedure with the following model:

$$y_{ijk} = \beta_0 + L_i + BG_j + S_k + \epsilon_{ijk},$$

where y = the trait of interest; β_0= the intercept; L_i = the fixed effect of lactation group (1, 2, ≥3) i; BG_j = the fixed effect of breed composition group or sire group (SB_j); S_k = stage of lactation group (days in milk < 100 = early lactation stage, days in milk > 100 = late lactation stage) k; ϵ_{ijk}= random error.

RESULTS AND DISCUSSION

Table 4-1 reports the average of rumination, grazing and time spent in other activities for each breed composition group. In our study, the investigation into the general daily behavior patterns across these breed composition groups showed no significant differences in ruminating time, averaging 430 min across the three groups. ProCross cows had lower ($P < 0.10$) grazing time than GrazeCross cows, but similar to HO. No significant differences in other activities were observed. In Table 4-2, differences are reported by sire group, and no significant differences in rumination

time, grazing time, or other activities were observed. Rumination time averaged 429 min/d across all five sire breed groups, while grazing time averaged 547 min/d.

Pereira and Heins (2019) reported differences in rumination behavior between HO and crossbred cows in a 24-h period, in a study conducted at the same facility as our study. The study compared Procross and GrazeCross cows to HO and a group of H64 (Pure HO whose genetics is equivalent to the breed average in 1964). The study compared daily rumination time according to breed composition groups, herd management system (organic vs. conventional), and parity. Organic cows had access to pasture for 20 hours per day from May to October, consuming about 85% of their daily DMI from pasture and supplemented with 2.72 kg of organic corn daily. Conventional cows were fed a total mixed ration (TMR) year-round in a dry-lot during the summer and a compost barn during winter. Pereira and Heins (2019) reported that H64 ruminated significantly less than the other groups; organic H64 cows ruminated on average 7.2% less than HO, whereas conventional H64 cows ruminated on average 5.6% less than HO. Even in our study, HO was the highest in rumination time, but cows in the current study ruminated somewhat less (465.9 min/d) than the animals in the Pereira and Heins (2019) study. Comparing the crossbred groups, Procross ruminated the least and the result was consistent between our study (389.6 min/d for Procross and 427.2 min/d for GrazeCross) and the Pereira and Heins (2019) study (519.8 min/d for organic Procross and 512.4 min/d for organic GrazeCross). Aikman et al. (2008) demonstrated breed-specific differences in eating and ruminating behaviors in tie-stall housed HO and JE cows fed a TMR diet. HO in the cited study spent an average of 360 min/day eating and 624 min/day ruminating, while Jerseys spent 382 min/day eating

and 540 min/day ruminating. These values are higher than those reported in our study, where HO ruminated for 474 min/day and JE for 432 min/day. The differences in ruminating time between the two studies may reflect variations in feed type and presentation, as cows in Aikman et al. (2008) study were fed ad libitum TMR, formulated to meet the animals' metabolic energy needs, while our study focused on grazing cows under different pasture conditions. Furthermore, JE in their study exhibited a faster rate of digesta passage and greater fiber digestibility than HO, which could account for some of the behavioral differences observed between these two studies. Braun et al. (2015), using a noseband pressure sensor, found HO cows, housed in tie-stalls and fed a total mixed ration (TMR), to ruminate 13% longer than Brown Swiss cows but similar to Swiss Fleckvieh. This suggests that genetic factors may influence rumination behavior among different dairy cattle breed composition.

Our study also described hourly grazing (Figures 4-1 and 4-2) and ruminating (Figures 4-3 and 4-4) behaviors across both breed (Figures 4-1 and 4-3) and sire groups (Figures 4-2 and 4-4). While both breed composition and sire breed were significant during the initial investigation, after conducting multiple comparisons using Tukey's adjustments, no statistical differences in grazing times were found among breed composition or sire groups. For example: NO grazed more (18.70 min) than HO at 18:00 pm which was initially significant (P <.0001), but which was not different after applying a Tukey multiple comparison test (P = 0.20). This variation in grazing time coincided with the evening milking, suggesting that the order in which the animals were milked may have influenced the results.

We identified two peak periods for grazing activities shared among all groups with no significant statistical differences: one in the morning around 07:00 and another in the evening around 20:00. Interestingly, all groups stopped grazing around 08:00, consistent with the morning milking schedule at the farm. This event shows the underscoring impact of farm routine activities on daily cow activities. Rosa et al. (2024) investigated how altering milking times can significantly impact grazing and ruminating behaviors in dairy cows. They found that aligning grazing periods more effectively with the natural circadian rhythms of the cows (Casey and Plaut 2022) increased grazing time from 125.3 min/d to 183.9 min/d. However, rumination required an adaptation period, initially decreasing on the first day (from 178.33 min/d to 81.11 min/d), but subsequently increasing on the second day of testing (from 102.22 min/d to 167.22 min/d).

Hourly ruminating behavior patterns exhibited a clear peak around 11 pm, closely following the grazing peaks, with only subtle breed composition or sire differences (Figures 4-1 and 4-3). Rumination remained elevated through the early morning hours and was low during the middle of the day (10:00 to 21:00). This finding suggests a natural circadian rhythm in ruminating activity, consistent across different genetic lines. The peak in rumination late at night (23:00) that we identified in our study is consistent with the findings of Gregorini et al. (2013), who reported consistently higher rumination rates late at night across HO, JE and HO x JE, from 22:00 to 06:00 the following morning independently from animal age or breed composition.

Average hourly grazing and rumination patterns across all breed composition groups (Figure 4-5) and sire groups (Figure 4-6) have been reported.

