UNIVERSITY OF SÃO PAULO
COLLEGE OF ANIMAL SCIENCE AND FOOD ENGINEERING

LARISSA SCHNEIDER GHELLER

Optimizing dairy cow performance during transition period and early lactation: a study of the use of ultra-diluted complexes in diets and close-up feeding accuracy

Pirassununga
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Corrected Version

Doctoral thesis presented to the Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo for obtaining the title of Doctor in Science.

Concentration area: Animal Quality and Productivity

Advisor: Prof. Dr. Arlindo Saran Netto

Co-advisor: Dra. Márcia Saladini Vieira Salles

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UNIVERSIDADE DE SÃO PAULO
Faculdade de Zootecnia e Engenharia de Alimentos
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CERTIFICADO

Certificamos que a proposta intitulada "Efeito da inclusão de composto homeopático (PeriParto Transição®) na dieta de vacas leiteiras em período de transição", protocolada sob o CEUA nº 6516190121 (ID 001643), sob a responsabilidade de **Arlindo Saran Netto** *e equipe; Larissa Schneider Gheller* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo – FZEA/USP (CEUA/FZEA) na reunião de 24/02/2021.

We certify that the proposal "Effect of homeopathic compound inclusion (PeriParto Transição®) in dairy cows diet during transition period", utilizing 24 Bovines (24 females), protocol number CEUA 6516190121 (ID 001643), under the responsibility of **Arlindo Saran Netto** *and team; Larissa Schneider Gheller* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Animal Science and Food Engineering - (São Paulo University) (CEUA/FZEA) in the meeting of 02/24/2021.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 06/2021 a 12/2021 Área: Zootecnia

Origem:	Agência Paulista de Tecnologia dos Agronegócios						
Espécie:	Bovinos	sexo:	Fêmeas	idade:	2 a 7 anos	N:	24
Linhagem:	Holandesa e Jersolando			Peso:	500 a 700 kg		

Local do experimento: O experimento será realizado no Instituto de Zootecnia, localizado em Nova Odessa (SP).

Pirassununga, 24 de fevereiro de 2021

Profa. Dra. Cristiane Gonçalves Titto
Coordenadora da Comissão de Ética no Uso de Animais
Faculdade de Zootecnia e Engenharia de Alimentos da
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Universidade de São Paulo - FZEA/USP

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Defense Date: 02/02/2024

Thesis defense committee members

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Dr. Luiz Carlos Roma Junior
Institution: Instituto de Zootecnia (IZ)

Dra. Gabriela Aferri
Institution: Instituto de Zootecnia (IZ)

DEDICATION

I would like to dedicate this thesis to my parents, Edgar Gheller and Solange Schneider Gheller. Their unconditional support and wise guidance were the solid foundation that gave me the courage to pursue my dreams. Thank you for being my safe haven during this challenging journey.

To my dear brother, André Schneider Gheller, I express my gratitude. Even at a distance, his presence has been a constant source of inspiration, an example of dedication and strength throughout this academic journey.

To Gustavo Freu, my fiancé, I dedicate this thesis with all my heart. Your understanding, patience and love have been fundamental foundations that have made this journey less arduous. Every challenge was easier with your presence at my side.

This achievement is also yours, and I dedicate it to you with all my heart!

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To my loved brother André, whose presence I miss so much in my daily life, thank you for being the best brother anyone could have. The memories we made and will continue to make together have a very special place in my heart. Thank you for always being there no matter where I am in the world.

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“*Nobody told me it was impossible, so I did it!*”

- Jean Cocteau -

ABSTRACT

GHELLER, L.S. **Optimizing dairy cow performance during transition period and early lactation: a study of the use of ultra-diluted complexes in diets and close-up feeding accuracy**. 2024. 92p Thesis (Doctorate) – College of Animal Science and Food Engineering, University of São Paulo, Pirassununga, 2024.

The transition period from three weeks before to three weeks after calving in dairy cows is characterized by significant metabolic changes that can be monitored to prevent adverse events in cow health. Strategies to minimize adverse effects and ensure success in future lactations, including the use of ultra-diluted complexes and the evaluation of nutritional accuracy and its effects, require further investigation. In this context, this thesis has been divided into two chapters. In the first chapter, the administration of ultra-diluted (UD) complexes to cows during the transition period and early lactation was evaluated. Thirty multiparous pregnant cows were blocked and randomly assigned to either a placebo control group or a UD group. The results showed that although the use of UD did not affect the cows' milk production, those fed UD tended to have lower somatic cell counts. In addition, a trend toward improved liver health was observed in cows in the UD group. These results suggest that the use of UD may provide benefits to dairy cows when used during the transition period and early lactation. The second chapter focused on the evaluation of dry cow diets during the close-up period, investigating the accuracy and precision of nutrient composition and its effect on cow performance at the beginning of lactation. Close-up diet samples were collected every four weeks during six visits to 40 dairy farms. The diets formulated by the nutritionists on each farm were also collected. The diets offered did not exactly match the formulated diets, with considerable variability in nutrient levels. Our results showed that greater variability in non-fiber carbohydrates (NFC) negatively affected liver health in fresh cows. Variability in fat and NFC affected serum β-hydroxybutyrate concentrations, and variability in NFC affected blood glucose levels. Non-esterified fatty acid concentrations were influenced by variability in dry matter and crude protein content of the diets. The results highlight the importance of maintaining consistency between diet formulations and feeding practices to optimize dairy herd performance and health. Therefore, integrating these approaches can help improve cow health and performance while promoting sustainable dairy production practices.

Keywords: Transition period. Ultra-diluted compounds. Diet accuracy. Dairy cows.

RESUMO

GHELLER, L.S. **Otimizando o desempenho de vacas leiteiras durante o período de transição e início da lactação: um estudo sobre o uso de complexos ultradiluídos em dietas e da acurácia de dietas close-up.** 2024. 92f Tese (Doutorado) – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2024.

O período de transição, intervalo de tempo que compreende três semanas antes até três semanas depois do parto de vacas leiteiras, é marcado por alterações metabólicas significativas, que podem ser monitoradas e permitem a prevenção de eventos adversos na saúde das vacas. Estratégias para minimizar impactos negativos e garantir sucesso na futura lactação, como o uso de complexos ultradiluídos e a avaliação da acurácia da dieta e seus impactos, carecem de investigações mais profundas. Neste sentido, esta tese foi elaborada em dois capítulos. No primeiro capítulo foi avaliado o fornecimento de complexo ultradiluído (UD) para vacas durante o período de transição e início da lactação. Trinta vacas gestantes multíparas foram blocadas e distribuídas aleatoriamente em um grupo controle placebo ou um grupo UD. Os resultados mostraram que apesar do uso de UD não ter afetado a produção de leite das vacas, as vacas alimentadas com UD tenderam a apresentar menor contagem de células somáticas. Além disso, foi observada uma tendência de melhor saúde hepática nas vacas do grupo UD. Estes resultados sugerem que o uso de UD pode trazer benefícios para vacas leiteiras quando utilizado durante o período de transição e início da lactação. O segundo capítulo focou na avaliação das dietas de vacas secas durante o período *close-up*, investigando a acurácia e precisão da composição nutricional e seu impacto no desempenho das vacas no início da lactação. Em 40 fazendas leiteiras, amostras das dietas *close-up* foram coletadas a cada quatro semanas durante seis visitas. As dietas formuladas pelos nutricionistas de cada fazenda também foram obtidas. As dietas oferecidas não correspondiam exatamente às dietas formuladas, com notável variabilidade nos níveis de nutrientes. Nossos resultados mostraram que a maior variabilidade nos carboidratos não fibrosos (CNF) afetou negativamente a saúde do fígado de vacas recém-paridas. A variabilidade na gordura e no CNF influenciou as concentrações séricas de β-hidroxibutirato, e a variabilidade do CNF impactou os níveis de glicose no sangue. As concentrações de ácidos graxos não esterificados foram influenciadas pela variabilidade no teor de matéria seca e proteína bruta das dietas. Os resultados enfatizam a importância de manter a consistência entre as formulações das dietas e as práticas alimentares para otimizar o desempenho e a saúde do rebanho leiteiro. Portanto, a integração destas abordagens pode ajudar a melhorar a saúde e o desempenho das vacas, ao mesmo tempo que promove práticas sustentáveis na produção leiteira.

Palavras-chave: Período de transição. Compostos ultradiluídos. Acurácia de dietas. Vacas leiteiras.

SUMMARY

GENERAL INTRODUCTION

The transition period in dairy cows, from three weeks prior to calving to the first three weeks of lactation, is a critical period characterized by significant metabolic changes. During the transition from dry period to lactation, the cow's nutrient requirements increase to support milk production in the subsequent lactation. However, when nutrient intake is unable to meet demand, cows can develop a negative energy balance (NEB), which has a negative impact on the cow's performance.

Monitoring negative energy balance includes analysis of two markers of energy metabolism: non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB). High concentrations of these markers have been associated with the occurrence of negative health events, as well as a reduction in milk production. In addition, the transition period is marked by a series of inflammatory events that contribute to reduced liver function and overall health in cows.

Early detection of changes during the transition period is essential to prevent economic losses and improve cow performance. In this context, strategies to monitor and improve the metabolic health of cows, especially through nutritional management, play a key role in mitigating disorders and ensuring successful lactation. Although several studies have been published on nutritional strategies to improve the transition period of dairy cows, the use of ultra-diluted complexes in this context remained unexplored.

Ultra-diluted complexes, based on the principles of homeopathy, have been widely used in livestock production, especially under the concept of population homeopathy, which treats herds based on common symptoms. However, the efficacy of these compounds is quite controversial, with few studies conducted with high scientific rigor and conflicting results. For this reason, there is a significant gap in the understanding of the use of ultra-diluted complexes during the transition period, highlighting the need for further studies.

Meanwhile, the concept of precision nutrition has emerged as a key element in feeding dairy cows to improve their performance. Precision feeding aims to meet the nutritional needs of cows without excesses and/or deficiencies, optimizing nutrient utilization by cows, minimizing environmental impact, and improving profitability. Assessing the accuracy between the diet formulated and the diet consumed is extremely important because significant variation can occur at all stages of feed management.

Optimizing the dairy industry requires a broad approach to the challenges of the transition period, exploring innovative solutions such as ultra-diluted complexes and emphasizing precision in feed management practices. Integrating these approaches can help improve cow health and performance while promoting sustainable practices in dairy production. The following chapters of this thesis address these issues in detail, providing a comprehensive understanding and valuable insights into these critical components of the dairy industry.

1. CHAPTER 1 – LITERATURE REVIEW

1.1 TRANSITION PERIOD IN DAIRY COWS

The transition period encompasses the three weeks prior to calving and the first three weeks of lactation in dairy cows (GRUMMER, 1995). This period is characterized by various metabolic changes (e.g., increased nutrient demand) that occur in the cow to prepare her for calving and subsequent lactation (ARTUNDUAGA et al., 2018; DUFFIELD et al., 2003). During this phase, the cow transitions from a low metabolic demand (dry period) to a heightened nutritional demand, crucial for producing colostrum and increased milk volume (SANTOS; SANTOS, 1998). Therefore, the nutritional requirements during this period are higher than the nutrient intake of the cow. The high energy requirement associated with the reduction in dry matter intake results in the development of a NEB in cows (WANKHADE et al., 2017).

Traditionally, NEB during the transition period is monitored by the concentrations of NEFA and BHB, which are considered indicators of energy metabolism (DUFFIELD et al., 2009). During NEB, body fat mobilization occurs, which consists of the breakdown of triacylglycerols (TAG) stored in adipose tissue, resulting in the release of glycerol and NEFA into the bloodstream in an attempt to compensate for the energy deficit. The rise in plasma NEFA levels, combined with heightened liver blood flow, leads to increased hepatic NEFA uptake (REYNOLDS et al., 2003). Although NEFA release provides energy to tissues, the bovine liver has limited capacity to metabolize it into TAG. As the metabolic capacity reaches its limit, TAG accumulates in the liver, resulting in the non-utilization of acetyl-CoA in the tricarboxylic acid cycle and the conversion of TAG to ketone bodies such as acetone, acetoacetate, and BHB (DRACKLEY; OVERTON; DOUGLAS, 2001; KUHLA; METGES; HAMMON, 2016).

Excessive TAG accumulation in the liver impairs its normal function and is associated with NEFA concentrations in the blood (GOFF; HORST, 1997; HEUER et al., 2001). Cows that exhibit lipolysis are at greater risk of developing fatty liver syndrome (GOFF; HORST, 1997), which impairs the liver's gluconeogenic activity, resulting in lower blood glucose levels and decreased insulin secretion. This sequence of events leads to increased body fat mobilization, heightening fatty acid liver uptake, and contributing to escalated ketogenesis (GRUMMER, 1993). Imbalances between energy requirements and nutrient intake often lead to metabolic disruptions that negatively impact cow fertility and milk production (WANKHADE et al., 2017).

High levels of NEFA and BHB during the transition period have previously been negatively associated with disease occurrence and milk production in dairy cows (CHAPINAL et al., 2011, 2012; KERWIN et al., 2022a; MOYES et al., 2009; OSPINA et al., 2010a; ROBERTS et al., 2012). A study conducted across 72 North American dairy farms in New York and Vermont States evaluated pre- and postpartum NEFA and BHB concentrations and their association with negative health events during the first 30 days in milk (DIM; KERWIN et al., 2022a). The findings revealed that cows with high postpartum NEFA (≥ 0.46 mmol/L) and BHB (≥ 0.90 mmol/L) concentrations were more than three times more likely to be diagnosed with diseases such as metritis, abomasal displacement, and clinical ketosis. In addition, it was reported that cows with prepartum NEFA concentrations ≥ 0.17 mmol/L were 3.3 times more likely to be culled during the first 30 DIM (KERWIN et al., 2022a). A similar result was observed by Roberts et al. (2012), who conducted a retrospective cohort study involving around 6,000 cows from dairy farms in Canada and the United States. In this study, cows with NEFA concentrations ≥ 0.40 mmol/L and BHB concentrations ≥ 0.70 mmol/L in the week before calving were more likely to be culled in the first 60 DIM. High concentrations of NEFA (≥ 0.80 mmol/L) and BHB (≥ 1.2 mmol/L) in the first week after calving were also associated with an increased risk of culling during the first 60 DIM (ROBERTS et al., 2012). High NEFA concentrations (ranging from ≥ 0.27 to ≥ 0.60 mmol/L) during the prepartum period have been associated with negative health events such as metritis, abomasal displacement, and ketosis (CHAPINAL et al., 2011; OSPINA et al., 2010a). Cows with BHB concentrations of ≥1.0 mmol/L in the first week after calving were 13.6 times more likely to develop abomasal displacement, whereas those with BHB concentrations ≥ 1.2 mmol/L in the first week after calving were 4.7 times more likely to develop clinical ketosis (SEIFI et al., 2011). In addition, high levels of NEFA and BHB during prepartum and postpartum have also been associated with the development of mastitis at calving and early lactation (MOYES et al., 2009).

In multiparous cows, NEFA concentrations ≥ 0.50 mmol/L one week prior to calving resulted in a reduction of 1.6 kg/day in milk production during the first dairy herd improvement test (DHI; approximately 120 DIM; CHAPINAL et al., 2012). NEFA concentrations ≥ 0.70 mmol/L in postpartum multiparous cows were associated with a 1.8 kg/day decrease in milk production in the first DHI (CHAPINAL et al., 2012). When milk production was estimated for 305 DIM, high prepartum NEFA concentrations had a significant effect on total lactation milk production (HUZZEY et al., 2015; KERWIN et al., 2022a; OSPINA et al., 2010b). Ospina et al. (2010b) reported that NEFA concentrations ≥ 0.33 mmol/L were linked to a reduction of 683 kg of milk in the 305 DIM projection. Huzzey et al. (2015) reported that each 1 mmol/L increase in prepartum NEFA concentration was associated with a decrease of 1,465 kg of milk in the projection to 305 DIM. Additionally, Kerwin et al. (2022a) associated the production of 479 kg less milk (projected to 305 DIM) when prepartum NEFA concentrations were ≥ 0.17 mmol/L. In addition, milk production projected for 305 DIM was reduced by 393 kg at post-calving BHB concentrations ≥ 0.96 mmol/L (OSPINA et al., 2010b). According to Duffield et al. (2009), cows with postpartum serum BHB concentrations ≥ 1.40 mmol/L produced at least 1.9 kg less milk in the first milk control test.

During the transition period, dairy cows experience a reduction in liver function associated with increased inflammation and oxidative stress (BERTONI et al., 2008; BIONAZ et al., 2007; HORST; KVIDERA; BAUMGARD, 2021; TREVISI et al., 2012). Calving in cows is characterized by triggering a series of inflammatory conditions (CAPPA; TREVISI; BERTONI, 1989), which can act to reduce liver function and increase oxidative stress (BIONAZ et al., 2007; TREVISI et al., 2012). These traits negatively impact cow health since the liver plays a pivotal role in numerous physiological and biochemical processes, notably in glucose regulation and lipid metabolism (TACKE; LUEDDE; TRAUTWEIN, 2009). Studies indicate that cows often experience some degree of systemic inflammation after calving (BERTONI et al., 2008; TREVISI et al., 2010), and the intensity of this inflammation is associated with the occurrence of disease and a reduction in milk production (BRADFORD et al., 2015). In addition, during the transition period there is also a change in immune function, which has also been described as a risk factor for postpartum diseases such as hypocalcemia, retained placenta, metritis, abomasal displacement and mastitis (GARRO; MIAN; COBOS ROLDÁN, 2014; OSORIO et al., 2014; TREVISI et al., 2012; VAN SAUN, 2016; WU et al., 2017).

