UNIVERSIDADE FEDERAL DE VIÇOSA

Integrated approaches to dairy cow performance: nutrition, reproduction, and production systems

Kellen Ribeiro de Oliveira
Doctor Scientiae

VIÇOSA - MINAS GERAIS
2026

KELLEN RIBEIRO DE OLIVEIRA

Integrated approaches to dairy cow performance: nutrition, reproduction, and production systems

Thesis submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Polyana Pizzi Rotta

VIÇOSA - MINAS GERAIS
2026

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T
O48i
2026

Oliveira, Kellen Ribeiro de, 1997-
Integrated approaches to dairy cow performance: nutrition, reproduction, and production systems / Kellen Ribeiro de Oliveira. – Viçosa, MG, 2026.
1 tese eletrônica (135 f.): il. (algumas color.).

Texto em inglês.
Inclui anexo.
Orientador: Polyana Pizzi Rotta.
Tese (doutorado) - Universidade Federal de Viçosa, Departamento de Zootecnia, 2026.
Inclui bibliografia.
DOI: https://doi.org/10.47328/ufvbbt.2026.110
Modo de acesso: World Wide Web.

1. Bovinos de leite - Reprodução. 2. Bovinos de leite - Nutrição. 3. Bovinos de leite - Registros de desempenho. 4. Rúmen. I. Rotta, Polyana Pizzi, 1987-. II. Universidade Federal de Viçosa. Departamento de Zootecnia. Programa de Pós-Graduação em Zootecnia. III. Título.

CDD 22. ed. 636.2142

Bibliotecário(a) responsável: Alice Regina Pinto Pires CRB-6/2523

KELLEN RIBEIRO DE OLIVEIRA

Integrated approaches to dairy cow performance: nutrition, reproduction, and production systems

Thesis submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

APPROVED: February 27, 2026.

Assent:

Kellen Ribeiro de Oliveira
Author

Polyana Pizzi Rotta
Adviser

To my grandfather (in memorian), my person in the world.

ACKNOWLEDGMENTS

This work has been sponsored by the following Brazilian research agencies: Coordination for the Improvement of Higher Education Personnel (CAPES; Financing code 001), Minas Gerais State Foundation for Research Aid (FAPEMIG) and National Council of Scientific and Technological Development (CNPq).

This study was supported in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES), the National Council for Scientific and Technological Development (CNPq, 141066/2022-9), Lallemand Animal Nutrition, and Alta Genetics Brazil.

I am deeply grateful to the Universidade Federal de Viçosa, especially the Department of Animal Science, for fostering my passion for research and ruminant nutrition. UFV was my home for 10 years, and now it is time to simply express my sincere gratitude for everything it has given me.

First and foremost, I thank God for guiding my path. I also dedicate special thanks to my grandfather, Recenvindo Ribeiro (in memoriam), my angel, who inspired me throughout his life and helped cultivate my love for animals. To my mom, Flaviana, thank you for dreaming with me, for your unconditional love, and for all the sacrifices you made to make my academic journey possible. To my father, Robson, for his love, patience and for believing in me. To my entire family, thank you for your endless support.

I am profoundly grateful to my advisor, Polyana Rotta, who has been more than an academic mentor—she has been a source of friendship and inspiration. Thank you for continuously believing in me and in the power of education. Your guidance was essential throughout these nearly nine years. I also extend my gratitude to Professors Alex Lopes, Sebastião Valadares, Marcos Marcondes, and all the professors who guided me in the Departments of Veterinary Medicine and Animal Science.

I would also like to thank the University of Vermont and Professor João Costa for the opportunity to work with animal nutrition in the Green Mountain State while pursuing my dream of working with calves. Your support during this time was invaluable.

My sincere thanks to my committee members and co-authors – Tadeu da Silva, Roberto Sartori, Carlos Consentini, Tiago Ferreira, Hilário Mantovani, Lucas Mari, and Eric Chevaux – for the valuable experiences and for helping me grow as a researcher.

I am especially thankful to Luiz Eduardo for his patience, support, and emotional strength during the most challenging moments of this journey. I also express my appreciation to all the friends I made in Viçosa and Vermont – you made this path lighter and more meaningful.

To the students of Família do Leite, both undergraduate and graduate, thank you for your contributions. A special thank you to my interns – Camila, Vinicius, Guaxi, Daniel, Rafael, Mariana, Maria, Abraão, Laura, Motta, Robson, João Pedro, Mirella, Lívia, Bianca, Fernanda, and Marcos – and to my scholarship students – Poliana, Igor, Barbara, Carina, and João – your dedication and hard work were essential to this project.

To everyone who has been part of my journey at UFV and throughout my doctoral studies, thank you.

Last, but not least, I would like to express my sincere gratitude to all the animals who have contributed not only to this research, but to my journey and formation as a scientist. Working alongside you has shaped how I understand animal health, nutrition, welfare, and responsibility in dairy production. This thesis is also a reflection of what you have taught me.

Thank you all from the bottom of my heart.

ABSTRACT

OLIVEIRA, Kellen Ribeiro de, D.Sc., Universidade Federal de Viçosa, February, 2026. **Integrated approaches to dairy cow performance: nutrition, reproduction, and production systems**. Adviser: Polyana Pizzi Rotta.

Dairy cow performance in tropical production systems results from complex interactions among nutritional efficiency, postpartum health, reproductive management, and housing environment. Advancing sustainable dairy production therefore requires an integrated understanding of how these biological and management components influence productivity, resilience, and environmental outcomes. This thesis investigated key determinants of dairy system performance through four complementary studies combining controlled experimentation, large-scale retrospective analyses, and on-farm environmental assessment. The first study evaluated supplementation with the live yeast Saccharomyces cerevisiae CNCM I-1077 and demonstrated improvements in rumen degradability of fibrous feeds, enhanced nutrient utilization, and greater feed efficiency. These findings indicate that optimizing rumen kinetics can improve metabolic efficiency and help mitigate environmental stress without necessarily altering microbial diversity. The second study analyzed retained placenta in commercial dairy herds under tropical conditions using a large retrospective dataset. Retained placenta was associated with cows' breed, parity, and calving characteristics, and consistently resulted in reduced pregnancy per insemination, increased pregnancy loss, and greater risk of culling. These results confirm that postpartum disorders exert persistent effects on reproductive outcomes and herd longevity, highlighting the importance of preventive health strategies. The third study examined herd-level reproductive performance and showed that management intensity and housing system are major determinants of reproductive efficiency. More frequent reproductive monitoring was primarily associated with improved service rate and shorter intervals between inseminations, whereas compost barn and free stall systems had superior reproductive outcomes compared with dry lot systems. These findings emphasize that reproductive success in commercial herds is strongly related to management rather than fertility itself. The fourth study evaluated manure excretion dynamics in compost barn systems and demonstrated that the concentration of feces and urine within the bedding promotes composting and substantially reduces nitrogen requiring external treatment. This redistribution of nutrient flows enhances recycling efficiency and illustrates how housing design can simultaneously support environmental sustainability and animal welfare. Together, these studies demonstrate that improving dairy production in tropical environments requires a system-level approach integrating digestive efficiency, postpartum health, reproductive management, and production system design. Collectively, these findings highlight how nutrition, reproduction, management, and production system design shape productivity and sustainability in modern dairy systems.

Keywords: dairy systems; herd management; reproductive efficiency; rumen function; sustainability; tropical production

RESUMO

OLIVEIRA, Kellen Ribeiro de, D.Sc., Universidade Federal de Viçosa, fevereiro de 2026. **Abordagens integradas para o desempenho de vacas leiteiras: nutrição, reprodução e sistemas de produção**. Orientadora: Polyana Pizzi Rotta.

O desempenho de vacas leiteiras em sistemas de produção tropicais resulta de interações complexas entre eficiência nutricional, saúde pós-parto, manejo reprodutivo e sistema de produção. Assim, avanços na sustentabilidade e produtividade da pecuária leiteira dependem de uma compreensão integrada de como esses componentes biológicos e gerenciais influenciam simultaneamente a eficiência produtiva, a resiliência dos animais e impactos ambientais. Esta tese investigou temas-chave do desempenho de sistemas leiteiros por meio de quatro estudos complementares que combinaram experimentação controlada, análises epidemiológicas em larga escala e avaliações ambientais em nível de fazenda. O primeiro estudo avaliou a suplementação com a levedura viva Saccharomyces cerevisiae CNCM I-1077 e demonstrou aumento da degradabilidade ruminal de fibras, maior eficiência alimentar e melhor aproveitamento de nutrientes. Esses resultados indicam que a otimização da cinética ruminal pode aumentar a eficiência metabólica e atenuar efeitos ambientais adversos sem necessariamente alterar a diversidade microbiana. O segundo estudo analisou a retenção de placenta em rebanhos leiteiros comerciais sob condições tropicais utilizando banco de dados retrospectivo. A retenção de placenta foi associada a fatores de raça, paridade e características do parto, e apresentou efeitos consistentes na redução da prenhez por inseminação, aumento da perda gestacional e maior risco de descarte. Esses achados confirmam que distúrbios pós-parto exercem impactos persistentes sobre o desempenho reprodutivo e a longevidade produtiva das vacas, ressaltando a importância de estratégias preventivas de saúde. O terceiro estudo avaliou o desempenho reprodutivo retrospectivo em nível de rebanho e demonstrou que a intensidade do manejo e o sistema de produção são determinantes da eficiência reprodutiva. Maior frequência de monitoramento reprodutivo esteve associada principalmente ao aumento da taxa de serviço e à redução do intervalo entre inseminações, enquanto sistemas compost barn e free stall apresentaram melhores resultados reprodutivos quando comparados a sistemas de produção a céu aberto (dry lot). Esses resultados indicam que o sucesso reprodutivo em rebanhos comerciais é relacionado ao manejo, e não apenas pela fertilidade individual. O quarto estudo avaliou a dinâmica de excreção de nutrientes em sistemas compost barn e demonstrou que a compostagem reduz o nitrogênio que necessita de tratamento externo. Essa redistribuição do fluxo de nutrientes aumenta a eficiência de reciclagem e evidencia o papel do sistema de produção na promoção simultânea da sustentabilidade ambiental e do bem-estar animal. Em conjunto, os resultados demonstram que a melhoria do desempenho de sistemas leiteiros tropicais requer uma abordagem sistêmica que integre eficiência digestiva, saúde pós-parto, intensidade de manejo reprodutivo e sistema de produção. A integração entre nutrição, saúde, manejo reprodutivo e dinâmica ambiental evidenciada nesta tese reforça que a eficiência produtiva e a sustentabilidade dos sistemas leiteiros dependem de abordagens que considerem o sistema como um todo.

Palavras-chave: eficiência reprodutiva; função ruminal; manejo de rebanho; produção tropical; sistemas de produção de leite; sustentabilidade

SUMMARY

1. INTRODUCTION

Dairy production requires a combination of nutrition, reproduction, and management that results in biological efficiency and farm sustainability. Cows' performance cannot be understood through isolated physiological processes, but rather as the result of interconnected metabolic, reproductive, and management-driven mechanisms acting across multiple biological scales. Consequently, improving dairy system efficiency demands a multidimensional perspective that links rumen function, health, reproductive performance, and housing strategies.

At the nutritional level, dairy systems depend on fibrous feed, making the efficiency of fiber utilization a key determinant of productivity. Strategies that enhance ruminal fermentation may therefore help improve milk production (DEVRIES AND CHEVAUX, 2014), as well as ruminal and metabolic stability. Supplementation with *Saccharomyces cerevisiae* (SC), a live eukaryotic yeast, helps maintain ruminal pH, prevents metabolic disturbances, and serves as a growth factor for rumen bacteria, mainly cellulolytic bacteria (AMIN AND MAO, 2018), ultimately leading to improved nutrient use efficiency (AROWOLO AND HE, 2018) and fiber digestibility (JIANG *et al*., 2017). Because rumen function is linked to systemic metabolism, improvements in digestive efficiency may influence health and reproductive outcomes, particularly during early lactation, when cows face their greatest physiological challenges.

The transition period represents a critical biological window linking nutrition to reproduction (GRUMMER, 1995). Metabolic imbalances during this phase are strongly associated with postpartum disorders such as retained placenta (RP; LEBLANC, 2008), which impact health and future reproductive performance (VALLEJO-TIMARAN *et al*., 2021; TONI *et al*., 2015). These effects extend beyond individual cows, as prolonged days open and reduced reproductive efficiency directly impact herd longevity and profitability (LOUCA AND LEGATES, 1968; SASAKI *et al*., 2020).

Beyond individual physiology, reproductive success in tropical dairy herds is also influenced by management intensity and environmental conditions. Heat stress, common in tropical climates where the temperature–humidity index frequently exceeds stress thresholds, interferes with estrus expression, embryo survival, and metabolic adaptation. Additionally, differences in breed composition, management frequency, and reproductive monitoring strategies can affect fertility outcomes across herds (OLIVEIRA *et al*., 2019; CAMARGO *et al*., 2007). These factors highlight that reproductive performance results not only from cow biology but also from herd-level decision-making (DERKS *et al*., 2013; RITTER *et al*., 2021).

Housing and production systems integrate the biological and environmental aspects of dairy production. Compost barn systems are associated with greater cow comfort due to unrestricted lying space, reduced environmental impact, and a lower prevalence of hoof and limb lesions (BLACK *et al*., 2013). Production systems also influence cow comfort, estrus expression, heat stress exposure, and ultimately reproductive efficiency (BEWLEY *et al*., 2017; LESO *et al*., 2019). Conversely, dry lot systems are generally less intensive and may expose cows to greater climate variability, potentially impairing reproductive performance and complicating management practices.

Production efficiency must be evaluated not only in biological terms but also in environmental and sustainability contexts. Compost bedded pack is a sustainable system for dairy cows and allows for the reduction of manure released into the environment or the need for its treatment. The bedded pack can be removed and reused in agriculture without prior treatment (DAMASCENO *et al*., 2020).

Dairy cow performance results from the interaction of ruminal fermentation, metabolic adaptation, reproductive physiology, management, and production system structure. Therefore, the objective was to investigate dairy cow performance using a multidimensional framework that integrates ruminal fermentation efficiency, transition health with emphasis on retained placenta, herd-level reproductive performance, and production system sustainability.

REFFERENCES

AMIN, A. B.; MAO, S. Influence of yeast on rumen fermentation, growth performance and quality of products in ruminants: a review. *Animal Nutrition*, v. 7, p. 31-41, 2021. DOI: https://doi.org/10.1016/j.aninu.2020.10.005.

AROWOLO, M. A.; HE, J. Use of probiotics and botanical extracts to improve ruminant production in the tropics: a review. *Animal Nutrition*, v. 4, p. 241-249, 2018. DOI: https://doi.org/10.1016/j.aninu.2018.04.010.

BEWLEY, J. M.; ROBERTSON, L. M.; ECKELKAMP, E. A. A 100-year review: lactating dairy cattle housing management. *Journal of Dairy Science*, v. 100, p. 10418-10431, 2017. DOI: https://doi.org/10.3168/jds.2017-13251.

BLACK, R. A.; TARABA, J. L.; DAY, G. B.; DAMASCENO, F. A.; BEWLEY, J. M. Compost bedded pack dairy barn management, performance, and producer satisfaction. *Journal of Dairy Science*, v. 96, p. 8060-8074, 2013. DOI: https://doi.org/10.3168/jds.2013-6778.

DAMASCENO, F. A.; MONGE, J. L.; NASCIMENTO, J. A. C.; ANDRADE, R. R.; BARBARI, M.; SARAZ, J. A. O.; FERRAZ, G. A. S. Estimate of manure present in compost dairy barn systems for sizing of manure storage. *Agronomy Research*, v. 18, n. S2, p. 1213-1219, 2020. DOI: https://doi.org/10.15159/AR.20.105.

DERKS, M.; VAN WERVEN, T.; HOGEVEEN, H.; KREMER, W. D. J. Veterinary herd health management programs on dairy farms in the Netherlands: use, execution, and relations to farmer characteristics. *Journal of Dairy Science*, v. 96, p. 1623-1637, 2013. DOI: https://doi.org/10.3168/jds.2012-6106.

DEVRIES, T. J.; CHEVAUX, E. Modification of the feeding behavior of dairy cows through live yeast supplementation. *Journal of Dairy Science*, v. 97, p. 6499-6510, 2014. DOI: https://doi.org/10.3168/jds.2014-8226.

GRUMMER, R. R. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. *Journal of Animal Science*, v. 73, n. 9, p. 2820-2833, 1995. DOI: https://doi.org/10.2527/1995.7392820x.

GUNNINK, J. W. Retained placenta and leucocytic activity. *Veterinary Quarterly*, v. 6, p. 49-51, 1984. DOI: https://doi.org/10.1080/01652176.1984.9693909.

JIANG, Y.; OGUNADE, I. M.; ARRIOLA, K. G.; QI, M.; VYAS, D.; STAPLES, C. R.; ADESOGAN, A. T. Effects of the dose and viability of Saccharomyces cerevisiae. 2. Ruminal fermentation, performance of lactating dairy cows, and correlations between ruminal bacteria abundance and performance measures. *Journal of Dairy Science*, v. 100, p. 8102-8118, 2017. DOI: https://doi.org/10.3168/jds.2016-12371.

LEBLANC, S. J. Postpartum uterine disease and dairy herd reproductive performance: a review. *Veterinary Journal*, v. 176, p. 102-114, 2008. DOI: https://doi.org/10.1016/j.tvjl.2007.12.019.

LESO, L.; BARBARI, M.; LOPES, M. A.; DAMASCENO, F. A.; GALAMA, P.; TARABA, J.; KUIPERS, A. Invited review: compost-bedded pack barns for dairy cows. *Journal of Dairy Science*, v. 103, n. 2, p. 1072-1099, 2020. DOI: https://doi.org/10.3168/jds.2019-16864.

LOUCA, A.; LEGATES, J. E. Production losses in dairy cattle due to days open. *Journal of Dairy Science*, v. 51, p. 573-583, 1968. DOI: https://doi.org/10.3168/jds.S0022-0302(68)87031-6.

MAHNANI, A.; SADEGHI-SEFIDMAZGI, A.; ANSARI-MAHYARI, S.; GHORBANI, G. R.; KESHAVARZI, H. Farm and cow factors and their interactions on the incidence of retained placenta in Holstein dairy cows. *Theriogenology*, v. 159, p. 87-97, 2021. DOI: https://doi.org/10.1016/j.theriogenology.2020.10.007.

OLIVEIRA, C.; SERAPIÃO, R. V.; CAMARGO, A. J. dos R.; FREITAS, C. de; IGUMA, L. T.; CARVALHO, B. C.; CAMARGO, L. S. de A.; OLIVEIRA, L. Z.; VERNEQUE, R. da S. Oocyte origin affects the in vitro embryo production and development of Holstein (Bos taurus taurus) - Gyr (Bos taurus indicus) reciprocal cross embryos. *Animal Reproduction Science,* v. 209, 2019. DOI: https://doi.org/10.1016/j.anireprosci.2019.106165.

RITTER, C.; DORRESTEIN, L.; KELTON, D. F.; BARKEMA, H. W. Herd health and production management visits on Canadian dairy cattle farms: structure, goals, and topics discussed. *Journal of Dairy Science*, v. 104, p. 7996-8008, 2021. DOI: https://doi.org/10.3168/jds.2020-19833.

SASAKI, O.; TAKEDA, H.; NISHIURA, A. The economic value of days open in Holstein cows in Japan based on simulated changes in conception rate. *Animal Science Journal*, v. 91, 2020. DOI: https://doi.org/10.1111/asj.13342.

TONI, F.; VINCENTI, L.; RICCI, A.; SCHUKKEN, Y. H. Postpartum uterine diseases and their impacts on conception and days open in dairy herds in Italy. *Theriogenology*, v. 84, p. 1206-1214, 2015. DOI: https://doi.org/10.1016/j.theriogenology.2015.06.022.

VALLEJO-TIMARAN, D. A.; REYES, J.; GILBERT, R. O.; LEFEBVRE, R. C.; PALACIO-BAENA, L. G.; MALDONADO-ESTRADA, J. G. Incidence, clinical patterns, and risk factors of postpartum uterine diseases in dairy cows from high-altitude tropical herds. *Journal of Dairy Science*, v. 104, p. 9016-9026, 2021. DOI: https://doi.org/10.3168/jds.2020-18692.

CHAPTER 1: EFFECTS OF SACCHAROMYCES CEREVISIAE CNCM I-1077 ON FIBER DEGRADABILITY, TOTAL-TRACT DIGESTIBILITY, PRODUCTIVE PERFORMANCE, AND RUMEN MICROBIOTA OF DAIRY COWS

RUNNING HEAD: Live yeast supplementation for dairy cows

Effects of *Saccharomyces cerevisiae* CNCM I-1077 on rumen fiber degradability, total-tract digestibility, productive performance, and rumen microbiota of dairy cows

Kellen R. Oliveira[1], Alex L. Silva[1], Eric Chevaux[2], Lucas J. Mari[3], Poliana T. R. Salgado[1], Igor D. R. Ponciano[1], João. V. C. Rodrigues[1], Carina. S. Bittencourt[1], Fiorella L. Viquez-Umana[4], Hilario C. Mantovani[4], and Polyana P. Rotta[1*]

[1]Department of Animal Science, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil 36570-900

[2]Lallemand SAS, Blagnac, France F-31702

[3]Lallemand Brasil Ltda, Aparecida de Goiânia, Brazil 74923-090

[4]Department of Animal and Dairy Sciences, University of Wisconsin – Madison, Madison, WI 53706

[*]Correspondent author: polyana.rotta@ufv.br

ABSTRACT

This study evaluated the effects of supplementation with *Saccharomyces cerevisiae* strain CNCM I-1077 (**SC**) on rumen degradability, milk production, nutrient digestibility, and rumen microbiota in dairy cows. Two studies were conducted using a crossover design. In Study 1, eight non-lactating, rumen-cannulated Holstein cows (2.3 ± 1.22 lactations; average BW 599.6 ± 58.26 kg) were assigned to either a control or SC group (1 × 10^10 CFU/cow per day) for two periods of 38 days for in situ rumen degradation trials with different forage and non-forage fiber sources (**NFFS**). In Study 2, eight lactating Holstein cows (average parity 2.5 ± 1.71; average BW 608.6 ± 71.39 kg; average milk yield 30.1 ± 2.56 kg/day) were assigned to a control or SC group (2.0 × 10^10 CFU/cow per day) under moderate heat stress conditions (temperature-humidity index (**THI**): 68.4 ± 5.21), also across two periods of 31 days each. The trial protocol included detailed monitoring of feed intake, milk yield (**MY**) and milk composition, total tract digestibility, rumen microbiota, and rumen parameters. In Study 1, forage and NFFS degradability were analyzed separately, with cows within period as random effects and treatment (control or SC) and their interaction as fixed effects. In Study 2, cows were randomized by parity in blocks, with cows as random effects and treatment, time, and their interaction as fixed effects. Digestibility was analyzed considering only treatment as a fixed effect. The significance level was set at 0.05, and a trend was indicated if 0.05 > P > 0.10. Supplementation with SC CNCM I-1077 improved DM and NDF degradability independently of forage type or quality in both fast and slow degradable fractions, but not at the 12-hour incubation point. In NFFS, SC increased DM degradability only in the slow degradable fraction. In lactating cows, SC reduced DMI without affecting MY or composition. Feed efficiency improved in cows supplemented with SC, despite the absence of effects from SC supplementation on microbiota richness and taxonomic composition at the genus level in both

trials. Supplementation with live yeast SC CNCM I-1077 improved rumen degradability, nutrient availability, and feed utilization.

Key words: feed efficiency, fiber degradation, live yeast, milk yield

Nonstandard abbreviations used: ALF = alfalfa; BMR = brown midrib corn silage; CIT = citrus pulp; CON = control treatment; COT = cottonseed whole; DDG = dried distillers' grain; EE = ether extract; HQS = high quality corn silage; LQS = low quality corn silage; NDFI = neutral detergent fiber intake; NFFS = non-forage fiber source; OMI = organic matter intake; SBH = soybean hulls; SC = *Saccharomyces cerevisiae*; THI = temperature humidity index.

INTRODUCTION

Feeding live yeast to dairy cows has been studied for its potential to improve animal performance and support the rumen by stabilizing rumen pH. This stabilization occurs by reducing lactate production and stimulating lactate consumption, thereby preventing lactate accumulation and reducing the risk of SARA (Guedes et al., 2008; Khan et al., 2016; Razzaghi et al., 2023). Additionally, live yeast supplementation modulates the rumen bacterial population and optimizes fiber digestibility, even under tropical conditions (Sousa et al., 2018). Rumen degradation of forages grown under tropical conditions can vary significantly due to differences in plant cell wall composition associated with environmental factors (Ford et al., 1979; Machado et al., 2013). Therefore, the efficacy of feed additives such as live yeast may depend on the type of forage, especially when comparing tropical and temperate forages.

In vitro studies have shown that live yeast promotes the proliferation of lactate-consuming bacteria, including *Selenomonas ruminantium* and *Megasphaera elsdenii* (Amin and Mao, 2021). Additionally, by reducing the oxygen concentration in the rumen, yeast creates anaerobic conditions favorable for microbial activity (McAlister et al., 2011), resulting in increased VFA production and improved fermentation of low-quality forages (Elghandour et al., 2014).

Although previous research has shown that supplementation with the live yeast SC can increase milk fat content, MY, and overall production efficiency (Ondarza et al., 2010; DeVries and Chevaux, 2014), these effects are largely attributed to improved NDF digestibility (Guedes et al., 2008), and a gap remains in its application under tropical conditions. Previously, under heat stress, yeast administration has also been associated with a dose-dependent increase in energy-corrected MY, suggesting improved feed efficiency with increased SC doses (Perdomo et al., 2020).

However, no previous study has investigated the effects of live yeast SC supplementation in lactating dairy cows exposed to heat stress while consuming tropical corn silage-based diets. This study addresses this gap by evaluating cow performance under moderate heat stress and comparing the degradability of different feeds (tropical vs. temperate) in response to live yeast supplementation. This approach provides new insights into the interaction among yeast supplementation, feed composition, and environmental conditions, and has practical implications for dairy production systems in tropical regions.

Therefore, the objective of this study was to investigate the effects of SC supplementation on in situ degradation of DM and NDF in low- and high-quality corn silages, alfalfa, and NFFS. Additionally, we aimed to evaluate the effects of yeast supplementation on milk production and composition, total tract nutrient digestibility, rumen microbiota, and rumen parameters in lactating dairy cows exposed to moderate heat stress. We hypothesized that supplementation with SC CNCM I-1077 would improve rumen fermentation and increase forage DM and NDF degradation without significantly affecting the degradation of NFFS. We also expected that cows receiving SC supplementation would have higher milk and milk fat yields and greater production efficiency even under moderate heat stress.

MATERIAL AND METHODS

The studies were conducted at the Dairy Research Facility of the Department of Animal Sciences at the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil. Before initiation, all procedures were reviewed and approved by the Ethics Committee for the Use of Farm Animals at the Universidade Federal de Viçosa, as described in protocol 051/2021.

Study 1

Animals and Housing

Eight non-lactating Holstein dairy cows fitted with rumen cannulas, with an average parity of 2.3 ± 1.22 SD and an average BW of 599.6 ± 58.26 kg, were used. They were housed individually in stalls with rubber mattress bedding and had ad libitum access to water under neutral THI conditions (61.9 ± 1.5). Cows were randomly assigned to either a control group or a group supplemented with SC, using a two-period crossover design. Each period included 14 days of adaptation followed by 24 days of data collection.

Cows were housed individually in a single-stall system with mattress bedding and a ventilated system, with one fan for every two cows. The mattresses were cleaned daily and treated with hydrated lime to keep them dry. Water was provided ad libitum, and the stalls were flushed twice daily to ensure cleanliness.

Feeds and Treatment

The diet was formulated to meet the nutrient requirements of non-lactating cows according to NASEM (2021; Table 1) and was fed once daily at 0700 h as part of a restricted intake, individually for each cow. The TMR was similar for both treatments, with the only difference being the addition of SC CNCM I-1077. The diet contained 7.1 ± 2.11% of the feed retained on the 19-mm sieve, 60.6 ± 0.71% on the 8-mm sieve, and 32.3 ± 1.57% that passed the 8-mm sieve of the Penn State particle separator, expressed on a per-feed basis. After morning feeding, cows received 0.5 g/cow/day of Levucell SC20 (1×10^{10} CFU of *Saccharomyces cerevisiae* CNCM I-1077; Lallemand Animal Nutrition, Montréal, Quebec, Canada). *Saccharomyces cerevisiae* was diluted in 50 mL of water at 35°C and administered via cannula directly into the rumen, followed by manual mixing of the rumen contents. Control cows underwent the same procedure, with the cannula opened and the rumen contents mixed manually to apply the same aerobic stress to the rumen contents, but without the yeast supplement. Each experimental period consisted of 14 days of adaptation and 5 days of rumen and feed sampling to evaluate different NFFS, corn silages, and alfalfa. This was followed by three consecutive 120-hours in situ rumen degradability assays, separated by 2-day intervals.

Corn silage samples were collected weekly to analyze DM content and adjust the diet. Offered feed was sampled and dried for 72 hours at 55°C in a convection oven, then ground through 2-mm and 1-mm sieves using a Wiley mill (Wiley Mill, A.H. Thomas, Philadelphia, PA) for further analysis. In Study 1, corn silage was also sampled daily from day 15 to day 19, composited at the end of this period, dried as previously described, milled through 2-mm and 1-mm sieves, and analyzed for DM, OM, and CP according to AOAC (2006), and NDF according to Mertens (2002), with thermostable α-amylase added to the neutral detergents and autoclaved samples (Pell and Shofield, 1993).

Ruminal Sampling

From days 15 to 19 the rumen contents of each of the four rumen sacs were sampled by the same trained researcher. Four sampling points were defined in relation to morning feeding and yeast inoculation: 1) 0 hour – immediately after morning feeding; 2) 6 hours - after morning feeding; 3) 12 hours - after morning feeding; and 4) 24 hours - after morning feeding, immediately before morning feeding the next day.

Samples Characterization and in situ Degradability

The study included 6 brown mid-rib (**BMR**) corn silages from the United States, 6 tropical corn silages from Brazil, 4 alfalfa haylage (**ALF**) samples from the United States, and 4 non-forage fiber sources from Brazil: whole cottonseed (**COT**), soybean hulls (**SBH**), citrus pulp (**CIT**), and dried distillers grains (**DDG**), as listed in Table 2. Corn silages were clustered into two categories based on their composition – starch, CP, NDF, and DM content – as either low-quality corn silage (**LQS**) or high-quality corn silage (**HQS**), as described in Table 3.

The dried samples were ground through a 4 mm sieve using a Wiley mill (A.H. Thomas, Philadelphia, PA) and prepared for further incubation. Numbered nylon bags (16 × 10 cm, 50 µm pore size, Ankom Technology, Macedon, NY) were used to package the feeds for the degradability experiment. For forages, 3 g DM per bag was weighed, while for NFFS, 5 g DM per bag was weighed to obtain residues for further NDF analysis. Samples were previously dried in a ventilated forced-air system at 55°C for 72 h.

Each feed was incubated in triplicate in each cow via the rumen cannula, following the recommendations of Ørskov et al. (1980). To compare all samples, they were randomly incubated in three consecutive 120-hour rumen in situ degradability tests, with a two-day interval between tests. Incubation was performed in reverse order, and the bags were removed together at 120, 30, and 12 hours, starting two hours after feeding. The bags were grouped and placed on a steel chain (90 × 2 cm; Menezes et al., 2019) with a 300 g weight at the end to ensure continuous immersion in the rumen contents, totaling 400 g for the entire apparatus. For each group of bags from each cow to be washed together, non-incubated bags (blank samples) were added as a reference for time 0. Immediately after sampling, the bags were immersed in cold water and then washed in a washing machine for five cycles with water at room temperature until the water was clear. The bags were then dried in a ventilated forced-air oven at 55°C for 48 hours and then in an oven at 105°C for 2 hours before being weighed. The residues were ground through a 1 mm sieve using a Wiley mill for further analysis.

Microbiota Analysis

QIIME2 2024.10.0 (Bolyen et al. 2019) was used to process DNA sequences. The samples had a mean of 102,760 forward and reverse reads, with a minimum of 55,469 and a maximum of 144,741 reads per sample. First, DADA2 (Callahan et al. 2016) was used to group the sequences into ASVs, filter them based on quality, and build an ASV table with the count of ASVs per sample. A total of 22,214 ASVs were obtained, with a mean length of 416 bp. The sequences were then aligned with MAFFT, and a phylogenetic tree was built with FastTree using the "phylogeny align-to-tree-mafft-fasttree" plugin of QIIME2. Finally, taxonomy was assigned to the sequences using the Silva 133 database, trimmed to the V4 region of the 16S rRNA gene.

The ASVs table, phylogenetic tree, and taxonomy assignment were imported into phyloseq (McMurdie and Holmes 2013), and chloroplasts and mitochondria were removed. After filtering, the ASV count decreased to 22,201. The samples were then rarefied to 43,997 sequences for the first trial and 37,963 sequences for the second trial. This cutoff was determined based on the increase in species richness with the number of sequences per sample and the sample with the lowest sequence count.

Rarefied samples were used for alpha and beta diversity analysis. For alpha diversity, ASV richness and the exponential of the Shannon index were estimated using phyloseq. For beta diversity, Weighted UniFrac, Unweighted UniFrac, and Bray-Curtis distances between samples were estimated. Ggplot2 (Wickham 2016) was used to visualize the alpha and beta diversity results.