In Table 4-3, we report differences among sire breeds for performance and management traits. The overall p-value presented in the table is testing the global null hypothesis that breed group explained no variation in performance and was generally non-significant due to the inclusion of many groups with relatively few cows. However, pairwise comparisons among the more extreme groups were often significantly different. For example, BCS presents an overall non-significant p-value ($P = 0.32$) when compared across NO, VR, and JE. However, a pairwise comparison of HO and MO is significantly different ($P = 0.05$).

Because sire breed effects were generally larger than breed composition group (data not listed), we concentrated on sire breed with regards to performance differences. Significant pair-wise sire breed differences were observed in BCS, with 3.19 for HO and 3.53 for MO ($P = 0.05$); for the same breed comparison, fly scores were 0.94 for MO and 1.86 for HO ($P = 0.02$). Thermal imaging revealed VR cows displayed cooler leg temperatures (29.16°C) than the 32.49°C observed for NO ($P = 0.03$).

The importance of BCS spans several domains, including reproductive performance, milk production, and health and longevity, where deviations from optimal BCS can lead to diminished fertility, increased disease susceptibility, and adverse milk production outcomes. Consistent with Gallo et al. (2017), our findings placed Montbéliarde, a breed with a Simmental background, at the highest BCS (3.53). Our study aligns with Hazel et al. (2013) and further literature by Pereira et al. (2022) and

Piazza et al. (2023), showing MO and crossbreds generally exhibiting higher BCS compared to pure HO.

The results for pdNDF showed that NO was significantly different (P <0.02) from HO, MO and JES, suggesting that NO cattle exhibited lower NDF digestibility compared to the other breed compositions. NO also had significantly higher leg temperature (P = 0.034) than VR, and thermal imaging studies by Hardie (2016) and Montanholi et al. (2008, 2010) linked lower surface temperatures to improved feed efficiency. These results could indicate that NO -sired cows had lower feed efficiency but given the relatively small sample size, we are cautious about drawing firm conclusions in regard to sire breed or breed composition group. The fecal sampling and thermal imaging results do, however, suggest that both these novel traits could be valuable tools to monitor pasture performance and can be collected with relative ease.

Beecher et al. (2014) reported on digestibility and feed conversion efficiency across dairy cow genotypes, particularly highlighting significant differences between JE and HO cows. They observed higher digestibility rates in JE for dry matter (80.6%), organic matter (81.7%), nitrogen (82.4%), neutral detergent fiber (81.0%), and acid detergent fiber (74.4%) compared to HO (dry matter = 78.8%; organic matter = 79.5%; nitrogen = 79.8%; neutral detergent fiber = 78.6%; acid detergent fiber = 70.5%). However, our findings did not reflect significant differences in pdNDF between HO-sired (HOS) and JE-sired (JES) cows, indicating potential discrepancies and suggesting the influence of factors beyond mere genetic makeup. Additionally, our cows were JE-sired as opposed to purebred JE.

Milk yield (Table 4-3) was significantly different ($P = 0.06$) across sire breeds, where HOS showed the highest yield (21.01 kg) while JES showed the lowest (14.53 kg). Milk protein yield was not affected by breed composition ($P > 0.10$). In the pairwise comparison of milk component percentages, the Viking Red-sired (VRS) cows had a significantly higher milk fat percentage than the HOS cows ($P = 0.02$). Additionally, the MOS cows exhibited a significantly higher protein percentage compared to the HOS cows ($P < 0.001$). SCS also showed significant variation ($P = 0.01$) across sire breed groups, with Normande sired (NOS) having the lowest (1.36) and MOS and JES having the highest amount (4.37).

Consistent with findings from Houdek et al. (2024), our study noted that HOS generally had higher milk yield compared to JES. Ormston et al. (2022) observed that HO × JE crossbreds produced milk with higher fat, protein, and casein contents than pure HO. In our analysis, JES demonstrated higher fat (4.12% for JES vs. 3.51% for HOS, $P = 0.09$). These results were consistent with Coffey et al. (2017), who found that JE × HO cows had higher fat percentage (5.13% versus 4.58%) and protein percentage (3.80% versus 3.60%) than purebred HO cows. that JE × HO cows had higher fat percentage (5.13% versus 4.58%) and protein percentage (3.80% versus 3.60%) than purebred HO.

For BCS and Fly score the overall p-value are not significant (0.32 for BCS and 0.36 for Fly score) due to the analysis being caught up comparing NO, VR, and JE which are mostly identical. However, when we focus on conducting a pairwise comparison of HO and MO, the breed compositions are significantly different ($P = 0.05$ for BCS and $P = 0.05$ for Fly score).

Generally, correlations of grazing behavior with performance traits were not significant (Table 4-4). Cows with higher yield generally exhibited numerically increased grazing and rumination times, with a significant correlation observed between protein production and grazing time (r = 0.55) (P = 0.053). Similarly, a study from Iqbal et al. (2023) with grazing dairy cows (HO, JE, crossbreed HO x JE) reported a weak to moderate positive correlation between the time spent grazing and the animal production traits (milk yield (r = 0.34), fat (r = 0.43), protein (r = 0.22)). The study also discovered a strong positive correlation between rumination time and production traits (milk yield (r = 0.64), fat (r = 0.57), protein (r = 0.52)). The data in Table 4-4, along with the literature studies presented, suggest that cows with higher protein yield allocate more time to grazing in order to fulfill their nutritional requirements. This increase in grazing is associated with extended rumination time, as more grazed forage leads to prolonged digestion processes.

The idea that feed efficiency observations on pastured cows could be evaluated with relatively simple techniques is supported by a study from Løvendahl et al. (2018), who highlighted significant breed composition differences in feed efficiency traits, such as RFI, among HO, JE, and HO × JE cows. Their study indicates that direct observation of grazing behavior and automated recording devices like AfiCollar (Afimilk, Kibbutz Afikim, Israel) (https://www.afimilk.com/) are feasible for collecting feed efficiency data, with grazing time explaining up to 0.32% of the variance in milk yield and 0.49% in milk fat, while rumination time explained up to 6.73% of the variance in milk fat and 6.53% in milk solids.