When assessing the health status of cows, the use of blood biomarkers associated with inflammation, such as acute phase proteins, can be extremely important (BERTONI; TREVISI, 2013). Considering the potential non-uniform variations of these biomarkers when assessing various health disorders, employing multiple biomarkers can aid in result interpretation (CECILIANI et al., 2012). In recent years, indices have been developed with the aim of assessing the health of cows during the transition period (BERTONI; TREVISI, 2013). The liver activity index (LAI; BERTONI et al., 2008) and the liver functionality index (LFI; BERTONI et al., 2006) are examples of those created indices. While LAI considers negative acute phase proteins (albumin, cholesterol, and retinol) on days 7, 14, and 28 after calving, LFI calculation uses albumin, cholesterol, and bilirubin concentrations on days 3 and 28 after calving. Both indices are associated with inflammatory responses, with lower values indicating more severe inflammatory responses (BERTONI; TREVISI, 2013). Notably, LFI calculation is standardized for healthy cows, enhancing its reliability for intra- or inter-herd comparisons.

While both indices serve to evaluate cow condition at lactation onset, retinol analysis can often be costly, and waiting until day 28 of lactation might result in an unnecessary delay in identifying cows with issues (KERWIN et al., 2022b). Therefore, the liver health index (LHI) has recently been proposed (KERWIN et al., 2022b). This index uses albumin, cholesterol, and bilirubin concentrations from blood samples collected between 3 and 12 DIM and suggests that cows diagnosed with negative health events have a lower LHI compared to healthy cows. In addition, LHI was positively associated with milk production and pregnancy up to 150 DIM in multiparous cows, suggesting that cows with better postpartum performance have a higher LHI (KERWIN et al., 2022b).

As aforementioned, early identification of metabolic, inflammatory, and immunological changes during the prepartum and postpartum periods can help prevent economic losses due to disease treatment costs, reduced productivity, inadequate reproductive performance, and cow culling (SHIN et al., 2018). Nutritional strategies applied during this period aim to potentially influence metabolism positively, diminish inflammation and disease risks, and enhance cow productivity (DRACKLEY, 1999). There are numerous studies in the literature on the nutritional management of cows during the transition period (ARSHAD et al., 2020; CATTANEO et al., 2023; FEHLBERG et al., 2023; NEVILLE et al., 2022; OH et al., 2021; REZAEI ROODBARI et al., 2016; SUN et al., 2023). In this context, the use of yeast, rumen-protected lysine and choline, calcareous algae, capsicum oleoresin, conjugated linoleic acid and n-3 and n-6 polyunsaturated fatty acids during the transition period has been investigated.

One study evaluated the effects of live *Saccharomyces cerevisiae* supplementation during the transition period on rumen function, milk production, and metabolic and inflammatory conditions in dairy cows (CATTANEO et al., 2023). The findings revealed that administering live *Saccharomyces cerevisiae* led to increased dry matter intake and postpartum rumination time. In addition, an increase in milk production associated with an increase in milk protein content was observed. There was also an improvement in the modulation of rumen fermentation. Cows supplemented with yeast showed a shorter and less intense inflammatory response around calving, suggesting a possible improvement in liver function (CATTANEO et al., 2023).

Another study evaluated the effect of adding rumen-protected lysine to the diet of multiparous Holstein cows on their immunometabolic status (FEHLBERG et al., 2023). The results indicated that the inclusion of rumen-protected lysine in the diet of cows during the transition period resulted in improvements in biomarkers associated with liver function (as evidenced by increased concentrations of acute phase proteins), while at the same time reducing inflammatory status (FEHLBERG et al., 2023).

The effectiveness of supplementing rumen-protected choline in cows' diets during the transition period has been investigated (ARSHAD et al., 2020). A meta-analysis of 20 manuscripts published between 1984 and 2018 that investigated the inclusion of rumen-protected choline in the pre- and postpartum diets of dairy cows showed significant benefits. The addition of rumen-protected choline to cows' diets resulted in an increase in milk yield, milk yield corrected for energy, fat, and protein. Moreover, a tendency toward reduced risk of retained placenta and mastitis was observed in cows supplemented with rumen-protected choline during the transition period (ARSHAD et al., 2020).

Neville et al. (2022) evaluated the effects of adding calcareous marine algae on intake, milk production, energy balance, mineral metabolites, and inflammatory markers in dairy cows during the transition period. Their findings suggest that supplementing calcareous marine algae to dairy cows during this phase increases dry matter intake (both pre- and postpartum) and enhances energy-corrected milk production and milk fat content. Cows fed calcareous marine algae showed improved energy balance and reduced plasma concentrations of acute phase proteins after calving. The addition of calcareous marine algae to the diet also had a positive effect on NEFA concentrations. These results suggest that supplementation with calcareous marine algae may be a tool that can be used to improve the health and production of dairy cows during the transition period (NEVILLE et al., 2022).

A study was conducted to evaluate the effect of rumen-protected capsicum supplementation on the productive performance of cows during the transition period (OH et al., 2021). The results showed that protected capsicum improved the metabolic profile of cows and demonstrated a trend towards increased milk production and feed efficiency in dairy cows (OH et al., 2021). Other studies, such as those developed by Rezaei Roodbari et al. (2016) and Sun et al. (2023), have addressed the supplementation of rumen-protected conjugated linoleic acid and n-3 and n-6 polyunsaturated fatty acids for dairy cows during the transition period. The addition of conjugated linoleic acid resulted in increased milk production in cows, as well as increased plasma glucose and cholesterol concentrations and reduced loss of body condition score (REZAEI ROODBARI et al., 2016). Similarly, the addition of n-3 polyunsaturated fatty acids has been shown to increase dry matter intake, increase milk production, and reduce negative energy balance after calving (SUN et al., 2023).

It is known that the success of the transition period is directly related to the success of the entire lactation. Therefore, strategies to monitor and improve the metabolic health of dairy cows during this period have been discussed, highlighting the importance of nutritional management to manage metabolic stress and related disorders (CAIXETA; OMONTESE, 2021; HASSAN et al., 2022). Optimizing the dairy industry requires a broad approach to the challenges of the transition period, exploring innovative solutions such as ultra-diluted complexes and emphasizing precision in feed management practices. Integrating these approaches can help improve cow health and performance while promoting sustainable practices in dairy production. These aspects will be addressed in the following chapters of this thesis.

1.2 ULTRA-DILUTE COMPLEXES: CHARACTERIZATION AND USE

Ultra-diluted complexes are also known as homeopathic. The usage practice of these compounds was developed and established by the German physician Samuel Hahnemann (1755 – 1843). The method was based on the principle of "like cures like", proposing treatments based on inducing symptoms like those of the disease being treated in healthy individuals (CORREA; QUINTAS, 1994). In other words, Hahnemann suggested that in order to cure a disease, it is necessary to use a medicine that induces in healthy individuals’ symptoms similar to those of the disease to be treated (HAHNEMANN, 2001). After a few years of study, in 1796, Hahnemann published "Essay on a New Principle for Establishing the Curative Powers of Medicinal Substances," which mentions his main discoveries regarding the use of ultra-diluted medicines. Later in 1810, the book "Organon of the Art of Healing" was published, which is still considered the "Bible of Homeopathy" (GEMELLI; PEREIRA, 2018).

Homeopathy rests on four fundamental principles detailed in the book 'Organon of the Art of Healing' (TEIXEIRA, 2006). These principles are: 1) Law of similar - the administration of ultra-diluted substances to healthy individuals induces symptoms similar to those presented by sick individuals (NUNES, 2005; TEIXEIRA, 2010); 2) Experimentation on healthy individuals - the prescribed ultra-diluted compound must provoke in healthy individuals symptoms similar to those manifested by the patient (PUSTIGLIONE, 2017); 3) Administration of minimum doses - ultra-diluted substances must be diluted and dynamized in order to avoid toxicity and increase potency (CORRÊA et al., 2006); and 4) Indication of a single drug - each individual should receive a single drug because each substance acts in a specific way in the body (BENITES, 2005).

The substances employed in homeopathic practice are sourced from diverse natural kingdoms, encompassing plant, mineral, and animal materials, along with synthetic origins (FONTES, 2009; NOGUEIRA et al., 1986). Pharmaceutical products, serums, vaccines, and bacterial cultures can also be used to prepare ultra-diluted complexes (FARMACOPEIA, 2011; FONTES, 2009). In the course of preparing ultra-diluted complexes, involving dilution, dynamization, and extraction of active elements, inert substances adhering to purity standards set by the Brazilian Pharmacopoeia are used as vehicles and excipients. Key excipients include purified water, ethyl alcohol, glycerin, lactose, and sucrose. To a lesser extent, inert tablets such as globules, micro-globules, and tablets are also utilized (FONTES, 2011).

The pharmaceutical preparation of ultra-diluted complexes is the result of dynamization processes that involve dilution of the active ingredients followed by succussion or trituration. Three scales of dilution are used in homeopathic pharmacotechnicals: decimal, centesimal and fifty millesimal. The decimal scale is used for dilutions of 1:10, where one part of the active ingredient is diluted in nine parts of the inactive ingredient. This scale was created by Hering and is widely used in the United States and Germany (FONTES, 2011). The symbols created to identify this dilution are X (ten in Roman numerals), D, or DH - which stands for dynamization on Hering's decimal scale. The centesimal scale, developed by Hahnemann and mainly used in Brazil, consists of a dilution of 1:100, where one part of the active ingredient is diluted in 99 parts of the inactive ingredient. The centesimal scale is identified by C, a, no indication, or CH (Hahnemannian Centesimal). At the fifty millesimal scale, the dilution is prepared at a ratio of 1:50,000 between the active and inert ingredients and is identified by the symbols Q or LM (FONTES, 2011).

Despite consolidated practice, the mechanism of action behind the use of ultra-diluted compounds is still not fully understood (MISHRA et al., 2011). Various hypotheses have been proposed to support the action of homeopathy, one of which is the idea that these substances have a direct effect on the living organism and do not act on the etiological agent (CARILLO, 2008). The effect of the homeopathic compound on the organism, known as the primary effect, can induce secondary reactions that lead to changes in metabolic parameters (FONTES et al, 2005). Teixeira, Leal and Ceschin (2008) describe the primary action as the therapeutic, adverse, and side effects of conventional drugs, while the secondary action, characterized as the principle of homeopathic healing, is known as the "rebound effect", i.e. the manifestation of opposite reactions to diseases.

Ultra-diluted substances can be used alone or in combination, which characterizes ultra-diluted complexes. These combinations are often used to treat and/or prevent diseases by administering several substances with similar therapeutic principles (SILVA et al., 2011). This characteristic has led to the widespread use of ultra-diluted complexes in animal husbandry, since health problems in livestock are generally multifactorial and homeopathy makes it possible to treat disorders through the action of different agents (PINHEIRO et al., 2021). It is in this context that the concept of population homeopathy comes into play.

Population homeopathy refers to the application of homeopathy in herds (REAL, 2008). The application of population homeopathy is made possible by the concept of *genius epidemicus* described by Hahnemann, which refers to the behavior of many animals that is externalized as if it were only one (ARENALES, 2002). This principle arose due to the occurrence of diseases with the same cause (epidemics) in herds and led Hahnemann to recommend treatments based on the predominant symptoms in most of the population, leaving aside individual symptoms (REAL, 2008). Homeopathic medicines applied to herds aim to treat the *genius epidemicus* and are usually composed of two or more medicines (LOPES, 2004). In the individualized approach to herd treatment, all animals are considered a single organism, since they have similar characteristics (PEREIRA, 2012).

Population homeopathy, as described by Real (2008), is based on three principles. The first is to consider the herd as a single organism in which each animal contributes to the whole, regardless of its age and/or function, since all animals in a herd share the same environment, management, food, water, and often genetics. The second principle recognizes that the herd is in a constant state of disequilibrium and includes the concepts that the modernization of production systems harms natural behavior and induces organic imbalances, which are manifested by a reduction in productivity and the development of pathologies (DANTZER; MORMÈDE, 1984; REAL, 2008). The third principle, known as the modulating action of homeopathic, emphasizes the ability of homeopathic to act through the neuroendocrine system, promoting the elimination of toxins and restoring functional harmony, strengthening the body's defenses, and preventing the development of diseases caused by stressful factors (REAL, 2008).

The transition from traditional homeopathy to population-based approaches has been profoundly impactful, allowing a significant number of animals to benefit from homeopathic interventions. This practice brings advantages such as cost reduction, efficacy, and the absence of drug residues in meat and milk, making it appealing to producers (REAL, 2008). In addition, the administration of homeopathic compounds is simple because they can be incorporated into different vehicles, such as water, mineral salt, and feed (VARGAS et al., 2013). Also, as pointed out by Vargas et al. (2013), the use of homeopathy in disease control and prevention is associated with reduced stress in animals, contributing to animal welfare.

The use of ultra-diluted compounds to improve herd productivity has increased significantly. In relation to dairy cattle, the use of ultra-diluted compounds has been described in calves, heifers, and lactating cows (DA SILVA et al., 2021; DAY, 1986; ENBERGS; SENSEN, 2007; FORTUOSO et al., 2018; MORAIS, 2014). These studies highlight the potential use of ultra-diluted complexes in the occurrence of diarrhea, mastitis, endometritis, and control of ectoparasites.

The use of ultra-diluted complexes as a prophylactic alternative to minimize bacterial infection and prevent neonatal diarrhea in calves was evaluated by Fortuoso et al. (2018). Using the commercial product Dia 100® (REAL H, Campo Grande, Brazil), which is indicated for the prevention and/or cure of diarrhea, the authors reported that the ultra-diluted complex was 50% effective in controlling neonatal diarrhea in calves and reduced the use of antibiotics by 60% compared to the control group. In addition, use of the ultra-diluted complex reduced the number of bacterial pathogens in fecal samples, which improved intestinal health and enhanced nutrient absorption by the calves.

In a similar approach, Brazilian researchers evaluated the prophylactic use of the ultra-diluted complex on the health, metabolism and performance of weaned Holstein heifers and the residual effects until first lactation (SILVA et al., 2021). Although no beneficial effects of the ultra-diluted complex on heifer performance and first lactation were observed, the use of the homeopathic compound contributed to a reduction in the incidence of digestive disorders, the cumulative number of days with tick-borne diseases and the risk of culling heifers. Consequently, the study's findings suggest that employing the ultra-diluted complex could potentially decrease the necessity for antimicrobial therapy in heifers, particularly regarding tick-borne diseases and digestive issues (SILVA et al., 2021).

For adult animals, studies often focus on the treatment and control of conditions such as mastitis, endometritis and retained placenta. Based on 29 peer-reviewed publications from 1981 to 2014, Doehring and Sundrum (2016) conducted an analysis of the efficacy of using ultra-dilution for adult cows. The results of this study show that 51.8% of the trials showed positive effects on the prevention and cure of diseases of the reproductive system and mammary gland of cows. Positive effects on mammary gland health with the use of ultra-diluted compounds were also described by Aubry et al. (2013). In this study, mammary quarters with high electrical conductivity, identified by the automated milking system, were treated intramammary with an ultra-diluted compound administered twice daily for 2 consecutive days. Treatment of affected mammary quarters resulted in a reduction in milk electrical conductivity 4 – 7 days after the first treatment, accompanied by a return of animal production (AUBRY et al., 2013). However, as this was a pilot study and there was no control group, the authors suggested that further studies with high scientific rigor should be carried out to clarify this issue.

In a controlled clinical trial, Werner, Sobiraj and Sundrum (2010) evaluated the efficacy of the ultra-diluted complex treatment strategy for mild to moderate clinical mastitis in cows compared to antibiotic treatment (cloxacillin 1000 mg) and placebo. The study included 136 lactating dairy cows from four herds in Germany with 147 affected quarters, which were randomly assigned to one of the three treatment groups. The results showed a therapeutic effect of the ultra-diluted complex treatment in cases of mild and moderate clinical mastitis caused by environmental pathogens or negative cultures. This suggests that treatment with ultra-diluted substances could be an alternative to the use of antibiotics in cases of mild and moderate clinical mastitis. However, improved farm- and cow-specific diagnostics is an important prerequisite for the use of the ultra-diluted complex treatment strategy for mastitis treatment (WERNER; SOBIRAJ; SUNDRUM, 2010).

Homeopathic compounds have been explored for controlling ectoparasites in cattle. In a study conducted by Morais (2014), ultra-diluted compounds were investigated for their efficacy against ticks (*Rhipicephalus (Boophilus) microplus*) in dairy cattle. The research involved three cattle groups: T1, which received the ultra-diluted complex; T2, which was housed in the same paddock as T1 and received a placebo; and T3, which received a placebo and was housed in a separate paddock from T1 and T2. The results of this study showed a lower number of engorged teleogynes in the T1 group compared to the T2 and T3 groups. Therefore, it was concluded that the ultra-diluted compound was able to reduce tick infestation (MORAIS, 2014). Similarly, Paixão et al. (2021) reported control of parasitism by *Rhipicephalus (B.) microplus* and an increase in weight gain of cattle in a field trial using the ultra-diluted compound.