Statistical Analysis

The 12 corn silages (6 BMR and 6 Brazilian) were classified into quality groups using a composite nutritional index based on starch, CP, NDF, and DM. Starch and CP were treated as positive indicators of nutritive value, NDF as a negative indicator, and DM was evaluated relative to a target range of 32% to 38% to reflect adequacy for fermentation and preservation (Khan et al., 2012). Starch, CP, and NDF were standardized using z-scores, and DM contributed through a penalty proportional to its deviation from the target value. The final index was calculated as a weighted linear combination of these components, with weights defined a priori based on biological relevance rather than statistical optimization. Silages were ranked by the index, with the six highest-scoring samples classified HQS and the remaining six classified as LQS, representing a stratification based on relative nutritional ranking.

Statistical analysis was conducted using R (R Core Team, Version 2024.04.02) with the “lmer” function from the “lme4” package. Two groups were analyzed for the in situ test: forages (LQS, HQS, and alfalfa haylage) and non-forage fiber sources (NFFS: COT, SBH, CIT, and DDG). Cow within period was included as a random effect, while ingredient type (forage or non-forage fiber source), treatment, and their interaction were included as fixed effects. For the analysis of rumen pH, VFA, and rumen ammonia nitrogen, the same model was used, with time after feeding and treatment included as fixed effects. When necessary, mean values were subjected to post hoc analysis using the Tukey test in the "emmeans" function. Differences were considered significant if $P \leq 0.05$ and as a trend if $0.05 < P \leq 0.10$.

For microbiota analysis, the alpha diversity indexes (ASV richness and the exponential of Shannon) were first analyzed using model I for the first trial and model II for the second trial. Both models were fitted with an autoregressive residual correlation structure using the lme function from the nlme R package (Pinheiro et al., 2025). The normality of the residuals was assessed both graphically and with a Shapiro test. Homoscedasticity and a mean of zero for the residuals were checked graphically.

$$y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta_{ij}) + \gamma_k + \upsilon_l + \varepsilon_{ijkl} \ (model\ I)$$

$$y_{ijklm} = \mu + \alpha_i + \beta_j + (\alpha\beta_{ij}) + \tau_k + \gamma_l + \upsilon_m + \varepsilon_{ijklm} \ (model\ II)$$

y_{ijlkl} = response variable (ASV richness or exponential Shannon index)

μ = overall mean

α_i= treatment fixed effect i (control or SC supplementation)

β_j= fixed effect of time after feeding j

$\alpha\beta_{ij}$= interaction between treatment and time after feeding ij

γ_k= fixed effect of experimental period

τ_k= fixed effect of cow parity (included only on model II)

υ_l or υ_m= random effect of cow

ε_{ijkl}or ε_{ijklm}= residual error

Beta diversity distances (Weighted UniFrac, Unweighted UniFrac, and Bray-Curtis) were analyzed using models III and IV for the first and second trials, respectively. We performed a permutational multivariate analysis of variance (PERMANOVA) to compare distances between treatments using the adonis2 function from the vegan R package (Oksanen et al., 2022). We grouped by cow to account for it as a random variable. For the taxonomic composition of the samples by treatment, we used the R package ANCOMBC2 (Lin and Peddada, 2024) to identify differentially abundant taxa.

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \upsilon_l + \varepsilon_{ijkl} \ (model\ III)$$

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \tau_k + \gamma_l + \upsilon_m + \varepsilon_{ijklm} \ (model\ IV)$$

y_{ijlkl} = response variable for diversity index

μ = overall mean

α_i= treatment fixed effect i (control or SC supplementation)

β_j= fixed effect of time after feeding j

γ_k= fixed effect of experimental period

τ_k= fixed effect of cow parity (included only on model IV)

υ_l or υ_m= random effect of cow

ε_{ijkl}or ε_{ijklm}= residual error

Study 2

Animals and Housing

Eight lactating Holstein cows, four primiparous and four multiparous (parity = 2.5 ± 1.71 SD), were included in this study. The cows were ruminally cannulated, produced an average of 30.1 ± 2.56 kg of milk per day at 176 ± 26 DIM, and weighed 608.6 ± 71.39 kg. They were randomly assigned to two treatment groups: Control or supplemented with SC. The study used a crossover design with two periods, each lasting 32 days. Each period included 21 days of adaptation and 10 days of data collection (Figure S1; see Notes). The trial was conducted during the summer, with a mean THI of 68.4 ± 5.21, measured with a digital thermohygrometer (K29-5070H, Kasvi, Pinhais, Paraná, Brazil) installed in the center of the barn where the cows were housed.

Cows were housed individually in single stalls with mattress bedding and a ventilated system, using one fan for every two cows. The mattresses were cleaned daily and treated with hydrated lime to keep them dry. Water was provided ad libitum, and the stalls were flushed twice daily to ensure cleanliness.

Feeds and Treatment

The TMR was formulated to meet the nutrient requirements of lactating cows producing 32 kg of milk per day, as recommended by NASEM (2021; Table 2). At the start of the trial, a cereal mixture of concentrated ingredients was prepared. Before each feeding, this grain mixture was thoroughly mixed with corn silage, Tifton hay, and whole cottonseed. The feed supply was adjusted each morning to ensure that 10% of the total feed remained unconsumed (orts) based on the previous day's intake. Cows were fed individually ad libitum twice daily at 0700 and 1430 h, while milking occurred three times daily at 0630, 1400, and 2030 h.

The feed provided to the two treatment groups was similar. After the morning feeding, the rumen cannulas of cows in the SC group were opened to inoculate live yeast directly into the rumen, and the rumen contents were mixed manually. To control for possible oxygen exposure from opening the cannulas, the cannulas of cows in the control group were also opened and the rumen contents were mixed manually, but without adding the yeast supplement. The live yeast supplementation consisted of 2.0 × 10^10 CFU per cow per day of the same live yeast (SC CNCM I-1077) used in Study 1, which is the recommended dose for lactating heatstressed cows (Perdomo et al., 2019). Each trial period included a 21-day adaptation phase followed by a collection phase.

The particle size distribution of the diet and orts was determined using the Penn State Particle Separator. On an as-fed basis, the diet contained 11.2 ± 2.14% particles larger than 19 mm, 32.5 ± 1.87% particles between 8 and 19 mm, and 57.9 ± 3.27% particles smaller than 8 mm. In the orts, 33.0 ± 19.09% of the particles were larger than 19 mm, 25.3 ± 6.50% were between 8 and 19 mm, and 41.7 ± 14.90% were smaller than 8 mm. Corn silage samples were collected weekly, dried in a convection oven at 55°C for 72 hours to adjust nutrient content, and then ground through 2-mm and 1-mm sieves using a Wiley mill (A.H. Thomas, Philadelphia, PA) for further analysis.

Ruminal Measurements

The cows were fed twice daily. From days 27 to 31, the rumen contents of each of the four rumen sacs were sampled by the same trained researcher. Four sampling points were defined in relation to morning feeding and yeast inoculation: 1) 0 h – immediately after morning feeding; 2) 6 h after morning feeding and immediately before afternoon feeding; 3) 12 h after morning feeding and 6 h after afternoon feeding; and 4) 24 h after morning feeding, immediately before morning feeding the next day.

Milk Yield and Composition

The lactating cows were milked three times a day at 0630, 1400, and 2030 h. After the acclimatization period from days 22 to 24, the MY of each cow was recorded for three consecutive days during all three milking sessions using a digital scale. Milk samples were collected by homogenizing the milk, then taking 350 mL samples from each milking session. These samples were analyzed using the Lactoscan Ultrasonic Milk Analyzer (Milkotronic Ltda, Bulgaria), and the composition for each sample was calculated. The results were summed to obtain the total production and percentage. Based on the analysis results and MY data, the production levels of fat, protein, and lactose were calculated using the daily mean compound percentages and daily production of each compound.

Total Tract Digestibility

On the 26th and 27th days of the study, total fecal output from each cow was collected directly at the time of defecation to ensure samples were free of urine contamination. Samples were placed in individual buckets, and every 24 hours all collected feces were carefully weighed using a digital scale and then homogenized. At the end of each 24-hour period, samples of the total feces collected that day were taken. These samples were dried for 72 hours in a ventilated forced-air system. After drying, samples were ground using a 1-mm sieve (Willey mill, A.H. Thomas, Philadelphia, PA) for further analysis.

Feed and orts samples were collected on the days of fecal and rumen collections. These samples were dried for 72 hours in a ventilated forced-air system and then ground through 2 mm and 1 mm sieves (Willey mill, A.H. Thomas, Philadelphia, PA). During the same sampling period, the corn silage offered to the cows was collected from days 25 to 31 in Study 2. At the end of the sampling period, samples were composited, dried at 55°C for 72 hours in a ventilated forced-air system, and ground through 2-mm and 1-mm sieves using a Wiley mill.

The feed, orts, and fecal samples were analyzed for DM, OM, and CP according to AOAC (2006), and for NDF according to Mertens (2002), with autoclaving to neutral detergent using thermostable α-amylase (Pell and Shofield, 1993).

Microbiota Analysis

Microbiota data from Study 2 were processed and analyzed using the same bioinformatic and statistical pipeline described for Study 1, including sequence processing in QIIME2, ASV inference with DADA2, taxonomic assignment with the Silva database, filtering procedures, rarefaction, and alpha and beta diversity analyses in phyloseq.

Heat Stress Assessments

On two consecutive days, from day 26 to day 27 of the study, the animals' response to heat stress was assessed by three trained researchers. Rectal temperature was measured with a digital thermometer (TH1027, G-Tech, Londrina, Paraná, Brazil) with an accuracy of ±0.2 ºC at 1-hour intervals for 48 hours. At the same time, the evaluator monitored the respiratory rate by counting flank movements over 15 seconds and multiplying by 4 to obtain breaths per minute. According to Divers and Peak (2018), these are the most common indicators used to assess heat stress in dairy cows (Galán et al., 2018). Each cow had 48 values for each time period.

Statistical Analysis

Data were analyzed using the “lmer” function fitted by REML from the “lme4” package in R. Animals were randomized in blocks by parity. For the parameters DMI, OMI, CPI, NDFI, and milk, the model included the fixed effects of SC supplementation, time, and their interaction, with cow included as a random factor. In the pH analysis, time 0 was used as a covariate for the other times, following the same model as for animal performance.

Digestibility was compared only between treatment groups. Data for SCC were log-transformed and analyzed. Data were considered outliers if their internal residual was greater than |2.5|. If necessary, means were subjected to Tukey's test at a significance level of 0.05, and a trend was noted if $0.05 < P \leq 0.10$.

Similarly, using the “lmer” function in a binary analysis, odds ratio estimates were calculated for rectal temperatures above 39.17 ºC and respiratory rates above 40 movements per minute (Divers and Peak, 2018), comparing the SC group to the control group.

RESULTS

Study 1

The feeds showed different patterns of DM degradability during the 12 hours incubation ($P < 0.01$, Table 4), with ALF exhibiting higher DM degradability, while HQS and LQS had similar values. However, DM degradability after 12 hours was not affected by treatment ($P = 0.49$). At 30 hours of incubation, all evaluated feeds were similarly affected by treatment ($P = 0.01$), with SC improving DM degradability compared to their counterparts. Among the feeds, LQS had the lowest degradability at 30 h of incubation ($P = 0.02$), while the ALF group had the highest, and HQS tended to have higher degradability ($P = 0.02$). After 120 hours of incubation, the feeds showed different levels of DM degradability ($P < 0.01$). HQS and LQS were more degradable than ALF ($P < 0.01$). At this time, there was no treatment effect on feed degradation ($P = 0.11$).

For NDF degradability, treatment tended to increase degradability after 12 hours of incubation ($P = 0.07$). However, different patterns of NDF degradability were observed among the feeds (*P* < 0.01). Alfalfa showed higher NDF degradability compared to LQS and HQS (P < 0.01), which had similar degradability levels (*P* = 0.61). Additionally, NDF degradability of forages tended to be affected by treatment at 30 hours of incubation (*P* = 0.05). At 30 hours, ALF had the highest degradability, followed by HQS, with LQS showing the lowest degradability (*P* < 0.01). At 120 hours of incubation, SC supplementation also increased NDF degradability (*P* < 0.01) for all forages evaluated. Silages (LQS and HQS) showed similar NDF degradation patterns (*P* > 0.05), while ALF was less degraded at this time point.

For NFFS, the effect of feed was observed at all incubation times for both DM and NDF degradability (*P* < 0.01, Table 5). For DM degradability, at 12 hours, CIT degraded faster than COT and DDG (*P* < 0.01), which had similar degradation levels (*P* = 0.86); SBH had the lowest DM degradability (*P* < 0.01). At 30 hours, CIT still had the highest DM degradability (*P* < 0.01), followed by SBH, DDG, and COT, which had the lowest degradability. For NFFS, there was a trend toward an interaction between feed and treatment at 120 h for DM (*P* = 0.06; Table 5), with SC supplementation tending to increase COT degradation at this time point.

A similar pattern was observed for NDF degradability, with no treatment effects detected at any evaluated time (*P* = 0.24), but different patterns of feed degradation (*P* < 0.01). At 12 h of incubation, CIT had the highest NDF degradability (*P* < 0.01), differing from DDG and SBH. In contrast, COT had the lowest NDF degradability at this time point (*P* = 0.01).

At 30 hours, NDF degradability was highest for CIT, which differed from DDG and SBH, as both showed similar levels of degradation. COT had the lowest NDF degradation (*P* < 0.01). At 120 hours, DDG and SBH remained similar and showed the highest NDF degradation (*P* = 0.73), while CIT had lower degradation than the other two. Additionally, COT continued to have the lowest NDF degradation at this time point (*P* = 0.04).

Rumen pH was not affected by SC supplementation (*P* = 0.33) but fluctuated throughout the day (*P* < 0.01). The lowest pH occurred 6 hours after feeding (*P* < 0.01), increased 12 hours after feeding, and was highest 24 hours after feeding in both groups (Figure 2, $P < 0.01$).

There was no treatment effect on VFA production ($P > 0.27$, Figure 3). However, the time of sampling affected the concentrations of acetate, propionate, and butyrate in the rumen ($P < 0.01$). Acetate and propionate levels were lower only at 24 hours after feeding, while butyrate concentrations were lower at 6 and 24 hours post-feeding compared to 0 and 12 hours post-feeding.

Study 2

During most of the experimental period, THI values were above 68, indicating that the animals were under moderate heat stress (NASEM, 2021; Figure 1). Lactating cows supplemented with SC had lower DMI ($P = 0.04$; Table 6) and showed a trend toward lower OM intake ($P = 0.05$) and CP intake ($P = 0.08$). Neutral detergent fiber intake did not differ between treatments ($P = 0.27$). Neutral detergent fiber intake increased over the trial days ($P = 0.01$) but remained similar between treatments ($P = 0.30$).

Cows fed SC had higher total tract DM digestibility ($P = 0.02$; Table 6). Similar results between treatments were observed for total apparent digestibility of OM ($P = 0.82$), CP ($P = 0.73$), and NDF ($P = 0.14$).

Rumen pH was not affected by the treatment ($P = 0.79$). However, time influenced pH ($P < 0.01$), with the highest pH observed 24 hours after feeding (P < 0.01), followed by measurements at 6 and 12 hours after feeding. This indicates that cows had the highest rumen pH near feeding time (Figure 4).

The rumen concentrations of acetate (P = 0.10), propionate (P = 0.77), and butyrate (P = 0.65) did not differ between treatments but were influenced by sampling time ($P < 0.01$;

Figure 5). Acetate reached its lowest concentration 24 hours after morning feeding ($P < 0.01$). Butyrate showed a similar pattern, with the lowest levels at 0 and 24 hours after feeding ($P < 0.01$). Propionate peaked 12 hours after feeding (6 hours after the afternoon meal), but similar concentrations were observed immediately after the morning feeding (0 hour) and 6 hours after the morning feeding ($P < 0.01$). For all VFAs studied, the lowest concentrations occurred 24 hours after feeding ($P < 0.01$). The acetate to propionate ratio was not affected by treatment ($P = 0.30$) or time after feeding ($P = 0.56$).

Saccharomyces cerevisiae strain CNCM I-1077 supplementation did not affect rumen ammonia-nitrogen concentration expressed in mM ($P = 0.32$; Figure 6) or time after feeding ($P = 0.30$). No interaction between treatment and time was observed ($P = 0.43$). Similar results were obtained for rumen ammonia-nitrogen levels (mg/L), with no effect of treatment ($P = 0.44$), time ($P = 0.13$), or interaction between treatment and time ($P = 0.54$).

There was no difference in MY between treatments ($P = 0.12$, Table 7). There was also no difference in 4% FCM ($P = 0.47$), milk energy ($P = 0.76$), or energy-corrected milk between the SC and control treatments ($P = 0.46$).

No differences were found between the treatments in fat content ($P = 0.58$) or fat yield in kilograms ($P = 0.58$). There were also no differences in protein content ($P = 0.10$) or protein yield ($P = 0.92$) between the two groups. The percentage ($P = 0.92$) and yield ($P = 0.91$) of lactose production were also similar between SC and CON cows. The composition of solid yield ($P = 0.14$) and percentage ($P = 0.32$) also showed no difference between treatments (Table 7). However, SC-treated cows had lower SCC than CON cows ($P = 0.02$). Milk urea nitrogen tended to be lower in cows supplemented with SC than in CON cows ($P = 0.07$).

Calculations of milk production efficiency showed that cows receiving SC supplementation had higher values. Specifically, SC-supplemented cows consistently showed higher efficiency for MY and DMI ($P = 0.02$). A similar result was observed for FCM relative to DMI (*P* = 0.03). When efficiency was evaluated based on OM intake, comparable results were found, with significant ratios for MY (*P* = 0.02) and FCM (*P* = 0.03).

Regarding heat stress physiological responses, the odds of having a high rectal temperature (above 39.17 ºC) were not affected by SC supplementation (*P* = 0.20). In contrast, the odds of an elevated respiratory rate (greater than 40 movements per minute) were significantly reduced in SC-supplemented cows compared to controls (OR: 0.66; CI: 0.48–0.92; *P* = 0.01).

Microbiota Diversity

Microbial diversity, estimated by ASV richness and the exponential of the Shannon index, remains unchanged by the SC treatment. This result was consistent in both trials, even when diversity was analyzed at different time points after feeding (Figure 7).

Overall, there is no difference in the composition of the samples based on the beta diversity results (Figure 8). The difference between the samples based on the Weighted UniFrac distance is not significant in either trial. Although the Unweighted UniFrac and Bray-Curtis distances show a P-value below 0.05, the low percentage of variance explained by the principal coordinates analysis (PCoA) indicates these distances contribute little to the overall variability between samples.

Consistent with the results from the beta diversity analysis, there is no difference in taxonomic composition at the genus level between the treatment groups (Figure 9, Supplementary Table 1). The ancomBC2 analysis detected no differentially abundant taxa between treatment groups in either trial (Supplementary Table; see Notes). However, some taxa differed at the genus level between sampling times in the first trial (a genus from the Bacteroidales BS11 gut group, *P* = 0.04) and in the second trial (Kurthia, *P* = 0.005). There were also differentially abundant taxa at the genus level between periods in the first trial (a genus from the Bacteroidales BS11 gut group, *P* = 0.04; Rummeliibacillus, *P* = 0.001; and Bacillus, *P* = 0.004) and in the second trial (Candidatus Saccharimonas, P < 0.001; Anaerovibrio, *P* = 0.009; a genus from the family Veillonellaceae UCG-001, *P* = 0.002; and Kurthia, *P* = 0.005).

DISCUSSION

This study sequentially integrated two complementary approaches to assess the nature and extent of fiber degradability and digestibility in SC-supplemented cows. It first examined the ruminal degradability of common feed ingredients for dairy cows, then evaluated the response of milking cows to supplementation. In the in situ degradation study, non-lactating cows were used to measure rumen degradability of the forage and to avoid distortion of rumen dynamics caused by lactation (Schadt et al., 2014), while lactating cows were selected to evaluate performance and its relationship to total tract digestibility.

The better NDF degradability of HQS at 30 hours may be explained by its lower lignin content, which can facilitate access to and hydrolysis of insoluble carbohydrates by the rumen microbiota (Keith et al., 1979; Oba and Allen, 1999). This is usually attributed to the greater potentially degradable fraction and higher feed value of BMR (Luo et al., 2022). However, two tropical hybrids showed a similar fermentation pattern within the HQS cluster. These results highlight the need for tailored strategies to address the unique challenges of tropical forages, such as higher lignin content and the influence of climate on forage digestibility (Ford et al., 1979). However, DM degradability for both silage clusters was similar, and both differed from ALF, which had higher undegradable DM at 120 hours of incubation.

Among the NFFS, COT had the lowest digestibility due to its high NDF content, derived mainly from the lint, which can promote rumination and potentially improve the rumen digestibility of other ingredients (Coppock et al., 1985; Pierce et al., 2024). COT can remain in the rumen with the fiber mat for rumination (Kononoff and Heinrichs, 2003), helping other forages stay in the rumen longer and be better digested, making it an important fiber source for ruminants.

The improved forages' DM and NDF degradability at 30 hours, as well as NDF degradability at 12 and 30 hours, were particularly beneficial given that the retention time of corn silage in the rumen is about 34.8 hours, as reported by Krämer et al. (2013) for a 50% forage diet typical of dairy cows. This improvement in degradability indicates greater efficiency in both the rapidly and slowly degrading fractions of available nutrients (Huhtanen et al., 2008).

Similarly, SC supplementation improved the degradability of NFFS at 120 hours, which is consistent with their slower passage rate being similar to or lower than that of conventional forages (Nocek and Russell, 1988; Firkins, 1997). The feeds evaluated in the current study showed an increase in the potential degradability of DM by SC for both forages and NFFS, indicating greater utilization potential in the rumen.

The improved degradability of forages and NFFS with SC supplementation supports previous findings (Guedes et al., 2008; Poppi et al., 2021) that highlighted the potential of strain CNCM I-1077 to enhance fiber degradation, particularly in forages. This may positively affect the remaining non-fermentable nutrients by promoting better utilization of corn silage or legumes.

Although no changes in rumen pH or VFA concentrations were observed, SC likely enhanced microbial activity and supported fiber degradation, consistent with previous findings (Jiang et al., 2017; Perdomo et al., 2019), despite the absence of differences in microbiota richness and genus-level composition. The lack of pH changes in this study may be due to the high forage content and low levels of rapidly fermentable carbohydrates, as rumen pH remained consistently above 6.5, minimizing acidotic conditions.

In lactating cows, SC supplementation resulted in improved total DM digestibility under moderate heat stress when fed a corn silage TMR and enhanced feed efficiency associated with lower DMI. Although MY and composition did not differ, SC cows had higher milk/DMI and FCM/DMI efficiency. These findings are not consistent with those of Ondarza et al. (2010) or Desnoyers et al. (2009), who reported a 1.2 kg/day increase in MY associated with increased DMI when cows were fed the same strain. Similarly, Takyia et al. (2024) demonstrated higher efficiency in SC cows than in control cows and an increase in FCM. Although this was associated with lower DMI and a trend toward lower OM and CP intake, the supplemented cows showed higher efficiency.

These cows maintained milk and solids production at lower intakes, indicating better digestibility of DM, while showing no effects on the digestibility of OM, CP, and NDF. This was unexpected, as Study 1 showed an improvement in NDF degradability in the rumen. Previous studies have also reported improved digestibility of DM and OM (Diepersloot et al., 2024), CP (Perdomo et al., 2020), and NDF (Sousa et al., 2018; Poppi et al., 2021) when the SC CNCM I-1077 strain was used.

The lower SCC observed in SC cows indicates improved udder health, which may be related to reduced production of inflammatory cytokines (Sun et al., 2021), despite the absence of VFA treatment effects. A previous study also linked supplementation with SC fermentation products to improved udder health in subclinical mastitis by limiting cell damage and increasing recovery through regulation of heat shock proteins (Vailati-Riboni et al., 2021). A limitation of this study was the lack of evaluation of inflammatory mediators related to mastitis.

When ruminants experience heat stress, they increase their respiration rate to facilitate evaporative cooling (Zhou et al., 2023). The higher respiration rate also affects the buffer system in the rumen, as more carbon dioxide is expelled (Bernabucci et al., 2010). Notably, cows receiving SC were less likely to have a respiratory rate exceeding the normal threshold of 40 movements per minute, even when they had an abnormal rectal temperature, compared to cows not receiving SC supplementation.

Despite these improvements in mitigating heat stress, SC did not alter rumen VFA concentration, rumen pH, or rumen ammonia-nitrogen levels. Although VFA results from production and absorption through the rumen epithelium, there is limited information on the actual amount produced in the rumen. Previous studies reported increased VFA production, nitrogen conversion, and rumen pH associated with SC strain CNCM I-1077 (Perdomo et al., 2020), supplementation of SC-based products in vivo (Zhu et al., 2017), or in vitro analysis (Elghandour et al., 2014). These results highlight the efficacy of SC in improving rumen efficiency and nutrient utilization.

Despite the lack of effects from SC supplementation on microbiota richness and genus-level taxonomic composition in both trials, lactating cows supplemented with SC had higher feed conversion for milk production.

CONCLUSIONS

Supplementation with *Saccharomyces cerevisiae* strain CNCM I-1077 has the potential to improve rumen degradability of DM and NDF from common dietary fiber sources in dairy cows, including both forage and non-forage fiber sources. This improvement leads to better nutrient availability, resulting in no change in milk production or composition, but improved somatic cell count and feed conversion. These results highlight the potential of this yeast strain as a valuable nutritional supplement to optimize feed conversion.

NOTES

We are grateful to the following Brazilian foundations for their help with this study: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; Brasilia, DF, Brazil), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Brasilia, DF, Brazil), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG; Belo Horizonte, MG, Brazil). We would also like to thank Lallemand Animal Nutrition for funding this study. Supplemental material for this article is available at https://doi.org/10.17632/64bbry89gz.1.

REFERENCES

Amin, A. B., and S. Mao. 2021. Influence of yeast on rumen fermentation, growth performance and quality of products in ruminants: A review. 2021. Anim. Nutr. 7:31–41. https://doi.org/10.1016/j.aninu.2020.10.005.

Arowolo, M. A., and J. He. 2018. Use of probiotics and botanical extracts to improve ruminant production in the tropics: A review. Anim. Nutr. 4:241–249. https://doi.org/10.1016/j.aninu.2018.04.010.

Assunção, A. S., T. E. da Silva, D. Quirino, M. O. Franco, E. Detmann. 2022. Variability among animals and incubation protocols for ruminant in situ degradation studies with tropical feeds. Animals 12: 1901. https://doi.org/10.3390/ani12151901.

AOAC International. 2006. Official Methods of Analysis. 18th ed. AOAC International, Gaithersburg, MD.

Bedford, A., L. Beckett, L. Harthan, C. Wang, N. Jiang, H. Schramm, L. L. Guan, K. M. Daniels, M. Hanigan, and R. White. 2020. Ruminal volatile fatty acid absorption is affected by elevated ambient temperature. Sci. Rep. 10:13092. https://doi.org/10.1038/s41598-020-69915-x.

Bernabucci, U., N. Lacetera, L. H. Baumgard, R. P. Rhoads, B. Ronchi, and A. Nardone. 2010. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. Animal. 4:7. https://doi.org/10.1017/S175173111000090X.

Coppock, C. E., J. R. Moya, J. W. West, D. H. Nave, J. M. Labore, and C. E. Gates. 1985. Effect of lint on whole cottonseed passage and digestibility and diet choice on intake of whole cottonseed by Holstein cows. J. Dairy Sci. 68:1198–1206. https://doi.org/10.3168/jds.S0022-0302(85)80947-4.

Desnoyers, M., S. Giger-Reverdin, G. Bertin, C. Duvax-ponter, and D. Sauvant. 2009. Meta-analysis of the influence of Saccharomyces cerevisiae supplementation on ruminal parameters and milk production of ruminants. J. Dairy Sci. 92:1620–1632. https://doi.org/10.3168/jds.2008-1414.

DeVries, T. J., and E. Chevaux. 2014. Modification of the feeding behavior of dairy cows through live yeast supplementation. J. Dairy Sci. 97: 6499-95. https://doi.org/10.3168/jds.2014-8226.

Diepersloot, E. C., M. R. Pupo, L. F. Ferraretto. 2024. Effect of monensin and live-cell yeast supplementation on lactation performance, feeding behavior, and total-tract nutrient digestibility in dairy cows. J. Dairy Sci. 107:4449-4460. https://doi.org/10.3168/jds.2023-24125.

Divers, T. J., and S. F. Peek. 2018. Clinical examination. Pages 2–17 in *Rebhun's Diseases of Dairy Cattle*. 3rd ed. S. F. Peek and T. J. Divers, ed. Elsevier.

Ding, G., Y. Chang, L. Zhao, Z. Zhou, L. Ren, and Q. Meng. 2014. Effect of Saccharomyces cerevisiae on alfalfa nutrient degradation characteristics and rumen microbial populations of steers fed diets with different concentrate-to-forage ratios. J. Anim. Sci. Biotech. 5:24. https://doi.org/10.1186/2049-1891-5-24.

Elghandour M. M. Y., J. C. V. Chagoyan, A. Z. M. Salem, A. E. Kholif, J. S. M. Castaneda, L. M. Camacho, and M. A. Cerrillo-Soto. 2014. Effects of *Saccharomyces cerevisiae* at direct addition or pre-incubation on in vitro gas production kinetics and degradability of four fibrous feeds. Ital. J. Anim. Sci.13:295-301. https://doi.org/10.4081/ijas.2014.3075.

Firkins, J. L. 1997. Effects of feeding nonforage fiber sources on site of fiber digestion. J. Dairy Sci. 80:1426-1437. https://doi.org/10.3168/jds.S0022-0302(97)76072-7.

Ford, C. W., I. M. Morrison, and J. R. Wilson. 1979. Temperature effects on lignin, hemicellulose and cellulose in tropical and temperate grasses. Aust. J. Agric. Res. 30:621-633. https://doi.org/10.1071/AR9790621.

Galan E., P. Llonch, A. Villagra, H. Levit, S. Pinto, and A. del Prado. 2018. A systematic review of non-productivity-related animal-based indicators of heat stress resilience in dairy cattle. PLoS ONE. 13: e0206520. https://doi.org/10.1371/journal.pone.0206520

Guedes, C. M., D. Gonçalves, M. A. M. Rodrigues, and A. Dias-da-Silva. 2008. Effects of a Saccharomyces cerevisiae yeast on ruminal fermentation and fibre degradation of maize silages in cows. Anim. Feed Sci. Tech. 145:27-40. https://doi.org/10.1016/j.anifeedsci.2007.06.037.

Huhtanen, P., A. Seppala, S. Ahvenjarvi, M. Rinne. 2008. Prediction of in vivo neutral detergent fiber digestibility and digestion rate of potentially digestible neutral detergent fiber: comparison of models. J. Anim. Sci. 86:2657-2669. https://doi.org/10.2527/jas.2008-0894.

Jiang, Y., I. M. Ogunade, K. G Arriola, M. Qi, D. Vyas, C. R. Staples, and A. T. Adesogan. 2017. Effects of the dose and viability of Saccharomyces cerevisiae. 2. Ruminal fermentation, performance of lactating dairy cows, and correlations between ruminal bacteria abundance and performance measures. J. Dairy Sci. 100:8102–8118. https://doi.org/10.3168/jds.2016-12371.

Jiang, Y., I. M. Ogunade, S. Qi, T. J. Hackmann, C. R. Staples, and A. T. Adesogan. 2017. Effects of the dose and viability of Saccharomyces cerevisiae.1. Diversity of ruminal microbes as analyzed by Illumina MiSeq sequencing and quantitative PCR. J. Dairy Sci. 100:300-342. https://doi.org/10.3168/jds.2016-11263.

Khan, N. A., T. A. Tewoldebrhan, R. L. G. Zom, J. W. Cone, and W. H. Hendriks. 2012. Effect of corn silage harvest maturity and concentrate type on milk fatty acid composition of dairy cows. J. Dairy Sci. 95: 1472-1483. http://dx.doi.org/ 0.3168/jds.2011-4701.

Khan, R. U., N. Shabana, D. Kuldeep, K. Karthik, T. Ruchi, M. A. Mutassim, I. A. Alhidary, and A. Zahoor. 2016. Direct-fed microbial: beneficial applications, modes of action and prospects as a safe tool for enhancing ruminant production and safeguarding health. Int. J. Pharm. 12:220-231. https://doi.org/10.3923/ijp.2016.220.231.

Keith, E. A., V. F., Colenbrander, V. L. Lechtenberg, and L. F. Bauman. 1979. Nutritional value of brown midrib corn silage for lactating dairy cows. J. Dairy Sci. 62:788-792. https://doi.org/10.3168/jds.S0022-0302(79)83326-3.

Krämer, M., Lund, P., and Weisbjerg, M. 2013. Rumen passage kinetics of forage- and concentrate-derived fiber in dairy cows. J. Dairy Sci. 96:3163-3176. https://doi.org/10.3168/jds.2012-6146.

Kononoff, P. J, and A. J. Heinrichs. 2003. The effect of corn silage particle size and cottonseed hulls on cows in early lactation. J. Dairy Sci. 86:2438-2451. https://doi.org/10.3168/jds.S0022-0302(03)73838-7.

Luo, C., D. Wang, N. Lu, H. Li, G. Liu, Z. Cao, H. Yang, S. Li, X. Yu, W. Shao, and W. Wang. 2022. Analysis of chemical composition, amino acid content, and rumen degradation characteristics of six organic feeds. Animals. 12:682. https://doi.org/10.3390/ani12060682.

