The effect of feeding whey permeate on production and rumen conditions in lactating Holstein dairy cows

By

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS 5

LIST OF ABBREVIATIONS 7

FOREWORD 9

CONTRIBUTION OF AUTHORS 10

LIST OF TABLES 11

LIST OF FIGURES 12

CHAPTER 1. GENERAL INTRODUCTION 13

CHAPTER 2. LITERATURE REVIEW 16

2.1 Introduction 16

2.2 Fermentation and Microbial Function 17

2.2.1 Fermentation of WP 17

2.2.2 VFA and Microbial Profile 18

2.2.3 VFA Production Rates 20

2.2.4 Microbial Protein Synthesis 21

2.2.5 Effects of WP on DMI 22

2.3 pH regulation 23

2.4 Ruminal Acid-Base Balance 24

2.5 Subacute Ruminal Acidosis (SARA) 24

2.6 Impacts of WP on Milk Production and Composition 25

2.6.1 Milk yield 25
2.6.2 Milk fat and fatty acids 26
2.6.3 Effect of WP on other Milk Solids 27
2.7 Conclusions 28
2.8 HYPOTHESIS AND OBJECTIVES 30
2.8.1 Hypothesis 30
2.8.2 Objectives 30
CHAPTER 3. The effect of feeding whey permeate on production and rumen conditions in lactating Holstein dairy cows 31
3.1 ABSTRACT 31
3.2 INTRODUCTION 33
3.3 MATERIALS AND METHODS 36
3.3.1 Experimental Design, Animal Husbandry, and Dietary Treatments 36
3.3.2 DMI and Feed Chemical Composition Analyses 39
3.3.3 Milk Yields and Milk Analyses 40
3.3.4 Apparent total tract digestibility 41
3.3.5 Rumen pH, Rumen Fluid Analysis, and Rumination time 41
3.3.6 Urine and Fecal Sample Collection and Analyses 42
3.3.7 Blood Collection 43
3.3.8 Statistical Analysis 43

3.4 RESULTS 44
3.4.1 Dry matter intake and milk production 44
3.4.2 Apparent total-tract digestibility of nutrients 48
3.4.3 Rumen fermentation and pH 49
3.4.4 Blood metabolites, fecal and urine samples 51
3.5 DISCUSSION 53
3.5.1 Effects on Rumen Fermentation 53
3.5.2 Effects on DMI and Milk Composition 55
3.6 CONCLUSION 59
CHAPTER 4. GENERAL DISCUSSION 60
4.1 Overview 60
4.2 Implications of using WP on gut health 60
4.3 Limitations and future directions 61
4.4 Industry Applications 62
REFERENCES 64
SUPPLEMENTARY TABLES 73

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LIST OF ABBREVIATIONS

ADC: apparent total tract digestibility coefficient

ADF: acid detergent fiber

AIA: acid insoluble ash

ATP: adenosine triphosphate

BHB: β-hydroxybutyrate

BHBA: β-hydroxybutyric acid

BUN: blood urea nitrogen

CP: crude protein

DM: dry matter

DMD: dry matter digestibility

DMI: dry matter intake

DWP: dry whey permeate

ECM: energy corrected milk

FA: fatty acid

MUN: milk urea nitrogen

NDF: neutral detergent fiber

NE_L: net energy of lactation

NH_3: ammonia

peNDF: physically effective fiber

SCC: somatic cell count

SCFA: short chain fatty acid

TMR: total mixed ration

UUN: urine urea nitrogen

VFA: volatile fatty acid

WP: whey permeate

FOREWORD

This thesis was written in accordance with the University of Manitoba Faculty of Graduate thesis guidelines and follows the Journal of Dairy Science manuscript style format. It consists of an abstract, introduction, materials and methods, results, discussion, and conclusion. At this time, it has not been submitted for publication. Referencing format follows the requirements of the Journal of Dairy Science.

CONTRIBUTION OF AUTHORS

M Buckler: Sample and data collection, result interpretation, preparation of the manuscript, subsequent editing; **G Dallago:** preliminary and final statistical analysis**,** reviewing and editing of the manuscript; **H Derakhshani:** reviewing and editing of the manuscript; **JC Plaizier:** experimental design, result interpretation, and preliminary statistical analysis**; R Gervais:** fatty acid analysis and interpretation, reviewing and editing of the manuscript; **M Steele:** reviewing and editing of the manuscript.