CONCLUSIONS

This preliminary study provided insight into how breed composition can impact milk production and grazing behavior of pasture-based dairy cows. While we did not find significant differences in hourly grazing and rumination time across different breed compositions, differences emerged in grazing time per day with ProCross cows spending less time grazing compared to GrazeCross, suggesting differences in behavioral patterns potentially linked to genetic makeup. Our study on dairy cow grazing and daily behavior patterns supports observations from existing literature into the natural animal circadian rhythms and the impact of farm routines on cow activities, highlighting two pronounced peaks in grazing activities and a consistent evening peak in ruminating behavior across various breed composition. This study demonstrates consistent behavior across breed groups and highlights that farm routines such as milking can disrupt cow behavioral patterns. Understanding behavior patterns could help develop management protocols that do not interfere with a cow's natural routines, which could increase cow performance and comfort.

ACKNOWLEDGMENTS

This work was supported by the USDA-NIFA-OREI competitive grant no. 2016-51300-25862 to the last author. The authors have not presented any conflict of interest.

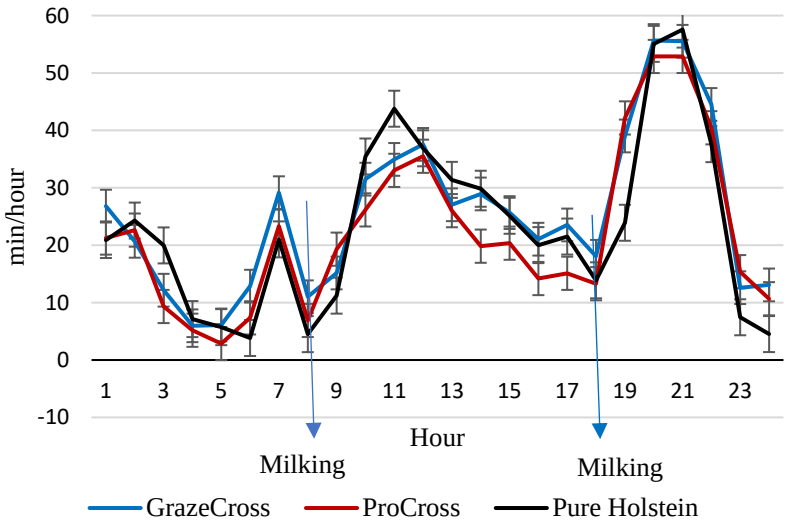

Figure 4-1: Hourly grazing patterns for pure Holsteins, GrazeCross, and ProCross cows

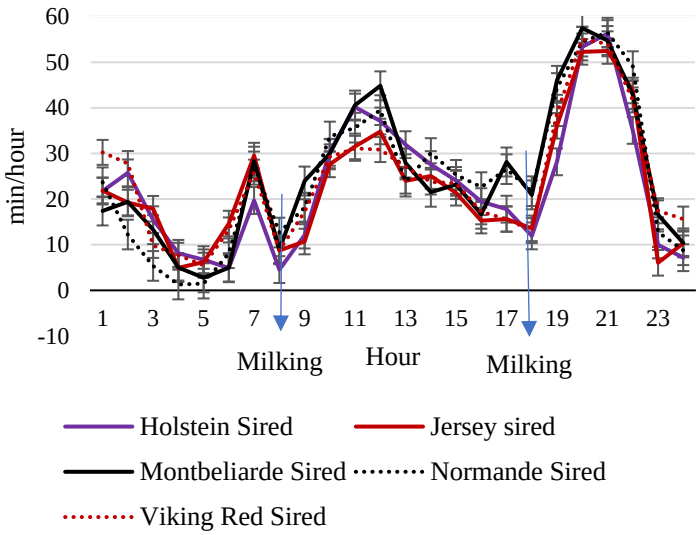

Figure 4-2: Hourly grazing patterns for Holstein, Jersey, Montbéliarde, Normande, and Viking Red sire groups

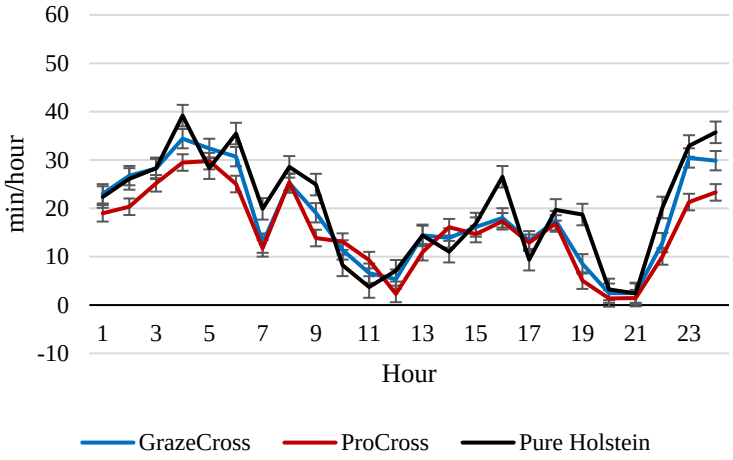

Figure 4-3: Hourly ruminating patterns for pure Holstein, GrazeCross, and ProCross cows