Although positive results have been reported, studies of ultra-diluted compounds are quite controversial. While studies evaluating the efficacy of ultra-diluted compounds in the treatment of mastitis and metritis show positive results, some authors report no benefit from the use of homeopathy (ARLT et al., 2009; EBERT et al., 2017). According to Doehring and Sundrum (2016), the divergent results of studies on ultra-diluted compounds may be due to the nature of the studies, which are usually conducted under very specific conditions without being repeated in a comparable way, indicating the lack of a proper scientific approach (EBERT et al., 2017). Therefore, the results of these studies cannot be generalized and should be considered as unique cases. Another factor is that homeopathic treatment is context sensitive, i.e., clinical signs, pathogens involved, behavior, constitution of the homeopathic compound, and environmental conditions. It is therefore clear that scientific evidence of the efficacy of homeopathy is still lacking (ALBRECHT, 2013).

In this context, given the limited number of studies with ultra-diluted compounds developed with high scientific rigor and the contradictory results between these studies, the need to advance the frontier of knowledge in this area becomes evident. This need is even more evident when we establish a relationship between the use of ultra-diluted compounds and the transition period of dairy cows, since there are no studies in the literature that evaluate the use of ultra-diluted compounds in this specific context. It is known that the transition period is a critical phase in the life of dairy cows (GRUMMER, 1995; KNEGSEL et al., 2014; MULLIGAN; DOHERTY, 2008). Therefore, it is necessary to evaluate strategies capable of mitigating the changes and/or disturbances that dairy cows face during this period, providing conditions for cows to overcome these challenges. The first work carried out, which evaluates the use of ultra-diluted complex to dairy cows during the transition period (GHELLER et al., 2023) is presented in Chapter 2 of this thesis.

1.3 ASSESSMENT OF PRECISION AND ACCURACY OF FEED MANAGEMENT PRACTICES FOR BETTER COW PERFORMANCE

The livestock industry faces a significant challenge in meeting the growing demand for animal products. Several initiatives have been developed to improve animal performance, and nutrition appears to be a critical factor. Improving animal performance requires taking advantage of technological advances and available nutritional resources. In this context, “precision nutrition” seems to have a positive impact on animal health and performance.

Precision nutrition has become increasingly important in the dairy industry in recent decades. The basic principles of this concept focus on meeting the nutritional needs of cows without excesses or deficiencies. The goal is to optimize nutrient utilization, minimize the environmental impact of excreta, and improve the overall profitability of animal production (CARNEIRO; SANTOS; ALMEIDA, 2021). Diet consistency can be assessed by either precision or accuracy. Precision refers to the degree of variation between rations and includes both chemical and physical composition. Conversely, accuracy refers to the variability between the actual ration fed to cows and the theoretical ration (PERRICONE, 2019). Overall, nutritionists formulate theoretical rations using specialized software to meet the nutritional requirements of cows. However, the actual diet delivered may differ from the formulated diet.

It is widely accepted in the dairy industry that there are at least five stages in the formulation and delivery of rations. The first stage involves formulating a ration that meets the nutritional needs of the cows or pens. The second step is to enter this formulated ration, complete with current dry matter (DM) values, into feed management software. The third step is loading the ration into the feed wagon, and the fourth step is delivering the ration to the cows. The final stage, the fifth, is represented by the actual ration consumed by the cows (ROSSOW; ALY, 2013).

During each stage in the process of producing the final ration, an increase in variation in nutrient content can occur, which can be influenced by feed management practices. Therefore, the diet that reaches the cow’s trough and is effectively consumed can vary depending on the DM content of the ingredients, the method of loading the ingredients into the feed wagon, the mixing and unloading process, or it can still be altered by selection by the cows (ROSSOW; ALY, 2013). When a total mixed ration (TMR) is sorted, cows may end up consuming a diet with a different nutrient composition than what was originally delivered (LEONARDI; ARMENTANO, 2003), and it has been shown to affect production at the herd level (SOVA et al., 2013).

Several studies have been conducted worldwide to evaluate diet variability (e.g., precision or accuracy) and its effect on dairy cow performance (CARNEIRO; SANTOS; ALMEIDA, 2021; SOVA et al., 2014; TAYYAB et al., 2018). Sova et al. (2014) evaluating the accuracy of TMR**,** reported that greater variability in net lactation energy (NE_L) content was associated with a decrease in milk production efficiency. In addition, the accuracy of TMR fed to lactating cows showed that variability in TMR composition can significantly impact dairy cow productivity (SOVA et al., 2014).

The accuracy of the TMR offered to high-yielding cows from 20 commercial dairy herds in Castro, Paraná, Brazil, was also evaluated (CARNEIRO; SANTOS; ALMEIDA, 2021). The results showed significant differences in the diets formulated, fed, and apparently consumed due to various factors related to diet management and forage processing. Particle size distribution often deviated from recommended values, especially for elongated forage particles. Uniformity of TMR also showed considerable variation between farms. Diet uniformity has a significant effect on apparent intake of the diet, making the selection process more pronounced in diets with lower mixing quality (CARNEIRO; SANTOS; ALMEIDA, 2021). Therefore, the results of this study highlighted the importance of monitoring and improving TMR homogeneity.

A study carried out in Minnesota examined the interaction between feed management practices and diet characteristics in 50 dairy herds (ENDRES; ESPEJO, 2010). The authors compared the DM, neutral detergent fiber (NDF) and crude protein (CP) content of the analyzed ration with the formulated ration based on bunk samples. The results showed an association between variations in NDF content over time (due to sorting) and reduced milk production, which was probably due to herds with poorer feed management, indicating that variability in NDF could contribute to decreased milk production (ENDRES; ESPEJO, 2010). However, it's important to note that this association could also be influenced by herds with higher NDF levels in the TMR.

To evaluate the effect of feed management on cow nutrient status and milk production, and to determine if nutrients can serve as indicators of feed management practices, weekly TMR samples were collected and analyzed at five commercial dairies in California (ROSSOW; ALY, 2013). Differences between the nutrient analyses of these samples and the nutrients in the formulated rations were evaluated. Results showed that differences in nutrients delivered to cows were associated with differences in both milk and milk fat production, and these differences were attributed to different forage management practices (ROSSOW; ALY, 2013). Recognizing that feed management is a key factor influencing nutrient delivery and milk production, its incorporation into the feed formulation should be considered.

A study conducted on 50 UK farms aimed to characterize the particle size distribution and physically effective fiber content of grass silage, corn silage, and mixed rations (TAYYAB et al., 2018). The results showed that the particle size distribution observed in the herds studied was significantly higher than the North American guidelines for forages and mixed rations. In addition, inadequate consistency in the mixture and a high degree of selection in the mixed rations were found in most of the herds, raising concerns about the impact of these factors on cow performance (TAYYAB et al., 2018).

In a symposium report, Stone (2008) emphasized the importance of minimizing excessive diet variability to avoid negative effects on cow production and health. The author also highlighted that a) diet variability can be reduced through feed analysis and careful selection of diet ingredients, considering both type and quantity; b) proper sampling of feed is essential; c) feeders play a critical role in improving the consistency and uniformity of the consumed diet and should be trained in various aspects of feed management; d) precision in the preparation of feed batches is essential; e) the inclusion of concentrates and premixes can improve accuracy; f) the use of electronic feed recording systems makes it easier to monitor batch preparation; and g) sorting by cows can have a significant impact on the consumed ration, and measures such as controlling forage length (less than <5 cm), ensuring adequate distribution of midsize particles in the ration, and incorporating water or wet ingredients can help mitigate sorting problems.

Based on the above considerations, it is reasonable to speculate that ensuring a consistent ration is a critical factor in maximizing cow performance. Therefore, chapter 3 of this thesis characterized (composition of ingredients and nutrients) the diets of dry cows during the close-up period; determined the accuracy between the nutrient composition of the formulated diet and the diet offered to the cows; and determined if the greater variability between the formulated and offered diets reflects poorer early lactation outcomes (e.g., body condition score, blood metabolites, and milk production) for dairy cows.

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2. CHAPTER 2 – THE PERFORMANCE AND METABOLISM OF DAIRY COWS RECEIVING AN ULTRA-DILUTED COMPLEX IN THE DIET DURING THE TRANSITION PERIOD AND EARLY LACTATION

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The performance and metabolism of dairy cows receiving an ultra-diluted complex in the diet during the transition period and early lactation

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ABSTRACT

This study evaluated the effects of feeding an ultra-diluted complex to dairy cows during the transition period and early lactation. Thirty multiparous pregnant dairy cows were blocked and randomly assigned to either a placebo control (CON) group or ultra-diluted complex (UD) group. The CON group received a placebo (basal diet + 40 g/cow/day of expanded silicate), while the UD group received the ultra-diluted complex (basal diet + 40 g/cow/day of PeriParto Transição–RealH, composed of ultra-diluted substances + vehicle: expanded silicate). Cows were evaluated from 30 days before the expected calving date until 60 days in milk (DIM) for sample and data collection. Post-partum dry matter intake (DMI) was not affected by the treatment. Cows fed UD had higher DMI relative to BW. Feeding UD increased milk lactose content and decreased milk protein content. Cows fed UD had lower somatic cell counts in the third and fourth week of lactation. Cows fed UD showed a tendency for higher liver health index. Using UD during the transition period and early lactation may benefit liver and udder health of dairy cows with no detrimental effect on milk performance.

Keywords: homeopathy; liver health; periparturient; stress; udder health

RESUMO

Este estudo avaliou os efeitos da adição de complexo ultradiluído na dieta de vacas leiteiras durante o período de transição e início da lactação. Trinta vacas leiteiras, gestantes e multíparas, foram blocadas e distribuídas aleatoriamente em grupo controle placebo (CON) ou grupo complexo ultradiluído (UD). O grupo CON recebeu placebo (dieta basal + 40 g/vaca/dia de silicato expandido), enquanto o grupo UD recebeu o complexo ultradiluído (dieta basal + 40 g/vaca/dia de PeriParto Transição – RealH, composto de substâncias ultradiluídas + veículo: silicato expandido). As vacas foram avaliadas desde 30 dias antes da data prevista para o parto até os 60 dias em lactação (DEL) para amostragem e coleta de dados. O consumo de matéria seca (CMS) pós-parto não foi afetado pelo tratamento. Vacas alimentadas com UD apresentaram maior CMS em relação ao peso corporal. A alimentação com UD aumentou o teor de lactose do leite e diminuiu o teor de proteína do leite. Vacas alimentadas com UD apresentaram menor contagem de células somáticas na terceira e quarta semana de lactação. Vacas alimentadas com UD tenderam a apresentar melhores índices de saúde hepática. O uso de UD durante o período de transição e início da lactação pode beneficiar a saúde do fígado e do úbere das vacas leiteiras, sem nenhum efeito prejudicial da produção de leite.

Palavras-chave: homeopatia; saúde hepática; periparturiente; estresse; saúde do úbere.

2.1 INTRODUCTION

Dairy cows’ transition period (TP) is the interval comprising 3 weeks before up to 3 weeks after calving (GRUMMER, 1995). TP is characterized by significant metabolic changes that occur to prepare the cow for calving and future lactation (ARTUNDUAGA et al., 2018; DRACKLEY, 1999; HORST; KVIDERA; BAUMGARD, 2021). The transition from the dry period, a period of low metabolic demand, to lactation, a period of high nutrient demand for colostrum synthesis and increased milk production, can lead to metabolic imbalances with consequent impairment of immune function (SANTOS; SANTOS, 1998). In addition, if cows are unable to adequately cope with the changes during this period, liver damage, systemic inflammation, and energy metabolism problems may be triggered (BERTONI et al., 2008). These changes often result in an increased risk of health problems that can negatively affect the performance and well-being of dairy cows (BERTONI et al., 2008; BIONAZ et al., 2007; HAMMON et al., 2006). Ensuring a successful transition to lactation is extremely important to optimize the performance of dairy cows (CAIXETA; OMONTESE, 2021). In this scenario, the adoption of nutritional strategies and management measures can be helpful in minimizing adverse effects on the metabolism of dairy cows undergoing the TP.

Several nutritional approaches have been studied to improve the transition of dairy cows from the dry period to the subsequent lactation (FEHLBERG et al., 2023; SUN et al., 2023). Among these approaches, the use of compounds of natural origin (BOSCO STIVANIN et al., 2019; CHENG et al., 2018; GESSNER et al., 2020) has received considerable attention, especially due to the global trend of reducing the use of antimicrobials in the livestock production system (BALLOU; DAVIS; KASL, 2019). In this context, employing ultra-diluted compounds becomes an interesting and safe alternative to antibiotics (PINHEIRO et al., 2021). The ultra-diluted compounds, also known as homeopathic compounds, were posited by Samuel Hahnemann in the 18th century, and are based on the principle “like cures like”, which suggests that substances that cause symptoms in healthy individuals can be used to treat similar symptoms in sick individuals (CORRÊA et al., 2006; HAHNEMANN, 2001). The use of ultra-diluted compounds involves the use of highly diluted and dynamized substances to avoid toxicity and stimulate the body’s healing responses (CORRÊA et al., 2006; TEIXEIRA, 2009). Even though the mechanisms of action behind the ultra-diluted compounds are not fully understood (MISHRA et al., 2011), previous research has already revealed that the use of ultra-diluted compounds in newborn calves was able to decrease the number of episodes of neonatal diarrhea (FORTUOSO et al., 2018), has the potential to reduce the occurrence of digestive disorders and reduce the number of days affected by tick-borne diseases in weaned calves, as well as minimize the risk of culling heifers (DA SILVA et al., 2021). On the other hand, other studies did not demonstrate the effect of the use of ultra-diluted compounds in the treatment of mastitis (EBERT et al., 2017) and prevention of endometritis (ARLT et al., 2009).

Despite significant scientific advances, studies with ultra-diluted compounds have shown highly controversial results (DOEHRING; SUNDRUM, 2016). Given the conflicting results and the limited number of studies with high scientific rigor using ultra-diluted compounds, there is clear evidence that we need to advance the frontiers of knowledge in this area. To our knowledge, no studies have evaluated the effects of ultra-diluted compounds in the diet of cows during TP. To fill this gap, the aim of this study was to investigate the effects of an ultra-diluted complex on the productive performance and metabolic profile of dairy cows during TP and early lactation. The ultra-diluted complex used in this study was developed for managing the stress-related issues and liver health of dairy cows during the TP and consists of microminerals and homeopathic medications. According to Boericke (1901), the compounds in the ultra-diluted complex used in our study can act by promoting cow productivity, providing preventive and curative benefits for enteritis and liver problems, as well as acting on calving ease, fertility problems, and post-traumatic recovery of cows. We hypothesized that cows receiving ultra-diluted complex in the diet have better performance and metabolic profile during TP and early lactation.

2.2 MATERIALS AND METHODS

This study was conducted from June to October 2021 at the facilities of the Dairy Cattle Research Center, belonging to the São Paulo Agency for Agribusiness Technology, Animal Science Institute, located in Nova Odessa (São Paulo, Brazil). This study was approved by the Committee on Ethics in the Use of Animals of the Faculty of Animal Science and Food Engineering of the University of São Paulo, under number 651619012. Assuming a pooled standard deviation of 4 units, the study would require a sample size of 9 for each group (i.e., a total sample size of 18, assuming equal group sizes), to achieve a power of 80% and a level of significance of 5% (two sided), for detecting a true difference in means between the test and the reference group of 5.34 (i.e., 1.65–3.69) units, according to Zhou et al. (2016).

2.2.1 Experimental design, treatments, and diets

This study was a randomized, and placebo-controlled trial. Thirty crossbred (Holstein × Jersey) dairy cows, multiparous (3.7 ± 1.8 lactations, 545.6 ± 67.6 kg of body weight; BW, 2.60 ± 0.42 of body condition score; BCS), pregnant and expected to calve 30 days after the beginning of the evaluations were enrolled in this study. Cows were divided into blocks according to BCS, and parity. The cows were weighted using a walking-through scale (Toledo, MGR 3000 Campo, São Bernardo do Campo, Brazil). Body condition score determination was performed according to the method proposed by Edmonson et al. (1989). The cows were randomly assigned to the following treatments: (a) placebo control (CON, basal diet with the addition of vehicle only–expanded silicate 40 g/cow/day); and (b) UD (basal diet with the addition of 40 g/cow/day of the ultra-diluted complex (Peri-Parto Transição–RealH) composed by *Aletris acemose* 10^{-12} + *Aristolochia* 10^{-14} + *Arnica montana* 10^{-14} + *Arsenicum album* 10^{-14} + *Bellis perenis* 10^{-14} + *Berberis vulgaris* 10^{-12} + *Calcium carbonicum* 10^{-30} + *Carboneum tetrachloricum* 10^{-30} + *Cardus marianus* 10^{-12} + *Chelidonium majus* 10^{-12} + *China officinalis* 10^{-12} + *Chionantus virginica* 10^{-30} + *Cimicifuga racemosa* 10^{-60} + *Colibacilinum* 10^{-18} + *Colocynthis* 10^{-18} + *Croton tiglium* 10^{-14} + *Eberthinum* 10^{-18} + *Enterococinum* 10^{-18} + *Ferrum metallicum* 10^{-18} + *Gossypium* 10^{-14} + *Hypericum perforatum* 10^{-60} + *Ignatia amara* 10^{-60} + *Iodum* 10^{-14} + *Leptandra virginica* 10^{-12} + *Mercurius vivus* 10^{-14} + *Myrica cerifera* 10^{-14} + *Natrum muriaticum* 10^{-60} + *Oophorinum* 10^{-12} + *Paratyphoidinum* 10^{-18} + *Phosphorus* 10^{-14} + *Podophylinum peltaltum* 10^{-30} + *Pulsatilla* 10^{-14} + *Ruta graveolens* 10^{-14} + *Silicea terra* 10^{-400} + *Sulphur* 10^{-18} + *Symphytum officinale* 10^{-60} + *Tireoidinum* 10^{-14} + vehicle (expanded silicate, enough quantity for 1 kg). The UD and the placebo were added during concentrate preparation to achieve an in-take of 40 g/cow/day according to the manufacturer's instructions. The ultra-diluted complex used in this study was prepared according to the Materia Medica described by Boericke (1901). To achieve blinding of research and farm personnel, treatments were designated as A and B. Only the professor/advisor knew the treatment assignments and was not involved in treatment allocation and administration, field collection, or data analysis. Data were unblinded at the time of article writing.