Machado, P. A. S., S. C. Valadares Filho, E. Detmann, S. A. Santos, R. F. D. Valadares, C. Ducatti, P. P. Rotta, and L. F. Costa e Silva. 2013. Development of equations to estimate microbial contamination in ruminal incubation residues of forage produced under tropical conditions using 15N as a label. J. Anim. Sci. 91:3836-3846. https://doi.org/10.2527/jas2012-5636.

McAllister, T. A., K. A. Beauchemin, A. Y. Alazzeh, J. Baah, R. M. Teather, and K. Stanford. 2011. Review: The use of direct fed microbials to mitigate pathogens and enhance production in cattle. Can. J. Anim. Sci. 91:193-211. https://doi.org/10.4141/CJAS10047.

Menezes, A. B., S. C. Valadares Filho, M. C. Pacheco, P. Pucetti, J. M. Pereira, P. P. Rotta, and J. S. Caton. 2019. Single point ruminal incubation times necessary to estimate rumen degradable protein content in concentrate feeds. Transl. Anim. Sci. 2019.3:1686–1690. https://doi.org/10.1093/tas/txz058.

Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beaker or crucibles: collaborative study. J. AOAC Int.85:1217-1240. PMID: 12477183.

Moallem, U., H. Lehrer, L. Livshitz, M. Zachut, and S. Yakoby. 2009. The effects of live yeast supplementation to dairy cows during the hot season on production, feed efficiency, and digestibility. J. Dairy Sci. 92:343–351. https://doi.org/10.3168/jds.2007-0839.

Nocek, J. E., and J. B. Russell. 1988. Protein and energy as an integrated system: relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. J. Dairy Sci. 71:2070. https://doi.org/10.3168/jds.S0022-0302(88)79782-9.

Oba, M. and M. S. Allen. 1999. Effects of brown midrib 3 mutation in corn silage on dry matter intake and productivity of high yielding dairy cows. J. Dairy Sci. 82:135–142. https://doi.org/10.3168/jds.S0022-0302(99)75217-3.

Ondarza, M. B., C. J. Sniffen, L. Dussert, E. Chevaux, J. Sullivan, and N. Walker. 2010. Case study: Multiple-study analysis of the effect of live yeast on milk yield, milk component content and yield, and feed efficiency. Prof. Anim. Sci. 26:661–666. https://doi.org/10.15232/S1080-7446(15)30664-1.

Ørskov E. R., F. D. DeB Hovell, and F. Mould. 1980. The use of nylon bag technique for the evaluation of feedstuffs. Trop. Anim. Prod. 5:3.

Pell, A. N., and P. Schofield. 1993. Computerized monitoring of gas production to measure forage digestion in vitro. J. Dairy Sci. 76:10631073. https://doi.org/10.3168/jds.S0022-0302(93)77435-4.

Perdomo, M. C., R. S. Marsola, M. G. Favoreto, A. Adesogan, C. R. Staples, and J. E. P. Santos. 2020. Effects of feeding live yeast at 2 dosages on performance and feeding behavior of dairy cows under heat stress. J. Dairy Sci. 103:325-339. https://doi.org/10.3168/jds.2019-17303.

Pierce, R. B., Y. A. Adeniji, R. Bomberger, S. R. Goodall, and K. J. Harvatine. 2024. Effect of feeding increasing levels of whole cottonseed on milk and milk components, milk fatty acid profile, and total-tract digestibility in lactating dairy cows. J. Dairy Sci. 107:2916-2929. https://doi.org/10.3168/jds.2023-23944.

Poppi, A. C., G. Lazzari, A. L. M. Gomes, R. M. do Prado, R. T. R. Almeida, D. M. Zanzarin, E. J. Pilau, C. C. Jobim, L. J. Mari, E. Chevaux, F. Chaucheyras-Durand, A. T. Adesogan, J. L. P. Daniel. 2021. Effects of feeding a live yeast on rumen fermentation and fiber degradability of tropical and subtropical forages. J. Sci. Food Agric. 101: 6220–6227. https://doi.org/10.1002/jsfa.11273.

Razzaghi, A., M. Malekkhahi, and A. F. Brito. 2023. Lactation performance, milk fat output, and nutrient digestibility responses to the addition of liquid molasses or yeast culture in dairy cows fed super-conditioned corn. J. Dairy Sci. 106:6080–6093. https://doi.org/10.3168/jds.2022-22768.

Sales, G. F. C., B. F. Carvalho, R. F. Schwan, L. F. Vilela, J. A. M. Meneses, M. P. Gionbelli, and C. L. S. Ávila. 2021. Heat stress influence the microbiota and organic acids concentration in beef cattle rumen. J. Therm. Biol. 97:102897. https://doi.org/10.1016/j.jtherbio.2021.102897.

Siegfried, R., H. Rückemann, and G. Stumpf. 1984. Method for the determination of organic acids in silage by high performance liquid chromatography. Landwirtsch Forsch. 298–304.

Schadt, I., D. R. Mertens, P. J. Van Soest, G. Azzaro, G. Licitra. 2014. Stage of lactation and corresponding diets affect in situ protein degradation by dairy cows. J. Dairy Sci. 97:7995-8007. http://dx.doi.org/ 10.3168/jds.2014-7987.

Soufizadeh, M., R. Pirmohammadi, Y. Alijoo, and H. K. Behroozyar. 2018. Indigestible neutral detergent fibers: Relationship between forage fragility and neutral detergent fibers digestibility in total mixed ration and some feedstuffs in dairy cattle. Vet Res. Forum. 9: 49-57. PMID: 29719664

Sousa, D. O., C. A. Oliveira, A. V. Velasquez, J. M. Souza, E. Chevaux, L. J. Mari, L. F. P. Silva. 2018. Live yeast supplementation improves rumen fibre degradation in cattle grazing tropical pastures throughout the year. Anim. Feed Sci. 236:149-158. https://doi.org/10.1016/j.anifeedsci.2017.12.015.

Sun, F., Y. Cao, C. Cai, S. Li, C. Yu, J. Yao. 2016. Regulation of Nutritional Metabolism in Transition Dairy Cows: Energy Homeostasis and Health in Response to Post-Ruminal Choline and Methionine. PLoS ONE 11: e0160659. https://doi.org/10.1371/journal.pone.0160659.

Takyia, C. S., R. G. Chesini, A. C. Freitas, N. T. S. Grigoletto, D. J. C. Vieira, G. Poletti, N. P. Martins, O. P. Sbaralho, N. Roth, T. Acedo, C. Cortinhas, and F. P. Rennó. 2024. Dietary supplementation with live or autolyzed yeast: Effects on performance, nutrient digestibility, and ruminal fermentation in dairy cows. J. Dairy Sci. https://doi.org/10.3168/jds.2023-24194.

Vailati-Riboni, M., D. N. Coleman, V. Lopreiato, A. Alharthi, R. E. Bucktrout, E. Abdel-Hamied, I. Martinez-Cortes, Y. Liang, E. Trevisi, I. Yoon, J. J. Loor. 2021. Feeding a Saccharomyces cerevisiae fermentation product improves udder health and immune response to a *Streptococcus uberis* mastitis challenge in mid-lactation dairy cows. J. Anim. Sci. Biotech. 12: 62. https://doi.org/10.1186/s40104-021-00560-8.

Zhou, M., P. W. G. Groot Koerkamp, T. T. T. Huynh, and A. J. A. Aarnink. 2023. Evaporative water loss from dairy cows in climate-controlled respiration chambers. J. Dairy Sci. 106:2035-2043. https://doi.org/10.3168/jds.2022-22489.

Zhu W., Z. Wei, N. Xu, F. Yang, I. Yoon, Y. Chung, J. Liu, J. Wang. 2017. Effects of Saccharomyces cerevisiae fermentation products on performance and rumen fermentation and microbiota in dairy cows fed a diet containing low quality forage. J. Anim. Sci. Biotechnol. 81:36. https://doi.org/10.1186/s40104-017-0167-3.

Table 1. Ingredients and chemical composition of the experimental diet used in Study 1

Item	% DM
Ingredient	
Corn silage	87.5
Soybean meal	4.91
Ground corn grain	3.38
Dried distilled grain	2.14
Urea	1.23
Mineral mix	0.28
Limestone	0.24
Sodium bicarbonate	0.14
Sodium chloride	0.11
Ammonium sulfate	0.09
Chemical composition, DM	
Dry matter	33.5
Crude protein	12.6
Rumen degradable protein	10.1
Rumen undegradable protein	2.5
Metabolizable protein	8.57
Neutral detergent fiber	40.9
Starch	15.0
Ether extract	2.35

Table 2. Ingredients and chemical composition of the experimental diet used in Study 2

Item	% DM
Ingredient	
Corn silage	50.5
Finely ground corn grain	15.1
Dried distilled grain	10.0
Cottonseed whole	8.87
Soybean meal	8.41
Tifton hay	4.42
Vitamin and mineral mix[1]	1.43

Sodium bicarbonate	0.74
Magnesium oxide	0.36
Urea	0.25
Chemical composition, DM	
Dry matter	45.3
Crude protein	16.0
Rumen degradable protein	11.0
Rumen undegradable protein	5.0
Neutral detergent fiber	41.4
Starch	22.0
Ether extract	3.92

[1]Mineral mix composition = 200 g/kg of calcium, 15 mg/kg of cobalt, 700 mg/kg of cupper, 15g/kg of sulfur, 43 g/kg of phosphorus, 35 mg/kg of iodine, 40 g/kg of magnesium, 1 g/kg of manganese, 20 mg/kg of selenium, 90 g/kg of sodium, 2.6 g/kg of zinc, 240,000 UI/kg of A vitamin, 87,000 UI/kg of D_3 vitamin, and 1,750 UI/kg of E vitamin.

Table 3. Chemical composition of feeds (%) used to estimate rumen degradability in cows receiving *Saccharomyces cerevisiae* CNCM I-1077 supplementation or control in Study 1

Feed	Forage group	Origin	DM[1], %	CP[2] %DM	Ash %DM	NDF[3] %DM	EE[4] %DM	Starch %DM
BMR 1	HQS[5]	USA	36.7	7.06	9.5	37.2	0.4	35.8
BMR 2	LQS	USA	27.7	7.15	4.2	42.7	1.4	23.5
BMR 3	HQS[6]	USA	32.1	8.45	3.4	32.0	2.1	29.4
BMR 4	HQS	USA	35.1	7.19	3.2	30.2	1.3	46.3
BMR 5	HQS	USA	33.9	6.81	2.9	30.3	1.6	25.6
BMR 6	LQS	USA	37.2	7.11	3.1	34.4	1.2	27.2
Haylage 1	Alfalfa	USA	43.6	18.4	9.6	36.1	0.65	0.0
Haylage 2	Alfalfa	USA	27.9	20.5	11.0	39.4	0.5	0.0
Haylage 2	Alfalfa	USA	45.3	12.9	9.7	38.5	0.9	0.0
Haylage 4	Alfalfa	USA	43.7	18.9	9.8	39.3	0.7	0.0
Corn silage 1	LQS[6]	Brazil	38.8	6.54	2.9	30.4	1.2	33.0
Corn silage 2	HQS	Brazil	31.9	8.01	3.8	35.6	3.1	21.0
Corn silage 3	HQS	Brazil	33.4	7.72	4.1	29.0	1.8	23.7
Corn silage 4	HQS	Brazil	36.7	6.43	2.8	39.5	1.6	14.1
Corn silage 5	LQS	Brazil	36.3	7.74	5.0	41.1	0.5	18.1
Corn silage 6	LQS	Brazil	30.9	7.37	3.8	37.6	1.1	30.5
Soybean hulls	NFFS[7]	Brazil	88.3	8.71	4.0	63.2	1.3	0.3
Dried distillers grains	NFFS	Brazil	92.8	44.8	2.5	48.1	8.4	0.6
Citrus pulp	NFFS	Brazil	90.8	7.21	8.6	25.6	1.9	2.8
Cottonseed whole	NFFS	Brazil	88.0	21.9	3.5	48.4	11.2	0.9

[1]Dry matter.
[2]Crude protein.
[3]Neutral detergent fiber.
[4]Ether extract.
[5]HQS: High quality corn silage.
[6]LQS: low quality corn silage.
[7]Non-forage fiber source.

Table 4. Rumen degradability of tropical corn silage, brown midrib corn silage, and alfalfa in cows fed or not fed *Saccharomyces cerevisiae* strain CNCM I-1077

	Treatment						*P* -value[1]		
	Control			SC[2]					
	HQS[3]	ALF[4]	LQS[5]	HQS[3]	ALF[4]	LQS[5]	Treatment	Feed	T × F
DM degradability, %									
12 h	9.2 ± 1.00[b]	25.8±1.20[a]	10.5 ± 1.01[b]	10.6 ± 1.00[b]	26.4±1.21[a]	10.2 ± 1.00[b]	0.49	<0.01	0.69
30 h	75.5 ± 1.00[ab]	78.1±1.15[a]	74.4 ± 1.00[b]	78.6 ± 1.00[ab]	78.5±1.12[a]	76.8 ± 0.9[b]	0.01	0.02	0.34
120 h	85.8 ± 0.90[a]	81.3±1.00[b]	84.0 ± 0.81[a]	87.6 ± 0.88[a]	81.3±1.00[b]	85.8 ± 0.81[a]	0.11	<0.01	0.52
NDF degradability, %									
12 h	26.5 ± 1.37[b]	42.7±1.59[a]	24.4 ± 1.38[b]	28.2 ± 1.36[b]	45.5±1.57[a]	25.2 ± 1.37[b]	0.07	<0.01	0.74
30 h	49.5 ± 1.44[b]	54.3±1.66[a]	45.8 ± 1.41[c]	52.7 ± 1.43[b]	56.5±1.62[a]	46.5 ± 1.40[c]	0.05	<0.01	0.57
120 h	72.6 ± 1.08[a]	60.4±1.19[c]	72.2 ± 1.00[a]	74.6 ± 1.05[a]	63.6±1.19[c]	74.1 ± 1.00[a]	0.01	<0.01	0.81

[1]*P*-value refers to treatment (T), feed (F), and their interaction (T × F).
[2]*Saccharomyces cerevisiae* strain CNCM I-1077 supplied at 1× 10^{10} CFU day $^{-1}$.
[3]HQS: High quality corn silage
[4]ALF: Alfalfa.
[5]LQS: Low quality corn silage
[a,b,c]Lowercase letters indicate difference for feeds.

Table 5. Rumen degradability of non-forage fiber sources in cows supplemented or not supplemented with *Saccharomyces cerevisiae* strain CNCM I-1077

	Treatments								*P*-value[1]		
	Control				SC[2]						
	SBH[3]	CIT[4]	COT[5]	DDG[6]	SBH	CIT	COT	DDG	Treatment	Feed	T × F
DM degradability, %[7]											
12 h	46.3±1.53[c]	82.0±1.53[a]	49.7±1.61[b]	50.6±1.53[b]	46.2±1.53[c]	81.6±1.53[a]	51.5±1.61[b]	49.8±1.53[b]	0.94	<0.01	0.77
30 h	71.2±1.37[b]	96.3±1.37[a]	57.8±1.37[d]	65.2±1.37[c]	66.8±1.37[b]	95.7±1.47[a]	56.7±1.37[d]	65.9±1.47[c]	0.29	<0.01	0.24
120 h	97.4±0.65[a]	96.5±0.60[a]	71.5±0.60[b]	97.0±0.60[a]	97.6±0.60[a]	96.6±0.60[a]	74.4±0.60[b]	97.4±0.60[a]	0.04	<0.01	0.06
NDF degradability, %											
12 h	37.5±2.39[c]	69.4±2.39[a]	22.9±2.58[d]	54.9±2.39[b]	36.2±2.39[c]	66.1±2.39[a]	21.2±2.58[d]	52.0±2.39[b]	0.24	<0.01	0.97
30 h	63.8±2.20[b]	89.6±2.20[a]	29.9±2.20[c]	62.7±2.20[b]	59.7±2.20[b]	88.9±2.37[a]	35.1±2.20[c]	64.7±2.37[b]	0.70	<0.01	0.19
120 h	96.2±1.86[a]	89.3±1.72[b]	55.7±1.72[c]	96.4±1.72[a]	97.2±1.72[a]	89.4±1.72[b]	58.5±1.72[c]	97.1±1.72[a]	0.33	<0.01	0.88

[1]*P*-value refers to treatment (T), feed (F), and their interaction (T × F).
[2]*Saccharomyces cerevisiae* strain CNCM I-1077 supplied at 1× 10^{10} CFU day^{-1}.
[3]Soybean hulls.
[4]Citrus pulp.
[5]Cottonseed whole.
[6]Dried distilled grain.
[7]Time 0 was not compared between treatments.
[a,b,c]Lowercase letters indicate a difference for feeds.

Table 6. Means and standard errors of the means (SEM) for total intake, total tract apparent digestibility, and feed efficiency in lactating cows supplemented or not supplemented with live yeast *Saccharomyces cerevisiae* CNCM-1077

Item	Control	SC[1]	SEM[2]	*P*-value		
				Treatment	Day	Treatment × Day
Total intake, kg						
Dry matter	21.4	20.0	0.58	0.04	0.20	0.30
Organic matter	20.2	18.9	0.55	0.05	0.28	0.20
Crude protein	4.4	4.1	0.32	0.08	0.80	0.99
Neutral detergente fiber	8.5	8.0	0.22	0.27	0.01	0.30
Apparent total-tract digestibility, %						
Dry matter	66.0	67.1	0.44	0.02	-	-
Organic matter	68.0	68.3	0.80	0.82	-	-
Crude protein	80.1	80.3	0.52	0.73	-	-
Neutral detergente fiber	53.3	56.1	1.33	0.14	-	-

[1] Cows supplemented with live yeast *Saccharomyces cerevisiae* CNCM-1077.
[2]Standard error of mean.

Table 7. Means and standard error of means to milk composition and efficiency of milk production in lactating cows supplemented with live yeast *Saccharomyces cerevisiae* CNCM-1077 (SC) or not supplemented

Item	Control	SC[1]	SEM[2]	P-value		
				Treatment	Day	Treatment × Day
Milk yield, kg/d						
Milk	27.5	29.6	1.28	0.12	0.70	0.87
FCM 4%[3]	26.5	27.7	1.79	0.47	0.55	0.32
Milk energy[4]	26.5	27.2	1.73	0.76	0.65	0.26
ECM[5]	26.3	27.6	1.57	0.46	0.76	0.56
Milk component yield, kg/d						
Fat	1.01	1.06	0.033	0.64	0.46	0.19
Protein	0.90	0.92	0.056	0.92	0.84	0.57
Lactose	1.34	1.34	0.049	0.91	0.88	0.30
Total solids	3.26	3.37	0.184	0.14	0.66	0.13
Milk composition						
Fat, %	3.58	3.58	0.196	0.58	0.42	0.15
Protein, %	3.17	3.11	0.001	0.10	0.71	0.39
Lactose, %	4.74	4.71	0.000	0.92	0.92	0.65
Solids, %	11.5	11.4	0.275	0.32	0.45	0.19
SCC, cells/mL[6]	4.09	3.91	0.824	0.02	-	-
MUN, mg/dL	16.5	15.4	0.65	0.07		
Efficiency of milk production, kg/kg						
Milk/DMI	1.33	1.49	0.08	0.02	0.81	0.81
FCM/DMI	1.24	1.40	0.09	0.03	0.64	0.44
Milk/OMI	1.41	1.57	0.09	0.02	0.43	0.79
FCM/OMI	1.32	1.48	0.09	0.03	0.64	0.44

[1]SC: cows supplemented with live yeast *Saccharomyces cerevisiae* CNCM-1077.
[2] Standard error of mean.
[3]FCM4% = $\frac{291.14 \times Milk\ production\ in\ kg + 10944 \times Milk\ production\ in\ kg \times percentual\ of\ fat)}{728.88}$.
[4]Milk energy = $12.51 * Fat\ (kg) + 7.41 * Protein(kg) + 5.32 * Lactose\ (kg)$.
[5]ECM = $0.252 \times Milk\ yield\ (kg) + 12.3 \times Fat\ (kg) + 7.77 \times Protein\ (kg)$.
[6]Somatic cell count, logarithmic scale.

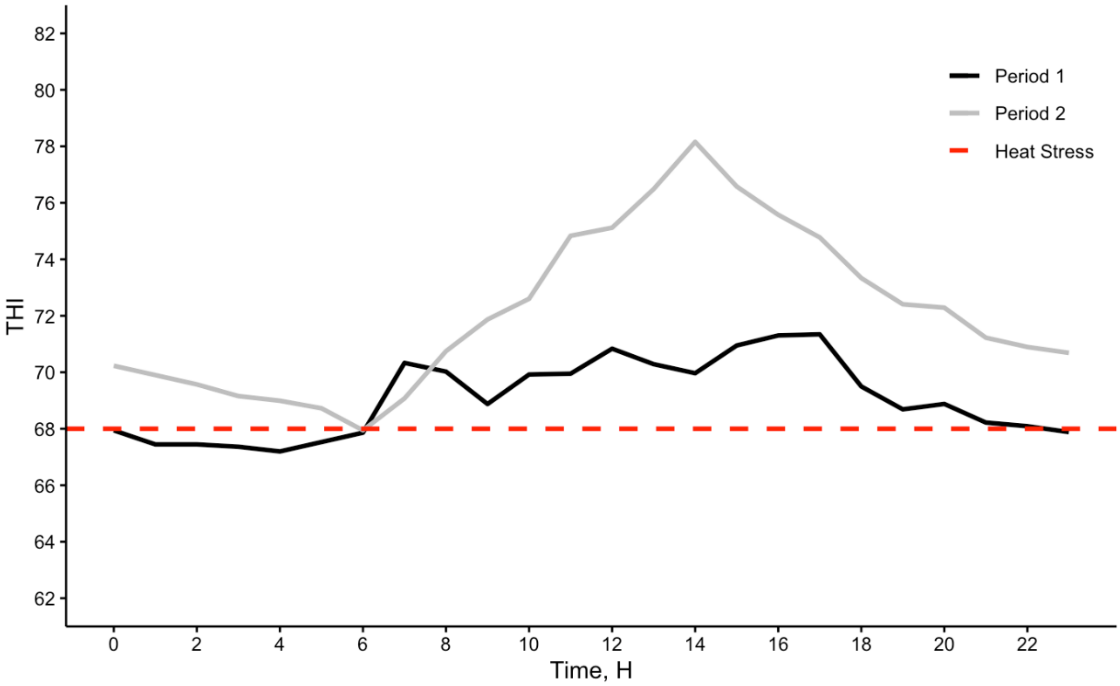

Figure 1. Temperature-humidity index (THI) recorded during Study 2.

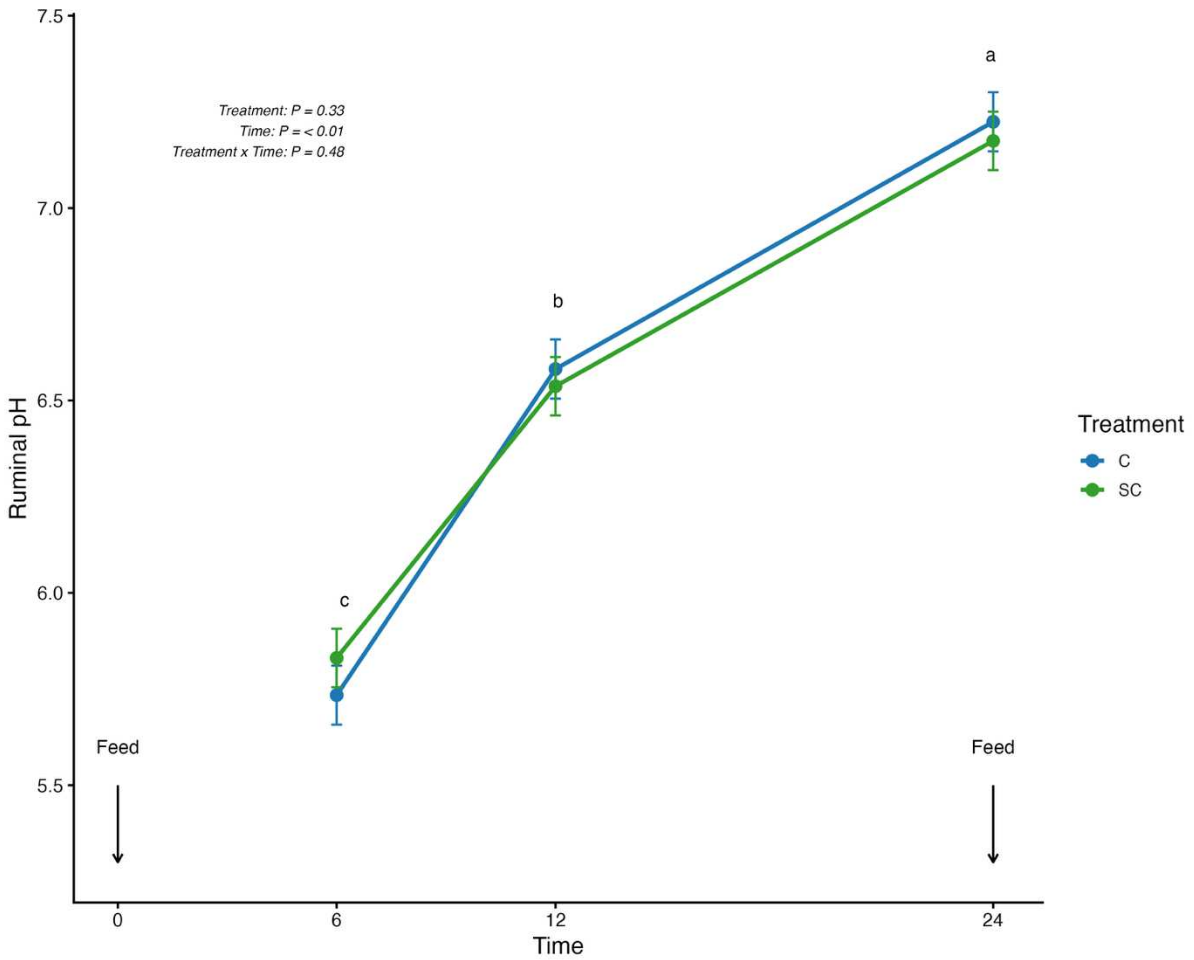

Figure 2. Rumen pH in relation to daytime hours in cows supplemented with *Saccharomyces cerevisiae* strain CNCM I-1077 (SC) or the control in Study 1.

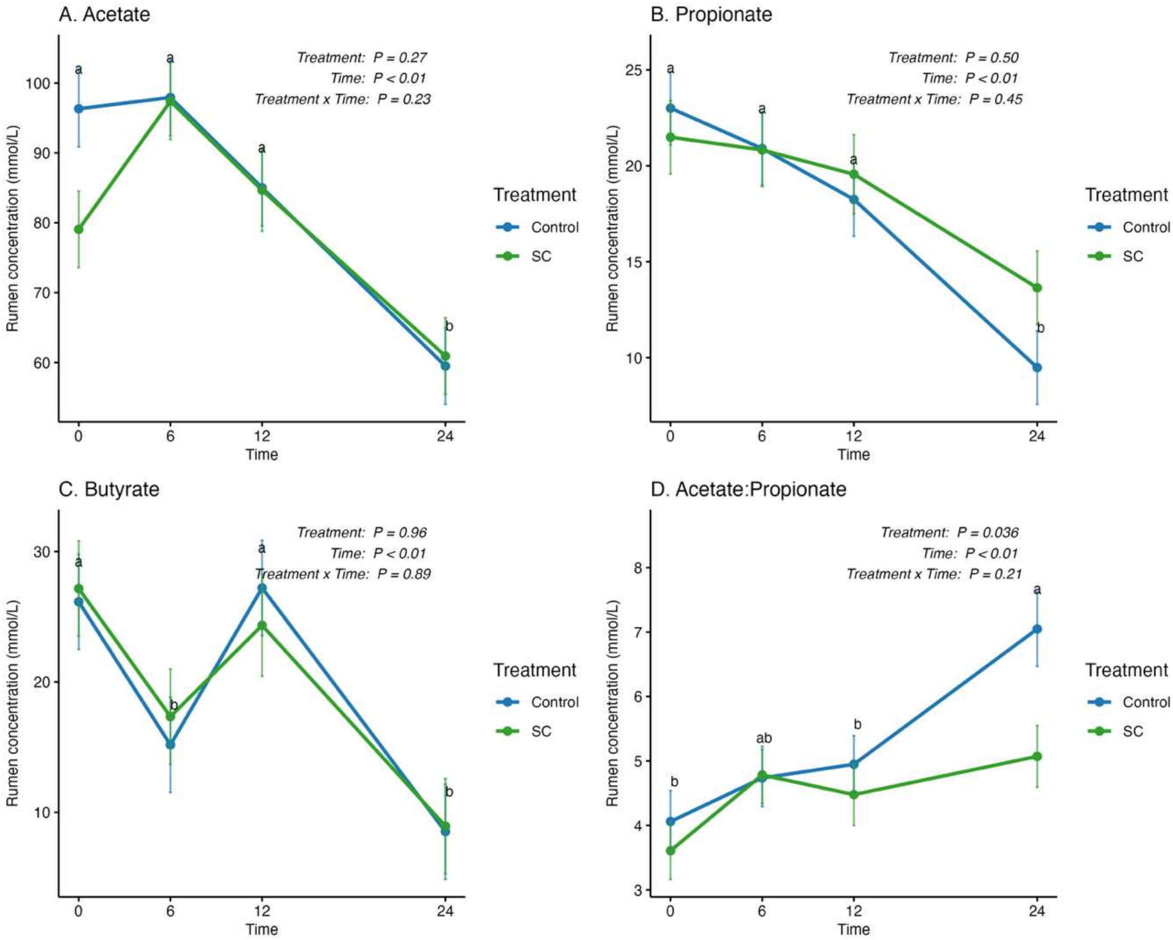

Figure 3. Concentration of volatile fatty acids (VFA) in the rumen fluid during the day in cows supplemented with *Saccharomyces cerevisiae* strain CNCM I-1077 (SC) or the control in Study 1. A. Acetate concentration; B. Propionate concentration; C. Butyrate concentration; and D. Proportion of acetate and propionate. The lower-case letters represent the effects of time after feeding.

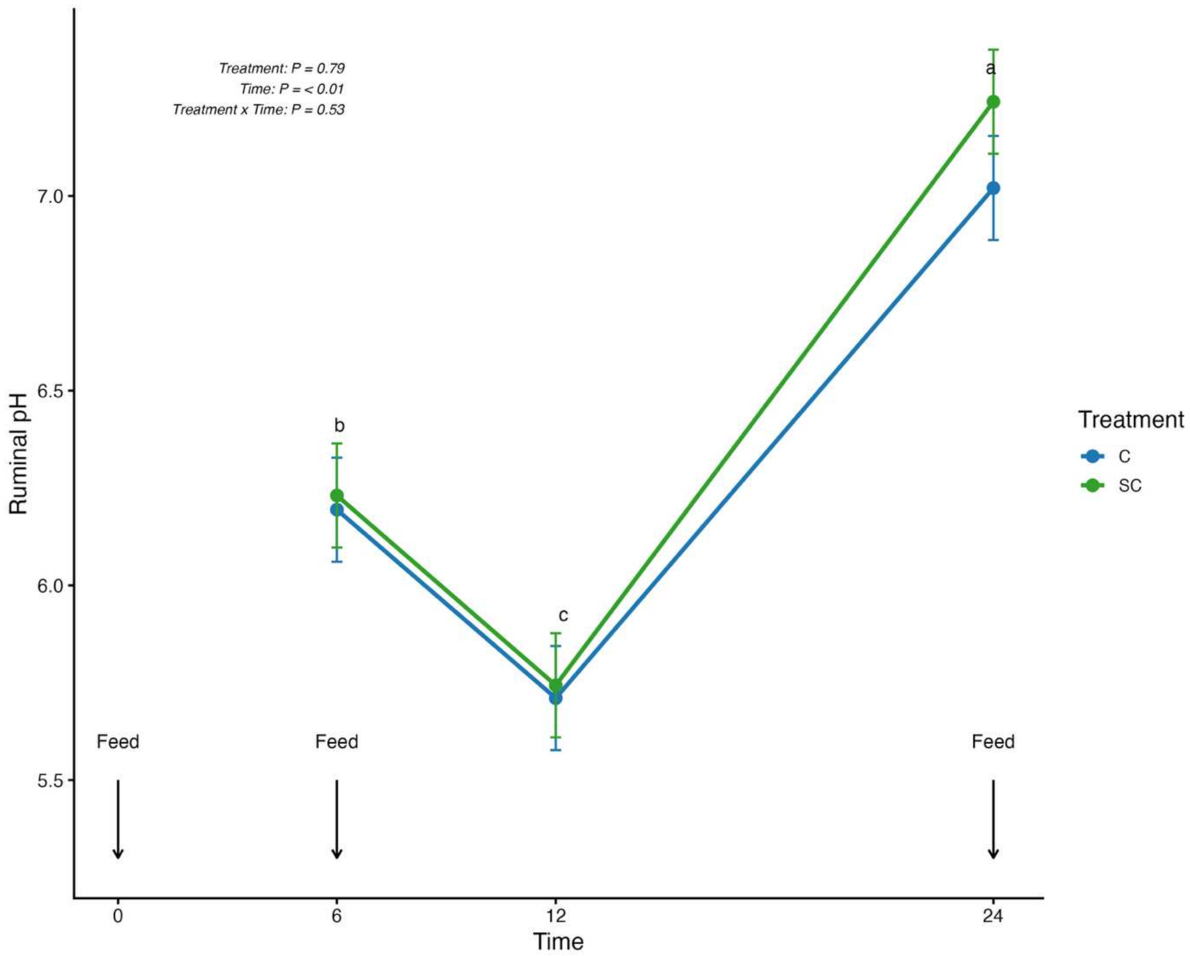

Figure 4. Rumen pH six hours after morning feeding (6 h), twelve hours after morning feeding and four hours after afternoon feeding (12 h), and 24 h after feeding or immediately before morning feeding of lactating cows supplemented with live yeast Saccharomyces cerevisiae CNCM-1077 (SC) or control in Study 2.