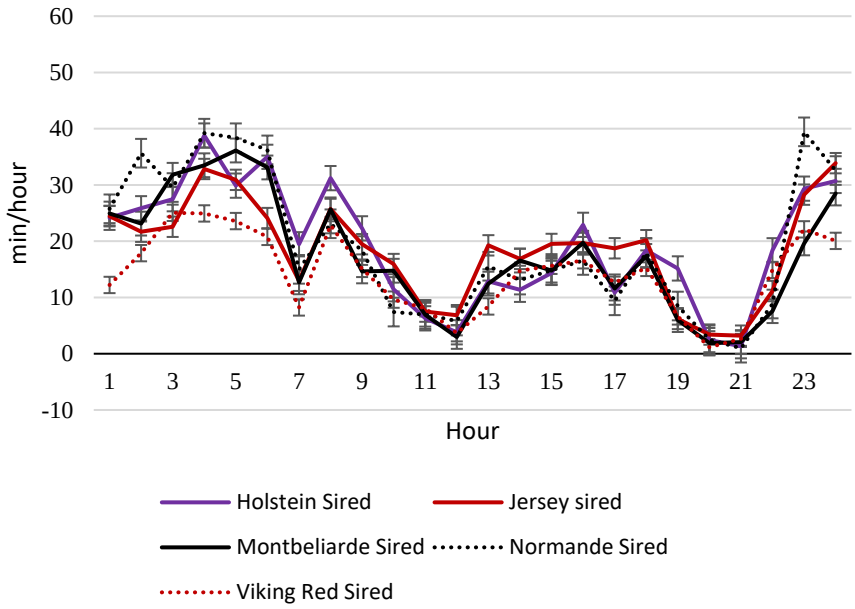

Figure 4-4: Hourly ruminating patterns for Holstein, Jersey, Montbéliarde, Normande, and Viking Red sire groups

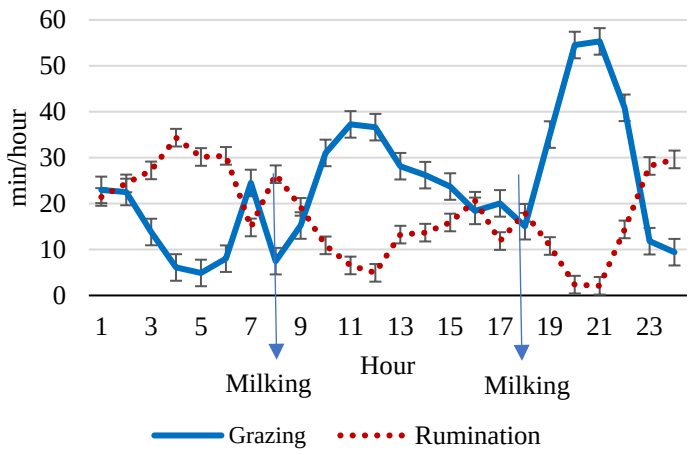

Figure 4-5: Average hourly grazing and rumination patterns across all breed composition groups

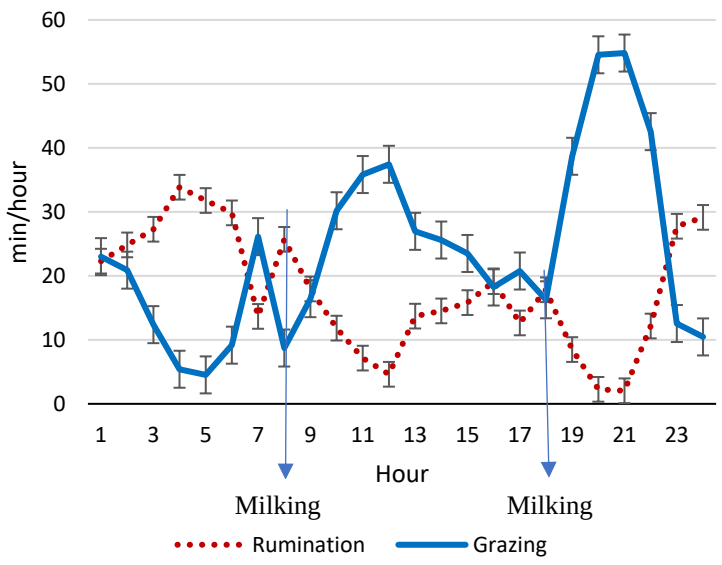

Figure 4-6: Average hourly grazing and rumination patterns across all sire breeds

Table 4-1: Least square means of 24-hour rumination time, grazing time and other behavior time by breed composition group[1]

Behavior	HO	PC	GC	P
Rumination (min/day)	465.9±33.47	398.6±36.56	427.2±57.73	0.62
Grazing (min/day)	548.9±29.42[a,b]	501.5±18.69[a]	574.1±17.23[b]	0.05
Other (min/day)	405.3±62.84	494.0±39.80	404.6±36.46	0.27

[1]HO=Holstein, PC = ProCross, GC = GrazeCross

Note: Different superscript letters (a, b) indicate significant differences between groups at $P < 0.10$.

Table 4-2: Least square means of 24-hour rumination time, grazing time and other behavior time by sire breed group[1]

Behavior	HOS	JES	MOS	NOS	VRS	P
Rumination (min/day)	474.09±42.97	432.05±52.63	420.04±52.63	463.35±52.63	357.37±37.57	0.36
Grazing (min/day)	531.61±27.46	531.46±33.63	529.55±33.63	616.89±33.63	523.04±24.32	0.29
Other (min/day)	404.46±48.10	434.05±58.91	454.60±58.71	336.44±58.91	512.96±42.10	0.25

[1]HOS=Holstein sired, MOS=Montbéliarde sired, NOS=Normande sired, VRS=Viking Red sired, JES=Jersey sired

Table 4-3: Sire breed least-squares-means for body condition score (BCS), fly score, surface temperature, potentially degradable NDF (pdNDF), and milk production and composition.