Cows were evaluated from 30 days before expected calving until 60 days after calving. During the prepartum period (30 days before calving until the day of calving), cows were housed in collective paddocks with shaded areas. After calving, the cows were moved to a lactating cow paddock equipped with automatic feeding system where the cows were identified by electronic tags and had access to the feed (Intergado Ltd.a., Contagem, MG, Brazil).

Throughout the experimental period, the cows were fed twice a day (7 a.m. and 2 p.m.) and had ad libitum access to water. Cows were also evaluated daily for ruminal acidosis signs (e.g., fecal changes, red limbs and hooves).

The basal diets before and after calving were formulated according to NRC (2001) recommendations. The diets offered to the cows were the same throughout the experimental period. Ingredients and chemical composition of the basal diet are presented in Table 1. Cows in both groups received the same basal diet except for the placebo or UD complex addition. Cows were fed CON or UD from 30 days pre-calving until 30 days post-calving. Sorghum silage was used as a unique roughage. Sorghum silage and concentrate were provided as a total mixed ration (TMR).

Table 1 – Ingredients and chemical composition of experimental diets.

Experimental Diets		
Ingredient (g/kg DM)	Prepartum	Postpartum
Sorghum silage [1,2]	716	600
Ground corn	112	219
Soybean meal 48% CP	112	156
Urea	10.7	4.50
Bicalcium phosphate	-	4.20
Limestone	-	3.60
Mineral premix [3,4]	45.1	8.20
Salt	-	2.70
Ultra-diluted complex [5,6]	4.20	1.80
Chemical composition [7,8] (g/kg DM)		
Dry matter, g/kg as fed	333	403
Ash	75.0	66.0
Organic matter	925	934
Crude protein	125	172
Ether extract	24.7	26.1
Neutral detergent fiber	528	413
Acid detergent fiber	375	276
Starch	166	238
Non-fiber carbohydrate [9]	290	361
Total digestible nutrients [10]	592	616
Lignin	62.4	47.4
NE_L, Mcal/kg [10]	1.33	1.49

[1] Prepartum silage chemical composition (DM basis): 303 g/kg as-fed, 87 g/kg CP, 594 g/kg NDF, and 150 g/kg starch.

[2] Postpartum silage chemical composition (DM basis): 300 g/kg as-fed, 86.0 g/kg CP, 593 g/kg NDF, and 159 g/kg starch.

[3] Each kg of prepartum contained: 35 IU Biotin, 260 g Ca, 40 g Cl, 15 mg Co, 505 mg Cu, 15 mg Cr, 110 g S, 200 mg F, 20 g P, 30 mg I, 10 g Mg, 1500 mg Mn, 15 mg Se, 25 g Na, 2015 mg Zn, 200,000 IU Vitamin A, 20,000 IU Vitamin D3, 725 IU Vitamin E, 500 mg sodium monensin.

[4] Each kg of postpartum contained: 240 g Ca, 30 g Co, 1010 mg Cu, 80 g S, 400 mg F, 60 mg I, 20 g Mg, 3000 mg Mn, 30 mg Se, 60 g Na, 4030 mg Zn, 400,000 IU Vitamin A, 40,000 IU Vitamin D3, 1450 IU Vitamin E, 1100 mg sodium monensin.

[5] Placebo control (CON) diet, addition of vehicle (expanded silicate).
[6] Ultra-diluted complex (UD) diet, addition of ultra-diluted complex composed of *Aletris acemose* 10^{-12} + *Aristolochia* 10^{-14} + *Arnica montana* 10^{-14} + *Arsenicum album* 10^{-14} + *Bellis perenis* 10^{-14} + *Berberis vulgaris* 10^{-12} + *Calcium carbonicum* 10^{-30} + *Carboneum tetrachloricum* 10^{-30} + *Cardus marianus* 10^{-12} + *Chelidonium majus* 10^{-12} + *China officinalis* 10^{-12} + *Chionantus virginica* 10^{-30} + *Cimicifuga racemosa* 10^{-60} + *Colibacilinum* 10^{-18} + *Colocynthis* 10^{-18} + *Croton tiglium* 10^{-14} + *Eberthinum* 10^{-18} + *Enterococinum* 10^{-18} + *Ferrum metallicum* 10^{-18} + *Gossypium* 10^{-14} + *Hypericum perforatum* 10^{-60} + *Ignatia amara* 10^{-60} + *Iodum* 10^{-14} + *Leptandra virginica* 10^{-12} + *Mercurius vivus* 10^{-14} + *Myrica cerifera* 10^{-14} + *Natrum muriaticum* 10^{-60} + *Oophorinum* 10^{-12} + *Paratyphoidinum* 10^{-18} + *Phosphorus* 10^{-14} + *Podophylinum peltaltum* 10^{-30} + *Pulsatilla* 10^{-14} + *Ruta graveolens* 10^{-14} + *Silicea terra* 10^{-400} + *Sulphur* 10^{-18} + *Symphytum officinale* 10^{-60} + *Tireoidinum* 10^{-14} + vehicle (expanded silicate, enough quantity for 1 kg).
[7] Estimated by NRC (2001), for prepartum cows, 550 kg body weight, eating 3 kg of concentrate per day.
[8] Estimated by NRC (2001), for fresh cows, 550 kg body weight, 3.5% fat in milk, 30 kg/d of milk yield and 2.98% true protein in milk, eating 10kg of concentrate per day.
[9] Estimated according to Hall (2000).
[10] Estimated according to NRC (2001).

2.2.2 Dry matter and nutrient intake

During the prepartum period, the cows were housed in collective paddocks, which did not allow us to evaluate the individual dry matter intake (DMI) during this period. Thus, DMI was only evaluated during the postpartum period.

After calving, DMI was recorded daily using automatic feeders (Intergado Ltda, Contagem, MG, Brazil). Feed supply was adjusted to maintain a feeding rate target orts between 5 and 10% of the offered feed (on as-fed basis). Samples of sorghum silage and orts were collected daily throughout the experimental period. Weekly samples were pooled to form one sample per treatment per experimental week. Concentrate ingredient samples were collected whenever concentrate was prepared at the feed mill.

The feed and orts samples were pre-dried in a forced-air oven at 55 °C for 72 h and processed in a Wiley mill (MA340, Marconi, Piracicaba, Brazil) using a 1 mm sieve. These samples were then analyzed for dry matter (DM) (method 930.15; AOAC, 2000), crude protein (CP) (N × 6.25; method 984.13; AOAC, 2000), ether extract (EE) (method 920.39; AOAC, 2000), ash (MM) (method 942.05; AOAC, 2000) and neutral detergent fiber (NDF) using alpha amylase without the addition of sodium sulfite (VAN SOEST; ROBERTSON; LEWIS, 1991). Non-fibrous carbohydrate (NFC) content was calculated according to Hall (2000), where NFC (g/kg) = 1000 − [(CP + EE + MM + NDF)].

Feed samples were also analyzed for starch content using an enzymatic degradation method (Amyloglucosidase®, Novozymes, Curitiba, PR, Brazil) and absorbances were measured on a semi-automatic spectrophotometer (SBA-200, CELM®, São Caetano do Sul, SP, Brazil) according to Hendrix (1993).

2.2.3 Milk yield and composition

Cows were milked twice daily (7 a.m. and 4 p.m.) and milk production was recorded electronically (Delpro®, DeLaval, Tumba, Sweden) during the whole experimental period. Milk samples proportional to the two milkings of the day were collected at weeks 1, 2, 4, 6, and 8 after calving. The samples were stored in tubes containing 2-bromo-2-nitropropane-1-3-diol, homogenized, and sent to the laboratory for analysis. Concentrations of fat, protein, and lactose were determined by infrared absorption (Bentley 2000®). In addition, somatic cell count (SCC) was analyzed by flow cytometry using the Somacount 300® (Bentley Instruments Inc., Chasca, MN, USA).

Milk yield was corrected for 3.5% fat (FCM) according to described by Sklan et al. (1992).

2.2.4 Colostrum yield and quality

All cows were followed through calving and the first milking was performed within two hours of calving. The quantity and quality of colostrum produced were recorded. Colostrum quality was measured using an optical Brix refractometer (Sper Scientific 300001 Refractometer, Brix 0–32%). Briefly, approximately 50 µL of colostrum was placed on the prism of the optical refractometer, where Brix readings were taken. Estimation of immunoglobulin concentration was performed by exposing the refractometer to a point of light that allowed the identification of a blue line on the scale, dividing it into light and dark colors (BIELMANN et al., 2010). All readings were performed by the same evaluator.

2.2.5 Blood metabolites

Blood samples (~10 mL) were collected on days 30 (actual: 29.3 ± 2.96) and 7 (actual: 8.9 ± 2.85) before the expected calving date, on the calving day (d0), and on days 7 and 30 after calving. Blood samples were collected by puncture of the jugular vein using a vacutainer, always before the morning feed. Vacuolated tubes with clot activator to obtain serum and tubes with sodium fluoride to obtain plasma were used.

Immediately after sampling, the samples were centrifuged at 2000× *g* for 15 min, and the serum and plasma were kept frozen until further analysis. Glucose (plasma samples), cholesterol, urea, liver enzymes [aspartate aminotransferase (AST) and gammaglutamyltransferasase (GGT)], bilirubin, calcium, and phosphorus concentrations were analyzed in serum samples using commercial biochemistry kits according to the manufacturer's instructions (glucose: Ref. 133-1/500; total cholesterol: Ref. 76-2/100; urea: Ref. 104-4; AST: Ref. 109-4/30; and GGT: Ref. 105-2/30; total bilirubin: Ref. 94-1/104; total calcium Ref. 90-2/60; phosphorus Ref. 12-200; Labtest, Lagoa Santa, Brazil). The measurements were performed in an automatic biochemical analyzer (Mindray, BS 120, Shenzhen, China).

Acute phase proteins (Immunoglobulin A, ceruloplasmin, transferrin, albumin, haptoglobin, and immunoglobulin G) were measured in serum samples. Serum protein was determined by the biuret method using a commercial kit (Total Protein Ref. 99-100; Labtest, Lagoa Santa, Brazil). The separation of protein fractions was performed by acrylamide gel electrophoresis with sodium dodecyl sulfate (SDSPAGE), according to Laemmli (1970). After fractionation, the gel was stained with Coomassie blue solution (50.0% methanol, 40.0% water, 9.75% glacial acetic acid, and 0.25% Coomassie blue) for 10 min. The gel was then placed in a 7.0% acetic acid solution to remove excess dye until the protein fractions were clear. Acute phase protein concentrations were determined using a computerized densitometer.

Plasma concentrations of non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) were measured in plasma samples using enzymatic kits (RANDOX Laboratories-Life Sciences Ltd. Crumlin, UK; BHB: Ranbut-Ref. RB1007; AGNE: Nefa Ref. FA115). The measurements were determined using an automatic system for biochemistry (SBA 200, CELM, Barueri, SP, Brazil).

The liver health index (LHI) was also calculated to characterize the liver function and inflammatory status of the cows. The LHI was calculated according to Kerwin et al. (2022), using the individual concentrations of albumin, cholesterol and bilirubin and the mean and standard deviation of the collected population, according to the following equation:

$$LHI = \left[(Albumin - \mu_{Albumin})/\sigma_{Albumin}\right] + \left[(Cholesterol - \mu_{Cholesterol}/\sigma_{Cholesterol})\right] \\ - \left[(Bilirrubin - \mu_{Bilirrubin}/\sigma_{Bilirrubin})\right]$$

where μ is the overall sampling population mean and σ is the overall sampling population standard deviation.

2.2.6 Statistical analysis

Data were analyzed with SAS (version 9.4, Statistical Analysis System Institute Inc., Cary, NC, USA). Normality of residuals and homogeneity of variances were checked using PROC UNIVARIATE. Six cows were removed from the study due to twin calving (n = 1; 1

from CON group and 0 from UD group) or involuntary culling (n = 5; 2 from CON group and 3 from UD group), leaving data from 24 cows (12 from CON group and 12 from UD group) for statistical analysis.

Data of DM and nutrient intake, milk production and composition, BW, and blood variables, were analyzed as repeated measures over time using PROC MIXED according to the following model:

$$Y_{ijk} = \mu + B_i + T_j + D_k + (T \times D)_{jk} + e_{ijk},$$

where: Y_{ijk} is the dependent variable; µ is the overall mean; B_i is the block random effect; T_j is the treatment fixed effect; D_k is the sampling day fixed effect; $(T \times D)_{jk}$ is the interaction between treatment and sampling day; and e_{ijk} is the residual error. A first-order autoregressive covariance structure [AR (1)] was used for the dry matter and nutrient intake, and milk yield and composition data. This covariance structure is suitable for data collected at equal intervals and assumes decreasing correlations as a function of time. For BW and blood variables, the spatial power covariance matrix [sp(pow)] was used, which is specific for data collected at different time intervals. Weight at the beginning of the experiment was used as a covariate, tested for all variables, and kept in the model when $p \leq 0.05$. Data on the blood sample collected on day −30 (actual: 29.3 ± 2.96) relative to the calving date was used as a covariate and forced into the models.

The SCC was log-transformed according to Schukken et al. (2003) to attend the data normal distribution:

$$SCC = Log_2\left({}^{SCC}/_{100}\right) + 3.$$

Colostrum-related data were analyzed using PROC MIXED according to the following model:

$$Y_{ij} = \mu + B_i + T_j + e_{ij}$$

where: Y_{ij} is the dependent variable; µ is the overall mean; B_i is the block random effect; T_j is the treatment fixed effect; and e_{ij} is the residual error.

Values are presented as least squares means. All analyses were considered significant if $P \leq 0.05$ and tendency if $P > 0.05$ and ≤ 0.10.

2.3 RESULTS

During the study period, four cows were treated for clinical mastitis (2 from CON and 2 from UD), two cows were treated for retained placenta (1 from CON and 1 from UD) and one cow (from UD group) was treated for hypocalcemia. None of the cows showed any signs of ruminal acidosis during the study period.

Cows included in this study were 3.7 ± 1.8 lactations, 545.6 ± 67.6 kg BW and 2.60 ± 0.42 ECC at the beginning of the evaluations. No differences in cows' weight were observed between treatments ($P = 0.459$). Cows fed UD had higher DMI relative to body weight (Table 2; $P = 0.034$) during early lactation, especially in the first 4 weeks ($P = 0.049$). The CP intake tended to be higher ($P = 0.064$) for cows receiving UD during the TP, especially between weeks 5 and 8 of lactation ($P = 0.053$). DM, organic matter, NDF, and EE intake were not affected ($P \geq 0.186$) by treatment. No interactions between treatment and time were found for the variables evaluated ($P \geq 0.119$).

Table 2 – Nutrient intake and body weight of dairy cows in control or ultra-diluted complex fed during transition and early lactation.

Variable	Treatments [1]		SEM [2]	*P*-value		
	CON	UD		Treat	Time	Treat × Time
Postpartum intake, kg/d						
Dry matter						
1 - 60d	18.2	19.9	0.79	0.186	<.0001	0.433
Week 1 – 4	16.3	16.7	0.86	0.755	0.001	0.454
Week 5 – 8	20.7	22.5	0.90	0.198	0.183	0.378
Dry matter, % BW [3]						
1 - 60d	3.53	3.94	0.13	0.034	<.0001	0.197
Week 1 – 4	2.94	3.40	0.15	0.049	<.0001	0.119
Week 5 – 8	4.13	4.46	0.17	0.149	0.082	0.320
Organic matter						
1 - 60d	17.0	18.6	0.74	0.194	<.0001	0.434
Week 1 – 4	15.2	15.6	0.80	0.761	0.001	0.454
Week 5 – 8	19.4	21.0	0.84	0.208	0.184	0.375
Crude protein						
1 - 60d	3.13	3.54	0.13	0.064	<.0001	0.400
Week 1 – 4	2.90	3.03	0.15	0.569	0.001	0.440
Week 5 – 8	3.48	3.91	0.14	0.053	0.282	0.302
Neutral detergent fiber						
1 - 60d	7.81	7.70	0.37	0.828	<.0001	0.393
Week 1 – 4	6.51	6.57	0.41	0.918	<.0001	0.249
Week 5 – 8	8.70	9.19	0.39	0.406	0.086	0.375
Ether extract						
1 - 60d	0.47	0.52	0.02	0.195	<.0001	0.345
Week 1 – 4	0.43	0.44	0.02	0.825	0.002	0.396
Week 5 – 8	0.54	0.58	0.02	0.182	0.172	0.472
BW [4]	521.73	510.06	19.97	0.459	<.0001	0.792

[1] CON: cows received placebo treatment (expanded silicate, 40 g/cow/day); UD: cows received ultra-diluted complex (PeriParto Transição–RealH, 40 g/cow/day).

[2] Standard error of the mean.

[3] Ratio between DMI and body weight.
[4] Body weight. The measurements were performed on days 30 (actual: 29.3 ± 2.96) and 7 (actual: 8.9 ± 2.85) before the expected calving date, on the calving day (d0), and on days 7 and 30 after calving.