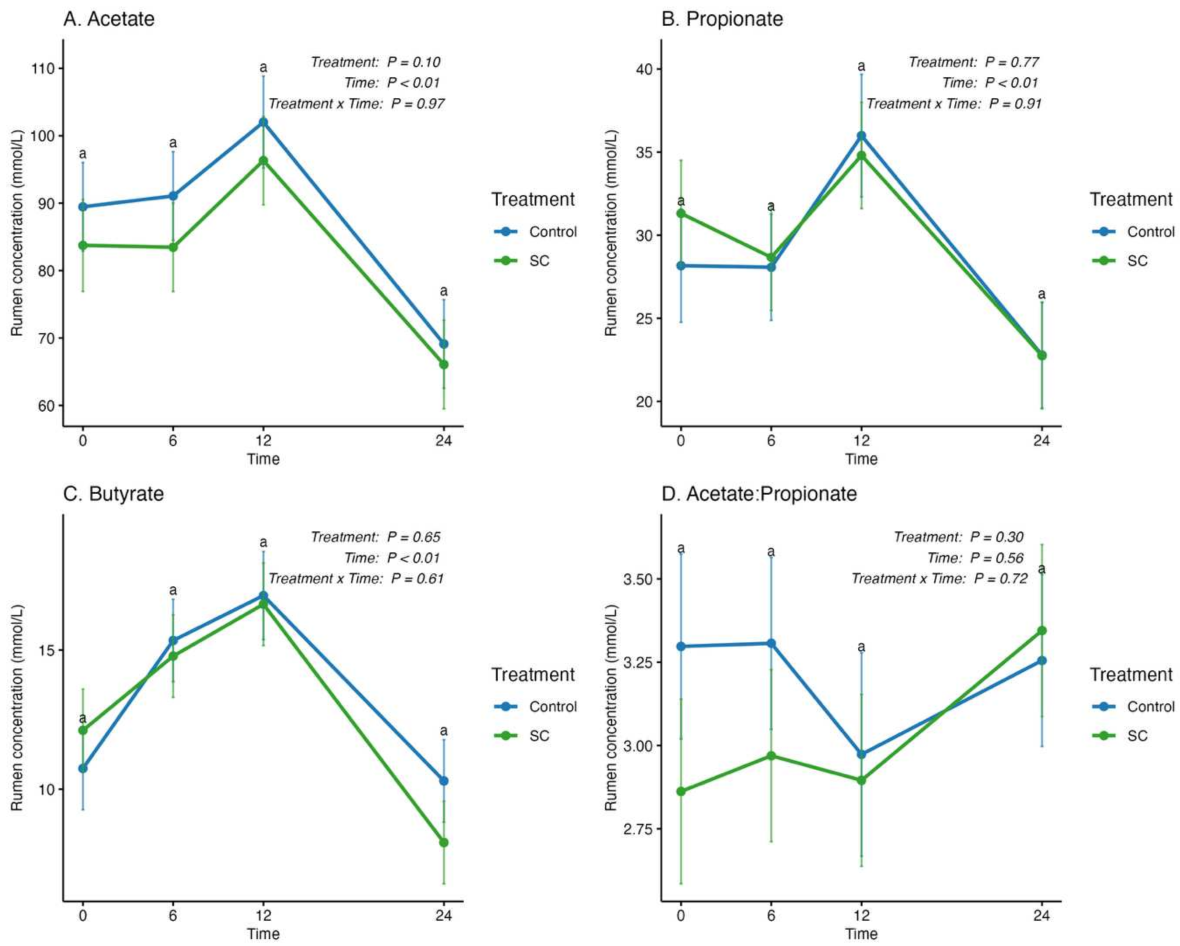

Figure 5. Rumen concentration (mmol/L) of volatile fatty acids (VFA) in Study 2. A. acetate concentration; B. propionate concentration; C. butyrate concentration; and D. proportion of acetate and propionate. The lowercase letters represent the effects of time after feeding.

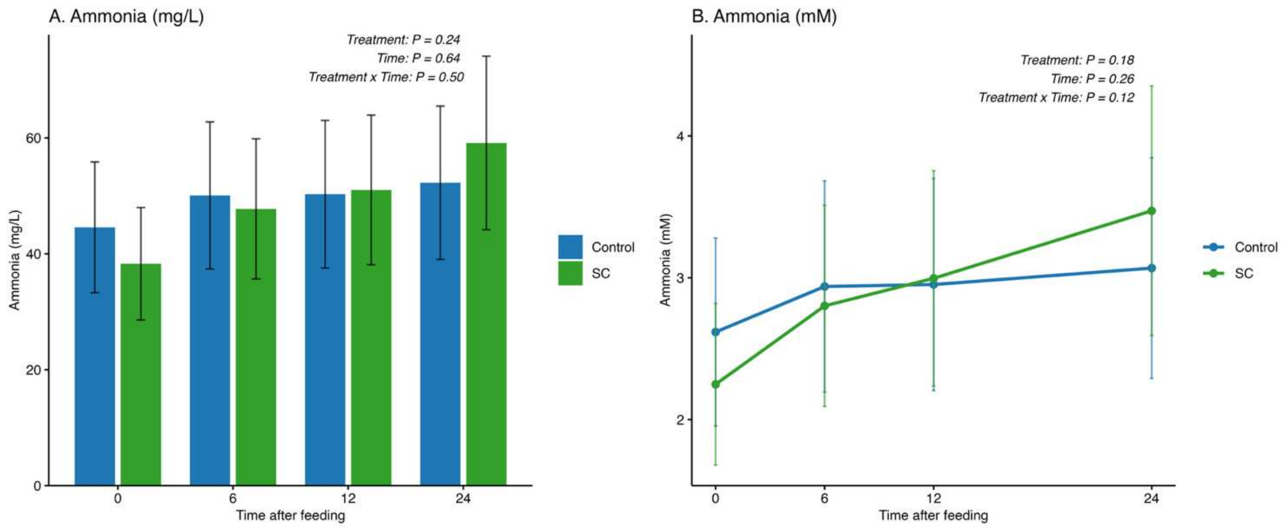

Figure 6. Ruminal ammonia concentration (A) in mM and (B) in mg/L across 0, 6, 12, and 24 h after morning-feeding. Points and bars represent estimated marginal means ± 95% CI from linear mixed models including treatment, hour, and their interaction, with cow nested within period as a random effect.

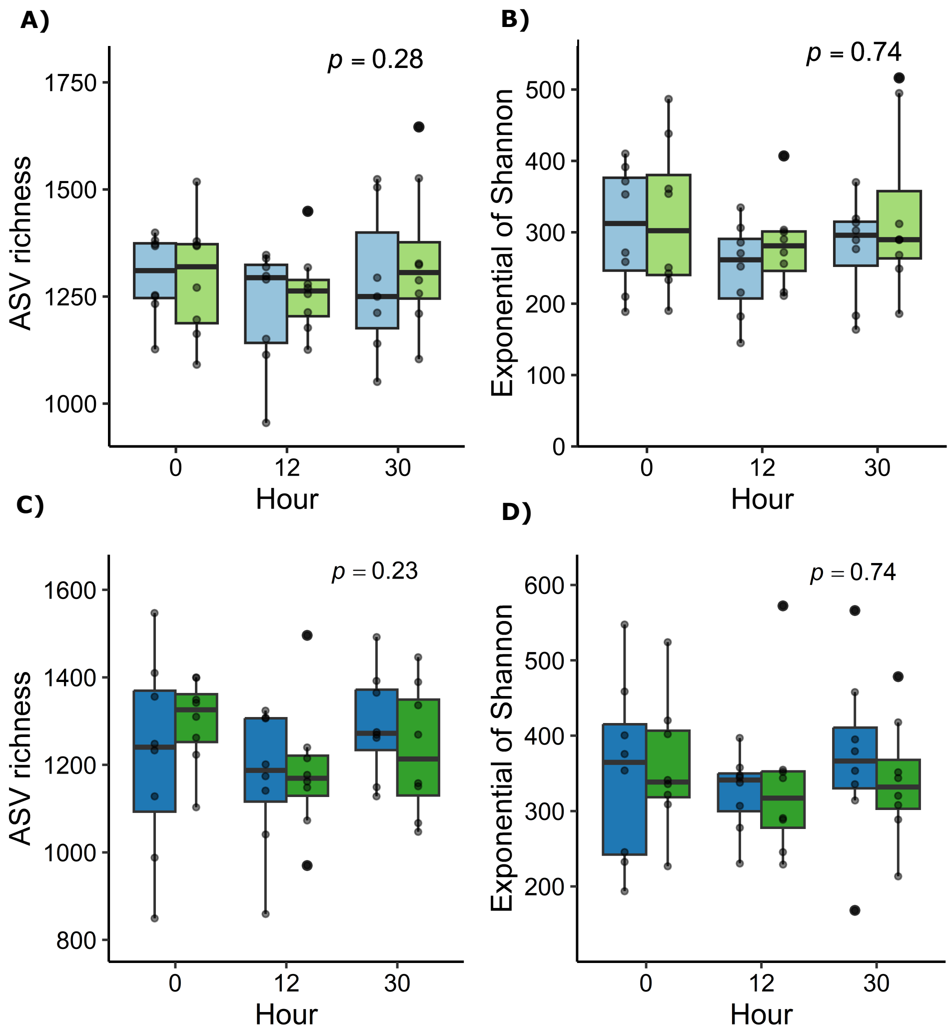

Figure 7. Effect of treatment groups and hours after feeding on the alpha diversity indexes of the samples of the first and second trial. Panels A and C show the ASV richness of each treatment, and Panels B and D show the Exponential of Shannon. Panels A and B correspond to the first trial, panels C and D to the second trial.

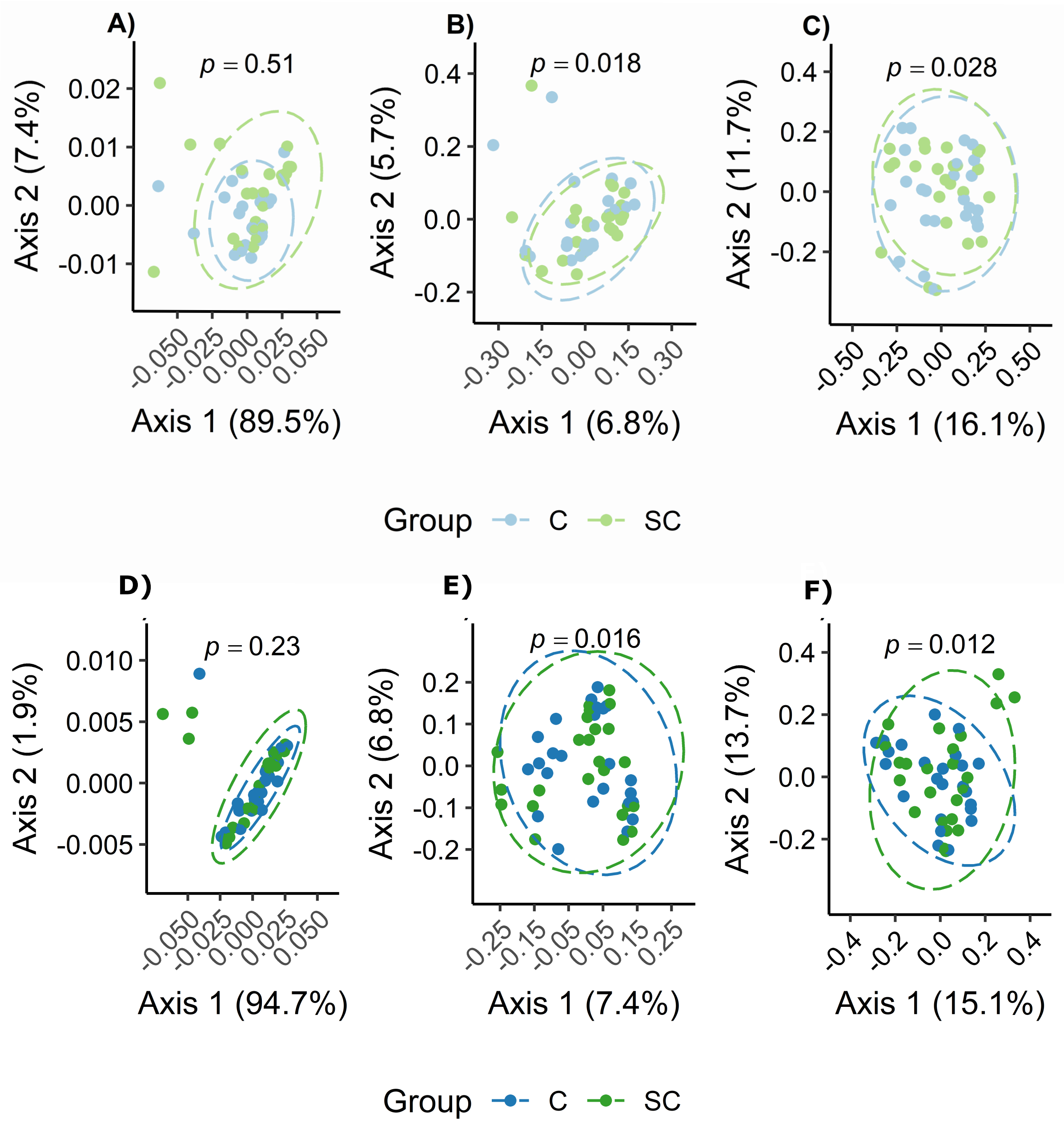

Figure 8. Effect of treatment groups on the beta diversity of the samples collected in the first and second trials. The distance is represented in a principal coordinates analysis plot (PCoA), colored based on the treatment groups. Panels A and D show the Weighted UniFrac distance between samples, Panels B and E show the Unweighted UniFrac distance between samples, and Panels C and F show the Bray-Curtis distance between samples. Panels A, B, and C correspond to the first trial, panels D, E, and F to the second trial.

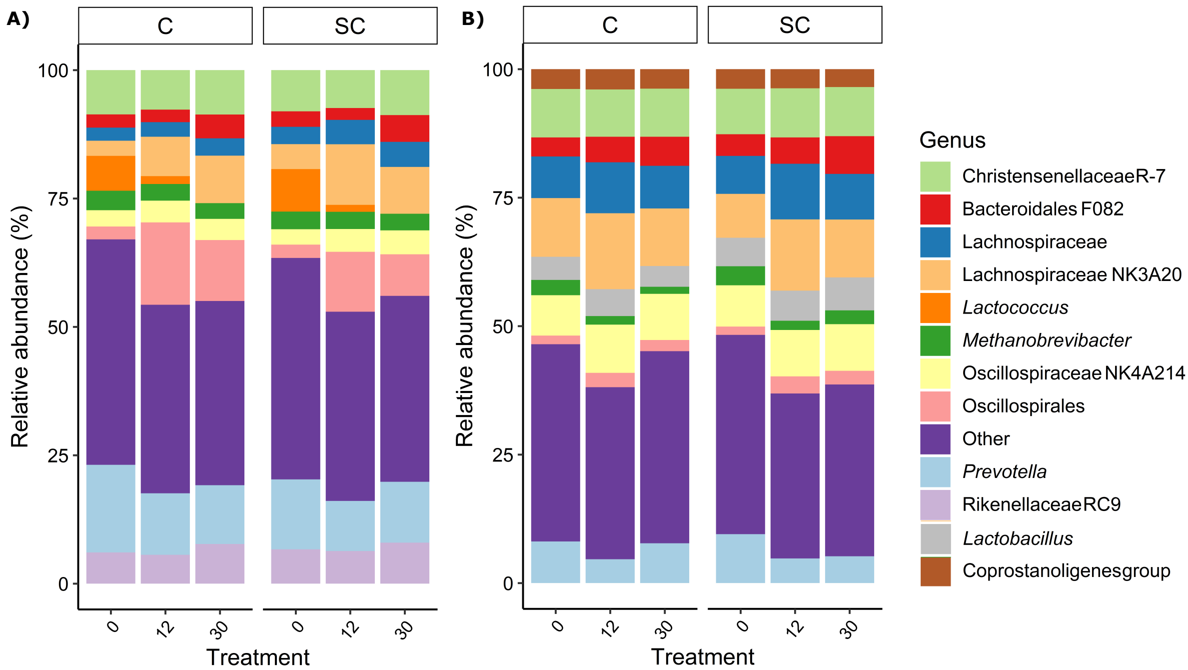

Figure 9. Taxonomic compositions of the treatment groups in the first and second trials, divided by hour after feeding. Taxa with an abundance below 1% were grouped as "Other". Panel A correspond to the first trial and panel B to the second trial

CHAPTER 2: RETAINED PLACENTA IN DAIRY COWS: AN EVALUATION OF RISK FACTORS AND REPRODUCTIVE PERFORMANCE

*Formatted following the guidelines of Theriogenology Journal

RETAINED PLACENTA IN DAIRY COWS: AN EVALUATION OF RISK FACTORS AND REPRODUCTIVE PERFORMANCE

K. R. Oliveira[1], T. E. da Silva[2], J. H. C. Costa[2], T. M. Ferreira[3], A. L. Silva[1], C. E. C. Consentini[4], R. Sartori[5], and P. P. Rotta[1*]

[1]Department of Animal Science, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil 36570-900.

[2]Department of Animal and Veterinary Sciences, University of Vermont, Burlington, VT 05401.

[3]Alta Genetics do Brasil, Uberaba, Minas Gerais, Brazil 38055-010.

[4]GlobalGen vet science, Jaboticabal, São Paulo, Brazil 14887-360.

[5]Department of Animal Science, ESALQ, University of São Paulo, Piracicaba, São Paulo, Brazil 13418-900.

[*]*Corresponding author: P. P. Rotta, phone number: +55 31 99449 1703, current mailing address: Department of Animal Science, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil, 36570-900, e-mail: polyana.rotta@ufv.br*

ABSTRACT

Retained placenta (RP) is a common postpartum disorder in dairy cows and is consistently associated with impaired fertility and increased reproductive losses. This study aimed to identify environmental, management, and genetic factors associated with RP and to evaluate its effects on reproductive performance and culling in commercial dairy farms under tropical conditions. A retrospective dataset including 138,175 calvings from 437 commercial dairy herds after data validation, from 2021 to 2023 was analyzed. Retained placenta was defined as the failure to expel the fetal membranes within 24 h after calving. Generalized linear mixed models were fitted using the glmmTMB package in R, with farm and cow included as random effects when appropriate. A total of 289,391 artificial insemination (AI) records were available for pregnancy per AI (P/AI) analyses, and 57,628 confirmed pregnancies were used for pregnancy loss (PL) analyses. Fixed effects included parity, breed, type of artificial insemination (AI), season, and year. Farms had Holstein and Holstein × Gyr (HG) crossbred cows in free stall, compost barn, or dry lot systems. Twin births, male calves, abortions, premature, higher parity, and the Holstein breed were associated with increased odds of RP. Crossbred 1/2 Holstein × Gyr (HG) cows had lower odds of RP compared to Holstein cows. Retained placenta was associated with reduced odds of P/AI at first and overall inseminations and with increased odds for PL, consistently across breeds, parity groups, and AI strategies. Cows affected by RP also had a higher risk of culling during lactation. These findings highlight the multifactorial nature of RP and its persistent negative association with fertility and herd longevity in tropical dairy systems.

Keywords: artificial insemination, fertility, pregnancy loss, retained placenta

1. Introduction

Retained placenta (**RP**) is one of the most frequent postpartum disorders in dairy cows and is typically defined as the failure of the fetal membranes to detach within 24 h after calving [1, 2]. The incidence of RP in cattle is influenced by multiple factors, including physiological and metabolic conditions, environmental and nutritional aspects, breed composition, seasonal variation, calving complications (e.g. twins, abortions, induced parturition) and increasing maternal age [3, 4]. Genetic background and production intensity further modulate the risk of RP, particularly in high-producing cows [5].

These factors may affect immunological functions that are critical for placental expulsion, such as neutrophils activity and calcium homeostasis [6, 7]. Additionally, difficult calvings, such as in multiple births, can cause uterine trauma and inflammation, leading to metabolic disturbances, tissue damage, and dysfunction, which may disrupt the process of membrane separation [8, 9].

The incidence of RP varies between 3% to 10% in most studies, but can reach higher levels under tropical conditions, where heat stress and metabolic challenges due oxidative stress [10]. Holstein × Gyr (HG) crossbred cows show greater tolerance to a hot environment compared with Holstein cows, which may favor postpartum recovery and potentially reduce the risk of retained placenta [11]. These advantages have been more consistently documented in grazing systems [12,13] and in studies evaluating reproductive performance [14–16].

Although RP has been extensively studied in temperate regions, information from tropical, confined dairy systems remains limited. Heat stress, management heterogeneity, and the widespread use of Holstein × Gyr crossbred cows may alter both RP incidence and its reproductive consequences under tropical conditions. Retained placenta has been associated with subsequent uterine disease [17], reduced P/AI, increased PL [18, 19], prolonged calving intervals [20], and increased culling risk[21, 22]. However, many previous studies relied on single-herd datasets or analytical approaches that did not account for herd-level clustering. Therefore, there is limited information regarding the impact of RP on reproductive performance across herds differing management practices, breed composition, and AI strategies.

Therefore, the objectives of this study were to identify environmental, management and genetic factors associated with RP and to evaluate the association between RP and its impact on P/AI, PL, and culling risk in dairy cows on commercial dairy herds, using mixed-effects models. We hypothesized that RP incidence would be associated with calving-related events, parity, season, and breed. In addition, we expected that cows experiencing RP would persistently impair reproductive performance, such as lower P/AI and higher PL, regardless of breed.

2. Material and Methods

2.1. Study design and data collection

The retrospective observational cohort study used cow-level data collected voluntarily through the "Concept Plus Dairy" program (Alta Genetics Brazil), which includes 961 farms in 10 Brazilian states: Alagoas, Ceará, Goiás, Minas Gerais, Santa Catarina, São Paulo, Paraná, Pernambuco, Rio de Janeiro, and Rio Grande do Sul. The program collects data from dairy farms across Brazil and compiles it into a national reference for the dairy industry. Participation in this Alta Genetics' program is voluntary and not connected to any commercial relationship. In return, participating veterinarians and farmers receive training, access to the Brazilian benchmark, and are ranked among the participating farms. Only herds maintaining continuous veterinarian assistance for at least one year and an annual average higher than 50 lactating cows were included.

The initial dataset comprised 240,506 calving events, 1,270,642 AI, and 467,853 pregnancies, from a total of 197,384 cows from first and later lactation cows recorded between January 2021 and December 2023 (Figure 1). Farmers and veterinarians regularly accessed the data through Ideagri software (Rúmina, Belo Horizonte, Brazil) or Alta Gestão software (Alta Genetics Inc., Uberaba, Brazil), both farm management platforms used to record production and reproduction. Information included calving records, AI, and pregnancy diagnosis. For the analysis of PL, only services with a positive previous diagnosis and potential follow-up through to

260 days after AI were retained, considering subsequent reproductive record (pregnancy re-check, calving, abortion, or reinsemination). Data on other peripartum diseases (e.g. metritis or ketosis) were not available, which was considered a limitation.

In addition, information on herd size and farm characteristics, production systems, and frequency of reproductive management were previously collected by Alta Genetics of Brazil using standardized forms completed by the veterinarians directly responsible for reproductive management on the participating farms.

Farms were only included in the study if they had received continuous veterinary care for at least 1 yr. Among all eligible farms, 52 contributed data continuously across all three years of observation (2021–2023), whereas the remaining farms contributed data for one or two years. Retained placenta was assessed during this period and defined as the failure to expel fetal membranes within 24 h after calving. Veterinarians from each farm consistently assess the data, with a commitment to training the farm staff to record RP data.

After data quality control and removal of incomplete or duplicated records, the final dataset included 138,175 calvings from 437 herds for RP analysis, 289,391 AI events for P/AI evaluation, and 57,628 pregnancies for PL analysis.

2.2. Animals, herds and management

Breeds were classified as Holstein (57.4%) or Holstein × Gyr crossbred cows (HG). Crossbred cows were categorized according to the proportion of Holstein genetics as 7/8 HG (14.3%), 3/4 HG (18.5%), or 1/2 HG (9.8%), indicating approximately 87.5%, 75%, and 50% (F1) Holstein genetic composition, respectively. Production systems included compost barn (68%), free stall (19.5%), and dry lot (12.5%). Records lacking information in the production system were excluded, resulting in the removal of 21,840 calving records, 274,965 AI records, and 106,632 pregnancy records. Herd size was expressed as the average number of lactating cows during the year, divided into less than 100 lactating cows, between 100 and 200 lactating cows, and more than 200 lactating cows. Information regarding diets was not available; therefore, nutritional management could not be evaluated.

Use of TAI at first service ranged from 0-100% across farms (mean = 83.4%). Cows were first inseminated between 38 and 81 DIM, out of the determined voluntary waiting period of 60 DIM for most farms. Heat abatement measures varied: 52.08% of farms used fans in the barns, 16.14% had water sprinklers in the feeding alley, and 48.44% used both sprinklers and fans as a cooling strategy in holding pens. Of these, 10.75% applied cooling twice a day, 46.23% three times a day, and 21.50% more than three times a day.

Table 1 shows the descriptive statistics of the final dataset used to perform the analysis, including the raw mean RP incidence and the number of farms included for each risk factor. These raw RP values are not adjusted for potential confounding variables — such as farm, season, parity, or breed composition — and should be interpreted with caution. The differences observed in Table 1 may not reflect true associations that have been formally examined in further statistical analyses.

2.3. Modelling and Statistical Analyses

The statistical analyses were performed using the R (R Core Team, version 2024.04.02). Calving dates were used to assign a corresponding season for analysis. Duplicate records on the same cow (including duplicate farms and parities) were removed, resulting in 171 duplicate calvings. In addition, only complete cases for each model were used. After these adjustments, the final dataset included 138,175 calving records from 437 farms to assess the risk factors associated with RP.

Calving types were defined as 1) normal if the calving process was without complications, 2) assisted for any calving that required intervention, 3) stillbirth for calves that had died by 24 h after birth, 4) premature if the calf was born between 260 and 270 d of gestation, and 5) abortion if a dead calf was born within 270 d after service (Committee on Bovine Reproductive Nomenclature, 1972).

To evaluate overall P/AI, 289,391 AI records from 139,162 cows across 437 farms with complete information were analyzed, considering only AI with diagnosis. The analysis of P/AI at first service included 139,222 AI events from 124,047 cows across 437 farms. Data for PL were retrieved from 57,628 pregnancy records from 49,852 cows and 174 farms. Only pregnancies with sufficient follow-up information to determine pregnancy outcome (maintenance to calving or pregnancy loss) within 260 days after AI were included in the analysis, i.e., from January 2021 to December 2023, were considered. Pregnancies were followed until calving, confirmed PL, or up to 260 days after AI. Pregnancies without sufficient follow-up information to determine outcome within this period were excluded from the PL analysis.

Calving outcomes were defined according to the Committee on Bovine Reproductive Nomenclature (1972). Abortion was defined as the delivery of a dead fetus at $\leq$ 260 d of gestation. Premature calving was defined as parturition occurring after 260 d of gestation, resulting in the birth of a live calf between 261 and 270 d. Stillbirth was defined as a calf born dead at or after 270 d of gestation. Pregnancy diagnoses were performed by the herd veterinarian using transrectal ultrasonography or rectal palpation, according to each farm's routine reproductive management schedule. The first diagnosis occurred at a mean of 32.1 d after AI, and the confirmation exam occurred at 68.0 d, pregnancies were rechecked once to three times. Pregnancy loss was identified based on (1) a negative pregnancy diagnosis following a prior confirmed pregnancy, (2) veterinarian records indicating abortion or fetal loss, or (3) reinsemination following a confirmed pregnancy. To minimize misclassification, only pregnancies with at least one subsequent reproductive record (pregnancy recheck, calving, abortion, or reinsemination) within the follow-up window were included in the PL analysis. Only farms with veterinarians performing weekly and bi-weekly reproductive visit schedules and using AI services were included due to the accuracy of their PL records.

Pregnancy loss was modeled as a binary outcome (1 = pregnancy loss; 0 = pregnancy maintained to ≥ 260 d of gestation) rather than a time-to-event variable because its ascertainment depended on subsequent pregnancy diagnosis or reproductive records, rather than on the exact timing of fetal loss. As the precise date of pregnancy loss could not be reliably determined for all cases, a binary classification approach was considered the most appropriate and conservative analytical strategy. A generalized mixed model with a binomial distribution and logit link function was used, including RP and its interactions with parity, breed, type of service, and AI number as fixed effects. Cows nested within farms were included as random effects to account for herd-level clustering and repeated observation within cows.

Pregnancy loss was recorded observed between 28- and 260-d post-AI, exclusively in cows that had previously been diagnosed with pregnancy. These time limits were established based on the date of first diagnosis and in accordance with the Committee on Bovine Reproductive Nomenclature [23], which defines PL as occurring before 260 d of gestation (after which it is classified as premature calving). Pregnancy loss (PL) was defined as the loss of a previously confirmed pregnancy between 28 and 260 days of gestation.

Although a time-to-event approach could be used to model pregnancy loss, the exact timing of pregnancy loss was not consistently available across herds, as losses were identified based on periodic pregnancy diagnosis, veterinary records, or reinsemination dates. Therefore, a binary mixed-effects model was considered more appropriate to evaluate the overall association between retained placenta and pregnancy loss while accounting for herd- and cow-level clustering.

Generalized linear mixed models were developed using the glmmTMB function from the glmmTMB package. For each outcome, an initial full model including all candidate exploratory variables was specified, with farm included as a random intercept to account for herd-level clustering. Only observations with complete data for all variables included in each model were retained for analysis. Fixed-effect selection was performed using a backward and order-dependent stepwise procedure implemented in the buildglmmTMB function from the buildmer package. Model simplification was guided by minimization of the Akaike information criterion (AIC), and biologically relevant terms were retained when supported by data.

For the RP model, the selected fixed effects included parity, twin calvings, season, calf sex, calving type, production system, herd size based on the mean of lactating cows during the year (less than 100, 100 to 200, or more than 200 lactating cows), breed, and year of data collection. In this model, RP was modeled as a binary outcome (1 = RP, 0 = non-RP), assuming a binomial distribution with a logit link function, with cows nested into the farm as random effects. No evidence of overdispersion was detected (dispersion ratio = 0.98; $P = 0.86$).

To evaluate the impact of RP on P/AI, GLMMs were fitted for first and subsequent performances, categorized as first, second, and third or subsequent AI. Fixed effects included RP and its interactions with type of artificial insemination (estrus or timed artificial insemination [**TAI**]), breed, semen type (sex-sorted or conventional), and parity. Cows nested on the farm were fitted as random effects. No overdispersion was detected in this model (dispersion ratio = 1.00; $P = 0.49$).

The PL model included fixed effects of parity, breed, type of service, AI number, and type of AI, and the interactions with RP. Random effects were specified for farm and cow, which was nested within the farm, to account for repeated measures and herd-level variation, without overdispersion (dispersion ratio = 1.03; $P = 0.14$).

Statistical inference for fixed effects in all GLMMs was based on Wald tests. Global significance of multi-level predictors was assessed using type III Wald chi-squared tests implemented via Anova function from the car package. Post hoc comparisons were performed using estimated marginal means obtained with the emmeans package. Contrasts comparing cows with and without RP within levels of categorical predictors were evaluated using Wald z tests on the log odds ratio scale. Odds ratio (OR) and 95% confidence intervals were derived by back-transformation of model estimates. For RP, P/AI, and PL, marginal means were back-transformed to the probability scale. Estimated marginal means were averaged over the levels of the fixed effects and were weighted proportionally according to the observed sample sizes. Pairwise comparisons were conducted using the cld function from the multcomp package with a significance level of 0.05. Trends ($0.05 < P \leq 0.10$) were not reported.

Time to culling was evaluated using survival analysis. The risk set included all cows with at least one recorded calving during the study period and complete information for RP status, parity, breed, and production system. Time origin was defined as the date of the last observed calving. Follow-up time was calculated as the interval from the last calving to the culling date when available. Cows without a recorded culling date were right censored at the end of the observational period, defined as the last date with follow-up available in the dataset. Records with inconsistent dates resulting in negative follow-up times were excluded.

Each cow contributed a single observation based on its most recent lactation within the study window. Proportional hazards assumptions were assessed using Schoenfeld residuals. Because non-proportionality was detected for parity, breed, and production system, these variables were included as stratification factors, allowing baseline hazards to vary across strata. The association between RP and culling risk was evaluated using a stratified Cox mixed effects model coxme, including farm as a random intercept. Given minor residual deviation from proportional hazards for RP, hazard ratios are interpreted as average effects over the follow-up period. The final culling dataset included 137,690 cows with 37,080 culling events observed.

3. Results

3.1. Risk Factors and Incidence of Retained Placenta

The adjusted overall incidence of RP was 2.6%. The risk of RP increased steadily with the number of lactations from 2.2% in primiparous cows to 5.1% in 7th lactation cows (Table 2). The odds of RP increased with parity, twin births, male calves, assisted calvings, abortions, and premature calvings. Holstein cows had higher odds of RP compared with 3/4 HG and 1/2 HG crossbred cows, whereas it was similar to 7/8 HG cows. Season was also associated with RP occurrence, whereas year, production system, and farm size were not.