	Sire Breed[1]					
	HOS	JES	MOS	NOS	VRS	P-value
BCS (n)[2]	3.19±0.11[a]	3.46±0.18[a,b]	3.53±0.12[b]	3.43±0.10[a,b]	3.42±0.13[a,b]	0.32
Fly Score (%)[3]	1.86±0.26[a]	0.96±0.38[b]	0.94±0.27[b]	1.33±0.25[a,b]	1.03±0.31[b]	0.36
Leg temperature (°C)	30.16±1.00[a,b]	29.57±1.53[a,b]	30.70±1.15[a,b]	32.49±0.98[a]	29.16±1.25[b]	0.18
Rump temperature (°C)	37.10±1.11	34.03±1.69	35.01±1.27	35.94±1.09	34.33±1.37	0.93
Flank temperature (°C)	36.47±1.20	35.66±1.84	35.75±1.38	34.55±1.18	34.55±1.49	0.90
pdNDF (%)	9.79±1.08[a]	9.91±1.64[a]	11.31±1.23[a]	14.56±1.06[b]	12.92±1.34[a,b]	0.02
Milk (kg/d)	21.01±1.67[a]	14.53±2.70[b]	17.97±1.93[a,b]	17.63±1.82[a,b]	18.72±2.21[a,b]	0.59
Fat (kg/d)	0.73±0.08	0.64±0.12	0.73±0.09	0.67±0.08	0.81±0.10	0.48
Protein (kg/d)	0.58±0.05[a,b]	0.44±0.08[a]	0.61±0.06[b]	0.53±0.06[a,b]	0.55±0.07[a,b]	0.56
Fat (%)	3.51±0.17[a]	4.12±0.28[b]	4.22±0.20[c]	3.81±0.19[a,b,c,d]	4.26±0.23[d]	0.31
Protein (%)	2.75±0.11[a]	2.96±0.17[a,c]	3.44±0.12[b]	3.02±0.12[c]	2.96±0.14[a,c]	0.01
MUN (mg/dl)[4]	13.03±0.73[a,b]	14.14±1.18[a,b]	13.96±0.85[a]	12.02±0.80[b]	14.47±0.97[a]	0.40
SCS (n)[4]	3.03±0.72[a,b]	4.17±1.16[a]	4.38±0.83[a]	1.36±0.78[b]	3.26±0.95[a,b]	0.06

[1]HOS=Holstein sired, MOS=Montbéliarde sired, NOS=Normande sired, VRS=Viking Red sired, JES=Jersey sired
[a,b,c,d]Different superscript letters within rows indicate significant differences between groups at $P < 0.10$.
[2]BCS scale 1-5 (Wildman et al., 1982) [3]Fly score scale 1-4 (Basiel et al., 2021)
[4]MUN = milk urea nitrogen; SCS = somatic cell score.

Table 4-4: Correlations between rumination, grazing, and other time and production and performance of grazing dairy cows

	----------(min/day)----------		
	Rumination	Grazing	Other
Milk Production (kg/d)	0.34	0.43	-0.46
Milk Fat Yield (kg/d)	0.22	0.31	-0.32
Milk Protein Yield (kg/d)	0.33	0.55*	-0.52
Milk Fat (%)	-0.30	-0.20	0.28
Milk Protein (%)	-0.22	0.14	0.05
MUN (mg/dl)	-0.37	-0.26	0.35
SCS (n)	-0.05	-0.35	0.17
Thermal Image Leg (ºC)	0.11	0.65	-0.43
Thermal Image Rump(ºC)	-0.52	-0.06	0.39
Thermal Image Flank (ºC)	-0.22	0.01	0.16
Fly Score (%)	0.24	-0.27	0.01
BCS (n)	0.14	-0.39	0.08
pdNDF (%)	-0.49	0.03	0.35

*$P < 0.10$.

Note: BCS = body condition score, pdNDF= potentially degradable NDF, SCS = somatic cell score, MUN = milk urea nitrogen

Chapter 5

Contrast of immune cell lineage, hair, dam and pooled colostrum genotypes in a newborn calf

ABSTRACT

Immune cells in a neonatal bovine could originate from the calf itself, maternally derived cells, or cells absorbed from colostrum. Blood samples are often used to determine telomere length (TL) because they are relatively easy to obtain in a minimally invasive manner, but blood contains a mix of cell types. When collecting blood samples from very young animals to determine TL, it's important to consider that immune cells might not be produced by the animal itself but could be absorbed from an external source, potentially biasing the results. The objective of this study was to compare the genotypes of immune cells of varying lineage in a one-day-old calf with genotypes obtained from its own hair sample, dam hair sample, and the consumed colostrum which was pooled from multiple cows. Polymorphonuclear cells (PMN) were separated from peripheral blood mononuclear cells (PBMC) with PBMC cells further separated into T-cell, B-cell, and monocyte populations with flow cytometry. Samples were subsequently genotyped for 139,376 single nucleotide polymorphisms (SNPs). We assumed the calf hair sample represented the true genotype of the calf and employed chi-squared tests to examine differences in hair and other genotypes. The call rate for calf genotypes ranged from 96.2% for T-cells to 98.6% for hair and PMN. Analysis of the cell lineage genotypes

demonstrated a generally high concordance with the assumed true genotype, although there were mismatches of 2, 37, 56, and 656 loci with the hair genotype in the PMN, B-cell, monocyte, and T-cell populations, respectively. Notably, the T-cell genotype exhibited higher heterozygosity (33.9%) compared to other cell types (33.0%), and only the T-cell genotype showed significant differences from the hair genotype ($P < 0.05$). The colostrum sample displayed a low call rate (78.3%) and a high level of heterozygosity (65.4%). Further analysis of the non-concordant T-cell genotypes revealed that 82 matched the dam genotype, 147 matched the colostrum genotype, and 105 matched both. Among the 322 genotypes that did not match either source, 70 lacked a colostrum genotype and there were 241 instances where the T-cell genotype was heterozygous, while the dam and colostrum genotypes were homozygous for opposing SNPs. Colostrum and T-cell genotypes were consistent with DNA contamination, as evidenced by elevated non-call rates, heterozygosity, and discrepancies with the assumed true genotype. Despite the apparent effects of genotype contamination on T-cell genotypes, the overall concordance with hair was 96.2% and T-cells contribute a small amount of the total DNA from blood samples. Therefore, immune cells isolated from the peripheral blood of newborn calves should provide mostly accurate genotypes with a small percentage of incorrect genotype calls due to contamination from colostrum cells.