LIST OF TABLES

Table 1. Ingredient and chemical composition (% DM) of the treatment groups 37

Table 2. The effect of feeding whey permeate on dry matter intake, milk yield, and milk composition in lactating Holstein dairy cows 46

Table 3. Effect of WP inclusion in lactating dairy cow diets on proportions of de novo and C16 milk fatty acids (g/100g of FA) 47

Table 4. Effect of WP inclusion in lactating dairy cow diets on apparent total tract digestibility coefficients of nutrients 48

Table 5. Influence of dry WP supplementation on ruminal fermentation products and pH 50

Table 6. Effects of dry WP supplementation on blood serum concentrations of metabolites 51

Table 7. Influence of dry WP supplementation on urine and fecal parameters 52

LIST OF FIGURES

Figure 1. Study outline, indicating date and time during each period that samples were taken. Figure generated using BioRender. 38

CHAPTER 1. GENERAL INTRODUCTION

Sourcing byproducts that can be utilized as a replacement for traditionally produced crops, such as barley can facilitate a sustainable dairy industry, supporting optimal performance while maintaining animal health status. As traditionally grown crops become more important to feed the growing human population, sourcing alternative feedstuffs to replace these starches is important in maintaining sustainability within the dairy industry.

Dairy cow diets require carbohydrates at inclusion rates of 60-70% as a primary energy source to support milk production and metabolic function (NASEM, 2021). It is recommended that starch components not exceed 30% dry matter (DM) basis of carbohydrates to avoid the incidence of metabolic disorders (Ravelo et al., 2022b). Partial replacement of starch with sugars to meet this carbohydrate requirement has been shown to improve milk fat, maintain ruminal pH, and improve feed digestibility (Broderick and Radloff, 2004; DeFrain et al., 2004; Broderick et al., 2008). Common sources of sugar products used in North America include citrus pulp, beet pulp, beet or cane molasses, and byproducts from cheese processing, such as whey permeate (WP) (Penner, 2015). The byproduct WP is yielded from the cheese making process through the ultrafiltration of whey. It is high in lactose (>75%), additionally containing 3-8% protein, 8-20% ash, 3-5% moisture, and <1.5% fat (Foegeding et al., 2011). This WP is highly digestible, and as such, presents an opportunity to partially replace the carbohydrate source from traditionally grown crops. The digestibility of feedstuffs, influenced by both source and processing can have a strong impact on ruminal pH. For example, corn has a digestibility rate of 72-89.9%, while barley ranges from 80.7-84.6%, and oats digest at rates of 92.7-94.0% within the rumen, with variability occurring due to processing and treatment (Gomez et al., 2016). However, feeding diets high in starch are highly associated with an increased risk of ruminal acidosis, with starches

that have a higher digestibility rate being associated with the highest acidosis risk (Plaizier et al., 2008; Gomez et al., 2016).

Previous research has demonstrated that increasing dietary sugar content improves rumen microbial efficiency, hence converting fermentable energy into microbial biomass which contributes towards maintaining ruminal pH (Penner et al., 2009; de Ondarza et al., 2020). By feeding rapidly fermentable carbohydrates, microbes are able to more effectively capture ruminally degradable N, due to preference over glucose as an energy source, in turn decreasing urinary urea N excretion (de Ondarza et al., 2017, 2020). Supporting this, numerous studies have shown that substituting sugars for starch in the diets of lactating Holstein cows does not cause a significant change in ruminal pH (DeFrain et al., 2004; Hoover et al., 2006; Broderick et al., 2008; Chibisa et al., 2015).

Ruminant diets are primarily digested by hydrolytic enzymes produced by microbes, resulting in the production of ammonia (NH_3), methane, carbon dioxide, and VFAs, such as acetate, propionate, and butyrate (Hua et al., 2022; Mahboubi et al., 2026). The composition of these VFAs may vary depending on the starch to NDF ratio within the diet (Dijkstra, 1994). VFAs are the primary drivers of milk synthesis and milk composition (Mahboubi et al., 2026). Acetate and butyrate support milk fat production (Urrutia et al., 2019), while an increased ratio of propionate to acetate is often linked to increased milk yield (Seymour et al., 2005). Feeding diets with higher levels of sugars has often been associated with increased butyrate production, leading to an increase in milk fat production (Oba, 2011). These observations suggest that replacing starch with sugar may alter ruminal fermentation patterns, thereby providing an opportunity to improve milk composition parameters.

The existing literature recommends dietary sugar in the form of WP at inclusion rates of up to 6% (Chibisa et al., 2015) and lactose at levels of up to 14.3% (DeFrain et al., 2004); however, few studies have comprehensively assessed responses across ruminal pH, VFA profiles, and fatty acid (FA) composition when feeding WP as a replacement for barley starch. Therefore, the objective of this study was to evaluate the effects of partially replacing barley starch with WP in isoenergetically and isonitrogenously formulated diets on milk composition, milk production, dry matter intake (DMI), ruminal fermentation capacity, and overall animal health.