Feeding UD increased (Table 3; $P = 0.024$) milk lactose content (%) between 5 and 8 weeks after calving. There was an interaction between treatment and time ($P = 0.0001$) for milk protein content, with cows fed UD having lower milk protein content, especially during the first 4 weeks of lactation. An interaction between treatment and time (Figure 1; $P = 0.001$) was also found for SCC, with cows fed UD having lower SCC at 3 and 4 weeks post calving. No treatment effect ($P \geq 0.119$) or interaction between time and treatment ($P \geq 0.138$) were found for milk yield, FCM, fat, protein and lactose yield, milk fat content and the ratio between DMI and milk yield and between DMI and FCM. The quantity and quality of colostrum produced did not differ ($P \geq 0.102$) between CON and UD group cows.

Table 3 – Milk yield and composition of dairy cows in control or ultra-diluted complex fed during transition and early lactation.

Variable	Treatments [1]		SEM [2]	*P*-value		
	CON	UD		Treat	Time	Treat × Time
Milk, kg/d						
1 - 60d	28.4	29.2	1.39	0.428	<0.0001	0.646
Week 1 – 4	27.9	29.0	1.42	0.309	<0.0001	0.738
Week 5 – 8	28.9	29.8	1.38	0.410	0.119	0.204
3.5% FCM, kg/d [3]						
1 - 60d	30.3	29.4	1.37	0.592	0.008	0.874
Week 1 – 4	30.3	29.2	1.43	0.523	0.019	0.896
Week 5 – 8	30.2	29.6	1.43	0.714	0.733	0.693
Fat, kg/d						
1 - 60d	1.11	1.04	0.053	0.365	0.390	0.765
Week 1 – 4	1.13	1.03	0.062	0.289	0.256	0.740
Week 5 – 8	1.09	1.05	0.054	0.575	0.874	0.895
Protein, kg/d						
1 - 60d	0.88	0.88	0.040	0.934	<0.0001	0.254
Week 1 – 4	0.92	0.92	0.041	0.832	0.0001	0.341
Week 5 – 8	0.85	0.84	0.044	0.700	0.955	0.537
Lactose, kg/d						
1 - 60d	1.24	1.30	0.064	0.238	<0.0001	0.898
Week 1 – 4	1.21	1.28	0.070	0.187	<0.0001	0.748
Week 5 – 8	1.28	1.32	0.069	0.428	0.233	0.541
Fat, %						
1 - 60d	3.90	3.63	0.146	0.119	0.800	0.719
Week 1 – 4	4.02	3.62	0.209	0.128	0.491	0.537
Week 5 – 8	3.80	3.64	0.145	0.382	0.966	0.968
Protein, %						
1 - 60d	3.14	3.03	0.070	0.313	<0.0001	0.0001

Week 1 – 4	3.30	3.21	0.072	0.371	<0.0001	0.002
Week 5 – 8	2.97	2.86	0.069	0.301	0.060	0.076
Lactose, %						
1 - 60d	4.40	4.51	0.039	0.055	<0.0001	0.138
Week 1 – 4	4.36	4.45	0.044	0.183	<0.0001	0.145
Week 5 – 8	4.42	4.57	0.041	0.024	0.755	0.311
Efficiency, *Milk:DMI* [4]	1.63	1.60	0.065	0.749	<.0001	0.409
Efficiency, *FCM:DMI* [5]	1.69	1.63	0.071	0.551	<.0001	0.458
Colostrum						
Yield, kg	4.25	5.63	0.66	0.102	-	-
Quality, °brix	25.6	27.7	1.20	0.232	-	-

[1] CON: cows received placebo treatment (expanded silicate, 40 g/cow/day); UD: cows received ultra-diluted complex (PeriParto Transição–RealH, 40 g/cow/day).
[2] Standard error of the mean.
[3] Fat corrected milk at 3.5%, calculated according to Sklan et al. (1992).
[4] Milk yield to dry matter intake ratio.
[5] 3.5% Fat-corrected milk to dry matter intake ratio.

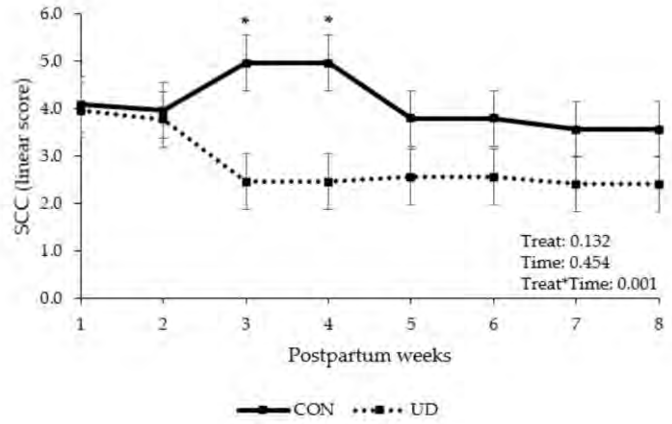

Figure 1 – Somatic cell count (SCC) linear score of dairy cows in control or ultra-diluted complex fed during transition period and early lactation. CON: cows received placebo treatment (expanded silicate, 40 g/cow/day); UD: cows received ultra-diluted complex (PeriParto Transição–RealH, 40 g/cow/day). Error bars are SEM (standard error of the mean). Asterisks represent differences between CON and UD where $P \leq 0.05$.

There was a tendency for interaction between time and treatment (Figure 2; $P = 0.065$) for plasma BHB concentration, with cows fed UD tending to have higher BHB concentrations on day 30 after calving. On the other hand, no treatment effects or interactions between treatment and time were observed for NEFA (Figure 2; $P \geq 0.829$), biochemical profile variables (Table 4; $P \geq 0.247$), and acute phase proteins (Table 5; $P \geq 0.213$). Cows fed UD tended to have a higher LHI (Figure 3; $P = 0.098$).

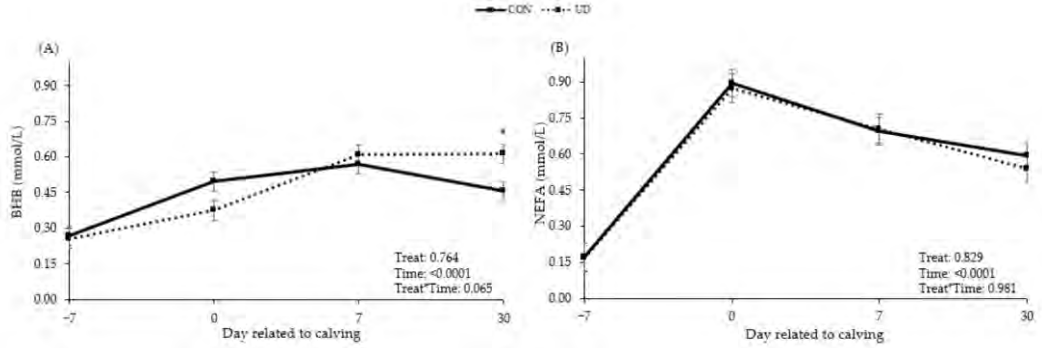

Figure 2 – Plasma concentrations of beta-hydroxybutyrate (BHB; (A)) and non-esterified fatty acids (NEFA; (B)) of dairy cows in control or ultra-diluted complex fed during transition period and early lactation. CON: cows received placebo treatment (expanded silicate, 40 g/cow/day); UD: cows received ultra-diluted complex (PeriParto Transição–RealH, 40 g/cow/day). Error bars are SEM (standard error of the mean). Asterisk represents statistical tendency between CON and UD where $P > 0.05$ and ≤ 0.10.

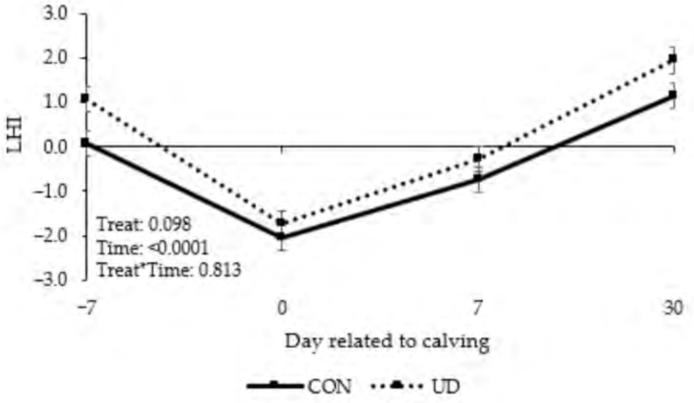

Figure 3 – Liver health index (LHI) of dairy cows in control or ultra-diluted complex fed during transition period and early lactation. CON: cows received placebo treatment (expanded silicate, 40 g/cow/day); UD: cows received ultra-diluted complex (PeriParto Transição–RealH, 40 g/cow/day). Error bars are SEM (standard error of the mean).

Table 4 – Blood biochemical profile of dairy cows fed control or ultra-diluted complex during transition and early lactation.

Variable [1]	Treatments [2]		SEM [3]	*P*-Value		
	CON	UD		Treat	Time	Treat × Time
AST (U/L) [4]	75.45	73.72	2.345	0.590	<0.0001	0.743
Bilirubin (µmol/L)	2.389	2.295	0.227	0.394	<0.0001	0.916
Calcium (mg/dL)	9.304	9.563	0.159	0.257	<0.0001	0.953
Cholesterol (mg/dL)	72.79	79.71	4.085	0.247	<0.0001	0.375
Phosphorus (mg/dL)	5.396	5.582	0.148	0.382	0.0009	0.292
GGT (U/L) [5]	22.63	23.44	0.572	0.334	0.050	0.536
Glucose (mg/dL)	67.19	69.89	3.956	0.474	<0.0001	0.700
Total protein (g/dL)	6.692	6.815	0.098	0.388	<0.0001	0.402
Urea (mg/dL)	28.60	27.28	1.195	0.445	<0.0001	0.674

[1] Blood samples were collected on days −30 (covariate), −7, 0, +7, and +30 days relative to calving.

[2] CON: cows received placebo treatment (expanded silicate, 40 g/cow/day); UD: cows received ultra-diluted complex (PeriParto Transição–RealH, 40 g/cow/day).

[3] Standard error of the mean.
[4] Aspartate aminotransferase.
[5] Gama glutamil transferase.

Table 5 – Acute phase proteins blood concentrations of dairy cows fed control or ultra-diluted complex during the transition period and early lactation.

Variable [1]	Treatments [2]		SEM [3]	*P*-Value		
	CON	UD		Treat	Time	Treat × Time
Albumin (g/L)	49.93	44.60	0.554	0.413	<0.0001	0.673
Ceruloplasmin (mg/dL)	50.13	54.81	4.748	0.358	0.036	0.808
Haptoglobin (mg/dL)	48.09	51.11	4.179	0.618	<0.0001	0.363
IgA (mg/dL)	71.20	71.79	2.393	0.835	<0.0001	0.213
Transferin (mg/dL)	184.44	188.44	7.854	0.748	0.1186	0.901
IgG (mg/dL)	1495.01	1575.73	63.19	0.377	<0.0001	0.395

[1] Blood samples were collected on days −30 (covariate), −7, 0, +7, and +30 days relative to calving.
[2] CON: cows received placebo treatment (expanded silicate, 40 g/cow/day); UD: cows received ultra-diluted complex (PeriParto Transição–RealH, 40 g/cow/day).
[3] Standard error of the mean.

2.4 DISCUSSION

This study provides the first scientific insights into the use of UD in the diet of transition and early lactation cows. Cows receiving UD tended to have a higher LHI than cows in the CON group. Higher post-calving LHI scores have been reported with better performance of cows throughout lactation, which is associated with reduced disease incidence, increased milk production, and a higher probability of pregnancy in the first 150 DIM (KERWIN et al., 2022). Studies evaluating liver function index by albumin, cholesterol, and retinol concentrations reported lower milk production in early lactation in cows with impaired liver function (TREVISI et al., 2012; ZHOU et al., 2016). However, although cows in the CON group had a lower LHI in our study, milk yield did not differ significantly between treatments.

Feeding UD resulted in a tendency to higher plasma levels of BHB at 30 days postpartum. Ketone elevation after calving is a normal adaptive response, but accumulation is not (DUFFIELD et al., 2009). Hyperketonemia in dairy cows is defined when the plasma BHB concentration is equal to or greater than 1.2 mmol/L (CHAPINAL et al., 2012; GORDON et al., 2017; SANTSCHI et al., 2016; SUTHAR et al., 2013). In addition, a cut-off point of 0.62 mmol/L for hyperketonemia has already been considered (FÜRLL, 2005). Mahrt et al. (2015), who evaluated the prevalence of hyperketonemia in dairy cows up to 42 days of lactation, found an average prevalence of 11.8% (ranging from 9.6% during the first two weeks of lactation to 14.6% between the fifth and sixth weeks of lactation). Even considering the cut-off point given by Fürll (2005), where our cows would be at the upper limit considered, we concluded that the cows in our study did not show any degree of hyperketonemia, as the highest BHB level found in this study was 0.610 mmol/L. Although cows may be at risk for hyperketonemia up to the sixth week of lactation (MAHRT; BURFEIND; HEUWIESER, 2015), we believe that the BHB tendency in our study may have occurred by chance, as BHB levels are quite sensitive and may vary for several reasons, including daily variations in body fluids (NIELSEN; INGVARTSEN; LARSEN, 2003).

Cows fed UD during transition and early lactation had higher ratio between DMI and BW, especially during the first 4 weeks of lactation. Assuming that the cows on the UD treatment had better metabolic conditions during early lactation due to better LHI, this may have resulted in an increased DMI relative to BW (SINGH et al., 2020; VAN HOEIJ et al., 2019). Although we found no significant effects related to fatty acids, cows in the CON group may have suffered some degree of hepatic fatty acid oxidation, which is directly related to decreased appetite and inhibition of feeding behavior (ALLEN; BRADFORD; OBA, 2009). Also, cows receiving UD treatment tended to have higher CP intake, which may be considered a reflection of the increase in DMI relative to BW.

Regarding milk yield and components, lactose and protein content were affected by treatment. Milk produced by cows fed UD had higher lactose content, especially between weeks 5 and 8 of lactation, while milk from UD-fed cows had lower protein content, especially during the first 4 weeks of lactation. The lactose content of cow’s milk can be influenced by udder health (MARTINS et al., 2020), metabolism, and energy balance (TELEVIČIUS et al., 2021). Although we did not find a significant effect on milk yield, higher lactose content may result in increased milk yield because lactose acts as an osmotic component in milk, stimulating secretion of water into milk via the bloodstream (VILAS BOAS et al., 2017). Also, because cows fed UD had higher DM:BW, they may have had higher ruminal production of propionate and consequently more precursors for gluconeogenesis (WANG et al., 2016), since glucose is the main precursor for lactose synthesis in the mammary gland (ZHAO, 2014). The lower protein content in milk can be explained by the dilution effect, because although we found no significant effect on milk yield, cows from the UD treatment produced numerically more milk than cows from the CON treatment.

Cows receiving the UD treatment had lower SCC than cows in the CON group, especially between weeks 3 and 4 after calving. SCC in milk has been used as an indicator of udder health and immune status (HAAS et al., 2007; RENEAU, 1986). Since UD treated cows had a better LHI, we can speculate that these cows also had a better immune response in early lactation. Improving the cow’s immune system have been associated with reduced SCC in milk

(SPANIOL et al., 2015; WARKEN et al., 2018). Additionally, high SCC has been reported by changing the milk composition (MARTINS et al., 2020), which could also explain the differences in lactose and protein content found in our study. However, changes in milk composition are dependent on the type of pathogen causing mastitis (MARTINS et al., 2020), but this assessment was beyond the scope of our study. The relations between UD complex and cows' immune system need to be further investigated.

Our study focused on presenting results related to performance and metabolism of dairy cows receiving UD during the TP but has some limitations. The UD used in this study is composed of many ultra-diluted substances, making it difficult to identify isolated effects of each component. Due to the small number of cows involved in this study, the results should be viewed and interpreted with caution as this may have reduced the statistical power to detect differences between treatments. Future larger and more detailed studies evaluating the use of UD in the diets of transition and early lactation cows could use this work as a starting point. In addition, although our results did not improve the understanding of the mechanisms of action behind the use of UD, they will contribute to the scientific community; to our knowledge this is the first study evaluating the use of UDs during TP and early lactation of dairy cows.

2.5 CONCLUSIONS

UD use during the TP and early lactation did not affect cow production. However, the use of UD may have beneficial effects when it comes to udder and liver health. Our results offer fresh perspectives on the impact of UD use on the metabolism and performance of dairy cows during early lactation. Our findings can serve as a great starting point for future research evaluating the potential use of ultra-diluted complexes during the TP.

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Institutional Review Board Statement: This study was approved by the Committee on Ethics in the Use of Animals of the Faculty of Animal Science and Food Engineering of the University of São Paulo on February 24, 2021 (protocol number 651619012).

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data of this study will be made available by the authors (corresponding author) to any qualified researcher.

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3. CHAPTER 3 - ACCURACY AND PRECISION OF DIETS FED TO CLOSE-UP COWS ON DAIRY FARMS AND ITS ASSOCIATION WITH EARLY LACTATION PERFORMANCE

MANUSCRIPT PREPARED ACCORDING TO JOURNAL OF DAIRY SCIENCE GUIDELINES

INTERPRETIVE SUMMARY

Accuracy and precision of diets fed to close-up cows on dairy farms and its association with early lactation performance. By Gheller et al. This observational study characterized close-up diets fed on commercial dairy farms and explored potential associations of the accuracy and precision of those diets with fresh cow performance. Corn silage and straw were the predominant forage sources used in the close-up dry cow diets, while soybean meal and canola meal were the main concentrate ingredients. Overall, the diets provided did not accurately represented the formulated diets. Diet variability, both between fed and formulated diets and from visit-to-visit during the close-up period, was associated with markers of metabolism and production in early lactation. These findings emphasize the importance of consistency between diet formulations and feeding practices to optimize dairy herd performance and health.