INTRODUCTION

Passive immunity from colostrum immunoglobulin absorption provides the primary pathogen defense for newborn calves (Chase et al., 2008; Vlasova and Saif, 2021). Innate immune and adaptive immune cells also work together to provide immunity (Marshall et al., 2018) and have multiple sources of origin, including production directly by the calf, or absorbed through colostrum (Chase et al., 2008; Vlasova and Saif, 2021). Maternal microchimerism, where maternal cells transfer to the fetus during gestation, may play a role in neonate immune function in humans (Borges et al., 2023). This appears to be an unlikely occurrence in cattle and other ruminants due to a less invasive placenta (Gash et al., 2019), though exceptions are known to occur with cloning (Hiendleder et al., 2004).

The innate immune system provides first line of cell mediated defense and includes neutrophils, macrophages, dendritic cells, natural killer (NK) cells, and innate lymphoid cells (ILCs) which are involved in the recognition and elimination of pathogens (Turvey, and Broide, 2010). On the other hand, the adaptive immune system is characterized by the ability to recognize and remember specific pathogens and includes T-cells and B-cells, which are involved in cellular and humoral immunity, respectively (Bonilla and Oettgen 2010).

Absorption of immune cells through colostrum or maternal microchimerism could result in calf genotyping errors and bias newborn TL estimates. Therefore, the objective of our study was to explore whether immune cells originated from a day-old calf's own immune system, the dam, or were absorbed through colostrum by contrasting immune

cell genotypes with those from the calf's hair, dam, and colostrum fed to the calf that was pooled from multiple cows prior to feeding.

MATERIALS & METHODS

Data

DNA was extracted from a hair sample of the calf, a hair sample from her dam and from the colostrum fed to the calf which was pooled from several cows and frozen before feeding. The colostrum fed to the calf did not include the dam's colostrum. Common protocol for PSU colostrum feeding consists of testing the colostrum obtained at milking from cows from Pennsylvania State University dairy and pooled by source into large batches, tested for IgG to meet quality thresholds, and then frozen. Calves are then fed thawed colostrum (Lopez et al., 2020).

Samples preparation and genetic analysis

Approximately 15 ml of blood was collected from the jugular vein of the subject into EDTA vacutainer tubes and separated into cell lineages using methods we have previously employed (Vasudevan et al., 2017). Briefly, the blood was diluted in a 1:1 ratio using 1X PBS-EDTA solution and was kept on ice to prevent T-cellular degradation. Polymorphonuclear cells (PMN) were separated from peripheral blood mononuclear cells (PBMC) over a Ficoll gradient using Ficoll Paque Plus (GE Healthcare Life Sciences, Chicago, Illinois). Borosilicate tubes were prepared, each containing 3 ml of Ficoll Paque Plus solution, and the diluted blood was gently layered on top of the

Ficoll solution in the tubes. The tubes were centrifuged at 1800 RPM for 30 minutes at 25° C to separate peripheral blood mononuclear cells (PBMCs) from the PMN fraction. The PBMC and PMN fractions were collected in separate 50 ml conical tubes containing 15 ml of cold 1X PBS-EDTA.

Antibodies for B-cells (mouse anti Bovine CD21; BioRad, Hercules, California, USA), monocytes (mouse anti-human CD14; BioRad, Hercules, California, USA), and T-cells (mouse anti-human CD3; Thermo-Fisher, Waltham, MA USA) that were validated for use in bovine cells and conjugated to PE (phycoerythrin), PE-AF750 (phycoerythrin-alex Fluor 750), and FITC (fluorescein isothiocyanate), respectively, were used to facilitate cell separation by flow-cytometry. PBMC were divided into 5 aliquots containing between 5 and 20 million cells per 1 ml tube. The negative control did not contain antibodies, one aliquot included all three antibodies, and the remaining three aliquots each included a single antibody.

A Pearson chi-squared test was used to determine whether there is a statistically significant difference between the expected frequencies and the observed frequencies of genotypes for each cell type (B-cells, T-cells, PMN and monocytes), and assuming that the hair sample represented the calf's true genotype.

The calf genotype was analyzed using Neogen’s GeneSeek Genomic Profiler (GGP) Bovine 150K, provided by Neogen Corporation (2020).

RESULTS AND DISCUSSION

A total of 140,668 SNPs were distributed across all autosomes and the X chromosome. Among hair genotypes, 136,623 were found to match with the B-cell sample, 133,039 with the T-cell sample, 136,587 with the monocytes sample, and 137,288 with the PMN sample (Table 5-1). The analysis excluded SNPs from the mitochondrial genome and the Y chromosome. The call rate for calf genotypes ranged from 96.2% for T-cell to 98.6% for hair and PMN, whereas the dam hair sample was 98.5% and the colostrum sample was 78.3%. Table 5-1 contrasts the genotypes called for the calf's T-cell, B-cell, monocytes, PMN, and hair. On the diagonal are the number of not called SNPs, above the diagonal are the number of SNP with the same genotype call as the calf's hair, and below the diagonal are the number of SNP with a mismatched genotype. T-cells (5237) had more non-calls compared to the other cell types, ranging from 2042 for PMN to 2261 for monocytes. Cell lineage genotypes mostly returned high concordance with the assumed true genotype, with 2, 37, 56, and 656 mismatches of hair genotype with PMN, B-cell, monocyte, and T-cells, respectively. Only the T-cell genotype was different from the hair genotype ($P < 0.05$).