CHAPTER 2. LITERATURE REVIEW

2.1 Introduction

Whey is a by-product of cheese and curd production, resulting in 9kg of wet whey generated per kilogram of cheese produced (Kosikowski, 1979). When whey undergoes the process of ultrafiltration, it is separated into WP and protein concentrate, with the latter consisting of lactalbumin and lactoglobulin (Lynch and McDonough, 1979; El-Shewy, 2016). There are high levels of lactose in WP (minimum of 75% on a DM basis), which is accompanied by nonprotein nitrogen, amino acids, vitamins, minerals and salts (Lynch and McDonough, 1979; Parashar et al., 2016). This nutrient profile generates an ideal concentrated feed additive for many agricultural species due to its sugar content, providing a readily fermentable source of energy. Whey composition can vary significantly dependent on factors such as milk source (cow, sheep, or goat), whey type (sweet or acidic), the type of cheese produced, lactation stage, animal diet, or season of the year (Favaro et al., 2025). There are two main types of whey including acidic and sweet whey; with whey permeate most commonly originating from sweet whey (Alsaed et al., 2013). Acid whey is obtained from the acidification of milk during the production of yoghurt, casein, and soft cheeses and has a pH of 3.57 to 4.34 (Alsaed et al., 2013; Deshmukh et al., 2024). Sweet whey on the other hand is made from rennet, or curd cheese formation and has a pH of 6.02 to 6.58 (Alsaed et al., 2013; Deshmukh et al., 2024).

The Canadian per capita cheese consumption has increased 12% from 2014 to 2024, rising from 13.59 kg to 15.59 kg respectively, with growth driven primarily from specialty cheese consumption (AAFC, 2025). Therefore, there is growing interest to identify innovative, economical, and ecologically sound methods of converting WP into a value-added product (O'Donoghue and Murphy, 2023). The primary uses of WP at present include animal feed,

fertilizer, or utilization by the food industry (Favaro et al., 2025). There are many uses for WP in the food industry, including use in beverages, bakery applications, and confectioneries (Soumati et al., 2023). However, lactose utilization remains restricted in many products due to concerns involved with lactose intolerance for some consumers (O'Donoghue and Murphy, 2023). While these uses continue to be beneficial for the food industry, it also presents the dairy industry with a product high in energy and minerals, notably calcium, potassium, and sodium, with minimal restrictions (O'Donoghue and Murphy, 2023).

Whey is considered the dairy industry's most significant environmental pollutant, with waste accounting for over 50% of the total volume produced (Besediuk et al., 2024). Oftentimes, WP ends up being disposed of, in turn incurring additional costs for its disposal fee in landfills (Favaro et al., 2025). Processes such as anaerobic digestion and aerobic treatment are required to remove the organic load to allow for environmentally safe disposal (Azkarahman et al., 2025). The organic matter content of WP is high, hence leading to ecological concerns when spreading WP on fields or utilizing it as a fertilizer due to its high biochemical and chemical oxygen demand (Favaro et al., 2025). However, WP represents a significant amount of additional energy and minerals that are being disposed of (Besediuk et al., 2024), in turn providing an opportunity for the agricultural industry to reincorporate this byproduct back into the diets of animals.

2.2 Fermentation and Microbial Function

2.2.1 Fermentation of WP

The degradation of WP is faster than that of starches, resulting in an increased fermentation rate within the rumen (Ravelo et al., 2023). Starch is fermented at rates of 2-50%/h, dependent on grain type and processing method (Sniffen et al., 1992). On the other hand, sugars, such as

WP, are fermented much faster, at ≥300%/h. These differences in fermentation rate impact the location of digestion, allowing sugars to be more easily fermented within the rumen, and with some starches fermenting slower, hence passing through the rumen and into the small intestine (Sniffen et al., 1992; Huntington et al., 2006; Harmon, 2009). While the digestion rate of WP is faster than that of starch, it has been hypothesized that bacteria are able to store sugars as microbial glycogen reserves for later energy use, hence not impacting the rumen's pH immediately (Van Houtert, 1993; Penner and Oba, 2009). Increasing sugar content in the diet in the form of molasses, beet pulp, WP, or citrus byproducts can help to improve an animal's efficiency by providing a more readily available energy source for the microorganisms present and by stimulating rumen epithelial cells to absorb nutrients and process feedstuffs more effectively (Oba et al., 2015; de Ondarza et al., 2020; Penner, 2015).