DRY COW DIET ACCURACY AND EARLY LACTATION PERFORMANCE

Accuracy and precision of diets fed to close-up cows on dairy farms and its association with early lactation performance

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ABSTRACT

Ensuring a consistent ration is critical for maximizing lactating cow performance, but this is not known for dry cows. The objectives of this observational study were to: 1) characterize close-up dry cow diets fed on commercial farms in Ontario, Canada, 2) describe the accuracy of the nutrient composition between the formulated close-up diet and the diet offered to the cows, 3) describe the precision of the close-up diets across time, and 4) to explore potential associations of that accuracy and precision with blood metabolic parameters and milk yield of cows in early lactation. Forty free-stall dairy farms were visited once every 4 wk, for a total of 6 visits to each farm, from April to October 2022. At each visit, samples of the close-up diet were collected and analyzed for dry matter (DM) content and chemical composition. Close-up diet formulations were also obtained by each farm's nutritionists. During each visit, fresh cows (0 to 14 days in milk [DIM]) had blood samples taken for blood metabolites. The same cows were also monitored for milk production up to 120 DIM. Multivariable models were used to analyze associations between variability (in relation to the formulated diet and across time) of nutrients in the close-up diet, as measured by coefficient of variation (CV), and outcomes in fresh cows. Corn silage (67.6% of farms) and straw (24.3% of farms) were the predominant primary forage sources used in the close-up dry cow diets. Soybean meal (37.8% of farms) and canola meal (18.9% of farms) were the main ingredients used as primary concentrate sources. Overall, the diets offered did not accurately represent the formulated diets. With the exception of net energy for lactation (NE_L), the CVs for the other nutrients were all greater than 5%. Diet variability, both between fed and formulated diets and from visit to visit during the close-up period, was associated with metabolic markers and dairy cow production. Lower variability in non-fiber carbohydrates (NFC) between the fed and formulated diets was associated with better liver health index scores. Visit-to-visit variability in fat% and NFC% were associated with β-hydroxybutyrate (BHB) concentrations, while NFC% variability was associated with blood glucose levels. Non-esterified fatty acids (NEFA) concentrations were associated with visit-to-visit variability in DM% and CP%. These results underscore the importance of maintaining consistency between diet formulations and feeding practices over time to optimize early-lactation dairy herd performance and health.

Key words: diet accuracy, close-up period, fresh cow performance.

RESUMO

Garantir uma dieta consistente é fundamental para maximizar o desempenho das vacas em lactação, mas isso não é conhecido para vacas secas. Os objetivos deste estudo observacional foram: 1) caracterizar as dietas de vacas secas durante o período close-up fornecidas em fazendas comerciais em Ontário, Canadá, 2) descrever a acurácia da composição entre as dietas close-up formuladas e as dietas oferecidas às vacas, 3) descrever a precisão das dietas close-up ao longo do tempo e 4) explorar potenciais associações dessa acurácia e precisão com parâmetros metabólicos sanguíneos e produção de leite das vacas no início da lactação. Quarenta fazendas leiteiras free-stall foram visitadas uma vez a cada 4 semanas, totalizando 6 visitas em cada fazenda, de abril a outubro de 2022. Em cada visita, amostras da dieta close-up foram coletadas e analisadas quanto ao teor de matéria seca (MS) e composição química. As formulações das dietas close-up também foram obtidas através do nutricionista de cada fazenda. Durante cada visita, vacas recém-paridas (0 a 14 dias em lactação [DEL]) tiveram amostras de sangue coletadas para análise de metabólitos sanguíneos. As mesmas vacas também foram monitoradas quanto à produção de leite até 120 DEL. Modelos multivariados foram utilizados para analisar associações entre a variabilidade (em relação à dieta formulada e ao longo do tempo) dos nutrientes na dieta close-up, medida pelo coeficiente de variação (CV), e os resultados em vacas recém-paridas. A silagem de milho (67,6% das fazendas) e a palha (24,3% das fazendas) foram as fontes primárias de forragem predominantes utilizadas nas dietas de vacas secas durante o período close-up. O farelo de soja (37,8% das propriedades) e o farelo de canola (18,9% das propriedades) foram os principais ingredientes utilizados como fontes primárias de concentrado. No geral, as dietas ofertadas não representavam com acurácia as dietas formuladas. Com exceção da energia líquida para a lactação (NE_L), os CVs para os demais nutrientes foram todos superiores a 5%. A variabilidade da dieta, tanto entre dietas fornecidas quanto formuladas e de visita para visita durante o período de close-up, foi associada a marcadores metabólicos e à produção de vacas leiteiras. A menor variabilidade nos carboidratos não fibrosos (CNF) entre as dietas fornecidas e formuladas foi associada a escores mais elevados no índice de saúde hepática. A variabilidade de visita a visita na % de gordura e % de CNF foi associada às concentrações de β-hidroxibutirato (BHB), enquanto a variabilidade% de CNF foi associada aos níveis de glicose no sangue. As concentrações de ácidos graxos não esterificados (AGNE) foram associadas à variabilidade de visita a visita na % de MS e % de PB. Esses resultados ressaltam a importância de manter a consistência entre as formulações da dieta e as práticas alimentares ao longo do tempo para otimizar o desempenho e a saúde do rebanho leiteiro no início da lactação.

Palavras-chave: acurácia da dieta, período close-up, desempenho de vacas recém-paridas.

3.1 INTRODUCTION

Feeding a dairy herd begins with formulating diets to meet the nutritional requirements of the cows at a specific stage of their production cycle. However, the diet that is delivered to the cows at the feed bunk and is effectively consumed can vary depending on the DM content of the ingredients, the method of loading the ingredients into the feed wagon, the mixing and unloading process, or it can be further altered by selection (sorting) by the cows (ROSSOW; ALY, 2013). Discrepancies between offered and formulated diets may have a negative effect on cow performance (BACH, 2024; STONE, 2008). Therefore, ensuring a consistent ration is a crucial factor in maximizing cow performance.

The consistency of the diets fed to cows can be evaluated in terms of accuracy and precision. Accuracy represents the degree to which the offered diet differs from the formulated diet, while precision refers to the degree of variation between the fed diets (JAMES; COX, 2008). Sova et al. (2014) evaluated the accuracy and precision of TMR fed to lactating cows on commercial farms and reported that day-to-day variability in composition of TMR was associated with cow performance. Specifically, greater daily variability in NE_L content was associated with reduced DMI, milk yield, and milk production efficiency (SOVA et al., 2014). In addition, it was observed that the TMR fed to lactating cows generally did not accurately reflect the formulated diets (SOVA et al., 2014). In a more recent study, it was demonstrated that the variation in the amounts of ingredients in the TMR fed to lactating cows compared to the formulated amounts of those ingredients in the TMR was quadratically related to milk production, indicating that as variation in dietary ingredients increased, milk production was lower (BACH, 2024). These results highlight the importance of maintaining consistency in the composition of the TMR provided to maximize cow productivity. Furthermore, understanding the variability between the fed and formulated diets can also help identify areas for improvement on-farm.

Most studies evaluating the variability of diets have been focused on lactating cows (BACH, 2024; CARNEIRO; SANTOS; ALMEIDA, 2021; TAYYAB et al., 2018; TRILLO; LAGO; SILVA-DEL-RÍO, 2016). However, it is possible that dietary variation may have even greater impacts for dry cows. The idea that effective nutritional management during the dry period is a pre-requisite for a successful start to subsequent lactation has become widely accepted. For example, Kerwin et al. (2023a) demonstrated that optimizing feed bunk space and providing adequate levels of metabolizable energy to cows during the prepartum period was associated with reduced prevalence of elevated non-esterified fatty acids (NEFA), BHB, and haptoglobin levels postpartum. In addition, those factors were also associated with decreased disease incidence, greater milk production, and an improved risk of pregnancy at first service. However, it is not known whether the accuracy between the diet offered, and the diet formulated during the close-up period, as well as the consistency in that diet fed over time, might affect the performance of cows in the subsequent lactation.

Therefore, the objectives of this observational study were to: 1) characterize close-up dry cow diets fed on commercial farms in Ontario, Canada; 2) describe the accuracy of the nutrient composition between the formulated close-up diet and the diet offered to the cows; 3) describe the precision of the close-up diets across time, and 4) explore potential associations of that accuracy and precision with blood metabolic parameters and milk yield of cows in early lactation.

3.2 MATERIAL AND METHODS

All the procedures of this study followed the guidelines of the Canadian Council on Animal Care (2009) and were approved by the University of Guelph Animal Care Committee (Protocol #3963).

3.2.1 Farm selection and visits

As part of a larger study investigating cows' stressors during the transition period on Ontario dairy farms (WAGEMANN FLUXÁ et al., 2023), 40 free-stall dairy farms (20 automated milking systems (AMS) and 20 parlor milking) located in southwestern Ontario, Canada were enrolled in this study. To be eligible to participate in the study, farms had to meet the following criteria: a) have a milk production monitoring program (DHI or milking machine recording); b) milk predominantly Holstein cows; c) have reproductive data available; and d) be located within a 2-h drive of Guelph, Ontario, Canada. For those farms that met the selection criteria, producers were contacted via email to verify their willingness to participate in the study. This process continued until 20 AMS farms and 20 parlors farms were included in the study. The average number (mean ± SD) of lactating cows on the farms included in this study was 190.3 ± 138.8 (median = 137, ranging from 60 to 550).

Farms were visited once every 4 wk (n = 6 visits) between April and October 2022 for sampling and data collection. On 2 farms, the first visit was skipped due to a late response from the farmer. Two farms were visited per day (one farm in the morning and other in the afternoon), with the time of day between visits varying (e.g., first visit in the morning, second visit in the afternoon, third visit in the morning, etc.). All visits were performed by a group of 2 to 4 researchers. Producer consent was received before farm visits and data collection. At the first visit, a survey on herd management, facilities, demographics, and nutritional management was conducted orally with the farm owner or manager.

3.2.2 Feed sampling and analyses

At each farm visit, feed samples of the close-up dry cow diet were collected. Samples were collected according to the guidelines suggested by Sova et al. (2014). Briefly, samples were collected from 6 to 10 spots from the feed bunk. After collection, samples were mixed to ensure a representative sample from the entire length of the feed bunk. During the first farm visit, information about the feeding schedule of the close-up cows was gathered to ensure that the feed sample collected was from a freshly mixed and delivered diet (< 1 h from delivery). When the researchers were unable to collect feed samples of freshly mixed feed because the time of feed delivery differed from the time of the visit, farm personnel collected the samples. The farm personnel were trained to collect samples following the aforementioned protocol. A total of 229 close-up dry cow diet samples were collected. For farms (n = 4) that top dressed pellets and/or supplements to their dry cows, calculations were performed, using known proportions of amounts fed, to combine those with the partial mixed ration to determine the complete diet, to be able to compare results.

After collection, all feed samples were frozen at -20˚C until further analysis. For DM determination, samples were thawed for 24 h and then oven-dried at 60˚C for 48 h. After drying, samples were ground to pass through a 1-mm sieve (Model 4 Wiley Laboratory Mill, Thomas Scientific, Swedesboro, NJ). The dried and ground samples were sent to the A & L Laboratory Services Inc. (London, ON, Canada) for analysis of ash (550°C; AOAC International, 2000, method 942.05), ADF (AOAC International, 2000, method 973.18), NDF with heat-stable α-amylase and sodium sulfite (AOAC International, 2000, method 2002.04), CP (N × 6.25; AOAC International, 2000, method 990.03; Leco FP-628 Nitrogen Analyzer, Leco Corp., St. Joseph, MI), starch (heat-stable amylase and amyloglucosidase; AOAC International, 2000, method 996.11), sugar (HALL, 2000), crude fat (EE, using pet ether; AOAC International, 2000, method 920.39), minerals (using aquaregia digestion inductively coupled plasma atomic emission spectroscopy), and calculation of net energy, using NRC (2001) equations.

3.3.3 Formulated diets

With the agreement of each farmer, the nutritionist responsible for diet formulation on each farm was contacted to obtain information on the diets formulated for the close-up cows; this information was collected retrospective to the period in which the visits were conducted. Out of 40 farms included in the study, nutritionists from 37 of the farms provided information about the close-up dry cow diets. The number of close-up dry cow diets sent by the nutritionists for the time period associated with the farm visits ranged from 1 to 10, with an average of 3 formulations per farm. Upon receipt of the diets, a screening process was carried out to determine which of the formulated diets received were actually being fed to the cows at each visit. Of the 228 close-up dry cow diet samples, 203 samples had a corresponding formulated diet that was sent by the nutritionists. The nutrient and ingredient composition of the diets were recorded and summarized for each farm visit. The main ingredients used in the dry cow diets during the close-up period (primary and secondary sources of forage and concentrate) are presented in Table 1. In addition, the average composition of the formulated and fed diets is presented in Table 2.

3.3.4 Fresh cows outcomes

At each farm visit, fresh cows were assessed for BCS, blood samples were collected, and milk yield and composition data were recorded. On each farm, up to 12 cows between 0 and 14 DIM (actual: 7 ± 4.2 DIM) were selected for sampling. For farms with more than 12 fresh cows (up to 14 DIM), 12 cows were randomly selected. BCS were assessed using the methodology proposed by Wildman (1982), which consists of a scale from 1 to 5 points with intervals of 0.25. All BCS scores were performed by the same evaluator.

Blood samples were collected from the coccygeal vessels using 10-mL vacutainer blood serum collection tubes. Samples from cows between 0 - 4 DIM were analyzed for NEFA, glucose, and BHB, while samples from cows between 5 - 14 DIM were analyzed for NEFA, cholesterol, albumin, total bilirubin, glucose, and BHB. Glucose and BHB measurements were obtained immediately after sampling using a drop of blood and a glucose test strip or ketone test and read on a Freestyle Precision Neo device (Abbott Diabetes Care, Saint Laurent, QC, Canada) as validated by Wittrock et al. (2013) for glucose and Kanz et al. (2015) for BHB. Glucose concentrations obtained by reading the Freestyle Precision Neo device (Abbott

Diabetes Care, Saint Laurent, QC, Canada) were corrected using the equation proposed by Wittrock et al. (2013), which is: mmol/L = [0.6 + (0.86 × glucometer reading)].

After collection, blood samples for serum isolation were immediately refrigerated and transported to the laboratory. At the laboratory, the samples were kept at room temperature for 1 h to allow coagulation and to facilitate fibrinogen breakdown and then centrifuged at 1,500 × g for 15 min at 18ºC to obtain the serum. Serum samples were stored in duplicate microtubes (~1.5 mL each), frozen at -20ºC, and sent to the Animal Health Laboratory at the University of Guelph for analysis of NEFA (reagent provided by Randox Laboratories, Crumlin, UK), cholesterol (reagent provided by Roche Diagnostics, Indianapolis, IN), albumin (reagent provided by Roche Diagnostics, Indianapolis, IN), and total bilirubin (reagent provided by Roche Diagnostics, Indianapolis, IN) using a photometric test on the Roche Cobas 6000 c501 instrument (Roche, Basel, Switzerland). The concentrations of albumin, cholesterol, and bilirubin were used to calculate the liver health index (LHI) as proposed by Kerwin et al. (2022) using the following equation: $\text{LHI} = [(\text{Alb} - \mu_{\text{Alb}})/\sigma_{\text{Alb}}] + [(\text{Chol} - \mu_{\text{Chol}})/\sigma_{\text{Chol}}] - [(\text{Bili} - \mu_{\text{Bili}})/\sigma_{\text{Bili}}]$, where Alb is the individual serum concentration of albumin, Chol is the individual serum concentration of cholesterol, Bili is the individual serum concentration of total bilirubin, μ is the mean of the sampled population, and σ is the standard deviation of the sampled population.

Milk production data were also collected from cows selected for blood collection. For DHI registered farms (n = 38) (Lactanet, Sainte-Anne-de-Bellevue, QC, Canada), milk production information and data were collected for all sampled cows for up to 120 DIM. For non-DHI farms (n = 2), milk production data were recorded and collected (also for all sampled cows up to 120 DIM) using Lely Horizon software (Lely Industries N.V., Maassluis, the Netherlands).

3.3.5 Calculations and statistical analyses

Differences between the fed and formulated dietary nutrient content, based on samples taken at each visit and corresponding ration formulation for the dry cow diet being fed when that visit occurred, were calculated for each farm by subtracting the formulated value from the laboratory analysis value. For each nutrient, the coefficient of variation (CV) between the formulated diet and the fed diet was calculated. The CV was calculated by dividing the standard deviation (SD) between the formulated value (at each visit) and the average analyzed value (at each visit) by the average of these values (fed and formulated at each visit) and expressed as a percentage. Visit-to-visit diet variability of the fed diet (expressed as CV) was calculated for all nutrients for each farm from the values obtained in the laboratory analyses within of the feed samples from each farm during the 6 visits. The CV (expressed as a percentage) was obtained by dividing the SD of each nutrient over the 6 visits by the average of these values over the 6 visits.

Statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC). Prior to analysis, all data were assessed for normality using the UNIVARIATE procedure. The average nutrient composition of the formulated and fed (offered) diets over the 6 visits to each farm were analyzed using the MIXED procedure in SAS and were considered statistically different if $P < 0.05$. The composition of the formulated diet was replicated for subsequent visits for farms that did not frequently change the close-up diet.