The frequency of homozygous and heterozygous genotypes when T-cells deviated from the hair genotype is shown in Table 5-2. In each case, one allele matched the calf's hair genotype, and in 22 cases, the hair genotype was heterozygous, while the T-cell genotype was homozygous.

Mismatched genotypes for T-cells were analyzed to determine how many of these were identical to the dam, the colostrum, both, or neither. Forty-nine percent of the T-cell mismatches differed from both dam and colostrum (322 observations), 22% were

identical to the colostrum, 16% were in common with both dam and colostrum, while 13% were identical to the dam. Seventy of the genotypes that did not match the dam or colostrum were missing a colostrum genotype; of the remaining 252, nearly all (246) had a heterozygous T-cell genotype but a homozygous genotype for hair that matched the colostrum and dam genotype.

Heterozygosity was higher for the T-cell genotype (33.9%) than for the other cell types (33.0%). The colostrum sample had a low call rate (78.3%) and a high degree of heterozygosity (65.4%). The higher non-call rate, higher rate of mismatches, and a greater degree of heterozygosity could indicate that the calf's T-cell population was partly derived from cells acquired from colostrum.

The combination of high heterozygosity and elevated non-called rate is evidence of DNA contamination. In our study, the potential mixing of DNA from multiple animals in the pooled colostrum sample could act like DNA contamination. For this reason, we will refer to the sample contamination as genotype contamination to distinguish it from typical sources of contamination from sample handling, etc. Calf genotype contamination can occur when external DNA is introduced to the calf’s system through colostrum ingestion or maternal microchimerism. DNA contamination can lead to reduced call rates in genotyping assays (Zajac et al., 2019). Contamination can also skew allelic ratios and lead to an overestimation of heterozygosity (Frantz et al., 2003; Pedersen and Quinlan, 2017; Zajac et al., 2019). This can have implications for assessing genetic diversity and population structure, as well as identifying inbred individuals or populations. The colostrum sample is expected to have a high degree of DNA contamination as it was pooled from multiple cows; thus, the high degree of heterozygosity and lower call rates

are not unexpected. The higher heterozygosity and lower call rate of the T-cell sample coupled with a higher mismatch rate with the hair genotype likely reflects a low-level degree of DNA contamination from cells ingested from colostrum and, less likely, maternally derived cells.

The occurrence of fetal microchimerism with fetal DNA detectable in maternal blood or tissues has been demonstrated in cattle (Lemos et al., 2011). However, the natural occurrence of maternal microchimerism is not well established. Hiendleder et al (2004) reported the presence of surrogate dam mitochondrial DNA in fetal blood following cloning and in-vitro fertilization (IVF), but not multiple ovulation and embryo transfer. The authors attributed the maternal microchimerism to placental disruptions known to occur with bovine cloning and IVF. Evidence of fetal microchimerism was demonstrated following cloning in goats, but not natural mating and neither cloning nor natural mating resulted in detectable maternal microchimerism (Gash et al., 2019). We observed 44 instances where the T-cell genotype matched the dam genotype and there was a called colostrum genotype. Almost all were associated with a heterozygous T-cell genotype (43), and in 10 of those cases, the hair genotype and colostrum genotype were homozygous for opposite alleles. The remaining could provide some evidence of maternal microchimerism but are more likely the result of the effects of DNA contamination on genotype calls as discussed above.

CONCLUSIONS

To summarize, genotypes from different cell lineages were nearly identical to those obtained from hair, suggesting blood from newborn calves will result in minimal genotyping errors. However, T-cell genotypes were more likely to not be called and significantly different than expected when compared to the assumed true genotype, suggesting some degree of DNA contamination from colostrum.

ACKNOWLEDGMENTS

This work was supported by the US-Israel Binational Agricultural Research and Development Fund # US-5000-1 to the last author. The authors have not presented any conflict of interest.

Table 5-1: The number of non-called genotypes (diagonal), concurrent genotypes (above diagonal), and mismatched genotypes (below diagonal) from hair, B-cell, T-cell, monocyte, and PMN samples in a day-old calf

	Hair	B	T	M	PMN
Hair	2049	136623	133039	136587	137288
B	37	2244	133375	136867	136633
T	656	609	5237	133383	133045
M	56	37	580	2261	136594
PMN	2	33	653	51	2042

Table 5-2: Number of genotypes from PMN, B-cell, monocyte, and T-cell populations that are different from hair genotype including the total number, the number where one allele was identical to the hair genotype, the number that are heterozygous or homozygous

	total mismatched alleles	N 1 allele identical to hair	N homozygous	N heterozygous
B	37	37	6	31
T	656	656	22	634
M	56	56	9	47
PMN	2	2	0	2

Chapter 6

Conclusions and future directions

RESULT HIGHLIGHTS

Telomere Length

- Moderate heritability
- Potential role as biomarker for cow longevity in dairy genetic index based on TL association with cow and heifer livability
- Breed composition differences with Montbéliard showing the longest telomere length
- Potential small bias in collecting TL data from calves due to colostrum absorption

Breed composition differences in pasture behavior

- Clear grazing and ruminating hourly and daily patterns determined, with two distinct peaks in grazing (around 7 a.m. and 8 p.m.) and a consistent evening peak in rumination (around 11 p.m.) across breed composition
- No substantial breed composition differences in behavior

CONCLUSIONS

TL is a polygenic trait with moderate heritability in both cows and heifers with significant differences among breeds. Cows with longer telomeres also had genetic merit for longer livability. These results support existent literature as Seeker et al. (2018) and Ilska-Warner et al. (2019) reported moderate heritability estimates for TL in dairy cattle. Furthermore, our results align with Brown et al. (2012) and Laubenthal et al. (2016), who established the association between TL and longevity-related traits, including productive lifespan and survival. The significant association between longer TL and Montbéliarde-sired cows aligns with the improved survival rates in Montbéliarde crossbreds observed in Hazel et al. (2014) and Heins et al. (2012), suggesting that the longevity advantages of Montbéliarde-sired cattle may be partially explained by their longer telomeres. Most telomere length evaluations rely on blood samples, which contains many different cell types and can be contaminated with cells ingested from colostrum for newborn calves. We demonstrated that genotypes from different cell lineages gave similar results to genotypes from hair samples with some exception for T-cells.