The random effect of farm accounted for a substantial proportion of the variability in RP incidence (26%), indicating marked heterogeneity among herds. The incidence of RP varied between the 437 farms evaluated and ranged from 0.01% to 36.38%.

3.2. Pregnancy per AI at First Service According to Retained Placenta and Other Variables

At first AI, none of the factors interacted with RP ($P > 0.05$; Table 3). Although several factors were associated with fertility, including RP itself. Multiparous followed by secundiparous had the worst P/AI compared to primiparous. Crossbred 1/2 HG had higher P/AI compared to all the other breed groups. Conventional semen and timed AI were superior to sexed semen and estrus insemination, respectively. Fertility at first AI was seasonal, peaking in winter and declining in Summer and Fall ($P < 0.01$).

3.3. Overall P/AI According to Retained Placenta and Other Variables

Retained placenta was associated with reduced odds of P/AI at first service and across all inseminations (Table 4). No significant interactions were detected between RP and breed, parity, AI type, or semen type, indicating that the negative association between RP and fertility was consistent across all these factors. However, the negative influence of RP on P/AI was strongest at first AI and diminished with subsequent inseminations. Primiparous cows had highest fertility compared to secundiparous and multiparous cows, whereas 1/2 HG was also superior to the other breeds. Timed AI and conventional semen were associated with higher P/AI.

Inseminations during the Summer resulted in lower P/AI, followed by spring and fall, which were similar, while winter AI had the highest fertility.

3.4. Pregnancy Loss According to Retained Placenta and Other Variables

Retained placenta was associated with a 48% increase in the odds of pregnancy loss ($P = 0.01$; Table 5). No significant interactions were observed between RP and breed type of AI, semen type, or parity ($P = 0.33$).

Parity influenced PL risk, with higher losses in older cows. Parity influenced PL ($P < 0.01$), primiparous cows and cows in 2nd lactation were less likely to have PL than cows in ≥3rd lactation ($P < 0.01$).

In relation to breed, Holstein cows had similar losses to 3/4 HG and 7/8 HG crosses, whereas 1/2 HG cows had the lowest PL. Timed AI seems to increase PL, while semen type did not interfere with this index.

Regarding the number of AI, an interaction ($P = 0.01$) with RP was found. In cows with RP, the PL was similar ($P = 0.36$) for all services. However, in cows without RP, the 1st AI was associated with a lower PL than the ≥ 3rd AI ($P < 0.001$), while the 2nd AI was comparable to the other services. After the 1st service, RP-affected cows had 1.66 odds ratio to PL ($P < 0.01$), while at the 2nd AI it was 1.47 ($P < 0.01$), and at ≥ 3rd AI, RP increased the odds ratio to PL by 1.31 ($P = 0.01$).

Compared to 1st AI in cows unaffected by RP, the odds ratio of PL was similar at 2nd AI 1.06 (CI: 0.98–1.15; $P = 0.07$), and increased by 1.13 (CI: 1.11–1.16; P < 0.01) at 3rd or later AI. These results indicate that the negative influence of RP on PL decreased slightly with successive AI. In cows without RP, PL increased with each subsequent AI. Compared with the 1st AI, the odds ratio of PL increased by 1.07 (CI: 1.01–1.13; $P = 0.02$) with the 2nd AI and by 1.14 (CI: 1.08–1.19; $P < 0.01$) with the ≥3rd AI.

There was a season effect on PL cows inseminated during the Spring had the highest PL compared to those in Fall and Winter ($P < 0.04$); however, Spring and Summer PL were similar ($P = 0.57$). PL was similar in Fall, Winter, and Summer ($P > 0.05$).

3.5. Culling Rate According to Retained Placenta and Other Variables

The culling analysis included 137,690 cows and 37,080 culling events. In the stratified Cox mixed-effects model accounting for farm-level clustering, cows with RP were associated with a higher risk of culling compared to cows without RP (HR = 1.14; CI: 1.10 –1.18; $P < 0.001$).

4. Discussion

This study aimed to identify risk factors for RP and to evaluate its associations with reproductive performance and culling in commercial dairy herds. Using data from 437 herds in Brazil, comprising 138,175 calvings, 289,391 AI records, and 57,628 pregnancies, it was observed that RP incidence was associated with parity and was higher in cows experiencing twin births, male calves, abortions, premature, or assisted calvings. Holstein cows exhibited higher odds of RP than 1/2 HG crossbred. In addition to cow-level and calving-related factors, a substantial proportion of the variability in RP incidence was attributable to herd-level differences, with farm random effects explaining approximately 26% of the total variance. This finding highlights the heterogeneity among herds and suggests that unmeasured management, environmental, or record-related factors contribute meaningfully to RP occurrence.

Retained placenta is a common health problem on dairy farms that is negatively associated with reproduction, productivity, and profitability, and is often due to a suboptimal transition period [3]. The incidence of RP is 5 to 10% in Canada [3, 24], 9 to 15% in Iran [25], and 6.7 to

19.9% in US dairy herds [26, 27, 28]. Our data had a mean incidence of 2.6% in the adjusted model, despite this rate reaching up to 16.1% in cases of premature calving.

Parity and calving-related events emerged as the strongest factors associated with RP. The increasing odds of RP with advancing parity are consistent with previous reports and may reflect cumulative physiological demands across successive lactations, including repeated exposure to metabolic and uterine challenges [29]. However, because metabolic indicators, immune function, and uterine involution were not measured, these interpretations should be considered biologically plausible explanations rather than demonstrated mechanisms. Similarly, the markedly higher RP risk observed in cows experiencing twin births, abortions, premature calvings, stillbirths, or assisted calvings reinforces the well-established association between abnormal parturition events and impaired placenta expulsion [30, 25], emphasizing the role of dystocia and tissue trauma in RP [31, 32].

Male calves, which were generally heavier at birth, were associated with a 20% higher incidence of RP than female calves, a difference greater than previously reported [33]. The adoption of sex-sorted semen to produce female calves may thus have indirect benefits for postpartum recovery, relative to fetopelvic ratio [34]. Selection of sires with a lower birth weight could be a strategy, it was previously suggested, especially for cows calving for the first time, which is expected to reduce the incidence of difficult calvings and thus the likelihood of RP [35, 36].

Seasonal effects on RP were modest in magnitude but detectable, with slightly higher odds during warmer periods. Although heat stress has been associated with impaired immune function and postpartum disorders in previous studies [37, 10], the present dataset did not include direct indicators of thermal load or physiological stress. Therefore, seasonal differences observed here should be interpreted as descriptive associations rather than evidence of heat-stress mediated mechanisms. Previous studies [38] found lower RP incidence in the compost barn system compared to the free stall. Despite that, the absence of differences among production systems and herd size further suggests that RP risk in confined tropical herds may be influenced more by cow-level and calving-related factors than by the housing system itself.

Breed composition was also associated with RP risk. Holstein cows exhibited higher odds of RP compared with 1/2 HG crossbred, whereas differences were less pronounced for crossbred with higher Holstein genetic proportions. These findings are consistent with reports describing improved adaptation of Gyr-influenced genotypes to tropical environments [39, 40, 41], related to embryonic resistance [42]. Nonetheless, the present study cannot disentangle whether these differences are attributable to thermotolerance, metabolic resilience, immune competence, or management-related factors correlated with breed composition. Our data also showed a lower incidence of RP in 1/2 HG cows associated with higher fertility rates. Genetic selection for thermotolerance combined with heat stress mitigation could improve herd profitability in the future. This approach will reduce the impact of weather conditions on production and reproductive performance by increasing animal resilience [43].

Retained placenta was consistently associated with reduced P/AI at first service and across all inseminations. Importantly, no significant interactions were detected between RP and parity, breed, AI strategy, or semen type, indicating the negative association between RP and fertility was broadly consistent across production scenarios. Although the magnitude of the association decreased with successive inseminations, cows affected by RP continued to show reduced reproductive efficiency, suggesting that the consequences of RP may extend beyond the immediate postpartum period.

Cows with RP were observed to be more likely to lose pregnancies during gestation, supporting the hypothesis that subclinical endometrial inflammation related to RP can persist for several cycles [44, 30]. These consequences have a direct impact on the time to the next pregnancy and increase the costs of the dairy farm [45]. Conversely, in cows without RP, PL risk increased with the number of AI, being highest on the third or more attempts.

Cows with RP were also associated with higher odds for pregnancy loss. This association was observed across parities, breeds, and AI strategies, with limited evidence of effect modification. Because pregnancy loss was evaluated as a binary outcome and the precise timing of pregnancy loss was not consistently available, the present analysis does not allow inference regarding the gestational stage or biological processes underlying these losses. Despite that, health problems, which can ultimately lead to lower P/AI and higher PL are consistently described on literature [3, 17, 47], once RP leads to dysfunction and lower fertility [48]. Multiparous cows are particularly susceptible to poorer uterine conditions due to anatomical and physiological changes that can affect fertility [49, 50], which was seen in this data analysis when multiparous cows had higher RP incidence and worse fertility.

Some of these challenges can be mitigated by reproductive management strategies. For example, in this study, TAI protocols improved P/AI, especially in cows with RP. Optimized synchronization protocols, including presynchronizations and other modifications during the TAI protocol, have been shown to improve fertility and PL outcomes [51].

In addition to its reproductive consequences, RP was associated with a higher risk of culling during lactation. This finding reinforces the relevance of RP as an indicator of reduced herd longevity, independent of parity, breed, or production system. Although culling decisions are inherently multifactorial and may reflect unmeasured health, economic, or management considerations, the persistence of this association after adjustment for key covariates highlights the broader impact of RP on herd sustainability. Previous studies associated this higher uterine disease with culling risk and consequent lower likelihood of pregnancy [47, 52]. This emphasizes the importance of RP prevention not only for reproductive performance but also for maintaining productive life in tropical dairy herds.

Several limitations should be acknowledged. The retrospective nature of the dataset precludes causal inference, and information on nutritional management, body condition score, metabolic status, and concurrent postpartum diseases was unavailable. Pregnancy loss ascertainment relied on routine veterinary diagnoses and subsequent reproductive records. Nevertheless, the large sample size, inclusion of multiple herds across different regions, and use of mixed-effects models accounting for herd- and cow-level clustering strengthen the robustness and external validity of the observed associations.

In summary, these results reinforce that RP remains a multifactorial disorder in dairy systems, with long-term consequences for reproductive success and herd health. Prevention management strategies, such as improved heat mitigation, optimized nutritional management, and timely intervention in the transition period, are recommended to reduce the incidence of RP and mitigate the reproductive and longevity impacts in dairy herds.

5. Conclusions

Retained placenta was significantly associated with reduced pregnancy per AI, increased pregnancy loss, and higher culling risk in dairy cows managed under tropical conditions. Crossbred 1/2 Holstein × Gyr cows exhibited lower odds of RP compared to purebred Holsteins, whereas parity and calving-related events were major associated factors of RP occurrence. Although causal mechanisms cannot be inferred, the consistency of associations across herds highlights retained placenta as a key indicator of subsequent reproductive outcome. These findings emphasize the importance of preventive strategies during the transition period to mitigate long-term reproductive and productive consequences of RP in tropical dairy systems.

Funding source

We are grateful to the National Council for Scientific and Technological Development (CNPq, Brasília, Brazil; 141066/2022-9). We acknowledge Alta Genetics of Brazil for providing data for the analyses and truly appreciate the farmers and technicians that collaborated in recording data and were willing to participate in the study.

Contribution statement

K. R. Oliveira: Conceptualization, Methodology, Data curation, Investigation, Formal analysis, Writing – original draft, Writing – review and editing. **T. E. Silva**: Methodology, Data curation, Formal analysis, Writing – review and editing. **J. H. C. Costa**: Methodology, Data curation, Writing – review and editing, Supervision. **T. M. Ferreira**: Investigation, Data curation. **A. L. Silva**: Methodology, Data curation, Writing – review and editing, Supervision **C. E. C. Consentini**: Conceptualization, Methodology, Data curation, Validation, Writing – review and editing. **R. Sartori**: Conceptualization, Methodology, Data curation, Validation, Writing – review and editing. **P. P. Rotta**: Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review and editing.

References

[1] Van Werven T, Schukken YH, Lloyd J, Brand A, Heeringa HTj, Shea M. The effects of duration of retained placenta on reproduction, milk production, postpartum disease and culling rate. Theriogenology 1992; 37:1191–1203. https://doi.org/10.1016/0093-691X(92)90175-Q.

[2] Dervishi E, Zhang G, Hailemariam D., Dunn SM, Ametaj BN. Occurrence of retained placenta is preceded by an inflammatory state and alterations of energy metabolism in transition dairy cows. J. Anim. Sci. Biotechnol. 2016; 7:1–13. https://doi.org/10.1186/s40104-016-0085-9.

[3] LeBlanc SJ. Postpartum uterine disease and dairy herd reproductive performance: A review. Veterin. J. 2008; 176:102–114. https://doi.org/10.1016/j.tvjl.2007.12.019.

[4] Hossein-Zadeh NG, Ardalan M. Cow-specific risk factors for retained placenta, metritis and clinical mastitis in Holstein cows. Vet. Res. Commun. 2011; 35:345–354. https://doi.org/10.1007/s11259-011-9479-5.

[5] Venjakob PL, Heuwieser W, Borchardt S. Associations between days in the close-up group and milk production, transition cow diseases, reproductive performance, culling, and be-

havior around calving of Holstein dairy cows. J. Dairy Sci. 2023; 106:7056–7075. https://doi.org/10.3168/jds.2022-22642.

[6] Gunnink JW. Retained placenta and leucocytic activity. Vet. Q. 1984; 6:49–51. https://doi.org/10.1080/01652176.1984.9693909.

[7] Kimura K, Goff JP, Kehrli ME, Reinhardt TA. Decreased neutrophil function as a cause of retained placenta in dairy cattle. J. Dairy Sci. 2002; 85:544–550. https://doi.org/10.3168/jds.S0022-0302(02)74107-6.

[8] Beagley JC, Whitman KJ, Baptiste KE, Scherzer J. Physiology and treatment of retained fetal membranes in cattle. J. Vet. Intern. Med. 2010; 24:261–268. https://doi.org/10.1111/j.1939-1676.2010.0473.x

[9] Ribeiro ES, Gomes G, Greco LF, Cerri RLA, Vieira-Neto A, Monteiro PLJ, Lima FS, Bisinotto RS, Thatcher WW, Santos JEP. Carryover effect of postpartum inflammatory diseases on developmental biology and fertility in lactating dairy cows. J. Dairy Sci. 2016; 99:2201–2220. https://doi.org/10.3168/jds.2015-10337.

[10] Sammad A, Umer A, Shi R, Zhu H, Zhao X, Wang Y. Dairy cow reproduction under the influence of heat stress. J. Anim. Physiol. Anim. Nutr. 2020; 104:978–986. https://doi.org/10.1111/jpn.13257.

[11] Sakai S, Yagi M, Fujime N, Kuse M, Sakumoto R, Yamamoto Y, Okuda. K, Kimura K. Heat stress influences the attenuation of prostaglandin synthesis by interferon tau in bovine endometrial cells. Theriogenology 2021; 165:52-58. https://doi.org/10.1016/j.theriogenology.2021.02.005.

[12] Costa ANL, Feitosa JV, Montezuma PA, Souza PT, Araújo AA. Rectal temperatures, respiratory rates, production, and reproduction performances of crossbred Girolando cows under heat stress in northeastern Brazil Int. J. Biometeorol. 2015; 59:1647–1653. https://doi.org/10.1007/s00484-015-0971-4.

[13] Quirino, DF, Marcondes MI, Rennó LN, Correa PVF, Morais VCL, Cunha CS, Silva TDA, Silva AL, Miller-Cushon E, Rotta PP. Intake, performance, and feeding behavior of Holstein and Holstein × Gyr heifers grazing intensively managed tropical grasses during the rainy season. Animals 2022; 16. https://doi.org/10.1016/j.animal.2022.100613.

[14] Sartori R, Sartor-Bergfelt R, Mertens SA, Guenther JN, Parrish JJ, Wiltbank MC. Fertilization and early embryonic development in heifers and lactating cows in summer and lactating and dry cows in winter. J. Dairy Sci. 2002; 85:2803–2812. https://doi.org/10.3168/jds.S0022-0302(02)74367-1.

[15] Ferreira RM, Ayres H, Chiaratti MR, Ferraz ML, Araújo AB, Rodrigues CA, Watanabe YF, Vireque AA, Joaquim DC, Smith LC, Meirelles FV, Baruselli PS. The low fertility of repeat-breeder cows during summer heat stress is related to a low oocyte competence to develop into blastocysts. J. Dairy Sci. 2011; 94:2383–2392. https://doi.org/10.3168/jds.2010-3904.

[16] Wolfenson D, Roth Z. Impact of heat stress on cow reproduction and fertility. Anim. Front. 2019; 9:32–38. https://doi.org/10.1093/af/vfy027.

[17] Vallejo-Timaran DA, Reyes J, Gilbert RO, Lefebvre RC, Palacio-Baena LG, Maldonado-Estrada JG. Incidence, clinical patterns, and risk factors of postpartum uterine diseases in dairy cows from high-altitude tropical herds. J. Dairy Sci. 2021; 104:9016–9026. https://doi.org/10.3168/jds.2020-18692.

[18] Wiltbank MC, Baez GM, Garcia-Guerra A, Toledo MZ, Monteiro PLJ, Melo LF, Ochoa JC, Santos JEP, Sartori R. Pivotal periods for pregnancy loss during the first trimester of gestation in lactating dairy cows. Theriogenology 2016; 86:239–253. https://doi.org/10.1016/j.theriogenology.2016.04.037.

[19] Pinedo P, Santos JEP, Chebel RC, Galvão KN, Schuenemann GM, Bicalho RC, Gilbert RO, Rodriguez Zas S., Seabury CM, Rosa G, Thatcher WW. Early-lactation diseases and fertility in 2 seasons of calving across US dairy herds. J. Dairy Sci. 2020; 103:10560–10576. https://doi.org/10.3168/jds.2019-17951.

[20] Toni F, Vincenti L, Ricci A, Schukken YH. Postpartum uterine diseases and their impacts on conception and days open in dairy herds in Italy. Theriogenology 2015; 84:1206–1214. https://doi.org/10.1016/j.theriogenology.2015.06.022.

[21] Louca A, Legates JE. Production losses in dairy cattle due to days open. J. Dairy Sci. 1968; 51:573–583. https://doi.org/10.3168/jds.S0022-0302(68)87031-6.

[22] Sewalen A, Miglior F, Kistemaker GJ, Sullivan P, Van Doormaal BJ. Relationship between reproduction traits and functional longevity in Canadian dairy cattle. J. Dairy Sci. 2008; 91:1660–1668. https://doi.org/10.3168/jds.2007-0178.

[23] Committee on bovine reproductive nomenclature. Recommendations for standardizing bovine reproductive terms. Cornell Vet 1972; 62:216–36.

[24] Nicola I, Chupin H, Roy JP, Buczinski S, Fauteux V, Picard-Hagen N, Cue R, Dubuc J. Association between prepartum nonesterified fatty acid serum concentrations and postpartum diseases in dairy cows. J. Dairy Sci. 2022; 105:9098–9106. https://doi.org/10.3168/jds.2022-22014.

[25] Mahnani A, Sadeghi-Sefidmazgi A, Ansari-Mahyari S, Ghorbani GR, Keshavarzi H. Farm and cow factors and their interactions on the incidence of retained placenta in Holstein dairy cows. Theriogenology 2021a; 159:87–97. https://doi.org/10.1016/j.theriogenology.2020.10.007.

[26] Qu Y, Faden AN, Traber MG, Bobe G. Potential risk indicators of retained placenta and other diseases in multiparous cows. J. Dairy Sci. 2014; 97:4151–4165. https://doi.org/10.3168/jds.2013-7154.

[27] Vieira-Neto A, Duarte GA, Zimpel R, Thatcher WW, Santos JEP. Days in the prepartum group are associated with subsequent performance in Holstein cows. J. Dairy Sci. 2021; 104:5964–5978. https://doi.org/10.3168/jds.2020-18889.

[28] Menta PR, Machado VS, Piñeiro JM, Thatcher WW, Santos JEP, Vieira-Neto A. Heat stress during the transition period is associated with impaired production, reproduction, and survival in dairy cows. J. Dairy Sci. 2022; 105:4474–4489. https://doi.org/10.3168/jds.2021-21185.

[29] Lean IJ, Golder HM, LeBlanc SJ, Duffield T, Santos JEP. Increased parity is negatively associated with survival and reproduction in different production systems. J. Dairy Sci. 2023; 106:476–499. https://doi.org/10.3168/jds.2021-2167.

[30] Heppelmann M, Weinert M, Brömmling A, Piechotta M, Hoedemaker M, Bollwein H. The effect of puerperal uterine disease on uterine involution in cows assessed by Doppler sonography of the uterine arteries. Anim. Reprod. Sci. 2013; 143:1–7. https://doi.org/10.1016/j.anireprosci.2013.11.003.

[31] Echternkamp SE, Gregory KE. Effects of twinning on gestation length, retained placenta, and dystocia. J. Anim. Sci. 1999; 77: 39–47. https://doi.org/10.2527/1999.77139x.

[32] Kirkpatrick BW, Berry DP. A genomic analysis of twinning rate and its relationship with other reproductive traits in Holstein-Friesian cattle. J. Dairy Sci. 2025; 108:1686–1698. https://doi.org/10.3168/jds.2024-25236.

[33] Hess, M. K., A. S. Hess, D. J. Garrick. The effect of calf gender on milk production in seasonal calving cows and its impact on genetic evaluations. PLoS One 2016; 11. https://doi.org/10.1371/journal.pone.0151236.

[34] Tsaousioti A, Praxitelous A, Kok A, Kiossis E, Boscos C, Tsousis G. Association between dam and calf measurements with overall and fetopelvic dystocia in Holstein heifers. J. Dairy Res. 2023; 90: 261-268. https://doi.org/10.1017/s0022029923000468.

[35] Dekkers JCM. Optimal breeding strategies for calving ease. J. Dairy Sci. 1994; 77:3441-3453. https://doi.org/10.3168/jds.S0022-0302(94)77287-8.

[36] Mee JF. Prevalence and risk factors for dystocia in dairy cattle: A review. Vet. J. 2008; 176:93-101. https://doi.org/10.1016/j.tvjl.2007.12.032.

[37] Gernand E, König S, Kipp C. Influence of on-farm measurements for heat stress indicators on dairy cow productivity, female fertility, and health. J. Dairy Sci. 2019; 102:6660–6671. https://doi.org/10.3168/jds.2018-16011.

[38] Witkowska D, Ponieważ A. The effect of housing system on disease prevalence and productive lifespan of dairy herds—a case study. Animals 2022; 12. https://doi.org/10.3390/ani12131610.

[39] Mendonca LC, Carvalho WA, Campos MM, Souza GN, Oliveira SA, Meringhe GKF, Negrao JA. Heat stress affects milk yield, milk quality, and gene expression profiles in mammary cells of Girolando cows. J. Dairy Sci. 2025; 108:1039–1049. https://doi.org/10.3168/jds.2024-25498.

[40] Camargo LSA, Viana JHM, Ramos AA, Serapião RV, Sa WF, Ferreira AM, Guimarães MFM, Vale Filho VR. Developmental competence and expression of the Hsp 70.1 gene in oocytes obtained from *Bos indicus* and *Bos taurus* dairy cows in a tropical environment. Theriogenology 2007; 68:626-632. https://doi.org/10.1016/j.theriogenology.2007.03.029.

[41] Oliveira CS, Serapião RV, Camargo AJR, Freitas C, Igumac LT, Carvalho BC, Camargo LSA, Oliveira LZ, Verneque RS. Oocyte origin affects the in vitro embryo production and development of Holstein (*Bos taurus taurus*) - Gyr (*Bos taurus indicus*) reciprocal cross embryos. Anim. Rep. Sci. 2019; 209:106165. https://doi.org/10.1016/j.anireprosci.2019.106165.

[42] Pegorer MF, Vasconcelos JLM, Trincac LA, Hansen PJ, Barros CM. Influence of sire and sire breed (Gyr versus Holstein) on establishment of pregnancy and embryonic loss in lactating Holstein cows during summer heat stress. Theriogenology 2007; 67:692-697. https://doi.org/10.1016/j.theriogenology.2006.09.042.

[43] Garner JB, Douglas ML, Williams SRO, Wales WJ, Marett LC, Nguyen TTT, Reich CM, Hayes BJ. Genomic selection improves heat tolerance in dairy cattle. Sci. Rep. 2016; 6:34114. doi: https://doi.org/10.1038/srep34114.

[44] Sheldon IM, Williams EJ, Miller ANA, Nash DM, Herath S. Uterine disease in cattle after parturition. Vet. J. 2008; 176:115–121. https://doi.org/10.1016/j.tvjl.2007.12.031

[45] Mahnani A, Sadeghi-Sefidmazgi A, Ansari-Mahyari S, Ghorbani GR. Assessing the consequences and economic impact of retained placenta in Holstein dairy cows. Theriogenology 2021b; 175:61–68. https://doi.org/10.1016/j.theriogenology.2021.08.036.

[47] Carvalho MR, Penagaricano F, Santos JEP, DeVries TJ, McBride BW, Ribeiro ES. Long-term effects of postpartum clinical disease on milk production, reproduction, and culling of dairy cows. J. Dairy Sci. 2019; 102:11701–11717. https://doi.org/10.3168/jds.2019-17025.

[48] Chebel RC, Santos JEP, Reynolds JP, Cerri RLA, Juchem SO, Overton M. Factors affecting conception rate after artificial insemination and pregnancy loss in lactating dairy cows. Anim. Reprod. Sci. 2004; 84:239-255. https://doi.org/10.1016/j.anireprosci.2003.12.012.

[49] Baez GM, Barletta RV, Guenther JN, Gaska JM, Wiltbank MC. Effect of uterine size on fertility of lactating dairy cows. Theriogenology 2016; 85:1357–1366. http://dx.doi.org/10.1016/j.theriogenology.2015.04.022.

[50] Madureira AML, Poole RK, Burnett TA, Guida TG, Edwards JL, Schrick FN, Vasconcelos JLM, Cerri RLA, Pohler KG. Size and position of the reproductive tract impacts fertility outcomes and pregnancy losses in lactating dairy cows. Theriogenology 2020; 158:66–74. https://doi.org/10.1016/j.theriogenology.2020.08.022.

[51] Consentini CEC, Wiltbank MC, Sartori R. 2021. Factors that optimize reproductive efficiency in dairy herds with an emphasis on timed artificial insemination programs. Animals 11:301-331. https://doi.org/10.3390/ani11020301.

[52] Dubuc J, Duffield TF, Leslie KE, Walton JS, LeBlanc SJ. Effects of postpartum uterine diseases on milk production and culling in dairy cows. J. Dairy Sci. 2011; 94:1339–1346. https://doi.org/10.3168/jds.2010-3758.

Table 1. Descriptive statistics on the incidence of retained placenta (RP) by categorical risk factors in 437 commercial dairy farms.

	Number of calvings	Number of farms	Number of RP cases	Incidence of retained placenta (%)
Parity				
1	50,435	437	2,694	4.5
2	39,151	437	2,968	6.6
3	24,457	422	1,990	7.2
4	13,707	417	1,183	7.6
5	6,530	396	626	8.2
6	2,725	352	265	9.8
7	1,170	275	98	7.7
Breed[1]				
Holstein	77,638	388	6,064	6.8
7/8 Holstein × Gyr	19,603	383	1,410	6.6
3/4 Holstein × Gyr	26,494	376	1,620	5.8
1/2 Holstein × Gyr	14,440	269	730	6.9
Calf sex				
Female	82,897	445	5,435	5.7
Male	55,278	437	4,389	7.1
Calving type				
Normal	129,021	446	8,063	5.5
Assisted	4,565	206	667	18.1
Stillbirth	3,197	299	594	16.4
Premature	180	70	60	32.2
Abortion	1,212	188	440	26.5
Twin				
No	134,096	446	8,808	5.6

Yes	4,079	357	1,016	21.9
Season				
Fall	39,220	437	2,770	6.4
Winter	39,863	433	2,657	5.9
Summer	31,394	428	2,486	6.6
Spring	27,698	438	1,911	5.8
System				
Compost barn	92,598	274	6,775	7.2
Free stall	24,710	40	1,970	8.2
Dry lot	20,867	149	1,079	3.6
Farm size (lactating cows)				
Less than 100	10,856	156	642	4.6
100 to 200	28,944	163	1,817	6.3
More than 200	98,375	162	7,365	7.3
Year				
2021	45,635	375	3,491	6.2
2022	56,586	431	3,901	6.4
2023	35,954	324	2,432	5.3

These retained placenta values are not adjusted for potential confounding variables and should be interpreted with caution. The differences observed in Table 1 may not reflect true associations.
[1]Pure Holstein and Holstein × Gyr crossbreed cows. Fractions represent the Holstein genetics contribution.

Table 2. Incidence of retained placenta in dairy cows (mean ± standard error) and odds ratio (mean and confidence interval) from the adjusted generalized linear mixed model for parity, breed, calf sex, calving type, twin pregnancy, season, farm characteristics, and year of data collection across 437 dairy farms.

Overall	Number of calvings	Retained placenta, %	Odds ratio [CI 95%]	*P*-value
	138,175	2.6 ± 0.26		
Parity				< 0.001
1	50,435	2.2 ± 0.22[a]	[Ref.]	
2	39,151	2.5 ± 0.25[b]	1.15 [1.13 – 1.17]	
3	24,457	2.9 ± 0.29[c]	1.33 [1.29 – 1.37]	
4	13,707	3.3 ± 0.33[d]	1.53 [1.46 – 1.61]	
5	6,530	3.8 ± 0.38[e]	1.76 [1.65 – 1.88]	
6	2,725	4.4 ± 0.44[f]	2.03 [1.88 – 2.20]	
7	1,170	5.0 ± 0.51[g]	2.34 [2.13 – 2.58]	
Breed[1]				0.007
Holstein	77,638	2.8 ± 0.28[b]	[Ref.]	
7/8 Holstein × Gyr	19,603	2.6 ± 0.27[ab]	0.96 [0.89 – 1.03]	
3/4 Holstein × Gyr	26,494	2.5 ± 0.25[a]	0.88 [0.81 – 0.97]	
1/2 Holstein × Gyr	14,440	2.3 ± 0.25[a]	0.83 [0.74 – 0.93]	
Calf sex				< 0.001
Female	82,897	2.5 ± 0.24	[Ref.]	
Male	55,278	2.9 ± 0.29	1.20 [1.15 – 1.26]	
Calving type				< 0.001
Normal	129,021	2.5 ± 0.24[a]	[Ref.]	
Assisted	4,565	4.6 ± 0.49[b]	1.92 [1.75 – 2.10]	
Stillbirth	3,197	7.3 ± 0.76[c]	3.11 [2.81 – 3.46]	
Premature	180	16.1 ± 2.74[d]	7.60 [5.37 – 10.76]	
Abortion	1,212	15.8 ± 1.60[d]	7.43 [6.48 – 8.54]	

Twin				< 0.001
No	134,096	2.5 ± 0.24	[Ref.]	
Yes	4,079	9.2 ± 0.89	3.86 [3.55 – 4.20]	
Season				< 0.001
Fall	39,220	2.4 ± 0.24[a]	[Ref.]	
Winter	39,863	2.7 ± 0.26[bc]	1.10 [1.03 – 1.17]	
Spring	31,394	2.6 ± 0.25[ab]	1.06 [0.99 – 1.13]	
Summer	27,698	2.9 ± 0.28[c]	1.18 [1.12 – 1.26]	
System				0.105
Compost barn	92,598	2.6 ± 0.26	[Ref.]	
Free stall	24,710	3.0 ± 0.37	1.15 [0.98 – 1.37]	
Dry lot	20,867	2.4 ± 0.27	0.92 [0.79 – 1.06]	
Farm size (lactating cows)				0.300
Less than 100	10,856	2.2 ± 0.29	[Ref.]	
100 to 200	28,944	2.5 ± 0.27	1.13 [0.91 – 1.41]	
More than 200	98,375	2.7 ± 0.29	1.21 [0.95 – 1.55]	
Year				0.148
2021	45,635	2.7 ± 0.27	[Ref.]	
2022	56,586	2.6 ± 0.26	0.95 [0.90 – 1.00]	
2023	35,954	2.6 ± 0.26	0.95 [0.89 – 1.01]	

Values represent adjusted probabilities (%) and odds ratio (OR, 95% CI).
[1]Pure Holstein and Holstein × Gyr crossbreed cows. Fractions represent the Holstein genetics contribution.
[a,b,c,d,e,f,g]Means followed by different lowercase letters indicate differences ($P \leq 0.05$) based on pairwise comparisons.

Table 3. Association between retained placenta and pregnancy per artificial insemination (P/AI; %) at first artificial insemination after calving using generalized mixed model adjusted for other variables: parity, breed, type of artificial insemination, and semen retrieved from 437 dairy farms. Means, odds ratio, and confidence interval for each level are presented.