Data from early lactation cows were averaged by farm and visit to obtain herd-level averages for the outcomes of interest (blood parameters and milk production). Associations between the outcomes and variability (CV) of fed and formulated dietary nutrient content (DM, CP, starch, fat, ADF, NDF, NFC, and NE_L) were analyzed with the MIXED procedure, treating visit as a repeated measure. Farm was included in the model as the subject of the repeated statement. The covariance structure chosen in the repeated analysis was CS (compound symmetry), which was determined to be the most appropriate according to Schwarz's Bayesian information criterion. A similar model was also used for visit-to-visit variability in fed diet nutrient content, without including repeated measures. Herd-average parity, BCS, and DIM at sampling were included as potential covariates in the model. The CV of the nutrients were included as explanatory variables and first tested in univariable models, where only variables with $P > 0.25$ were kept to be included and tested in multivariable models (DOHOO; MARTIN; STRYHN, 2009). Once the possible explanatory variables were identified, the SAS CORR procedure was used to check for possible correlations between the variables to be included in the multivariable models. In cases where two variables were correlated ($r > 0.80$), the most biologically plausible variable was retained for inclusion in the multivariable model. Non-significant variables ($P > 0.10$) were removed from the multivariable model by backward elimination, starting with the variable with the highest P value, until only variables with $P < 0.10$ remained in the multivariable model. If confounding variables (nonsignificant variables that, if removed from the model, would individually change the coefficient of another variable by more than 25%) were identified, they would be retained in the multivariable model; however, this was not the case in our study. Significance was considered when $P < 0.05$ and tendencies if $P \leq 0.10$. Only those outcome variables associated with a nutrient variability factor are presented further.

3.4 RESULTS AND DISCUSSION

3.4.1 Formulated diet ingredient composition

Based on the diet formulations sent by the nutritionists of the farms participating in the study, all of the farms used two forage sources when feeding their close-up dry cows. Corn silage was the most commonly used forage, serving as both a primary (67.6% of farms) and secondary (29.7% of farms) forage source (Table 1). This forage source has been recognized as one of the most popular in North American dairy cows diets (HASSANAT; GERVAIS; BENCHAAR, 2017). It is important to note that corn silage is a high-energy ingredient (FERRARETTO; SHAVER; LUCK, 2018), necessitating the addition of lower energy-density ingredients, such as straw, to achieve dietary balance. Straw, which is characterized as a low-quality forage (JANOVICK; BOISCLAIR; DRACKLEY, 2011), plays a crucial role in moderating energy density, particularly that of close-up dry cow diets. In our study, approximately 80% of the farms used straw as either a primary (24.3% of farms) or secondary (56.8% of farms) source of forage in their close-up diets (Table 1). Other forage sources, such as haylage, hay, and soybean silage, were also included in dry cow diets, but in lesser proportions.

Regarding the concentrate ingredients used in the close-up dry cows diets, the main ingredients were protein sources (Table 1). Soybean meal was the main ingredient used, both as a primary ingredient (37.8% of farms) and as a secondary ingredient (24.3% of farms). Soybean meal is a widely used protein supplement in dairy cattle diets (GIDLUND et al., 2015; PAULA; BRODERICK; FACIOLA, 2020). Canola meal was the second most common ingredient in concentrates (Table 1), used both as a primary (18.9% of farms) and secondary (13.5% of farms) concentrate ingredient. Canola meal is a high-quality protein source, with significant productivity in Canada, making it the second most important field crop (BENCHAAR et al., 2021; STATISTICS CANADA, 2022). Distillers grains were used as a secondary ingredient in the concentrate on 13.5% of the farms. This ingredient is a by-product of bioethanol production, from grains such as corn, wheat, and barley (LIU, 2011), has a high protein content (SCHINGOETHE et al., 2009), and is relatively cheaper than other high-protein feeds (LEE et al., 2020). Other ingredients such as soybean hulls, oat hulls, ground corn, and wheat were also present in the concentrates, but in lesser proportions (Table 1). Five farms chose to use only one concentrate ingredient in the diet, and the concentrate ingredients used in 4 farms were not shared by the nutritionists.

3.4.2 Formulated and fed diet nutrient composition and variability

Average nutrient composition of the formulated and fed diets are presented in Table 2. It was observed that the levels of DM, CP, P, and S were all lower in the offered diets compared to the formulated diets. On the other hand, the offered diets had higher concentrations of fat, ADF, NFC, K, Fe, ash and NE_L compared to the formulated diets. The degree of agreement between the offered and formulated diets is presented in Table 3. Based on a CV of 5% as a reference for assessing the accuracy of the diets (SOVA et al., 2014), the fed diets did not accurately represent the formulated diets. Coefficients of variation greater than 5% were observed for all nutrients except NE_L (Table 3). The greatest variability between the offered and formulated diets was observed for Na, Cl, Ca, fat, and trace minerals, where the CV was greater than 15% (Table 3).

The offered diets exceeded the formulated diets for NFC, ADF, NDF, ash, fat, K, and NE_L, and were deficient for Mn, Zn, Cu, DM, CP, starch, Cl, Mg, Na, S, Ca and P (Table 3). Overall, although these differences were small, large variability was observed between farms (Table 3), suggesting different formulation strategies among nutritionists. The nutrient that was most overfed during the close-up period was Fe; similar results have been previously reported (CASTILLO et al., 2013; DUPLESSIS et al., 2021; SINCLAIR; ATKINS, 2015). Although the addition of Fe to mineral supplements for dairy cows is not common (DUPLESSIS et al., 2021), many feeds, especially forages, generally contain high concentrations of this mineral (CASTILLO et al., 2013).

The nutritionist formulated close-up dry cow diets an average of 14.4% CP, while the actual CP content of the sampled fed diets was 12.9%, indicating that most of the study farms were underfeeding CP relative to formulation. The discrepancy in the CP content of cows' diets during the close-up period may have a negative impact on future lactation performance. Researchers have demonstrated that feeding diets with CP levels between 14.6 and 15.1% CP during the close-up period was associated with better performance of cows in early lactation, as well as better metabolic status in the postpartum period (AKHTAR et al., 2021; FARAHANI; AMANLOU; KAZEMI-BONCHENARI, 2017). However, it should be noted that the total amount of protein consumed is potentially more important than the protein content of the diets

(VAN SAUN; SNIFFEN, 2014). Unfortunately, we were not able to record cow intake in this study to be able to compare actual total protein consumed to the formulated total.

The average visit-to-visit variability in the nutrient composition of the sampled fed diets is presented in Table 4. Similar to the variability between the formulated and offered diets, a CV greater than 5% was observed for almost all variables except NE_L. The highest visit-to-visit variability was observed for Na, Cl and microminerals, while the lowest variability was observed for DM, CP, ADF, NDF, NFC, K, ash, and NE_L (Table 4). A large variation in visit-to-visit variability between farms was also observed. Few studies have been done to address the precision and accuracy of diets fed to dairy cows, and the impact of this variability within herds remains poorly understood. Sova et al. (2014) evaluated the accuracy and precision of diets fed to lactating dairy cows in 22 commercial herds. In parallel with the results of our study, Sova et al. (2014) discovered that the diets fed to lactating cows did not accurately represent the diets formulated by nutritionists. In terms of dietary precision, although Sova et al. (2014) reported less day-to-day variability between the diets provided compared to the visit-to-visit variability observed in our study, the nutrients that varied the most in both studies were the same: Na, Cu, Fe, Zn, and Mn. In our study, NE_L was the nutrient with the lowest visit-to-visit variability, and the same result was reported by Sova et al. (2014) when assessing the precision of diets fed to lactating cows. Interestingly, when Rossow and Aly (2013) evaluated TMR samples collected weekly from close-up, fresh cow, and lactating cow pens, they demonstrated that the greatest variation in TMR composition between visits occurred in the diet fed to cows during the close-up period. Similar to our findings, the CVs of the nutrients in the diets provided in that study were greater than 5%. This variability was attributed to contamination of the loads with ingredients from other diets, since close-up pen feeding is usually done in only one load (ROSSOW; ALY, 2013), and on many farms the close-up group is not the first group to be fed.

James and Cox (2008) proposed that precision refers to the consistency of the diet offered over the days and accuracy refers to how well the diet fed represents the formulated diet; based on that definition, it can be concluded that the diets offered to the cows during the close-up period on the farms studied did not meet the criteria of precision and accuracy. The lack of accuracy and precision in diets can be attributed to operator error, equipment failure, the order in which ingredients are loaded, as well as mixing errors and a lack of operator knowledge of the variation that occurs within a silo (CARNEIRO; SANTOS; ALMEIDA, 2021; JAMES; COX, 2008; STONE, 2008). Bach (2024) also recently reported that TMR deviations may be due to errors in the weighing of one or more dietary ingredients. Although we did not record all of these details, it is recommended that this information is recorded in future studies. This will allow for a better understanding of the factors that most influence diet variability during the close-up period.

A limitation of this study is that there may have been a time lag between the diets formulation and sample collection for laboratory analysis. During this time, some ingredients may have changed nutritional value or even been replaced. In addition, formulation changes may have occurred a few days prior to our visit. Therefore, although we received the formulations from the nutritionists, and tried to match those to the diets we sampled, it is possible that the producers did not implement the changes immediately. As a result, the feed samples collected may not have represented the formulation proposed for the day of the visit. Despite this limitation, our results highlight the need to implement best practices in feed management, both in the process of mixing diets on farms and in monitoring the nutritional composition of the ingredients used in close-up diets. Regardless, proper guidance of feeding personnel, regular analysis of feed samples, premixing, and reformulation of diets in view of changes in DM can help reduce diet variability (STONE, 2008).

3.4.3 Associations between diet variability and fresh cow outcomes

The characteristics and metabolic and production outcomes of the early lactation cows that were sampled in this study are presented in Table 5. The associations of the variability between the diets fed and formulated during the close-up period and the herd-average outcomes at the beginning of lactation are presented in Table 6. Additionally, the associations of the visit-to-visit variability of the diets fed during the close-up period and the herd-average outcomes observed at the beginning of lactation are presented in Tables 7 and 8.

Regarding the variability between the fed and formulated diets, only the herd-average LHI was associated with the variability of any nutrient in the multivariate models (Table 6). Each 1-lactation increase in the herd-average parity of the sampled cows was associated with 0.32 points decrease in LHI (Table 6). The LHI was also associated with the herd-average DIM of the cows on the day of sampling; for each additional DIM, the LHI was increased by 0.17 units (Table 6). Furthermore, each percentage point (p.p) increase in NFC variability was associated with an 0.034 unit decrease in the LHI of the fresh cows (Table 6). This indicates that the lower the variability in NFC between the fed and formulated close-up diet, the better the liver health was of the fresh cows. It has been reported that overfeeding NFC during the dry period can result in increased lipid accumulation in the liver and greater ketogenesis, due to mobilization of body reserves (RICHARDS et al., 2020), as well as cause inflammation

(MCCARTHY et al., 2015). In addition, overfeeding NFC during the dry period can also lead to impaired insulin function (HOLTENIUS et al., 2003) and increased mobilization of adipose tissue in early lactation (MANN et al., 2015) in dairy cows. Alternatively, reducing energy intake during the dry period may help improve appetite and milk production after calving (JANOVICK; DRACKLEY, 2010), and thus may contribute to improved liver health.

The herd-average blood BHB concentration was associated with herd-average parity (Table 7), with each 1-lactation increase in herd-average cow parity was associated with a 0.16 mmol/L increase in BHB concentration. Each p.p increase in the fat visit-to-visit variability in the diets offered was associated with a 0.005 mmol/L increase in BHB concentration. Excessive dietary fat may increase energy intake during the prepartum period, leading to decreased DMI, which is associated with increased mobilization of triacylglycerol (TAG) from adipose tissue and accumulation of TAG in the postpartum liver (DRACKLEY et al., 2005; GRUMMER; MASHEK; HAYIRLI, 2004), resulting in the non-utilization of acetyl-CoA in the tricarboxylic acid cycle and the conversion of TAG to ketone bodies such as acetone, acetoacetate and BHB (DRACKLEY; OVERTON; DOUGLAS, 2001; KUHLA; METGES; HAMMON, 2016).

The visit-to-visit variability of NFC in the diets offered was also associated with herd-average blood concentrations of BHB and glucose after calving (Table 7). Each p.p. increase in the visit-to-visit variability of NFC in the diets offered was associated with a 0.013 mmol/L decrease in BHB concentrations. Meanwhile, cows tended to have a 0.010 mmol/L higher blood glucose concentration for each p.p increase in the visit-to-visit variability of NFC in the fed diets (Table 7). These results may seem counterintuitive; however, this could be related to the fact that NFC content of the fed diet was 4.5 percentage points higher than that formulated (Table 2). As discussed above, chronic overfeeding of NFC prepartum could result in greater fat mobilization and lesser DMI postpartum (HAISAN et al., 2021; KERWIN et al., 2023b), leading to higher BHB and lower blood glucose. Thus, more variability could have meant that farms were on many occasions, actually providing NFC levels closer to their formulated target, which could have translated into improved metabolic health.

Herd-average serum NEFA concentrations were associated with visit-to-visit variability in the CP content of the fed diets (Table 7), with each p.p increase in CP visit-to-visit variability associated with a 0.006 mmol/L decrease in NEFA concentration. Overall, the average CP content of the diets fed to the cows (12.9%) was lower than that of the formulated diets (14.4%; Table 2). Again, it possible that greater variability in dietary CP over time, would have meant that that would have been more opportunities for the target (formulated) CP level to have been achieved, and thus met the cows’ protein requirements to ensure proper energy metabolism

(Farahani et al., 2017; 2019). NEFA concentrations were also associated with the visit-to-visit variability of DM in the diets fed to the cows (Table 7), with each p.p increase in DM variability was associated with a 0.011 mmol/L decrease in herd-average NEFA concentration. The consistency of the dietary DM content may have affected the consistency of DMI, as lower DM% than formulated may have resulted in lesser DMI. In our study, the DM content of fed diets was lower (47.4%) than that of formulated diets (49.8%; Table 2). Thus, this again might suggest that with greater variability in DM content over time, cows may have often fed a diet closer to the formulation, and thus consumed more total DM in the close-up period, which is key for ensuring good metabolic health post-caving (PÉREZ-BÁEZ et al., 2019) and production (DRACKLEY; CARDOSO, 2014). Consistent with this, each p.p. increase in DM variability over time of the fed diets tended to be associated with 0.4 kg/d greater milk production up to 120 DIM (Table 8).

The visit-to-visit variability in fed dietary starch was associated with LHI (Table 7), whereby each p.p increase in variability tended to be associated with a 0.032 unit increase in fresh cow LHI. This would indicate a better LHI with greater variability in the starch content of the diets offered during the close-up period. This is surprising given that the diets offered showed considerable visit-to-visit variability in starch content, with CV's ranging from 3.3 to 31.2% (Table 4). Since the starch content of the fed diets was similar to the formulated diets (15.9 vs 16.4%; Table 2), it is not clear why this association with visit-to-visit variation was detected and further investigation is needed.

3.4.4 Study limitations

This study is observational in nature and was designed to identify associations between observations of dry cow diet variability (i.e. from formulation and over time) and the metabolic and production outcomes measured on the enrolled transition cows from each farm. Therefore, we were not attempting to infer causality, but rather to identify biologically plausible associations that may, and could, be explored in future studies. It is also important to note that the variability data in this study was based on diet samples collected every 4 wk, which may not have reflected the actual nutritional situation on the farm at the time of sample collection. For example, when Sova et al. (2014) evaluated the day-to-day variability of diets fed to lactating cows, they reported less variability in nutrients compared to our study. The lack of precision in the diets delivered can be attributed to operator error and equipment failure, as well as changes in the composition of the diets, and this may have been more pronounced in our study due to the longer time interval between collections. In addition, individual variability of each ingredient, errors in the sampling process, and batch preparation procedures, although not registered in our study, may also have contributed to the increased variability of the diets delivered.

3.5 CONCLUSIONS

Corn silage and straw were the predominant forage sources used in the close-up dry cow diets fed on commercial dairy herds in Ontario, while soybean meal and canola meal were the primary concentrate ingredients. High variability was observed between the diets formulated by the nutritionists and the diets actually delivered, highlighting the lack of accuracy in the delivery of most nutrients. The variability, both between fed and formulated and from visit-to-visit, in the diets fed during the close-up period were associated with the metabolic markers and production of early-lactation dairy cows. These results highlight the importance of consistency between diet formulations and actual feeding practices over time to optimize early-lactation dairy herd performance and health.