Our findings demonstrated clear grazing and ruminating hourly and daily patterns, with two distinct peaks in grazing (around 7 a.m. and 8 p.m.) and a consistent evening peak in rumination (around 11 p.m.), with no substantial differences across breed. These patterns highlight the natural circadian rhythms of cows, which are strongly influenced by farm routines such as milking schedules. These patterns are consistent with the findings of Rosa et al. (2024), who identified similar behavior patterns in their study of grazing systems. Furthermore, novel tools such as resistance to fly infestation, potentially

degradable neutral detergent fiber (pdNDF), and heat dissipation have potential to enhance selection programs for performance of grazing cows but need further development. Understanding breed composition differences could impact genetic and breeding strategies aimed at optimizing grazing efficiency. This dissertation aimed to fill this gap by investigating novel tools such as TL, fly resistance, pdNDF and thermal images and investigate the breed composition differences and whether these differences are a significant factor influencing grazing and ruminating behavior.

Overall, integrating TL into genetic selection indices has the potential to enhance herd health and reduce premature culling, contributing to the sustainability and prosperity of dairy farming. However, the practical implementation of this approach should be rigorously validated through further research. Understanding cows daily grazing and rumination behavior and how to implement farm management to exploit the natural peak can improve the animal feed efficiency by allowing a more complete utilization of nutrients and consequently generate economic growth by incrementing animal's milk yield. However, further research is required to validate this approach.

FUTURE PROSPECTIVES

Selecting for longer telomere length (TL) in cattle could enhance longevity and overall health. Longer TL has been associated with increased survival, productive lifespan, and resilience. Incorporating TL into breeding programs would lead to genetically predisposed cattle living longer, facing fewer health issues, and remaining productive for a more extensive portion of their lives, reducing the rate of health-related culling. To implement TL into selection programs, TL measurements must be incorporated into elite herd genomic evaluations. Practical steps would include the collection of blood from cattle owned by genetic companies for by TL determination using qPCR. This data can then be used to develop genomic predicted transmitting abilities for TL, similar to those already in place for milk yield or reproductive performance.

Aligning farm practices with cows' circadian rhythms for grazing and rumination could enhance feed efficiency, milk yield, and overall cow health by allowing the animals to graze and ruminate more efficiently. Incorporating novel tools like fly resistance, pdNDF, and heat dissipation, alongside routine traits such as BCS, into pasture-based system research is feasible and will help provide insight into optimizing animal performance on pasture. Selecting cows with lower pdNDF enhances nutrient absorption through more efficient digestion. Improving fly resistance will reduce animal stress, while minimizing disease transmission and blood loss, consequently ensuring a higher amount of nutrients toward milk yield. Furthermore, the measurement of heat dissipation identifies animals with superior metabolic efficiency, enabling better adaptation to the environment and maximizing energy allocation for milk yield.

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VITA

Tanya Muratori

EDUCATION

PhD in Animal Science, The Pennsylvania State University, University Park, PA, 2024
MSc in Safety and Quality in Animal Production, The Alma Mater Studiorum University of Bologna, Bologna (Italy), 2016
BS in Animal Production and Control of Wildlife, The Alma Mater Studiorum University of Bologna, Bologna (Italy), 2013
Minors: Animal Production

PROFESSIONAL EXPERIENCE

Graduate Research Assistant at Penn State, Advisor: Chad Dechow, 2018 - 2024
Food Safety & Quality Control Manager: C.R.M. srl Address: Via del Mercato, 67-41122 Modena (MO) (Italy), 2017 - 2018
Food Safety & Quality Control Manager: Fattoria San Rocco di Filippini Adolfo & Gorgio ss. Address: Via per Recovato n°102 – 41013 Castelfranco Emilia (MO) (Italy), 2017
Food Safety & Quality Control Manager: IAS srl - Stella 81. Address: Via Marconi 23, 29010 Alseno (PC) (Italy), 2016
Curricular Traineeship: Spalanzani salumi di Passini Giuseppe e c. snc. Address: 110 Piazza Cooperazione, 41058 Vignola, (MO) (Italy), 2014 – 2015
Curricular Traineeship: INFA National Institute of Artificial Insemination. Address: Via Gandolfi 16, 40057, Cadriano (BO) (Italy), 2012 – 2013

HONORS AWARD AND PUBLICATIONS

Obie and Mary Ann Snider Scholarship in Dairy and Animal Science, 2021 – 2022
Graduate Student Poster Competition NE ADSA, 3rd place, 2021
3 Minute Thesis Competition ADSA, 2nd place, 2020
Gamma Sigma Delta Honor Society of Agriculture, member, 2020 – Present.
Genetic aspects of haematological parameters in Italian Large White pigs; P-067 on the Italian Journal of Animal Science 2015; volume 14: supplement 1.

MANUSCRIPTS IN PREPARATION

Muratori, T., T. Ott, A. Shabtay, M. Cohen-Zinder, and C. Dechow. 2024. Contrast of immune cell lineage, hair, dam and pooled colostrum genotypes in a newborn calf. Livestock Science
Muratori, T., Heins, B. J., Soder, K., & Dechow, C. 2024. Grazing and rumination patterns in a mixed breed organic herd.