Item		Number of AI[1]	Retained placenta		Odds ratio	*P*-value		
			Yes	No	(Yes *vs.* No)[1]	Main effect	Interaction	Level
Overall		139,222	31.9	38.8	0.74 [0.70 - 0.77]	0.017	-	0.017
Parity						< 0.001	0.541	
	Primiparous	52,813	36.0	42.9[c]	0.75 [0.70 - 0.81]			< 0.001
	Secundiparous	38,832	32.6	39.2[b]	0.75 [0.69 - 0.81]			< 0.001
	Multiparous	47,577	27.0	34.2[a]	0.71 [0.66 - 0.77]			< 0.001
Breed[2]						< 0.001	0.365	
	Holstein	84,150	32.0	38.4[a]	0.75 [0.71 - 0.80]			< 0.001
	7/8 Holstein × Gyr	20,459	31.5	38.0[a]	0.75 [0.67 - 0.84]			< 0.001
	3/4 Holstein × Gyr	23,931	31.5	38.7[a]	0.73 [0.65 - 0.82]			< 0.001
	1/2 Holstein × Gyr	10,682	32.9	43.9[b]	0.62 [0.52 - 0.77]			< 0.001
Type of AI						0.001	0.675	
	Estrus	26,716	30.0	37.6[a]	0.71 [0.63 - 0.80]			< 0.001
	Timed-AI	112,506	32.3	39.1[b]	0.74 [0.71 - 0.78]			< 0.001
Semen						< 0.001	0.289	
	Conventional	120,254	32.2	39.4[a]	0.73 [0.70 - 0.77]			< 0.001
	Sex-sorted	18,968	30.1	35.3[b]	0.79 [0.69 – 0.90]			< 0.001
AI Season						< 0.001	0.496	
	Fall	42,323	31.1	37.7[a]	0.75 [0.69 - 0.81]			< 0.001
	Winter	37,755	34.7	40.9[c]	0.77 [0.71 - 0.84]			< 0.001
	Spring	28,875	31.3	39.2[b]	0.71 [0.64 - 0.78]			< 0.001
	Summer	30,269	29.9	37.4[a]	0.71 [0.65 - 0.78]			< 0.001

Values represent adjusted probabilities (%) and odds ratio (OR, 95% CI) comparing cows with retained placenta to cows without retained placenta.
[1]Artificial insemination.

[2]Pure Holstein and Holstein × Gyr crossbreed cows. Fractions represent the Holstein genetics contribution.

[a,b,c] Means followed by different lowercase letters within the same column indicate differences ($P \leq 0.05$) based on pairwise comparisons.

Table 4. Association between retained placenta and overall pregnancy per artificial insemination (P/AI; %) in an adjusted generalized mixed model for parity, breed, type of artificial insemination, semen, and artificial insemination number retrieved from 437 dairy farms. Means, odds ratio, and confidence interval for each level are presented.

Item		Number of AI[1]	Retained placenta		Odds ratio	*P*-value		
			Yes	No	(Yes *vs*. No)[1]	Main effect	Interaction	Level
Overall		289,391	35.4	38.3	0.88 [0.85 - 0.91]	< 0.001	-	< 0.001
Parity						< 0.001	0.211	
	Primiparous	107,806	40.0	40.5[c]	0.98 [0.91 - 1.06]			0.614
	Secundiparous	81,078	35.4	37.2[b]	0.92 [0.85 - 1.00]			0.051
	Multiparous	100,507	31.5	33.2[a]	0.93 [0.86 - 0.99]			0.048
Breed[2]						< 0.001	0.456	
	Holstein	175,504	35.0	38.2[a]	0.87 [0.84 - 0.91]			< 0.001
	7/8 Holstein × Gyr	42,386	35.8	37.5[a]	0.93 [0.86 - 1.01]			0.069
	3/4 Holstein × Gyr	49,923	35.5	37.9[a]	0.90 [0.84 - 0.98]			0.012
	1/2 Holstein × Gyr	21,578	37.8	41.8[b]	0.84 [0.74 - 0.97]			0.017
Type of AI[3]						< 0.001	0.147	
	Estrus	82,540	33.3	36.9	0.85 [0.81 - 0.91]			< 0.001
	Timed-AI	206,851	36.3	38.9	0.90 [0.86 - 0.93]			< 0.001
Semen						< 0.001	0.195	
	Conventional	261,673	35.5	38.5	0.88 [0.85 - 0.91]			< 0.001
	Sex-sorted	27,718	35.2	36.5	0.95 [0.85 - 1.05]			0.311
AI number						< 0.001	< 0.001	
	1	138,554	33.9[aA]	39.5[dC]	0.78 [0.75 - 0.82]			< 0.001
	2	91,301	36.5[bB]	38.0[cB]	0.94 [0.89 - 0.99]			0.015
	3 or more	59,536	37.5[bcB]	35.8[bA]	1.07 [1.01 - 1.14]			0.024
AI Season						< 0.001	0.975	
	Fall	85,584	35.0	40.9[b]	0.89 [0.85 - 0.94]			< 0.001
	Winter	76,922	38.0	40.6[c]	0.89 [0.84 - 0.94]			< 0.001

Spring	61,356	34.8	37.9[b]	0.88 [0.82 - 0.94]	< 0.001
Summer	65,529	33.7	36.5[a]	0.88 [0.83 - 0.94]	< 0.001

Values represent adjusted probabilities (%) and odds ratio (OR, 95% CI) comparing cows with retained placenta to cows without retained placenta.

[1]Odds ratio of the comparison between retained placenta (No vs. Yes) in a determined level of the fixed effect.
[2]Pure Holstein and Holstein × Gyr crossbreed cows. Fractions represent the Holstein genetics contribution.
[3]Artificial insemination.

[a,b,c,d] Means followed by different letters indicate differences ($P \leq 0.05$) based on pairwise comparisons considering interaction with retained placenta.

[A,B,C,D] Means followed by different uppercase letters within the same column indicate differences ($P \leq 0.05$) based on pairwise comparisons inside yes or no retained placenta group.

Table 5. Association between retained placenta and pregnancy loss (%) between 28 and 260 d of gestation adjusted in a generalized mixed model for parity, breed, type of artificial insemination, semen, and artificial insemination number. Data retrieved from 174 dairy farms. Means, odds ratio, and confidence interval for each level are presented.

Item	Number of observations	Retained placenta		Odds ratio	*P*-value		
		Yes	No	(Yes *vs.* No)[1]	Main effect	Interaction	Level
Overall	57,628	30.4	22.8	1.48 [1.38 - 1.58]	0.004		0.001
Parity					< 0.001	0.328	
Primiparous	23,474	29.4	21.9[a]	1.49 [1.32 - 1.67]			< 0.001
Secundiparous	16,108	31.5	22.6[a]	1.57 [1.40 - 1.77]			< 0.001
Multiparous	18,046	30.7	24.1[b]	1.39 [1.25 - 1.56]			0.001
Breed[2]					< 0.001	0.507	
Holstein	34,233	31.7	23.4[a]	1.52 [1.40 - 1.65]			< 0.001
7/8 Holstein × Gyr	8,708	28.7	22.7[a]	1.37 [1.15 - 1.63]			0.006
3/4 Holstein × Gyr	9,679	28.7	22.6[a]	1.38 [1.16 - 1.64]			0.007
1/2 Holstein × Gyr	5,008	27.6	19.0[b]	1.63 [1.27- 2.08]			0.001
Type of AI[3]					0.005	0.131	
Estrus	13,609	27.2	21.8[b]	1.34 [1.16 - 1.56]			0.003
Timed-AI	44,019	31.4	23.1[a]	1.52 [1.41 - 1.65]			< 0.001
Semen					0.321	0.685	
Conventional	51,752	30.4	22.7	1.49 [1.38 - 1.60]			< 0.001
Sex-sorted	5,876	30.2	23.4	1.41 [1.12 - 1.78]			0.010
AI number					< 0.001	0.014	
1	22,567	31.6[c]	21.7[aA]	1.66 [1.48 - 1.87]			< 0.001
2	13,646	30.3[c]	22.8[abAB]	1.47 [1.29 - 1.68]			< 0.001
3 or more	21,415	29.2[c]	23.9[bC]	1.31 [1.18 - 1.46]			0.009
AI Season					0.004	0.388	
Fall	14,340	30.4	22.3[a]	1.53 [1.34 - 1.74]			< 0.001

Winter	16,637	30.7	22.1[a]	1.54 [1.36 - 1.74]	< 0.001
Spring	13,942	31.9	23.7[b]	1.51 [1.33 - 1.73]	< 0.001
Summer	12,709	28.7	23.2[ab]	1.33 [1.15 - 1.53]	< 0.001

Values represent adjusted probabilities (%) and odds ratio (OR, 95% CI) comparing cows with retained placenta to cows without retained placenta.
[1]Odds ratio of the comparison between retained placenta (No vs. Yes) in a determined level of the fixed effect.
[2]Pure Holstein and Holstein × Gyr crossbreed cows. Fractions represent the Holstein genetics contribution.
[3]Artificial insemination.
[a,b,c] Means followed by different lowercase letters indicate differences ($P \leq 0.05$) based on pairwise comparisons considering interaction with retained placenta.
[A,B,C]Means followed by different lowercase letters within the same column indicate differences ($P \leq 0.05$) based on pairwise comparisons inside yes or no retained placenta group.

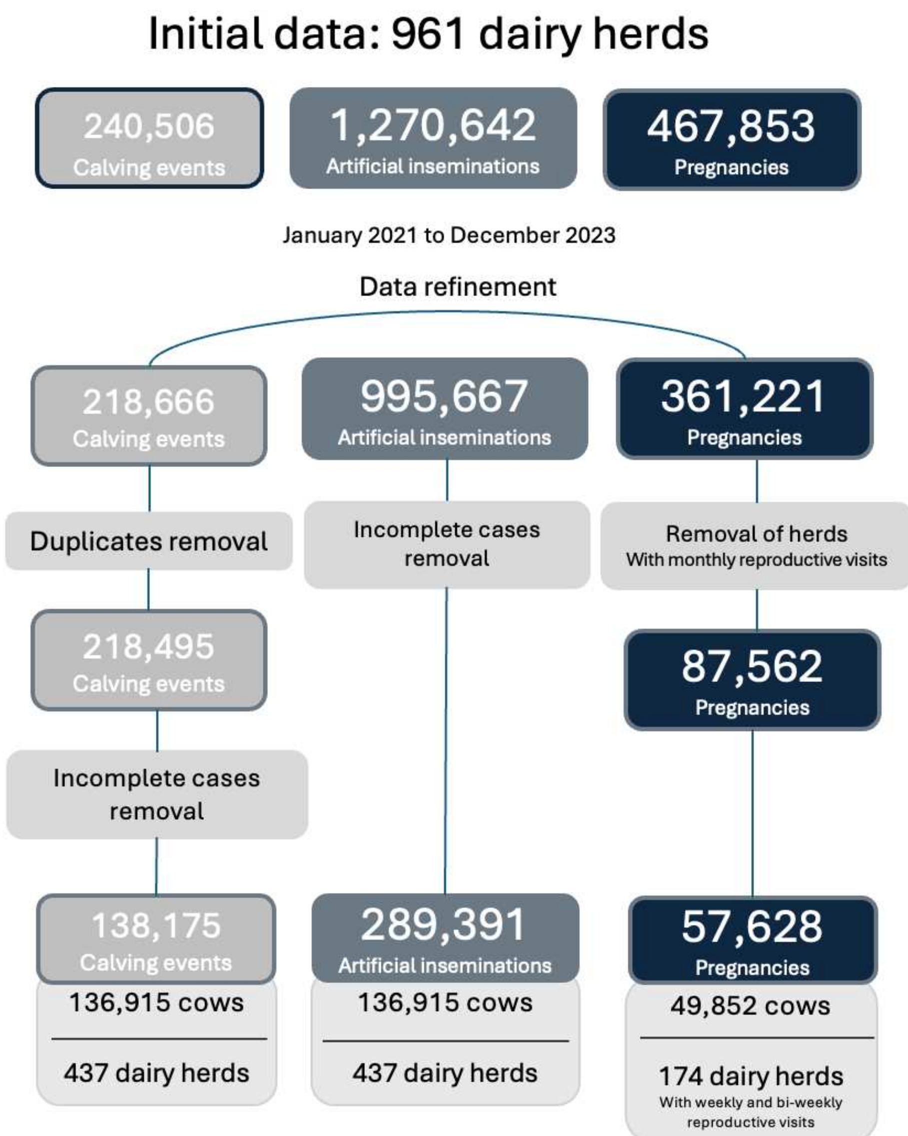

Figure 1. Flowchart illustrating the data handling and processing steps for assessing risk factors of retained placenta, pregnancy per artificial insemination, pregnancy loss in dairy cows from Brazilian farms.

CHAPTER 3: REPRODUCTIVE MANAGEMENT INTENSITY AND PRODUCTION SYSTEM ARE ASSOCIATED WITH HERD-LEVEL REPRODUCTIVE PERFORMANCE IN COMMERCIAL DAIRY HERDS

*Formatted following the guidelines of Theriogenology Journal

REPRODUCTIVE MANAGEMENT INTENSITY AND PRODUCTION SYSTEM ARE ASSOCIATED WITH HERD-LEVEL REPRODUCTIVE PERFORMANCE IN COMMERCIAL DAIRY HERDS

Kellen R. Oliveira[1], Tadeu E. da Silva[2], João H. C. Costa[2], Tiago M. Ferreira[3], Alex L. Silva[1], Carlos E. C. Consentini[4], Roberto Sartori[5], and Polyana P. Rotta[1*]

[1] Department of Animal Science, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.

[2]Department of Animal and Veterinary Sciences, University of Vermont, Burlington, Vermont.

[3]Alta Genetics do Brasil, Uberaba, Minas Gerais, Brazil.

[4] GlobalGen Vet Science, Jaboticabal, SP, Brazil.

[5] Department of Animal Science, ESALQ, University of São Paulo, SP, Brazil.

Abstract

This study evaluated the association between reproductive management frequency (RMF), production system, and herd-level reproductive performance in dairy herds. Herd-level retrospective data from 284 dairy herds from Brazil were analyzed and classified according to reproductive veterinary management frequency (monthly, bi-weekly, or weekly) and production system (compost barn, free stall, or dry lot). Reproductive management practices, reproductive performance indicators, and milk yield were assessed through a three-year retrospective observational analysis. Associations between management frequency or production system and herd-level outcomes were evaluated using generalized linear models and generalized linear mixed models when appropriate. Pairwise comparisons were conducted using estimated marginal means, and *P*-values were adjusted for multiple testing using Benjamini–Hochberg correction. Spearman correlation analysis assessed relationships between pregnancy rate and reproductive and productive indicators. More frequent reproductive veterinary visits were associated with higher service rates, shorter intervals between services, fewer days open, and higher pregnancy rates. In contrast, pregnancy per service did not differ across management frequencies. Free stall and compost barn systems showed greater adoption of reproductive technologies and superior reproductive performance compared with dry lot systems, while differences between compost barn and free stall systems were limited. Pregnancy rate was strongly correlated with service rate and pregnancy per service. In conclusion, herd-level reproductive performance was primarily associated with reproductive management frequency and production system. Weekly reproductive monitoring was associated mainly with service rate rather than pregnancy per artificial insemination (AI), while also compost barn and free stalls achieved better reproductive outcomes than dry lot systems.

Keywords: Management, Pregnancy rate; Veterinary assistance.

1. Introduction

Reproductive efficiency remains a major determinant of success in dairy herds, reflecting the combined effects of environmental conditions, postpartum health (Várhidi et al., 2024), estrus expression, management decisions, and consistency in reproductive management. Although reproductive physiology and management are widely studied, there is limited information on how the frequency of veterinary reproductive assistance interacts with production systems to influence herd-level reproductive outcomes in commercial dairy herds. This gap persists despite fertility being a high-priority topic for farmers (Derks et al., 2013; Ritter et al., 2021) and evidence that timely reproductive interventions can reduce the time to pregnancy diagnosis and improve overall herd reproduction (Armengol et al., 2023).

Production systems further influence cow comfort, estrus expression, heat stress exposure, and ultimately reproductive efficiency. Compost barn systems have been associated with greater cow comfort due to unrestricted lying space, reduced environmental impact (Oliveira et al., 2024), and a lower prevalence of hoof and limb lesions (Black et al., 2013). In contrast, dry lot systems are generally less intensive and may expose cows to higher heat stress and greater environmental variability, potentially impairing reproductive performance and complicating management practices.

The frequency of veterinary reproductive assistance is closely associated with earlier disease detection, improved classification of reproductive status, and more consistent implementation of evidence-based reproductive protocols. Although this relationship has been demonstrated in other species (Marynissen et al., 2025), empirical evidence linking the frequency of veterinary reproductive visits to herd-level reproductive outcomes in dairy cattle remains limited, particularly in commercial scale. Moreover, frequent veterinary involvement may improve record accuracy, reproductive planning, and strategic decision-making at the herd level. Frequent veterinary visits may also be associated with lower interval between services by improving pregnancy diagnosis efficiency and decision-making for re-insemination.

Therefore, this retrospective observational study aimed to evaluate multiple commercial herd reproductive performance. The objective was to assess how the frequency of veterinary reproductive assistance and the production system influence annual reproductive performance indicators in dairy herds. We speculate that a higher frequency of veterinary reproductive assistance and more intensive productive systems would be associated with higher service rate, shorter service intervals, and superior reproductive performance.

2. Material and Methods

This study was a retrospective observational analysis of commercial dairy herds. Data from 284 commercial dairy herds in ten Brazilian states (Figure 1) were evaluated retrospectively from July 2021 to June 2024. Herds were classified by production system as compost barn, free stall, or dry lot, and by frequency of veterinary reproductive assistance as weekly, bi-weekly, or monthly.

A total of 90,658 breeding cows were included in the analysis. The number of cows per herd ranged from 14 to 2,099, with cows having between one and seven calvings during the evaluated period (Table 1).

Data were obtained from the Concept Plus Program, developed by Alta Genetics Brazil, which conducts benchmarking analysis based on herd-level reproductive and productive indicators. Participating farmers and veterinarians completed structured questionnaires describing management practices. Additionally, herds provided individual reproductive records for at least one complete year, retrieved from its herd management software.

Data collection spanned three production years. However, only 17 herds were evaluated over three complete years, whereas 48 herds contributed data for two years and 137 herds for a single year. Therefore, the herd-year was considered the experimental unit, resulting in a total of 284 herd-years evaluated.

2.1. Data cleaning and herd eligibility

Reproductive records were initially provided at the individual cow and service levels, totaling 1,270,642 service records. As all reproductive and productive parameters were calculated at the herd-year level, only lactating breeding cows were considered for analysis. Consequently, 354,124 services from nulliparous cows were excluded. An additional 131,019 services from beef breeds or cows with unidentified breed classification were removed. Herds without production system information accounted for the exclusion of 52,749 services. To ensure accurate calculation of P/AI, 17,324 services lacking a recorded pregnancy diagnosis and 4,491 services from cows with more than eight lactations were excluded. Moreover, 55,373 services from herds that did not report the frequency of reproductive veterinary assistance or classified it as non-frequent were removed from the dataset.

For indicators requiring the number of cows eligible for breeding (e.g., service rate and pregnancy rate), data were calculated by the herd management using 21-days reproductive cycles. Service rate was calculated as the number of inseminations divided by the number of cows eligible for breeding during each 21- period. Pregnancy rate was calculated as the number of cows diagnosed as pregnant divided by the number of eligible cows within the same period.

Production systems were classified as compost barn, free stall, or dry lot. Veterinary reproductive assistance frequency was categorized as monthly, bi-weekly, or weekly. Breed composition across production systems, including purebred Holstein and Holstein × Gyr crossbreds (1/2, 3/4, and 7/8 Holstein), is present on Figure 2. The distribution of veterinary reproductive management frequency within each production system is presented in Figure 3.

After data aggregation and quality checks, 12,097 cows from 137 herds were excluded due to inconsistent records. Subsequently, 21,870 cows from 94 herds were removed because of incomplete information for the evaluated variables. Finally, herds with fewer than 10 cows were excluded, resulting in the removal of 23 cows from 4 herds. After all exclusion criteria were applied, the final dataset comprised 90,658 cows distributed across 284 dairy herds.

2.2. Calculations

For each herd year, the following variables were calculated: P/AI overall and by parity (primiparous, secundiparous, and multiparous), P/AI at first service, percentage of retained placenta, age at first calving (AFC), use of TAI, use of sexed semen (overall and at first AI), use of AI and embryo transfer (ET), average dry period length, voluntary waiting period (VWP) stipulated by the herd, interval from calving to first AI, and days open (DO), defined as the interval between calving and subsequent confirmed pregnancy.

Pregnancy rate and service rate were calculated by the herd management software based on 21-days reproductive cycles. The interval between services was calculated within lactation and parity by determining the number of days between consecutive services. The first service of each lactation was excluded from this calculation. Intervals were averaged at the herd-year level, with the production year defined as July to June.

Associations between herd management strategies, production systems, and reproductive and productive outcomes were assessed at the herd-year level using descriptive and inferential statistical approaches.

2.3. Statistical analysis

All statistical analyses were performed in R (R Foundation for Statistical Computing, Vienna, Austria). The herd–year was considered the experimental unit. Data were filtered to include only herds with consistent reproductive management frequency; records classified as "every 21 days" or "sporadic" were excluded from analyses comparing reproductive management frequency.

Distributional assumptions for continuous outcomes were evaluated using graphical inspection and descriptive statistics.

Descriptive statistics summarized the distribution of herd–year observations. The number of unique herd–year observations was determined by combining farm identification and year, and within-system proportions were calculated.

Associations between production system and herd-level reproductive and productive outcomes were evaluated using generalized Gaussian linear models (GLM), with production system as a fixed effect. Similarly, associations between reproductive management frequency and herd-level outcomes were assessed using GLM, with management frequency as a fixed effect. Separate models were fitted for each outcome. Because outcomes were expressed as herd-level annual indicators, models were fitted assuming Gaussian errors.

Given that the study design was based on aggregated herd-year data, individual-level covariates such as breed could not be included in the models. Thus, the predominance of crossbred cows in dry-lot systems should be considered when interpreting differences in productive and reproductive outcomes.

Type III tests were used to evaluate the overall effect of each factor. When significant, estimated marginal means were obtained using the emmeans package using the Sidak test, and pairwise comparisons among levels were conducted. Compact letter displays summarized multiple comparisons through multcomp package. To account for multiple testing across related outcomes, P-values were adjusted using false discovery rate control according to the Benjamini–Hochberg procedure.

To evaluate whether the voluntary waiting period differed across parity categories within management frequency, the voluntary waiting period was analyzed in a long-format dataset that included primiparous, secundiparous, and multiparous categories. A linear model with the interaction between management frequency and parity was fitted, and the interaction was interpreted using Type III tests and estimated marginal means within management frequency.

The mean interval between consecutive inseminations within lactation (excluding the first insemination of each lactation) was analyzed using a generalized linear mixed model with a Gamma distribution and log link to accommodate positive, right-skewed data. Management frequency and production system were evaluated as fixed effects in separate models, and herd was included as a random intercept to account for clustering of herd–year observations within each herd. Estimated marginal means were back-transformed to the original scale.

To examine relationships between pregnancy rate and other herd-level reproductive and productive variables, Spearman rank correlations were calculated between pregnancy rate and each predictor variable using complete-case pairs. Correlation coefficients (ρ) and corresponding P-values were reported. P-values from the correlation analyses were adjusted for multiple testing using false discovery rate control.

An adjusted significance level of $P \leq 0.05$ was used for inferential analyses.

3. Results

Descriptive analysis

Among the 284 evaluated herds, 73.6% housed lactating cows in compost barns, 7.4% in free stalls, and 19.0% in dry lots. Most cows were housed in compost barns (Figure 2), predominantly as purebred Holsteins, while crossbred cows were mainly distributed between compost barns and dry lot systems. Free stall systems housed mostly Holstein cows.

Most herds received reproductive veterinary assistance biweekly (48.9%), followed by weekly (35.9%) and monthly visits (15.2%). The use of AI is predominant in the herds, with only six herds reporting more than 90% ET use during the evaluated year. Regarding breed, 11 herds have 100% Holstein purebred breeding cows, and 13 herds had no Holstein purebred cows in their herds, 260 herds have both purebred Holstein and crossbred Holstein × Gyr cows.

Monthly veterinary visits were absent in free stall systems, while weekly visits were more common in free stall herds (Figure 3). Bi-weekly visits were most frequent in compost barn systems, whereas dry lot systems had the highest proportion of monthly visits.

Across herds, the stipulated VWP ranged from 15 to 80 days, while real VWP ranged from 3 to 271 d, and the interval between calving and first service was 68.3 days (Table 1). For the first AI after calving, 80% of herds used TAI, and P/AI ranged from 3.45% for multiparous cows to 38.6% for secundiparous cows, and 43.67% for primiparous cows during the first AI. Overall, 68.5% of services used TAI, resulting service rate, P/AI and pregnancy rate averaged 57.1%, 20.9%, and 20.7%, respectively. On evaluated herds, sexed semen use was in mean 20.2%.

Regarding typical period records, dry period ranged from 43.9 to 79.6 d, while days open ranged from 85.4 to 171.1 d. Retained placenta through herd-year varied from 0.0% to 33.9%. Parity average was 2.3 lactations for breeding cows, while AFC 26.9 months for them. At first lactation, mean MY was 8,111.5 kg and overall MY was 9,995.3 kg.

Reproductive management frequency

Herds receiving monthly veterinary visits had higher average parity ($P < 0.01$) and older AFC compared with herds visited bi-weekly, followed by weekly ($P < 0.01$; Table 2). Monthly herds also had a longer dry period than those with other management frequencies ($P < 0.01$).

Use of AI, TAI, and TAI at first service differed among management frequencies ($P < 0.01$). Monthly herds showed lower use of synchronization strategies ($P < 0.01$; Table 2), lower service rates ($P < 0.01$), longer days open ($P = 0.01$), and a longer interval between services compared to weekly herds ($P < 0.01$). Bi-weekly herds had intermediate values for these indicators.

The stipulated VWP was shorter in monthly herds than in weekly herds ($P < 0.001$), but the interval from calving to first service was significantly longer in monthly herds than in bi-weekly and weekly herds ($P < 0.01$). Similar patterns were observed when primiparous cows were evaluated ($P < 0.01$).

Pregnancy per AI, whether at first service or overall, did not differ among management frequencies across parity groups ($P \geq 0.63$). However, the pregnancy rate was associated progressively from monthly to bi-weekly to weekly herds, reflecting differences in service rate.

Use of sexed semen was higher in weekly herds ($P = 0.04$), whereas ET use did not differ among management frequencies ($P = 0.71$).

Production system

Compost barn and free stall systems showed lower parity ($P < 0.01$) and younger AFC compared with dry lot systems ($P < 0.01$; Table 3). Compost barn and free stall herds were similar in most reproductive management practices, including AI and TAI use, while dry lot herds had lower adoption of synchronization strategies ($P < 0.01$).

Stipulated VWP ($P = 0.01$) and the interval between calving and first AI ($P = 0.01$) were longer in dry lot systems compared with confined systems, particularly compost barns. This pattern was also observed in primiparous cows.

Pregnancy per AI did not differ among production systems when evaluated overall ($P = 0.49$) or by parity ($P \geq 0.26$). Service rate was highest in free stall herds, followed by compost barn, and lowest in dry lot herds ($P < 0.001$). Accordingly, free stall herds have the shortest interval between services, followed by compost barn and dry lot systems ($P < 0.01$). Pregnancy rate was higher in intensive confined systems compared with dry lot systems ($P = 0.01$).

When examining average milk production, free stall and compost barn herds had the highest overall average milk yield compared to dry lot herds ($P < 0.01$). For first-lactation cows, free stall herds had the highest milk yield, followed by compost barn and dry lot systems ($P < 0.01$).

Parameters correlated with pregnancy rate

Pregnancy rate is strongly and positively correlated with service rate and P/AI ($P < 0.01$; Table 4). High positive correlations were also observed with P/AI in primiparous ($P < 0.01$) and secundiparous cows ($P < 0.01$), as well as with P/AI at first service ($P < 0.01$). Moderate positive correlations were observed with MY at first lactation ($P < 0.01$), overall, MY ($P < 0.01$), TAI use ($P < 0.01$), and AI use ($P < 0.01$). In contrast, AFC ($P < 0.01$) and days open were negatively correlated with pregnancy rate ($P < 0.01$).

Retained placenta ($P < 0.01$), use of sexed semen ($P < 0.01$), and use of TAI ($P = 0.06$) showed weak correlations with pregnancy rate, whereas use of ET ($P = 0.79$), dry period length ($P = 0.68$), and herd parity ($P = 0.21$), were not significantly correlated with pregnancy rate after adjustment for multiple testing.

4. Discussion

This study suggests that both reproductive management and production systems are strongly associated with herd-level reproductive performance in dairy herds. Confined systems appeared to invest more intensively in reproductive programs, as indicated by a higher frequency of veterinary visits. In this context, free stall herds had the highest veterinarian visit frequency, followed by compost barn herds, which was associated with the service rate and MY for both the overall herd and primiparous cows.

Compost barn systems, as previously reported, are increasingly common production systems (Leso et al., 2020), largely due to the comfort provided by the free bed system and also its lower environmental impact (Oliveira et al., 2023), compared to conventional free stall housing. Dry lot systems were also prevalent in the evaluated population; however, they were characterized by lower management intensity, as evidenced by fewer veterinarian visits. This reduced intensity was associated with lower reproductive indices, including less use of TAI and lower service rates, reflecting less efficiency in breeding eligible cows.

The first artificial insemination (AI) postpartum is a critical factor in herd reproductive success and overall profitability (Inchaisri et al., 2010), as it is closely associated with postpartum disease incidence and treatment. The use of timed artificial insemination (TAI) protocols at first service has been shown to standardize hormonal conditions after uterine recovery, reduce reliance on estrus detection, and decrease days open (DO) (Moreira et al., 2001; Wiltbank et al., 2012; Cardoso Consentini et al., 2021).

Weekly veterinary visits were associated with lower AFC, which may suggest more intensive nutritional and reproductive programs, as well as higher replacement rates suggested by the presence of younger lactating cows. Heifers that calve at a younger age tend to have a longer lifespan, reducing culling before the second lactation (Bach, 2011), particularly because reproductive disorders remain one of the main causes of culling in dairy herds (Almeida et al., 2025).

A VWP close to 60 days has historically been recommended to allow adequate uterine involution and metabolic recovery postpartum (VanRaden et al., 2004; Miller et al., 2007; Hansson et al., 2025). Achieving this target remains challenging due to the negative energy balance associated with increasing production until peak yield, which generally occurs between 4 and 8 weeks postpartum and varies according to parity (Carvalho et al., 2019). Monthly herds had a longer interval from calving to first AI, approaching values suggested for high-producing or younger herds; however, this pattern is not supported by their productive profile, as these herds were characterized by lower milk yield and older cows.

Weekly visited herds showed greater uniformity in the interval from calving to first service and a shorter interval between services, reflecting more systematic and frequent reproductive monitoring. In contrast, monthly herds had greater variability, and primiparous cows were inseminated later. Delaying first service for primiparous cows may be beneficial, as it allows additional time for growth before the next calving (Stangaferro et al., 2018; Edvardsson Rasmussen et al., 2023). Bi-weekly herds exhibited intermediate patterns and inseminated parous cows earlier, which may be related to the known lower fertility of these cows compared to primiparous cows (Lean et al., 2023).

Pregnancy/AI was largely similar across management frequencies; however, differences in service rate, interval between services, and reproductive management practices were associated with marked differences in pregnancy rate and days open. These findings reinforce the concept that herd reproductive performance is primarily driven by management intensity rather than insemination success alone. Improvements in pregnancy rate directly influence the proportion of pregnant cows throughout the year, average days in milk, and MY, ultimately affecting farm income (Overton and Eicker, 2025).

Previous study has reported an increase of 1.6 days open for each additional kilogram of milk produced, while also demonstrating improved reproductive outcomes in herds assisted by full-time veterinarians (Mirzaie et al., 2024). In contrast, the present study showed that more frequent veterinary visits were associated with fewer days open and a higher 305-d MY herd average, highlighting the role of intensive management in mitigating the antagonism between milk production and reproduction.

Confined systems, including free stall and compost barn, were similar for most reproductive indices, suggesting that both housing systems can provide adequate comfort and management conditions. However, both systems outperformed dry lot, consistent with previous evidence that total mixed ration feeding systems enhance reinsemination efficiency of non-pregnant cows due to more intensive management (Chebel et al., 2025). This advantage was reflected in the shorter interval between services in the free stall herds, which consequently had the highest service rate, followed by the compost barn and dry lot systems. Cows managed under intensive feeding systems are more likely to be bred than those in pasture-based systems (Lean et al., 2023). However, pregnancy rate did not differ between confined systems, suggesting similar overall reproductive efficiency.

Confined systems, due to their advantageous reproductive outcomes, may be associated with adequate uterine recovery, as suggested by the interval from calving to first AI, and provide a favorable environment for the expression of fertility compared to dry lot systems. This advantage is reflected in the higher pregnancy rates observed. However, days open were not influenced by the production system, suggesting that management practices may override housing effects on this indicator.

5. Conclusion

Herd reproductive performance was strongly associated with reproductive management frequency and production system. More frequent veterinary visits were associated with reproductive efficiency mainly by higher service rate and lower interval between services, rather than by pregnancy per service. Confined systems supported more intensive reproductive management and achieved better reproductive outcomes than dry lot systems. Management intensity appears to be an important factor associated with herd-level reproductive efficiency.

Funding source

We are grateful to the National Council for Scientific and Technological Development (CNPq, Brasília, Brazil; 141066/2022-9). We acknowledge Alta Genetics of Brazil for providing data for the analyses and truly appreciate the farmers and technicians that collaborated in recording data and were willing to participate in the study.