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Table 1 – The major ingredients (primary and secondary sources of forage and concentrate) used by each herd in their dry cow diet during the close-up period

Item	n	% of farms
Primary major forage source		
Corn silage[1]	25	67.6
Hay	1	2.7
Haylage	1	2.7
Straw[2]	9	24.3
Data not provided[3]	1	2.7
Secondary major forage source		
Corn silage	11	29.7
Hay	1	2.7
Haylage	2	5.4
Straw[2]	21	56.8
Soybean silage	1	2.7
Data not provided[3]	1	2.7
Primary concentrate ingredient		
Canola meal	7	18.9
Ground corn	1	2.7
Corn gluten meal	1	2.7
Mixed grain[4]	1	2.7
Oats hulls	2	5.4
Protein blend[4]	1	2.7
Roasted soybeans	2	5.4
Soybean hulls	4	10.8
Soybean meal[5]	14	37.8
Data not provided[3]	4	10.8
Secondary concentrate ingredient		
Beet pulp	1	2.7
Canola meal	5	13.5
Grain distillers[6]	5	13.5
Ground corn	2	5.4
Soybean hulls	2	5.4
Soybean meal[7]	9	24.3
Wet brewers' grains	2	5.4
Wheat[8]	2	5.4
Only one concentrate ingredient	5	13.5
Data not provided[3]	4	10.8

[1] Corn silage (n = 23); corn silage blend (n = 1); BMR corn silage (n = 1).
[2] Straw (n = 6); chopped straw (n = 2); wheat straw (n = 1).
[3] No ingredients were included in the diets sent by the nutritionists.
[4] Unreported composition.
[5] Soybean meal (n = 11); high bypass soybean meal (n = 1); hi-pro soymeal (n = 2).
[6] Corn distillers (n = 4); hi-pro distillers (n = 1).
[7] Soybean meal (n = 8); high bypass soybean meal (n = 1).
[8] Wheat middling (n = 1); wheat shorts (n = 1).

Table 2 – Descriptive statistics (mean, SD, min and max) of diets fed and formulated to dry cows during the close-up period on 37 commercial dairy farms

Variable (% of DM, unless otherwise noted)	n	Formulated diets[1]			n	Fed diets[2]			SE	*P*-value
		Mean ± SD	Minimum	Maximum		Mean ± SD	Minimum	Maximum		
DM, %	191	49.8 ± 5.97	40.7	62.9	197	47.4 ± 7.18	33.6	76.6	0.48	<0.001
CP	199	14.4 ± 1.40	9.5	16.8	201	12.9 ± 2.26	7.5	20.3	0.13	<0.001
Starch	153	16.4 ± 3.14	10.0	23.5	200	15.9 ± 3.64	7.3	29.9	0.26	0.141
Fat	145	2.86 ± 0.70	2.1	4.8	201	3.04 ± 0.96	1.4	6.8	0.07	0.048
ADF	180	29.1 ± 3.05	21.5	34.8	201	29.9 ± 3.82	17.1	44.1	0.25	0.026
NDF	186	45.3 ± 3.49	35.7	53.0	201	45.7 ± 5.17	27.5	70.5	0.32	0.420
NFC	164	28.9 ± 3.44	20.1	36.1	201	33.4 ± 4.66	18.7	48.4	0.31	<0.001
Ca	197	0.87 ± 0.46	0.4	1.9	201	0.86 ± 0.42	0.3	2.6	0.03	0.855
P	197	0.36 ± 0.05	0.3	0.5	201	0.35 ± 0.07	0.2	0.6	0.00	0.041
K	197	1.13 ± 0.17	0.9	1.6	201	1.22 ± 0.23	0.8	1.9	0.02	<0.001
Mg	197	0.45 ± 0.11	0.3	1.2	201	0.43 ± 0.12	0.2	1.0	0.01	0.076
Na	185	0.15 ± 0.13	0.03	0.7	203	0.15 ± 0.13	0.0	0.8	0.01	0.656
S	182	0.29 ± 0.09	0.1	0.5	203	0.26 ± 0.11	0.1	0.6	0.01	0.017
Cu (mg/kg)	137	22.0 ± 8.44	9.5	59.0	201	21.2 ± 10.2	4.9	65.2	0.74	0.440
Fe (mg/kg)	108	212 ± 59.2	106.0	343.9	201	282 ± 126.8	73.2	999.3	9.02	<0.001
Zn (mg/kg)	131	94.9 ± 53.7	39.1	427.9	201	90.4 ± 43.9	21.1	272.2	3.79	0.411
Mn (mg/kg)	137	87.0 ± 40.8	33.5	333.6	201	85.6 ± 36.6	23.6	252.8	2.99	0.734
Cl	165	0.51 ± 0.22	0.2	1.0	178	0.47 ± 0.23	0.2	2.4	0.02	0.060
Ash	95	6.60 ± 1.80	3.9	10.5	201	7.36 ± 1.27	4.6	11.2	0.13	<0.001
NE_L (Mcal/kg)	162	1.42 ± 0.07	1.23	1.57	201	1.49 ± 0.07	1.2	1.7	0.01	<0.001

[1] Average value of formulated diets over the 6 visits to each farm (between April and October 2022), as provided by the nutritionist of each farm in the study.

[2] Average value of the diets fed to the cows over the 6 visits to each farm (between April and October 2022), calculated according to the diet samples collected at each visit.

Table 3 – Level of agreement between diets fed and formulated to dry cows in the close-up period on 37 commercial dairy farms (fed and formulated at each visit)

Variable (% of DM, unless otherwise noted)	n	Difference[1] between fed and formulated			CV[2] between fed and formulated		
		Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum
DM, %	185	-2.3 ± 5.85	-15.6	21.3	7.0 ± 5.79	0.1	27.0
CP	197	-1.4 ± 2.05	-7.1	5.6	10.8 ± 8.30	0.1	45.6
Starch	150	-0.5 ± 3.61	-10.2	14.2	11.3 ± 9.88	0.0	43.9
Fat	145	0.14 ± 0.85	-1.2	3.5	15.6 ± 12.35	0.0	54.4
ADF	178	0.8 ± 3.86	-16.0	11.1	7.3 ± 6.55	0.1	42.3
NDF	186	0.7 ± 4.93	-21.5	20.0	5.7 ± 5.11	0.0	39.8
NFC	164	4.3 ± 5.00	-6.6	22.2	11.7 ± 8.94	0.0	43.2
Ca	195	-0.01 ± 0.28	-0.9	0.9	16.9 ± 13.19	0.0	57.0
P	195	-0.01 ± 0.07	-0.2	0.1	10.1 ± 9.52	0.0	56.7
K	195	0.09 ± 0.23	-0.6	0.7	11.2 ± 8.06	0.0	36.3
Mg	195	-0.02 ± 0.14	-0.9	0.5	14.4 ± 12.72	0.0	84.1
Na	185	-0.02 ± 0.09	-0.4	0.4	27.7 ± 23.53	0.0	130.1
S	182	-0.02 ± 0.07	-0.3	0.2	13.1 ± 11.61	0.0	63.1
Cu (mg/kg)	135	-2.7 ± 8.46	-49.0	43.5	20.0 ± 16.34	0.0	100.6
Fe (mg/kg)	108	58.0 ± 97.76	-122.5	399.7	23.6 ± 17.80	0.2	82.9
Zn (mg/kg)	131	-6.6 ± 51.14	-377.7	91.0	19.9 ± 16.11	0.1	111.7
Mn (mg/kg)	135	-6.9 ± 36.37	-260.1	85.0	16.5 ± 13.20	0.0	90.3
Cl	148	-0.04 ± 0.25	-0.5	2.1	24.7 ± 16.61	0.0	109.3
Ash	95	0.4 ± 1.47	-2.6	3.7	13.5 ± 9.70	0.1	40.1
NE_L (Mcal/kg)	160	0.08 ± 0.09	-0.2	0.4	4.7 ± 3.34	0.0	17.0

[1] Nutritional composition of the diet fed - nutritional composition of the diet formulated; negative values indicate a deficiency between the diet provided and the diet formulated by the nutritionist.

[2] CV = coefficient of variation = (SD between the average of the fed and formulated values at each visit / average of the values fed and formulated at each visit) × 100.

Table 4 – Visit-to-visit variability in the nutrient composition of diets fed to dry cows during the close-up period across the visits conducted on 37 commercial dairy farms

Variable (% of DM, unless otherwise noted)	Mean[1]	SD[2]			CV[3]		
		Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum
DM, %	47.4	3.2 ± 1.65	1.2	9.3	6.8 ± 3.18	2.0	14.8
CP	12.9	1.2 ± 0.69	0.3	3.8	9.6 ± 5.75	2.2	27.6
Starch	15.9	1.9 ± 1.07	0.5	5.7	12.2 ± 6.76	3.3	31.2
Fat	3.04	0.5 ± 0.33	0.1	1.2	15.7 ± 10.56	1.9	53.5
ADF	29.9	2.0 ± 1.41	0.6	7.0	6.8 ± 4.39	1.9	22.7
NDF	45.7	3.0 ± 2.01	0.8	9.4	6.6 ± 4.18	2.1	20.6
NFC	33.4	2.9 ± 1.47	0.5	7.3	8.6 ± 4.47	1.4	21.0
Ca	0.87	0.1 ± 0.06	0.0	0.3	15.1 ± 7.30	3.8	38.7
P	0.35	0.0 ± 0.03	0.0	0.1	10.8 ± 7.15	2.9	28.3
K	1.22	0.1 ± 0.07	0.0	0.3	9.0 ± 4.41	2.5	18.4
Mg	0.43	0.1 ± 0.05	0.0	0.3	15.6 ± 8.56	4.6	38.4
Na	0.15	0.0 ± 0.04	0.0	0.2	29.0 ± 22.80	3.5	129.0
S	0.26	0.0 ± 0.02	0.0	0.1	12.3 ± 6.15	4.2	34.2
Cu (mg/kg)	21.2	5.7 ± 4.00	1.3	19.1	27.4 ± 14.78	6.9	58.8
Fe (mg/kg)	282.4	79.4 ± 59.72	16.3	303.5	27.0 ± 15.35	6.4	67.8
Zn (mg/kg)	90.4	20.1 ± 12.04	6.0	53.2	23.1 ± 11.29	6.6	45.9
Mn (mg/kg)	85.6	17.3 ± 12.00	2.5	51.6	20.1 ± 11.66	2.9	55.6
Cl	0.47	0.1 ± 0.15	0.0	0.9	18.1 ± 17.71	4.6	110.2
Ash	7.36	0.7 ± 0.27	0.2	1.3	8.9 ± 3.47	3.5	16.2
NE_L (Mcal/kg)	1.49	0.0 ± 0.03	0.0	0.1	2.7 ± 1.90	0.8	9.2

[1] Thirty-seven commercial dairy farms were visited every 4 wk for a total of 6 visits (between April and October 2022); at each visit, fresh samples of the diet fed to the cows during the close-up period were collected and the data were averaged for each farm.

[2] Standard deviation (SD) over the 6 visits.

[3] CV = coefficient of variation: = (SD over the 6 visits/ average value over the 6 visits) × 100.

Table 5 – Descriptive statistics of cow characteristics and early lactation outcomes for fresh cows from 37 commercial dairy farms in Ontario, Canada

Variable	Cow level data			Farm level data		
	Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum
Parity	2.4 ± 1.55	1.0	9.0	2.4 ± 0.39	1.7	3.8
DIM	7.2 ± 4.25	0.0	14.0	7.2 ± 0.84	5.6	9.1
BCS	3.2 ± 0.45	2.0	4.5	3.1 ± 0.17	2.8	3.4
BHB (mmol/L)	0.7 ± 0.54	0.1	5.4	0.7 ± 0.15	0.5	1.1
NEFA (mmol/L)	0.5 ± 0.33	0.1	2.2	0.5 ± 0.10	0.3	0.7
Glucose (mmol/L)	3.1 ± 0.61	1.1	9.3	3.1 ± 0.18	2.8	3.6
Liver health index (LHI)[1]	0.0 ± 2.16	-14.2	6.3	-0.2 ± 0.63	-1.6	1.1
Milk yield up to 120 DIM (kg/d)	43.7 ± 9.54	13.8	73.2	43.2 ± 4.81	31.4	50.3

[1] LHI = [(Alb − μAlb)/σAlb] + [(Chol − μChol)/σChol] − [(Bili − μBili)/σBili], where Alb is albumin, Chol is cholesterol, Bili is bilirubin, μ = the overall sampling population mean, and σ = the overall sampling population standard deviation.

Table 6 – Multivariable linear model variability[1] factors between fed and formulated diets during the close-up period associated with the liver health index (LHI)[2] of fresh cows between the 6 visits

Variable	LHI[2]		
	β[3]	SE[4]	*P*-value
Intercept	-0.31	0.051	
Parity	-0.32	0.113	0.006
DIM	0.17	0.053	0.001
CV[5] NFC	-0.034	0.014	0.016

[1] Diet variability, coefficient of variation (CV) in the nutrient composition of diets fed and formulated for dry cows in close-up period between the 6 visits.

[2] LHI = [(Alb − μAlb)/σAlb] + [(Chol − μChol)/σChol] − [(Bili − μBili)/σBili], where Alb is albumin, Chol is cholesterol, Bili is bilirubin, μ = the overall sampling population mean, and σ = the overall sampling population standard deviation.

[3] Estimated regression coefficient.

[4] Standard error.

[5] SD between the average of the fed and formulated values at each visit / average of the values fed and formulated at each visit.

Table 7 – Multivariable linear model for visit-to-visit variability[1] factors in diets fed during the close-up period associated with blood variables in fresh cows

Variable	BHB			Glucose			NEFA			LHI[2]		
	B[3]	SE[4]	*P*-value	B	SE	*P*-value	β	SE	*P*-value	β	SE	*P*-value
Intercept	0.39	0.158		3.03	0.054		0.64	0.043		-2.57	1.142	
Parity	0.16	0.060	0.013	-	-	-	-	-	-	-	-	-
DIM	-	-	-	-	-	-	-	-	-	0.28	0.151	0.073
CV[5] Fat	0.005	0.002	0.007	-	-	-	-	-	-	-	-	-
CV[5] NFC	-0.013	0.004	0.005	0.01	0.005	0.091	-	-	-	-	-	-
CV[5] DM	-	-	-	-	-	-	-0.011	0.005	0.038	-	-	-
CV[5] CP	-	-	-	-	-	-	-0.006	0.003	0.042	-	-	-
CV[5] Starch	-	-	-	-	-	-	-	-	-	0.032	0.015	0.045

[1] Diet variability, coefficient of variation (CV) of visit-to-visit variability in the nutrient composition of the fed diets.

[2] LHI (liver health index) = [(Alb − μAlb)/σAlb] + [(Chol − μChol)/σChol] − [(Bili − μBili)/σBili], where Alb is albumin, Chol is cholesterol, Bili is bilirubin, μ = the overall sampling population mean, and σ = the overall sampling population standard deviation.

[3] Estimated regression coefficient.

[4] Standard error.

[5] SD between what was fed over the 6 visits / average fed value over the 6 visits.

Table 8 – Multivariable linear model for visit-to-visit variability[1] factors in diets fed during close-up period associated with milk production up to 120 DIM

	Milk (kg/d) up to 120 DIM		
Variable	β^2	SE^3	*P*-value
Intercept	32.1	5.05	
Parity	3.5	1.95	0.084
CV^4 DM	0.4	0.23	0.096

[1] Diet variability, coefficient of variation (CV) of visit-to-visit variability in the nutrient composition of the fed diets.

[2] Estimated regression coefficient.

[3] Standard error.

[4] SD between what was fed over the 6 visits/ average fed value over the 6 visits.

4. OVERALL CONCLUSION

- The studies presented in Chapters 2 and 3 of this thesis have highlighted important aspects of dairy cow management during the transition period and early lactation.
- Chapter 2 showed that the use of an ultra-diluted complex can have positive effects on udder and liver health, although it does not affect milk production.
- Chapter 3 showed that the diets fed to cows during the close-up period did not accurately represent the formulated diets. There was also a lack of consistency in the diets offered, suggesting that more attention needs to be paid to feeding cows during the close-up period.
- Our results provide valuable information for understanding the factors that influence the health and performance of cows in early lactation.

ATTACHMENT A

animals

MDPI

Article

The Performance and Metabolism of Dairy Cows Receiving an Ultra-Diluted Complex in the Diet during the Transition Period and Early Lactation

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Simple Summary: The transition period is a critical phase for dairy cows, characterized by significant metabolic changes that occur to prepare the cow for calving and future lactation. This study evaluated the effects of an ultra-diluted complex on the productive performance and metabolic profile of dairy cows during the transition period and early lactation. Thirty multiparous pregnant cows were blocked and randomly assigned to either a placebo control (CON) group or an ultra-diluted complex (UD) group. Cows were evaluated from 30 days prior to calving through 60 days in milk. The addition of UD to the cows' diet had no direct effect on the cows' dry matter intake, but the cows in the UD treatment had a higher dry matter intake relative to their body weight. The group receiving UD showed a trend toward lower somatic cell counts, indicating better udder health. In addition, a trend toward better liver health was observed for cows in the UD group. These results suggest that the use of UD may be beneficial for dairy cows when used during the transition period and early lactation.

Abstract: This study evaluated the effects of feeding an ultra-diluted complex to dairy cows during the transition period and early lactation. Thirty multiparous pregnant dairy cows were blocked and randomly assigned to either a placebo control (CON) group or ultra-diluted complex (UD) group. The CON group received a placebo (basal diet + 40 g/cow/day of expanded silicate), while the UD group received the ultra-diluted complex (basal diet + 40 g/cow/day of PeriParto Transição–RealH, composed of ultra-diluted substances + vehicle: expanded silicate). Cows were evaluated from 30 days before the expected calving date until 60 days in milk (DIM) for sample and data collection. Post-partum dry matter intake (DMI) was not affected by the treatment. Cows fed UD had higher DMI relative to BW. Feeding UD increased milk lactose content and decreased milk protein content. Cows fed UD had lower somatic cell counts in the third and fourth week of lactation. Cows fed UD showed a tendency for higher liver health index. Using UD during the transition period and early lactation may benefit liver and udder health of dairy cows with no detrimental effect on milk performance.

Keywords: homeopathy; liver health; periparturient; stress; udder health

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1. Introduction

Dairy cows' transition period (TP) is the interval comprising 3 weeks before up to 3 weeks after calving [1]. TP is characterized by significant metabolic changes that occur