Contribution statement

K. R. Oliveira: Conceptualization, Methodology, Data curation, Investigation, Formal analysis, Writing – original draft, Writing – review and editing. **T. E. Silva**: Methodology, Data curation, Formal analysis, Writing – review and editing. **J. H. C. Costa**: Methodology, Data curation, Writing – review and editing, Supervision. **T. M. Ferreira**: Investigation, Data curation. **A. L. Silva**: Methodology, Data curation, Writing – review and editing, Supervision **C. E. C. Consentini**: Conceptualization, Methodology, Data curation, Validation, Writing – review and editing. **R. Sartori**: Conceptualization, Methodology, Data curation, Validation, Writing – review and editing. **P. P. Rotta**: Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review and editing.

References

Almeida, R. de, S. de Paula, M.M. Marquetti, M. Poczynek, D.F.F. da Silva, R.B. Navarro, A.A. Valloto, J.A. Horst, and V.B. Pedrosa. 2025. Longevity and Culling Reasons in Dairy Herds in Southern Brazil. Animals 15. https://doi.org/10.3390/ani15152232.

Armengol, R., L. Fraile, and A. Bach. 2023. Key performance indicators used by dairy consultants during the evaluation of reproductive performance during routine visits. Front Vet Sci 10. https://doi.org/10.3389/fvets.2023.1165184.

Bach, A. 2011. Associations between several aspects of heifer development and dairy cow survivability to second lactation. J Dairy Sci 94:1052–1057. https://doi.org/10.3168/jds.2010-3633.

Black, R.A., J.L. Taraba, G.B. Day, F.A. Damasceno, and J.M. Bewley. 2013. Compost bedded pack dairy barn management, performance, and producer satisfaction. J Dairy Sci 96:8060–8074. https://doi.org/10.3168/jds.2013-6778.

Busanello, M., D.G. Sousa, M. Poczynek, R. de Almeida, C.M.M. Bittar, F.A.C. Mendonça, and D.P.D. Lanna. 2022. Body growth of replacement dairy heifers from 3 distinct genetic groups from commercial Brazilian dairy herds. J Dairy Sci 105:3222–3233. https://doi.org/10.3168/jds.2021-21197.

Caraviello, D.Z., K.A. Weigel, P.M. Fricke, M.C. Wiltbank, M.J. Florent, N.B. Cook, K. V. Nordlund, N.R. Zwald, and C.L. Rawson. 2006. Survey of management practices on reproductive performance of dairy cattle on large US commercial farms. J Dairy Sci 89:4723–4735. https://doi.org/10.3168/jds.S0022-0302(06)72522-X.

Carvalho, M.R., F. Peñagaricano, J.E.P. Santos, T.J. DeVries, B.W. McBride, and E.S. Ribeiro. 2019. Long-term effects of postpartum clinical disease on milk production, reproduction, and culling of dairy cows. J Dairy Sci 102:11701–11717. https://doi.org/10.3168/jds.2019-17025.

Chebel, R.C., T. Gonzalez, A.B. Montevecchio, K.N. Galvão, A. de Vries, and R.S. Bisinotto. 2025. Targeted reproductive management for lactating Holstein cows: Economic return. J Dairy Sci 108:1584–1601. https://doi.org/10.3168/jds.2024-25525.

Consentini, C.E., M.C. Wiltbank, and R. Sartori. 2021. Factors that optimize reproductive efficiency in dairy herds with an emphasis on timed artificial insemination programs. Animals 11:1–30. https://doi.org/10.3390/ani11020301.

Derks, M., T. van Werven, H. Hogeveen, and W.D.J. Kremer. 2013. Veterinary herd health management programs on dairy farms in the Netherlands: Use, Execution, And relations to farmer characteristics. J Dairy Sci 96:1623–1637. https://doi.org/10.3168/jds.2012-6106.

Edvardsson Rasmussen, A., K. Holtenius, R. Båge, E. Strandberg, M. Åkerlind, and C. Kronqvist. 2023. A randomized study on the effect of extended voluntary waiting period in primiparous dairy cows on milk yield during first and second lactation. J Dairy Sci 106:2510–2518. https://doi.org/10.3168/jds.2022-22773.

Hansson, A., K. Holtenius, R. Båge, M. Lindberg, and C. Kronqvist. 2025. Effect of voluntary waiting period length on milk yield, fertility, and culling in high-yielding, second-parity cows. J Dairy Sci 108:13416–13424. https://doi.org/10.3168/jds.2025-26348.

Inchaisri, C., R. Jorritsma, P.L.A.M. Vos, G.C. van der Weijden, and H. Hogeveen. 2010. Economic consequences of reproductive performance in dairy cattle. Theriogenology 74:835–846. https://doi.org/10.1016/j.theriogenology.2010.04.008.

Kogima, P., T.A. Diesel, F.M.C. Vieira, A.L.B. Schogor, A.A. Volpini, G.J. Veloso, P.F.P. Ferraz, and M.L.A.N. Zotti. 2022. The Welfare of Dairy Cows in Pasture, Free Stall, and Compost Barn Management Systems in a Brazilian Subtropical Region. Animals 12. https://doi.org/10.3390/ani12172215.

Lean, I.J., H.M. Golder, S.J. LeBlanc, T. Duffield, and J.E.P. Santos. 2023. Increased parity is negatively associated with survival and reproduction in different production systems. J Dairy Sci 106:476–499. https://doi.org/10.3168/jds.2021-21672.

Marynissen, S., F. Mortier, L. Duchateau, P. Smets, S. Daminet, and D. Paepe. 2025. Exploring the Importance of Repeated Health Screening in Healthy Older Dogs. J Vet Intern Med 39. https://doi.org/10.1111/jvim.70166.

Miller, R.H., H.D. Norman, M.T. Kuhn, J.S. Clay, and J.L. Hutchison. 2007. Voluntary waiting period and adoption of synchronized breeding in dairy herd improvement herds. J Dairy Sci 90:1594–1606. https://doi.org/10.3168/jds.S0022-0302(07)71645-4.

Mirzaie, B., A. Sadeghi-Sefidmazgi, and M. Zhandi. 2024. A Survey Study on Management Practices Associated With Voluntary Waiting Period and Its Effects on Reproductive Performance of dairy cows in Iranian Dairy Farms. Vet Med Sci 10. https://doi.org/10.1002/vms3.70107.

Moreira, F., C. Orlandi, C.A. Risco, R. Mattos, F. Lopes, and W.W. Thatcher. 2001. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. J Dairy Sci 84:1646–1659. https://doi.org/10.3168/jds.S0022-0302(01)74600-0.

Overton, M.W., and S. Eicker. 2025. Associations between days open and dry period length versus milk production, replacement, and fertility in the subsequent lactation in Holstein dairy cows. J Dairy Sci 108:3764–3779. https://doi.org/10.3168/jds.2024-26055.

Ritter, C., L. Dorrestein, D.F. Kelton, and H.W. Barkema. 2021. Herd health and production management visits on Canadian dairy cattle farms: Structure, goals, and topics discussed. J Dairy Sci 104:7996–8008. https://doi.org/10.3168/jds.2020-19833.

Santos, L.G.C., J.C. dos S. Breda, F.M. Cerri, K.K.M. da C. Flaiban, E.J.F. Filho, and J.A.N. Lisboa. 2022. Effect of changes in body condition during the dry period, milk yield and genetic groups on the metabolic profiles of high-producing Holstein × Gir cows. Livest Sci 265. https://doi.org/10.1016/j.livsci.2022.105097.

Slade Oliveira, C., R. Varella Serapião, A.J. dos Reis Camargo, C. de Freitas, L. Tamy Iguma, B. Campos Carvalho, L.S. de Almeida Camargo, L. Zoccolaro Oliveira, and R. da Silva Verneque. 2019. Oocyte origin affects the in vitro embryo production and development of Holstein (Bos taurus taurus) - Gyr (Bos taurus indicus) reciprocal cross embryos. Anim Reprod Sci 209. https://doi.org/10.1016/j.anireprosci.2019.106165.

Stangaferro, M.L., R. Wijma, M. Masello, M.J. Thomas, and J.O. Giordano. 2018. Extending the duration of the voluntary waiting period from 60 to 88 days in cows that received timed artificial insemination after the Double-Ovsynch protocol affected the reproductive performance, herd exit dynamics, and lactation performance of dairy cows. J Dairy Sci 101:717–735. https://doi.org/10.3168/jds.2017-13046.

Stivanin, S.C.B., E.F. Vizzotto, J.F. Aires, A.R. Guadagnin, A.F. Bettencourt, F.S. Machado, M.M. Campos, T.R. Tomich, L.G.R. Pereira, and V. Fischer. 2024. Behavior and reactivity during milking of Holstein, Gyr and Girolando-F1 cows at the beginning of lactation. Journal of Veterinary Behavior. https://doi.org/10.1016/j.jveb.2024.08.002.

VanRaden, P.M., A.H. Sanders, M.E. Tooker, R.H. Miller, H.O. Norman, M.T. Kuhn, and G.R. Wiggans. 2004. Development of a national genetic evaluation for cow fertility. J Dairy Sci 87:2285–2292. https://doi.org/10.3168/jds.S0022-0302(04)70049-1.

Várhidi, Z., G. Csikó, Á.C. Bajcsy, and V. Jurkovich. 2024. Uterine Disease in Dairy Cows: A Comprehensive Review Highlighting New Research Areas. Vet Sci 11. https://doi.org/10.3390/vetsci11020066.

Wiltbank, M.C., A.H. Souza, P.D. Carvalho, R.W. Bender, and A.B. Nascimento. 2012. Improving fertility to timed artificial insemination by manipulation of circulating progesterone concentrations in lactating dairy cattle. Reprod Fertil Dev 24:238–243. https://doi.org/10.1071/RD11913.

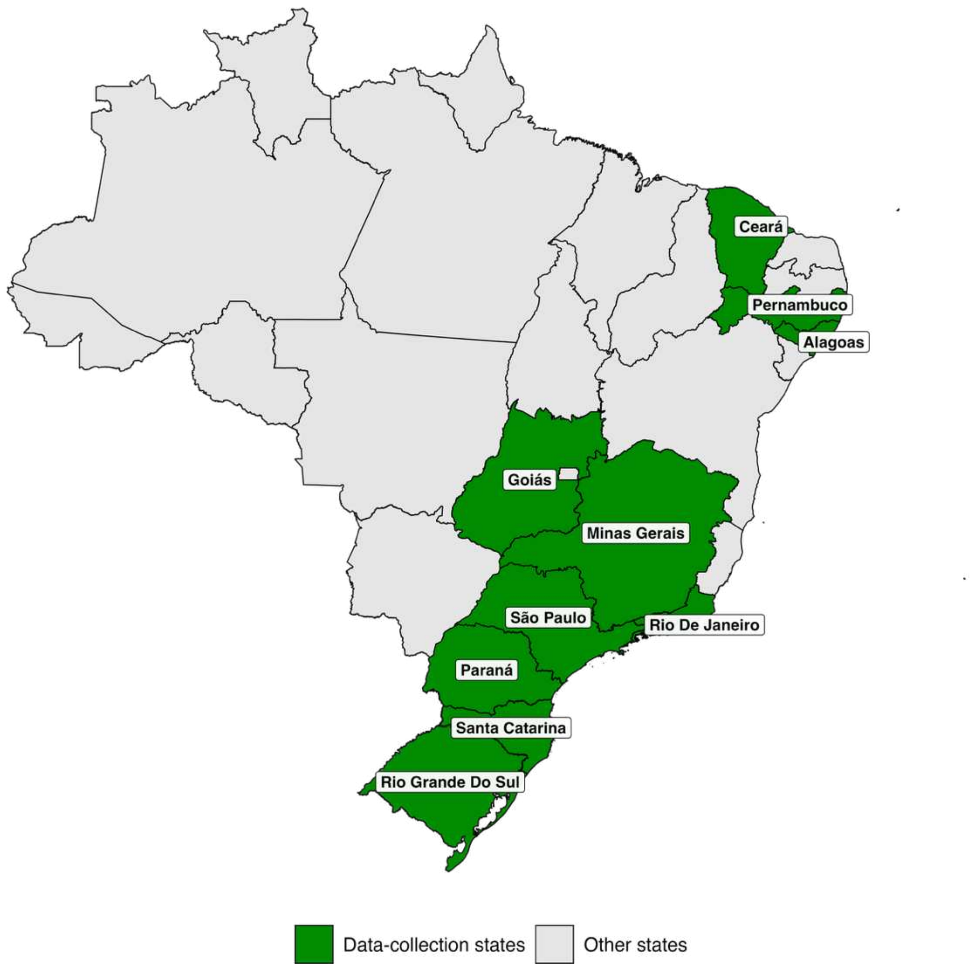

Figure 2. Geographic distribution of Brazilian states included in the reproductive performance analysis. States highlighted in green indicate those with available data used in the study.

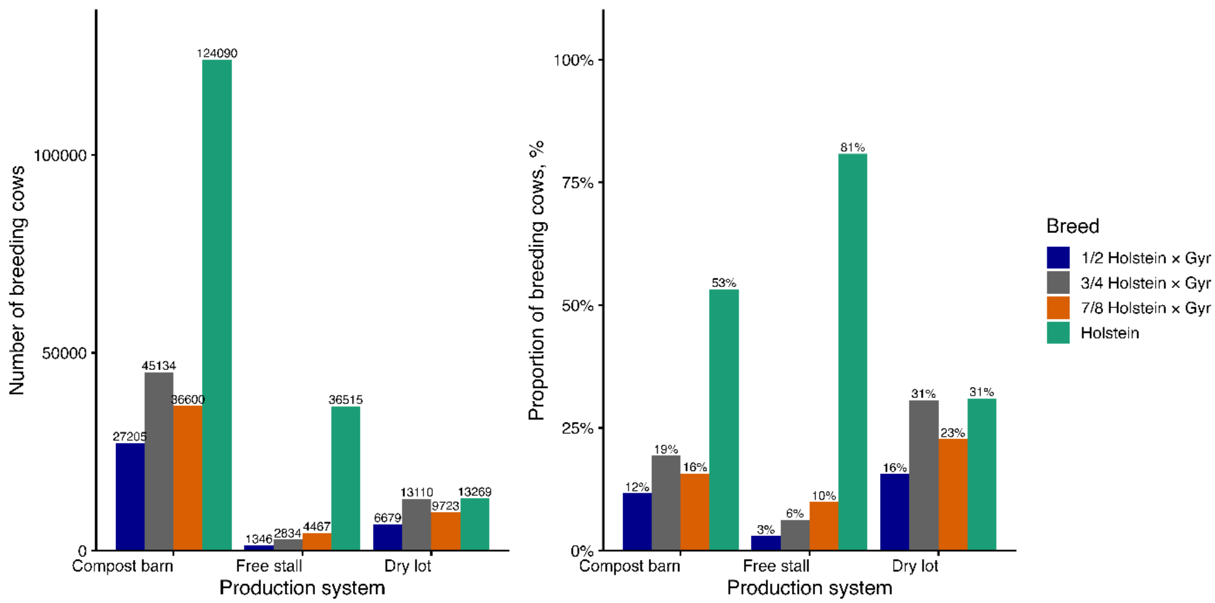

Figure 3. Distribution of cows' breeds across production systems. (A) number of cows, and (B) proportion of breeding cows per system and (%) according to production system and breed.

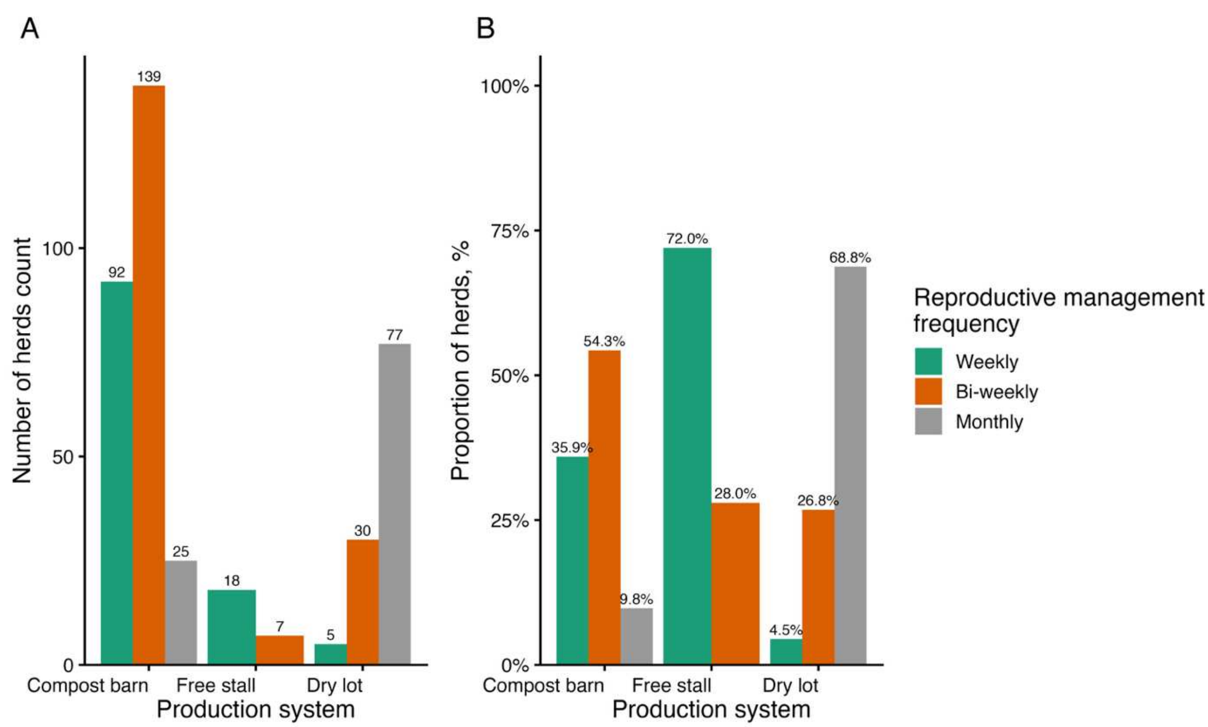

Figure 4. Distribution of reproductive veterinarian management frequency across production systems. (A) Number of herds count and (B) Proportion of herds (%) receiving weekly, bi-weekly, and monthly veterinary reproductive management

Table 1. Descriptive statistics of herd-level reproductive and productive performance indicators based on annual herd averages. Values are presented as minimum (Lower), maximum (Upper), mean, standard deviation (SD), and median.

Item	Lower	Upper	Mean	SD	Median
Number of breeding cows per herd	14	2,099	256.0	260.87	182.5
Days open	85.4	171.1	126.4	15.48	123.9
Age at first calving, months	22.6	38.4	27.6	3.10	26.9
Parity average	1.4	3.94	2.31	0.37	2.3
Dry period, d	43.9	79.6	57.3	4.68	57.1
Voluntary waiting period determined, d	15.0	80.0	50.9	8.31	52.0
Real waiting period, d	3.0	271.0	68.3	31.28	62.0
Timed AI use at first AI, %	0.0	100.0	80.1	25.05	90.2
Retained placenta, %	0.0	33.9	8.4	6.53	7.1
Sexed semen use, %	0.0	98.2	12.0	20.22	2.4
Artificial insemination use, %	0.0	100.0	91.5	19.31	99.3
Timed AI use, %	0.0	100.0	68.5	23.72	73.5
Embryo transfer use, %	0.0	100.0	5.1	17.80	0.0
Multiparous first AI Pregnancy/AI, %	3.4	58.3	35.7	10.16	37.0
Primiparous first AI Pregnancy/AI, %	10.0	75.0	43.7	10.63	43.9
Secundiparous first AI Pregnancy/AI, %	10.0	71.4	40.2	10.73	40.0
General first AI Pregnancy/AI, %	17.1	61.6	40.0	8.22	40.5
General Pregnancy/AI, %	18.1	54.4	36.3	7.10	36.0
Multiparous Pregnancy/AI, %	12.8	55.6	34.0	8.01	34.1
Primiparous Pregnancy/AI, %	20.9	63.6	39.0	8.43	38.6
Secundiparous Pregnancy/AI, %	16.2	69.6	36.9	8.64	36.7
Pregnancy rate, %	2.4	38.1	20.7	6.91	20.0
Service rate	21.7	93.1	57.1	12.73	57.0

Table 2. Herd-level reproductive and productive performance according to reproductive management frequency (weekly, bi-weekly, and monthly veterinary visits). Values are presented as least squares means ± standard error (SE), obtained from generalized linear models. Different superscript letters within a row indicate differences among management frequencies (*P* < 0.05).

Item	Weekly	Bi-weekly	Monthly	Pr (> Chisq)
Number of herds	102	139	43	
Parity, number	2.2 ± 0.04^{a}	2.3 ± 0.03^{a}	2.6 ± 0.05^{b}	< 0.001
Age at first calving, months	26.2 ± 0.30^{a}	27.6 ± 0.25^{b}	30.3 ± 0.43^{c}	< 0.001
Use of artificial insemination, %	94.7 ± 1.89^{b}	91.3 ± 1.62^{ab}	84.3 ± 2.91^{a}	0.017
Use of timed artificial insemination, %	74.7 ± 2.03^{b}	72.7 ± 1.74^{b}	40.2 ± 3.13^{a}	< 0.001
Use of embryo transfer, %	4.2 ± 1.77	6.0 ± 1.51	4.3 ± 2.72	0.706
Timed artificial insemination in the first service	88.7 ± 2.17^{c}	82.4 ± 1.86^{b}	51.9 ± 3.34^{a}	< 0.001
Dry period, days	56.3 ± 0.45^{a}	57.2 ± 0.39^{a}	60.1 ± 0.69^{b}	< 0.001
Voluntary waiting period, days	54.9 ± 0.76^{c}	49.4 ± 0.65^{b}	46.3 ± 1.18^{a}	< 0.001
Interval calving to first service, days	63.3 ± 3.02^{a}	66.7 ± 2.61^{a}	85.8 ± 4.76^{b}	< 0.001
Primiparous' interval calving to first service, days	63.3 ± 1.30^{a}	66.8 ± 1.14^{a}	78.3 ± 2.06^{b}	< 0.001
Retained of placenta, %	8.91 ±0.66	8.58 ± 0.55	6.43 ± 0.99	0.117
Use of sexed semen, %	16.16 ± 1.98^{b}	9.79 ± 1.70^{a}	9.06 ± 3.06^{ab}	0.036
Pregnancy per service on first AI[1], %	40.7 ± 0.82	39.7 ± 0.70	39.4 ± 1.26	0.632
Primiparous first AI pregnancy per service [1], %	44.6 ± 1.05	43.8 ± 0.90	40.9 ± 1.62	0.229
Secundiparous first AI pregnancy per service[1], %	41.1 ± 1.06	40.1 ± 0.91	38.6 ± 1.64	0.582
Multiparous first AI pregnancy per service[1], %	35.7 ± 1.00	34.8 ± 0.86	38.7 ± 1.56	0.160
Pregnancy per service, %	35.8 ± 0.76	36.4 ± 0.65	35.6 ± 1.17	0.778
Primiparous pregnancy per service, %	38.9 ± 0.84	38.8 ± 0.72	40.2 ± 1.29	0.681
Secundiparous pregnancy per service, %	37.4 ± 1.32	37.0 ± 0.73	36.6 ± 0.86	0.895

Multiparous pregnancy per service, %	32.9 ± 0.79	34.2 ± 0.68	35.9 ± 1.23	0.176
Service rate, %	64.7 ± 1.10^{c}	54.7 ± 0.94^{b}	46.8 ± 1.69^{a}	< 0.001
Pregnancy rate, %	23.4 ± 0.65^{c}	20.1 ± 0.56^{b}	16.7 ± 1.00^{a}	< 0.001
Interval between services	42.2 ± 0.69^{a}	45.3 ± 0.64^{b}	48.2 ± 1.12^{c}	< 0.001
Days open	122 ± 1.50^{a}	128 ± 1.29^{b}	131 ± 2.31^{b}	0.001
Attempts to pregnancy	2.9 ± 0.05^{b}	2.9 ± 0.04^{b}	2.5 ± 0.08^{a}	< 0.001

[1]Artificial insemination.

[2]Timed artificial insemination.

[3]Embryo transfer.

Table 3. Herd-level reproductive and productive performance according to production system (compost barn, free stall, and dry lot). Values are presented as least squares means ± standard error (SE), obtained from generalized linear models. Different superscript letters within a row indicate differences among production systems ($P < 0.05$).

Item	Compost barn	Free stall	Dry lot	Pr (> Chisq)
Number of herds	209	21	54	
Parity, number	2.3 ± 0.24[a]	2.2 ± 0.77[a]	2.6 ± 0.48[b]	< 0.001
Age at first calving, months	27.2 ± 0.21[a]	25.4 ± 0.66[a]	30.0 ± 0.40[b]	< 0.001
Use of artificial insemination, %	92.8 ± 1.32[b]	96.2 ± 4.16[b]	84.3 ± 2.59[a]	0.013
Use of timed artificial insemination, %	72.9 ± 1.53[b]	71.2 ± 0.48[b]	50.5 ± 3.01[a]	< 0.001
Use of embryo transfer, %	5.2 ± 1.23	0.0 ± 3.88	6.6 ± 2.42	0.416
Timed artificial insemination in the first service	84.0 ± 1.63[b]	88.0 ± 5.13[b]	61.9 ± 3.20[a]	< 0.001
Dry period, days	56.5 ± 0.31[a]	57.5 ± 0.96[a]	60.6 ± 0.60[b]	< 0.001
Voluntary waiting period, days	51.4 ± 0.57[b]	53.8 ± 1.79[b]	47.9 ± 1.10[a]	0.012
Interval calving to first service, days	65.3 ± 2.15[a]	63.5 ± 6.71[ab]	81.1 ± 4.18[b]	0.008
Primiparous' interval calving to first service, days	65.7 ± 0.98[a]	69.8 ± 2.99[ab]	71.5 ± 1.84[b]	0.056
Retained of placenta, %	8.8 ± 0.45	9.5 ± 1.41	6.3 ± 0.88	0.051
Use of sexed semen, %	12.1 ± 1.40	15.8 ± 4.42	9.8 ± 2.75	0.508
Pregnancy per service on first AI[1], %	40.7 ± 0.56	39.3 ± 1.78	37.6 ± 1.11	0.077
Primiparous first AI pregnancy per service [1], %	44.6 ± 0.73	43.5 ± 2.30	40.3 ± 1.43	0.056
Secundiparous first AI pregnancy per service[1], %	40.8 ± 0.74	41.1 ± 2.34	37.8 ± 1.46	0.255
Multiparous first AI pregnancy per service[1], %	36.0 ± 0.70	32.3 ± 2.22	35.6 ± 1.39	0.334
Pregnancy per service, %	36.4 ± 0.53	35.0 ± 1.67	35.1 ± 1.04	0.489
Primiparous pregnancy per service, %	39.1 ± 0.58	38.4 ± 1.85	38.9 ± 1.15	0.912
Secundiparous pregnancy per service, %	37.2 ± 0.60	34.5 ± 1.88	36.5 ± 1.18	0.405
Multiparous pregnancy per service, %	34.2 ± 0.55	30.9 ± 1.74	34.2 ± 1.10	0.266

Service rate, %	57.6 ± 0.84[b]	66.9 ± 2.67[c]	51.3 ± 1.66[a]	< 0.001
Pregnancy rate, %	21.1 ± 0.47[b]	23.6 ± 1.48[b]	18.2 ± 0.92[a]	0.008
Interval between services	44.7 ± 0.55[b]	40.6 ± 1.37[a]	46.1 ± 0.98[b]	0.005
Days open	126 ± 1.1	123 ± 3.3	129 ± 2.1	0.334
Attempts to pregnancy	2.8 ± 0.04	3.0 ± 0.12	2.7 ± 0.07	0.159

[1] Artificial insemination, [2] timed artificial insemination, [3] embryo transfer.

Table 4. Spearman correlation coefficients between pregnancy rate and reproductive and productive indicators in 284 dairy herds. Correlation coefficients (ρ) and corresponding *P*-values are shown. *P*-values were adjusted for multiple testing using control according to the Benjamini–Hochberg procedure.

Variable	Correlation	P-value
Service rate, %	0.77	< 0.001
Pregnancy per service, %	0.75	< 0.001
Secundiparous pregnancy per service, %	0.67	< 0.001
Primiparous pregnancy per service, %	0.62	< 0.001
Pregnancy per service on first AI[1], %	0.59	< 0.001
Secundiparous first AI pregnancy per service[1], %	0.56	< 0.001
Multiparous pregnancy per service, %	0.53	< 0.001
Voluntary waiting period, days	0.52	< 0.001
Primiparous first AI pregnancy per service [1], %	0.46	< 0.001
Milk yield in first lactation corrected for 305 d, kg	0.43	< 0.001
Multiparous first AI pregnancy per service[1], %	0.41	< 0.001
Use of timed artificial insemination, %	0.36	< 0.001
Timed artificial insemination in the first service	0.32	< 0.001
Retained of placenta, %	0.19	0.002
Use of sexed semen, %	0.19	0.002
Use of timed artificial insemination, %	0.11	0.064
Use of embryo transfer, %	-0.02	0.786
Dry period, days	-0.03	0.679
Parity, number	-0.08	0.206
Age at first calving, months	-0.49	< 0.001
Days open	-0.64	< 0.001

[1] Artificial insemination, [2] timed artificial insemination.

Interpretative summary

- More frequent reproductive veterinary visits were associated with herd reproductive efficiency primarily by higher service rate and reducing the interval between services.
- Pregnancy per artificial insemination was similar across management frequencies and production systems, suggesting that management intensity were related with reproductive performance at the herd level.
- Confined production systems supported more intensive reproductive management and achieved better reproductive outcomes than dry lot systems.

CHAPTER 4: REDUCED MANURE TREATMENT NEEDS WITH COMPOST-BEDDED PACK SYSTEMS IN DAIRY COWS

* Published on Sustainability

https://doi.org/10.3390/su162310408

sustainability MDPI

Article

Reduced Manure Treatment Needs with Compost-Bedded Pack Systems in Dairy Cows

Kellen R. Oliveira , Marcelo S. Rodrigues, Luís H. R. Silva, Poliana T. R. Salgado, Alex L. Silva and Polyana P. Rotta *

Department of Animal Science, Universidade Federal de Viçosa, Viçosa 36570-900, Brazil; kellen.oliveira@ufv.br (K.R.O.); marcelo.s.rodrigues@ufv.br (M.S.R.); luis.henrique@ufv.br (L.H.R.S.); poliana.salgado@ufv.br (P.T.R.S.); alex.lopes@ufv.br (A.L.S.)

* Correspondence: polyana.rotta@ufv.br

Abstract: The compost-bedded pack (CBP) system offers a sustainable solution for dairy farms by enhancing cow welfare and health while promoting environmental sustainability and improving manure management for agricultural reuse. This study aimed to evaluate the reduction in manure treatment required for agricultural use by analyzing manure excretion patterns in lactating cows. We compared seven Holstein and six Holstein × Gyr cows, divided into two CBP groups, and monitored their feces and urine behaviors over a 48 h period. Manure excretion was recorded across four farm areas: (1) feeding area, (2) resting area (composted bed), (3) path to the milking parlor, and (4) milking parlor. Both breeds predominantly excreted feces (45.03%) and urine (54.18%) in the resting area, which facilitated composting directly in the bedding. This resulted in a significant reduction in nitrogen requiring treatment, averaging 76.8–85.3 g per cow per day, accounting for 44–49% of total nitrogen excretion. The CBP system demonstrated its effectiveness in reducing environmental impact by minimizing nitrogen loss through volatilization and leaching, while also enhancing nutrient recycle in agriculture. These findings emphasize the CBP system's role in foresting sustainable dairy farming and environmentally friendly agricultural practices.

Keywords: compost-bedded pack; dairy cattle; environment; reuse; sustainability

Citation: Oliveira, K.R.; Rodrigues, M.S.; Silva, L.H.R.; Salgado, P.T.R.; Silva, A.L.; Rotta, P.P. Reduced Manure Treatment Needs with Compost-Bedded Pack Systems in Dairy Cows. *Sustainability* **2024**, *16*, 10408. https://doi.org/10.3390/su162310408

Academic Editors: Marcelo Henrique Otênio, Juliana Alves Resende and Alberto Delgado Dos Reis

Received: 1 November 2024
Revised: 18 November 2024
Accepted: 22 November 2024
Published: 27 November 2024

1. Introduction

Dairy farm management systems vary widely, including pasture-based, free stall, and compost-bedded pack (CBP) systems. One of the central challenges in all these systems is the effective management of bovine manure, a byproduct that requires careful treatment before being used in agriculture. Efficient manure management is essential for environmental sustainability, as improper handling can lead to nutrient runoff, greenhouse gas emissions, and the loss of valuable organic matter. The manure produced by lactating cows ranges from 50 to 70 kg per day [1,2], depending on the level of production and cow weight, which have a greater potential to affect the environment.

Among the dairy cows' systems, the CBP system stands out as a sustainable alternative. It offers a solution for manure issues by reducing the need for extensive treatment and allows for direct reuse of manure in farming, minimizing the processing required before agricultural application [1]. This approach aligns with the sustainability principles of "reduce, reuse, and recycle" by facilitating the recycling and repurposing of bedding materials in agricultural use, which in turn improves soil structure and bioactivity [3].

The selection of bedding materials, such as sawdust and wood shavings, plays a crucial role in the effectiveness of CBP systems. These materials promote aerobic activity and facilitate the compost maturation process [4–6], contributing organic matter to the soil [7] and improving its quality compared to conventional waste composts [8]. This dual benefit of composting and soil enhancement highlights the environmental advantages of CBP systems.

trial began, during which they were kept together to allow for social adjustment. The estimated dry matter intake (DMI) was 22.3 kg, with a crude protein content of 16.4%. Intake as a percentage of body weight was 3.30% for the Holsteins and 3.58% for the Holstein × Gyr crossbreeds.

Table 1. Ingredients and chemical composition of the experimental diet.

Item	
Ingredient, %DM Corn silage	74.83
Corn grain, dry	8.12
Dried distilled grain	3.51
Cottonseed, whole	4.68
Soybean meal	5.51
Hay	2.06
Sulfur flower	0.015
Sodium chloride	0.171
Bicalcium phosphate	0.107
Limestone	0.364
Mineral mix [1]	0.043
Sodium bicarbonate	0.107
Magnesium oxide	0.107
Yeast pre-mix [2]	0.105
Urea	0.259
Chemical composition, % DM Dry matter	45.1
Crude protein	16.4
Rumen degradable protein	11.9
Rumen undegradable protein	4.5
Neutral detergent fiber	35.6
Starch	25.9
Ether extract	4.24

[1] Mineral mix composition = 200 g/kg of calcium, 15 mg/kg of cobalt, 700 mg/kg of cupper, 15 g/kg of sulfur, 43 g/kg of phosphorus, 35 mg/kg of iodine, 40 g/kg of magnesium, 1 g/kg of manganese, 20 mg/kg of selenium, 90 g/kg of sodium, 2.6 g/kg of zinc, 240,000 UI/kg of A vitamin, 87,000 UI/kg of D3 vitamin, and 1750 UI/kg of E vitamin. [2] Yeast pre-mix = 35 g/kg beta-glucans, 15 g/kg zinc, 7500 mg/kg mannanoligosaccharide, 5000 mg/kg copper, 2500 mg/kg glucomannans, 120 mg/kg selenium, 80 mg/kg chrome, 4×10^{11} *Saccharomyces cerevisiae*.

2.2. Housing and Cooling

Cows were housed in a CBP with sawdust as the carbon source (Figure 1). This sawdust is a byproduct derived from pine wood shavings measuring 3–5 cm in size. The two pens, separated by breed were provided with a bedding area of 158 m^2. Holsteins were allocated 26.3 m^2 per cow, while Holstein × Gyr cows had 22.6 m^2 per cow. This space exceeds the typical range of 10 to 12 m^2 per cow recommended for dairy cows in Brazil and facilitated system observation.

The feeding area provided 35.6 m^2 of free space, with each cow having access to more than 1.4 m of trough feeding space. Four fans (Magnum, GEA Brasil, Campinas, Brazil) were installed in the bedding area to aid in drying the compost and cooling the cows. The bedded pack was stirred twice daily while the cows were in the milking parlor to maintain its quality and effectiveness.

Cows were milked three times daily at 600 h, 1330 h, and 2000 h. Before each milking session, cows were cooled using cycles of 1 min of sprinkling with a disc nozzle, followed by 5 min of ventilation with fans (Magnum, GEA Farm Technologies, Bönen, Germany). During the experiment, the ambient temperature ranged from 15.8 to 28.3 °C, while the temperature humidity index (THI) ranged from 60 and 77. These values were recorded by the meteorological station of the Universidade Federal de Viçosa, located 1.5 km from the CBP where the trial took place. THI values exceeded 68 half the time, indicating conditions of heat stress [2].

Figure 1. Experimental compost-bedded pack (CBP) system featuring bedding composed of a mixture of sawdust and manure. The CBP is equipped with fans positioned to enhance air circulation and promote optimal composting conditions.

2.3. Trial Evaluations

Before the trial began, cows were marked for easy identification using Celo Check marker paint (Weizur do Brazil, Brazil). Holsteins were numbered from 1 to 6, and Holstein × Gyr crossbreeds from 1 to 7.

The trial lasted 48 h, during which the location, urination, and defecation of each one of the thirteen animals was continuously monitored by trained observers across four designated areas: (1) feeding area (35.64 m^2), (2) resting place (composted bed, 158 m^2), (3) path to milking (272.57 m^2), and (4) milking parlors (9.92 m^2). Four observers recorded the specific locations of feces and urine disposal continuously from 0800 h on day one until 48 h later. The frequency of urination and defecation for each cow in each area was tallied at the end of each day.

On the second day, milk yield for each cow was measured during all three milking sessions using a DemaTron 70 control unit (GEA Group Aktiengesellschaft, Düsseldorf, Germany). A 350 mL milk sample was also collected using the VESTA Sampling System

(GEA Group Aktiengesellschaft, Düsseldorf, Germany) and analyzed for composition with the Lactoscan SP Ultrasonic Milk Analyzer (Milkotronic Ltd.a, Nova Zagora, Bulgaria). Milk yield was adjusted to energy-corrected milk (ECM), based on fat and crude protein, according to Reincke et al., 2018 [16]:

$$0.25 \times \text{kg Milk} + 12.2 \times \text{kg Fat} + 7.7 \times \text{kg Crude Protein} \quad (1)$$

2.4. N Excretion Calculations

Nitrogen (N) excretion was calculated based on N and DMI using equations from NASEM [2]:

$$\text{Urine N (g/d)} = 12.0\ (\pm 5.8) + 0.333\ (\pm 0.011)\ \text{N intake (g/d)} \quad (2)$$

urine N excretion based on N intake (Equation 14-5, NASEM, 2021, [2]).

$$\text{Fecal N (g/d)} = -18.5\ (\pm\ 3.59) + 10.1\ (\pm\ 0.169)\ \text{dry matter intake (kg/d)} \quad (3)$$

fecal N excretion (Equation 14-6; NASEM, 2021, [2]), based on dry matter intake in kg per day.

Additionally, we calculated total manure N excretion using a formula that incorporates diet, intake, and the yield and composition of milk: (Equation 14-4; NASEM, 2021, [2]).

$$\text{Manure N (g/d)} = (\text{DMI} \times \text{Diet crude protein})/0.625 - (\text{Milk} \times \text{Milk crude protein})/0.638 - 5 \quad (4)$$

2.5. Statistical Analysis

Data analysis was performed using R software [15], with specific packages employed for various statistical tests. Values such as body weight, days in milk, milk yield, energycorrected milk, and milk composition were analyzed using a linear mixed-effects model (lme) with the “lme4” package. In these models, breed was treated as a fixed effect, while individual cows were included as random effects to account for repeated measures. The models were checked for normality of residuals and homoscedasticity using diagnostic plots. Outliers were identified and removed using a threshold of 1.5 times the interquartile range. Additionally, confidence intervals were calculated for each effect, and effect sizes were derived using standardized coefficients (Cohen’s d). Comprehensive *p*-values were reported for all analyses.

For behavioral data related to excretion, normality and homoscedasticity assumptions were similarly verified, and outliers were excluded based on the same threshold of 1.5 times the interquartile range. Excretion behavior was analyzed by comparing the frequency of manure disposal between breeds using analysis of variance (ANOVA), implemented with the “aov” function in R. Data regarding the quantity of manure deposited in specific areas of the farm were compared using two-sample t-tests, with significance considered at $p \leq 0.05$ and trends reported for $0.05 < p \leq 0.10$. Where appropriate, Tukey’s post-hoc test was used to adjust for multiple comparisons.

Nitrogen excretion via feces, urine, and total manure was also analyzed using ANOVA to compare across breeds and days of collection. Interaction effects between breed and day of collection were included to assess whether differences in excretion patterns emerged over time. Mean comparisons were performed using the “emmeans” package, with significance set at $p \leq 0.05$, and effect sizes were calculated for the main effects. Results are presented as mean ± standard error (M ± SE) throughout the text.

In all analyses, comprehensive *p*-values, effect sizes, and 95% confidence intervals were reported to ensure transparency and validate the results. The assumptions of each statistical test were rigorously checked to ensure model reliability and robustness.

3. Results

Holstein cows demonstrated a similar milk yield compared to crossbred Holstein × Gyr cows (29.5 ± 1.61 vs. 26.0 ± 1.74 kg/d; Table 2). Both groups had comparable levels of total fat content and percentage of fat, as well as total protein.

Table 2. Mean (±SE) milk yield and composition of Holstein and crossbred Holstein Gyr.

Item	Holstein	Holstein × Gyr	*p*-Value
Milk yield, kg/d	29.5 ± 1.61	26.0 ± 1.74	0.1520
ECM [1], kg/d	28.0 ± 1.66	24.7 ± 1.80	0.1928
Fat, %	3.68 ± 0.139	3.55 ± 0.151	0.5327
Total fat, kg	1.08 ± 0.077	0.94 ± 0.083	0.2280
Protein, %	3.15 ± 0.034	3.25 ± 0.037	0.0537
Total protein, kg	0.93 ± 0.050	0.84 ± 0.054	0.2428

3.1. Manure Excretion Locations

Feces excretion in the walking area and milking parlor was similar (12.28% vs. 11.39%; $p = 0.5248$).

Urine excretion patterns were also concentrated in the resting area (54.18%; Figure 2, which was significantly more than in other areas ($p < 0.001$). These findings highlight the significance of the resting area as a critical zone for composting manure, where it can be reused as bedding material for the animals.

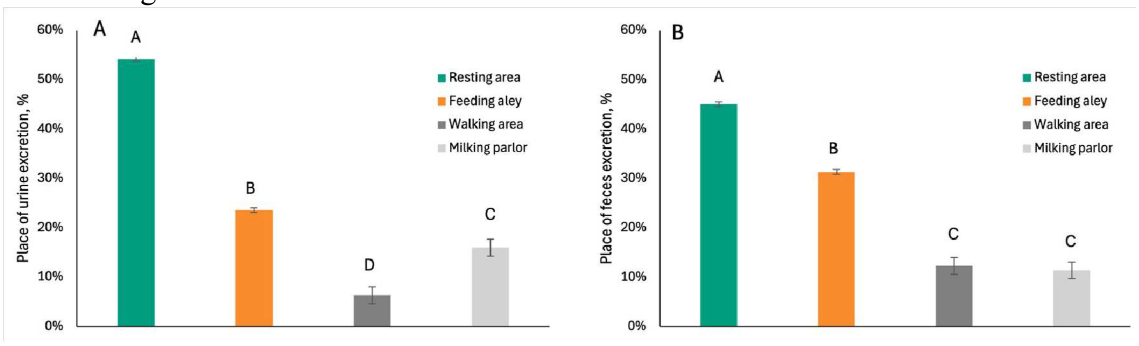

Figure 2. Mean (±SE) distribution of urine (**A**) and feces (**B**) excretion across different locations: resting area, feeding alley, walking area, and milking parlor. Means within different letters are significantly different ($p \leq 0.05$).

3.2. Behavior Patterns of Manure Excretion by Breed

Both Holstein and Holstein × Gyr breeds exhibited similar patterns of manure excretion across all areas within the CBP system. Feces excretion in the resting area, feeding alley, walking area, and milking parlor showed no significant breed differences ($p > 0.05$; Table 3). Urine excretion followed a similar trend, with both breeds excreting urine predominantly in the resting area and no differences between breeds. These results suggest that breed does not significantly influence manure deposition behaviors when cows are housed in a CBP system.

Table 3. Mean (±SE) comparative analysis of feces and urine excretion between lactating Holstein and Holstein × Gyr cows.

	Holstein	**Holstein × Gyr**	***p*-Value**
Feces excretion, %			
Resting area	46.5 ± 3.06	43.4 ± 3.15	0.4836
Feeding alley	29.9 ± 2.92	32.7 ± 3.01	0.5094
Walking area	13.5 ± 1.44	11.0 ± 1.49	0.2177
Milking parlor	10.0 ± 1.30	12.9 ± 1.34	0.1280
Urine excretion, %			
Resting area	53.1 ± 3.84	55.4 ± 3.96	0.6781
Feeding alley	22.5 ± 2.73	24.7 ± 2.81	0.5842
Walking area	6.6 ± 1.61	6.0 ± 1.61	0.8015
Milking parlor	17.9 ± 2.29	14.0 ± 2.36	0.2423

3.3. Reduction in N Excretion That Requires Treatment

Based on the milk yield and composition data for both breeds evaluated, Holstein and Holstein × Gyr cows exhibited comparable daily nitrogen (N) manure excretion rates (p = 0.2428; Table 4). Holstein cows excreted 435 ± 7.8 g of N daily, while the crossbred cows excreted 449 ± 8.42 g of N daily. However, both breeds demonstrated a significant reduction in the nitrogen excreted that requires treatment due to the high concentration of excreta deposited in the resting area (p = 0.4699). Holstein cows reduced 46.5% in feces that directly impact the environment or require treatment, which equates to 49.0 g/d of N per cow. In contrast, in crossbred Holstein × Gyr cows, 43.4% of their feces were deposited in the resting area of the CBP, resulting in a reduction of 47.8 g/d of N that would otherwise need treatment. Both breeds exhibited similar rates of N excretion in the resting area (p = 0.868) according to feces estimates.

Table 4. Mean (±SE) estimated daily nitrogen excretion and treatment-required nitrogen per cow for lactating Holstein and Holstein × Gyr cows.

	Holstein	**Holstein × Gyr**	***p*-Value**
N manure excretion [1]	435 ± 7.8	449 ± 8.4	0.2428
Reduction of N excretion that requires treatment, g/d			
Feces [2]	49.0 ± 5.04	47.8 ± 5.44	0.8680
Urine [3]	27.7 ± 3.88	37.5 ± 4.19	0.1005
Total [4]	76.8 ± 7.90	85.3 ± 8.53	0.4699
Reduction of N excretion that requires treatment, %	44.1 ± 4.53	49.0 ± 4.90	0.4699

[1] Based on milk yield and composition, Equation (4). [2] Based on dry matter intake, Equation (2). [3] Based on nitrogen intake, Equation (1). [4] Result of the sum of Equations (2) and (3).

Urine excretion in the resting area was similarly reduced (p = 0.1005) for both breeds, equating to 27.7 g/d less N per Holstein cow and 37.5 g/d for crossbred Holstein × Gyr cows. This reduction may positively impact the environment and lessen the need for further processing. In total, feces and urine excreted in the resting area account for 76.8 g/d of N for Holstein cows and 85.3 g/d for Holstein × Gyr cows, eliminating the necessity for treatment on dairy farms. This represents 44.1% of the total daily nitrogen excreted by Holstein cows and 49.0% for Holstein × Gyr cows, which is deposited directly for composting in the resting area of the CBP system. Overall, this indicates a reduction of 46.5 ± 3.34% of nitrogen, which is deposited in the resting area for composting.

4. Discussion

This study aimed to compare the manure excretion patterns of Holstein and Holstein × Gyr cows under identical conditions in a compost-bedded pack system (CBP). The results show that milk yield and composition were similar between the two breeds.

These findings align with earlier studies that evaluated Holstein cows under tropical conditions [17], and some research that reports higher fat and total solids in crossbred, while protein levels were comparable across breeds [18–20].

Manure deposition patterns in both Holstein and Holstein × Gyr were concentrated in the resting area, supporting previous findings on the CBP's effectiveness in managing waste. Both breeds demonstrated similar manure excretion behaviors, predominantly in the resting area, which facilitated the composting process. This concentration of waste helps significantly reduce the environmental burden by decreasing the amount of manure requiring treatment [21]. Our findings indicate a 44–49% reduction in nitrogen requiring treatment, which is slightly lower than the 75% reduction reported by some studies based on farmers' estimates [14,22]. The slight discrepancy could be attributed to the different methods used in previous studies, such as farmer estimates and calculus based on daily intake and observation. Further studies are needed to standardize nitrogen reduction rates across various production systems to provide a more comprehensive comparison.

The composting of manure within the CBP bedding not only stabilizes nutrients but also supports more sustainable agricultural practices [23]. Furthermore, our study highlights the effectiveness of the CBP system in reducing manure treatment needs, especially when comparing excretion patterns in specific areas of the barn. While producers typically estimate that 20 to 25% of the manure is deposited in the feed alley [24], our findings indicate that 23.56% of urine and 31.29% of feces were deposited in this area. This reinforces the CBP's efficiency in managing waste on dairy farms. Additionally, N from feces excreted in the resting area is composted on the cows' bed and reused in agriculture without further treatment, further enhancing sustainability.

Previous studies have focused on differing feeding patterns between Holstein and Holstein × Gyr heifers in pasture conditions, showing minimal differences in performance [25,26]. Crossbreeds with Zebu have also demonstrated greater thermotolerance than purebred Holsteins under tropical conditions [27]. However, limited data exist on the performance of those breeds when housed in confined systems during lactation. Our study fills this gap by demonstrating that both Holstein and Holstein × Gyr lactating cows exhibited similar manure excretion patterns when housed in a CBP system. The majority of excreta was deposited in the resting area, facilitating its incorporation into the bedding for subsequent composting. This behavior leads to a consistent reduction in the volume of manure requiring treatment across both breeds.

The reduction in N directly entering the soil—between 44 and 49%—equates to 76.8 to 85.3 g of N per cow per day, which could otherwise promote soil leaching. Manure is often applied to fields without proper treatment, and at inappropriate times, increasing the risk of nitrogen loss [23]. The composting significantly reduces N loss through ammonia volatilization and leaching when compared to chemical fertilizers in agricultural uses [28,29]. To achieve these benefits, cattle manure requires a minimum of 80 days of composting to reduce phytotoxicity, improve nutrient stability [30], and minimize the presence and spread of antibiotic-resistant bacteria [31] in the field. These composting practices are in line with the CBP system's removal and management of composted bedding once annually [4].

Addressing N management in agricultural practices is critical due to its potential for soil contamination. While the total N content in cattle manure is generally lower than in swine manure, composted cattle manure contains higher levels of total N compared to noncomposted manure [32]. The CBP system, as used for lactating cows, effectively reduces the amount of N entering the soil or requiring treatment for agricultural applications. This reduction helps prevent soil leaching and minimizes N loss through volatilization, making CBP a sustainable system for dairy farms.

Moreover, the use of composted cattle manure can significantly reduce the need for chemical fertilizers, improve corn grain yields, provide a more consistent nitrate supply, and reduce N losses in the field, promoting more sustainable agricultural practices [33]. Additionally, incorporating composted manure as an organic nutrient source can lower the costs associated with soil management [34], further emphasizing the environmental and economic benefits of using composted manure in farming systems.

The compost-bedded-pack (CBP) system is recognized for its environmental benefits, particularly its ability to reduce the volume of manure requiring treatment and limit direct manure deposition into the soil. By promoting efficient composting, the CBP system enhances nutrient retention, improves soil health, and decreases reliance on chemical fertilizers, thereby supporting more sustainable agricultural practices.

While this study provides valuable insights into manure excretion patterns and nitrogen reduction in Holstein and Holstein × Gyr cows within CBP system, several limitations must be acknowledged. The small sample size limits the generalizability of the findings, and observational biases may have influenced the accuracy of the data collection. Future research should address these limitations by using larger sample sizes, automated monitoring methods, and stricter control of environmental conditions to confirm and expand upon these results. Additionally, future studies could explore seasonal variations in manure excretion patterns and their effects on CBP system efficiency. Investigating how different seasons impact cows' behavior, manure deposition, and the composting process could lead valuable insights for optimizing CBP management throughout the year. Despite these constraints, this study offers meaningful contributions to sustainable manure management in dairy farming.

5. Conclusions

In our study, both Holstein and Holstein × Gyr cows exhibited similar excretion patterns when housed in a CBP system, with most of their waste deposited in the resting area. This behavior facilitated composting, leading to a substantial reduction in the amount of manure requiring treatment—45.03% for feces and 54.18% for urine. The concentration of waste in the CBP bedding allowed for enhanced nitrogen (N) retention at 46.5% and reduction in N loss through volatilization or leaching, which can otherwise occur when untreated manure is applied directly to fields.

These findings underscore the CBP system's effectiveness in managing manure while minimizing its environmental impact. By decreasing nitrogen emissions and incorporating organic matter back into the soil, the CBP system not only reduces the overall volume of farm waste but also contributes to healthier soil and improved biosecurity. The system offers a sustainable solution for dairy farming, fostering better waste management practices and environmental stewardship.

Author Contributions: Conceptualization, K.R.O. and M.S.R.; methodology, K.R.O., M.S.R. and P.P.R.; formal analysis, K.R.O. and A.L.S.; investigation, K.R.O., M.S.R., L.H.R.S. and P.T.R.S., writing— original draft preparation, K.R.O.; writing—review and editing, P.P.R.; supervision, P.P.R. and A.L.S.; project administration, P.P.R.; funding acquisition, P.P.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by CAPES PROEX (88887.844747/2023-00) and CNPq (141066/2022-9).

Institutional Review Board Statement: The animal study protocol was approved by the Committee of Animal Use Ethics Committee of the Department of Animal Science of the Universidade Federal de Viçosa, Minas Gerais, Brazil (protocol 35/2024).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Acknowledgments: We are grateful to the Dairy Research Facility of the Animal Science Department of Universidade Federal de Viçosa, Associação Brasileira de Criadores de Girolando, the donators of Holstein × Gyr animals for research use, and the research collaborators Bernardo Magalhães, Carina Bittencourt, João Vitor C. Rodrigues, and Thais A.S. Silva.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Damasceno, F.A.; Monge, J.L.; Nascimento, J.A.C.; Andrade, R.R.; Barbari, M.; Saraz, J.A.O.; Ferraz, G.A.S. Estimate of manure present in compost dairy barn systems for sizing of manure storage. *Agron. Res.* **2020**, *18*, 1213–1219. [CrossRef]
2. NASEM. *Nutrient Requirements of Dairy Cattle*, 8th ed.; National Academies Press: Washington, DC, USA, 2021.
3. Kuipers, A.; Galama, P.; Leso, L.; Bruegemann, K.; Klopcˇicˇ, M. A Composting Bedding System for Animals as a Contribution to the Circular Economy. *Processes* **2022**, *10*, 518. [CrossRef]
4. Barberg, A.E.; Endres, M.I.; Salfer, J.A.; Reneau, J.K. Performance and welfare of dairy cows in an alternative housing system in Minnesota. *J. Dairy Sci.* **2007**, *90*, 1575–1583. [CrossRef] [PubMed]
5. Janni, K.A.; Endres, M.I.; Reneau, J.K.; Schoper, W.W. Compost dairy barn layout and management recommendations. *Appl. Eng. Agric.* **2007**, *23*, 97–102. [CrossRef]
6. Llonch, L.; Castillejos, L.; Mainau, E.; Manteca, X.; Ferret, A. Effect of forest biomass as bedding material on compost-bedded pack performance, microbial content, and behavior of nonlactating dairy cows. *J. Dairy Sci.* **2020**, *103*, 10676–10688. [CrossRef]

7. Llonch, L.; Gordo, C.; López, M.; Castillejos, L.; Ferret, A.; Balanyà, T. Agronomic Characteristics of the Compost-Bedded Pack Made with Forest Biomass or Sawdust. *Processes* **2021**, *9*, 546. [CrossRef]
8. Leso, L.; Barbari, M.; Lopes, M.; Damasceno, F.; Galama, P.; Taraba, J.; Kuipers, A. Invited review: Compost-bedded pack barns for dairy cows. *J. Dairy Sci.* **2019**, *103*, 1072–1099. [CrossRef]
9. Galama, P.J.; Bokma, S.; van Dooren, H.J.; Ouweltjes, W.; Smits, M.; Driehuis, F. *Prospects for Bedded Pack Barns for Dairy Cattle*; Wageningen UR Livestock Research: Lelystad, The Netherlands, 2011.
10. Bewley, J.M.; Robertson, L.M.; Eckelkamp, E.A. A 100-year review: Lactating dairy cattle housing management. *J. Dairy Sci.* **2017**, *100*, 10418–10431. [CrossRef]
11. Bran, J.A.; Costa, J.H.C.; von Keyserlingk, M.A.G.; Hötzel, M.J. Factors associated with lameness prevalence in lactating cows housed in freestall and compost-bedded pack dairy farms in southern Brazil. *Prev. Vet. Med.* **2019**, *172*, 104773. [CrossRef]
12. Pilatti, J.A.; Vieira, F.C.M.; Rankrape, F.; Vismara, E.S. Diurnal behaviors and herd characteristics of dairy cows housed in a compost-bedded pack barn system under hot and humid conditions. *Animal* **2018**, *13*, 399–406. [CrossRef]
13. Black, R.A.; Taraba, J.L.; Day, G.B.; Damasceno, F.A.; Bewley, J.M. Compost bedded pack dairy barn management, performance, and producer satisfaction. *J. Dairy Sci.* **2013**, *96*, 8060–8074. [CrossRef] [PubMed]
14. Eckelkamp, E.A.; Taraba, J.L.; Akers, K.A.; Harmon, R.J.; Bewley, J.M. Understanding compost bedded pack barns: Interactions among environmental factors, bedding characteristics, and udder health. *Livestock Sci.* **2016**, *190*, 35–42. [CrossRef]
15. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2021; Available online: https://www.R-project.org/ (accessed on 22 August 2024).
16. Reincke, K.; Saha, A.; Wyrzykowski, L. The Global Dairy World 2017/18: Results of the IFCN Dairy Report 2018. IFCN Dairy Research Network: Kiel, Germany, 2018. Available online: https://ifcndairy.org/wp-content/uploads/2018/10/Dairy-ReportArticle-2018.pdf (accessed on 19 July 2024).
17. Oliveira, A.S. Meta-analysis of feeding trials to estimate energy requirements of dairy cows under tropical condition. *Anim. Feed. Sci. Technol.* **2015**, *15*, 30045–30046. [CrossRef]
18. Nóbrega, D.B.; Langoni, H. Breed and season influence on milk quality parameters and in mastitis occurrence. *Pesq. Vet. Bras.* **2011**, *31*, 1045–1052. [CrossRef]
19. Houaga, I.; Muigai, A.W.T.; Ng'ang'a, F.M.; Ibeagha-Awemu, E.M.; Kyallo, M.; Yassao, I.A.K.; Stomeo, F. Milk fatty acid variability and association with polymorphisms in SCD1 and DGAT1 genes in White Fulani and Borgou cattle breeds. *Mol. Biol. Rep.* **2018**, *45*, 1849–1862. [CrossRef]
20. Roessler, R.; Mpouam, S.E.; Schlecht, E. Genetic and nongenetic factors affecting on-farm performance of peri-urban dairy cattle in west Africa. *J. Dairy Sci.* **2019**, *102*, 2353–2364. [CrossRef]
21. Hao, X.; Larney, F.J. Greenhouse gas emissions during co-composting of cattle feedlot manure with construction and demolition (C&D) waste. *Front. Eviron. Sci. Eng.* **2017**, *11*, 15. [CrossRef]

22. Caldato, E.M.R.; Caldato, A.; Marcondes, M.I.; Rotta, P.P. Manual Técnico de Construção E Manejo de Compost Barn Para Vacas Leiteiras. Master's Thesis, Universidade Federal de Viçosa, Pró- Reitoria de Extensão e Cultura, Divisão de Extensão, Viçosa, Brazil, 2020.
23. Lazcano, C.; Gómez-Brandón, M.; Domínguez, J. Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure. *Chemosphere.* **2008**, *72*, 1013–1019. [CrossRef]
24. Barberg, A.E.; Endres, M.I.; Janni, K.A. Compost dairy barns in Minnesota: A descriptive study. *Appl. Eng. Agric.* **2007**, *23*, 231–238. [CrossRef]
25. Quirino, D.F.; Marcondes, M.I.; Rennó, L.N.; Correa, L.N.; Morais, V.C.L.; Cunha, C.S.; Silva, T.D.A.; da Silva, A.L.; Miller-Cushon, A.L.; Rotta, P.P. Intake, performance, and feeding behavior of Holstein and Holstein x Gyr heifers grazing intensive managed tropical grasses during the rainy season. *Animal.* **2022**, *16*, 100613. [CrossRef]
26. Abreu, M.; Cunha, C.; Costa, J.; Miller-Cushon, E.; Rotta, P.; Machado, A.; Morais, V.; Marcondes, M. Performance and Feeding Behavior of Holstein and Holstein × Gyr Crossbred Heifers Grazing Temperate Forages. *Trop. Anim. Health Prod.* **2022**, *54*, 100. [CrossRef] [PubMed]
27. Costa, A.N.L.; Feitosa, J.V.; Montezuma, P.A., Jr.; Souza, P.T.; Araújo, A.A. Rectal temperatures, respiratory rates, production, and reproduction performances of crossbred Girolando cows under heat stress in northeastern Brazil. *Int. J. Biometeorol.* **2015**, *59*, 1647–1653. [CrossRef] [PubMed]
28. Jia, X.; Yang, Q.; Dong, S.T.; Zhang, J.W.; Zhao, B.; Liu, P. Using manure for improving nitrogen fertilization and maize yield. *Exp. Agric.* **2021**, *56*, 901–914. [CrossRef]
29. Prados, L.F.; Chizzotti, F.H.M.; Cardoso, A.S.; Ruggieri, A.C.; Chizzotti, M.L.; Bretas, I.L.; Silva, B.C.; Rotta, P.P.; Silva, L.F.C.;
Valadares Filho, S.C. Prediction of nitrogen and phosphorus excretion for beef cattle. In *Nutrient Requirements of Zebu and Crossbred Cattle—BR-CORTE*, 4th ed.; Valadares Filho, S.C., Saraiva, D.T., Benedeti, P.B., Silva, F.A.S., Chizzotti, M.L., Eds.; Suprema: Visconde de Rio Branco, Brazil, 2023; pp. 321–338. [CrossRef]
30. Gómez-Brandón, M.; Lazcano, C.; Domínguez, J. The evaluation of stability and maturity during the composting of cattle manure. *Chemosphere* **2008**, *70*, 436–444. [CrossRef] [PubMed]
31. Gou, M.; Hu, H.W.; Zhang, Y.J.; Wang, J.T.; Hayden, H.; Tang, Y.Q.; He, J.Z. Aerobic composting reduces antibiotic resistance genes in cattle manure and the resistome dissemination in agricultural soils. *Sci. Total Environ.* **2018**, *612*, 1300–1310. [CrossRef]
32. Liu, S.; Wang, J.; Pu, S.; Blagodatskaya, E.; Kuzyakov, Y.; Razavi, B.S. Impact of manure on soil biochemical properties: A global synthesis. *Sci. Total Environ.* **2020**, *745*, 141003. [CrossRef]
33. Zhang, L.; Li, L.; Sha, G.; Liu, C.; Wang, Z.; Wang, L. Aerobic composting as an effective cow manure management strategy for reducing the dissemination of antibiotic resistance genes: An integrated meta-omics study. *J. Hazard. Mater.* **2020**, *386*, 121895. [CrossRef]
34. Zafari, A.; Kianmehr, M.H. Management and Reduction of Chemical Nitrogen Consumption in Agriculture. *Am. J. Plant Sci.* **2012**, *3*, 1827–1834. [CrossRef]

GENERAL CONCLUSION

Taken together, the four studies presented in this thesis demonstrate that productivity, reproductive success, and sustainability in tropical dairy systems are interdependent with nutritional efficiency, postpartum health, reproductive management, and manure handling strategies. Rather than acting as isolated components, these dimensions interact to determine how effectively dairy systems convert feed into milk, preserve cow health and longevity, and reduce nutrient losses to the environment.

In the first study, supplementation with Saccharomyces cerevisiae CNCM I-1077 improved the ruminal degradability of DM and NDF from important fiber sources used in dairy cow diets, including both forages and non-forage fiber sources. In lactating cows, this was reflected in greater total-tract DM digestibility, improved feed efficiency, lower DMI, and reduced somatic cell count, even though milk yield and milk composition were not altered. These results show that nutritional responses are not always expressed as more milk but may still represent meaningful improvements in feed efficiency to produce milk.

The second study showed that retained placenta remains an important problem in tropical dairy herds, not only because of its association with parity and calving-related events, but also because of its consequences for later performance. Cows with retained placenta had lower pregnancy per AI, greater pregnancy loss, and higher culling risk. These findings reinforce that events occurring around calving can continue to affect the cow beyond the postpartum period. Transition period is not just a short-term concern; it is closely related to reproductive success, longevity, and the overall efficiency of the herd.

The third study shows that reproductive performance at the herd level is strongly associated with management intensity. Herds with more frequent veterinary visits and more intensive production systems generally achieved better reproductive outcomes, mainly because they had higher service rates and shorter intervals between services. This suggests that reproductive efficiency is shaped not only by the biological condition of the cows, but also by how well the system is organized to detect, breed, and rebreed cows. When considered together, the second and third studies make it clear that good reproductive results depend on both cows' biological conditions postpartum and effective management practices.

The fourth study adds an environmental perspective by showing that, in compost-bedded pack systems, both Holstein and Holstein × Gyr cows deposited most of their feces and urine in the resting area, which favors composting and reduces the fraction of nitrogen that would otherwise require treatment or be lost directly to the environment. These findings highlight the role of housing and manure management in improving the sustainability of dairy production. In this case, the compost-bedded pack system was not only a housing strategy but also an effective way to retain nutrients and reduce manure treatment needs.

Together, the four studies correlate nutritional efficiency, postpartum health, reproductive management, and manure handling. Improved ruminal degradability and digestibility increase the efficiency with which nutrients are converted into productive output. Better postpartum status can reduce the reproductive losses that compromise herd performance. More intensive and structured management can translate biological potential into herd-level reproductive results. Finally, manure composting and nutrient recycling can improve the sustainability of the whole system.

Therefore, the central contribution of this thesis is to demonstrate that dairy systems depend on the integration of multiple biological and management processes. Across the four studies, the results consistently indicate that more efficient dairy systems are those able to use nutrients more effectively, minimize disorders and long-term consequences, and support management practices for intensive reproductive performance and manure handling.