2.2.2 VFA and Microbial Profile

A dairy cow's diet is primarily composed of concentrates and forages (Broderick and Radloff, 2004). Both feed types are vital for generating and maintaining fermentation processes by microbes in the rumen, which then generate VFAs as secondary products (Chambwe, 2019). There are five primary microorganisms present in the rumen that digest feedstuffs: bacteria, protozoa, viruses, archaea, and fungi (Hua et al., 2022). Bacteria are the main digestors of starch, and are responsible for converting it into glucose, then to pyruvate (Hua et al., 2022; Ricci et al., 2022). Pyruvate serves as an intermediate metabolite required for the production of three main VFAs: butyrate, acetate and propionate (Ricci et al., 2022). These VFAs produce up to 80% of the ruminant's total energy requirements, contributing towards milk production and growth (Mahboubi et al., 2026). During the production of VFAs, adenosine tri-phosphate (ATP) is generated, yielding energy for microbial growth and development (Van Houtert, 1993; Mahboubi

et al., 2026). Ruminal acetate synthesis occurs through the cleavage of pyruvate to acetyl-phosphate and formate, yielding acetate. Another pathway occurs through the breakdown of carbohydrates to pyruvate, which can be then converted to acetyl CoA, forming acetate (Van Houtert, 1993). Ruminal propionate synthesis occurs through the succinate and acrylate pathways. The succinate pathway begins with carbohydrates being catabolized to form pyruvate, which is then carboxylated using carbon dioxide to form oxaloacetate, subsequently reduced to malate (Van Houtert, 1993). These intermediates are then converted to succinate which is decarboxylated to propionate. The acrylate pathway instead occurs through the conversion of pyruvate to lactate, followed by the intermediates acrylyl CoA and propionyl CoA, which produce propionate (Van Houtert, 1993). Ruminal butyrate is primarily derived from acetate through two main pathways. In the first, two mol of acetate are converted to acetyl-CoA, to be then condensed to form 1 mol of acetoacetyl-CoA, which is further converted into butyrate (Van Houtert, 1993). The second pathway occurs through the carboxylation of acetyl-CoA to malonyl-CoA, which reacts with a second molecule of acetyl-CoA to produce acetoacetyl-CoA, subsequently forming butyrate (Van Houtert, 1993).

Protozoa compose 50% of the biomass of microorganism's present in the rumen (Newbold et al., 2015). Research has shown that protozoa help to stabilize the rumen and prevent pH and VFA fluctuations by feeding on bacteria and large starch particles (Denton et al., 2015). There are two main groups of protozoa present in the rumen: ectodiniomorphs and holotrichs, both of which being ciliate protozoa (Williams et al., 2020). Holotrichs are primarily present in the rumen of animals fed high non-structural carbohydrate-based diets, aiding in preventing sharp rumen pH fluctuations and hence, ruminal acidosis (Williams et al., 2020). On the other hand, entodinomorphs are primarily responsible for protein turnover in the rumen, referring to the

constant cycle of proteins being broken down into their component amino acids, then being replaced by brand new proteins (Newbold et al., 2015). These microorganisms digest starch by engulfing it and can later utilize that starch as an energy source (Hua et al., 2022). While the rate at which this process occurs varies depending on the starch type, the result is the same, with the production of acetate, butyrate, glycerol, hydrogen and carbon dioxide (Hua et al., 2022). Feeding diets high in starch from grain can alter the ruminal microbiome to favour amylolytic and lactic acid producing bacteria (Ravelo et al., 2023). As a result, diets high in rapidly fermentable carbohydrates, including sugars, may create conditions that limit fibrolytic activity if nitrogen availability becomes insufficient, as competing amylolytic populations increase their demand for available nitrogen (Hall, 2019).

2.2.3 VFA Production Rates

Lactose is hydrolyzed at a slower rate than sucrose or pure glucose, though this rate is still considerably faster than starch, meaning that it will not break down as quickly in the rumen compared to other types of sugar (Oba, 2011). The fermentation of WP occurs rapidly at rates of up to 300%/h; increasing overall VFA production within the rumen (Dijkstra, 1994). Sugar fermentation tends to favor butyrate (Ravelo et al., 2023), and as the production of butyrate increases, the molar proportions of acetate and propionate decrease in response (Hall, 2019). Rumen epithelial cells will favour the absorption of the three primary VFAs in the following order: butyrate > propionate > acetate (Seymour et al., 2005). Because rumen epithelial cells have been observed to favor butyrate over propionate and acetate (Seymour et al., 2005), this may allow for additional acetate and propionate to be available at a later time for metabolic function.

Feeding WP is associated with increased butyrate production in the rumen (Hall, 2019; Oba, 2011). Butyrate increases the development of ruminal papillae, thus increasing the absorptive surface area available for other nutrients (Hall, 2019). Butyrate can be absorbed both in the rumen and throughout the rest of the gastrointestinal tract (GIT), as it is preferably used by colonocytes as an energy source (Chen et al., 2025). Along with increasing absorptive surface area, DeFrain et al. (2004) conducted a study feeding lactose in place of corn at inclusion rates of up to 14.3%, and observed an increase in butyrate production, while the concentration of propionate decreased. Butyrate is a highly metabolizable FA, meaning it can easily be used by the animal as a precursor for *de novo* FA synthesis, occurring in the mammary gland (DeFrain et al., 2004). Because the molar proportions of acetate, propionate, and other lesser VFAs are altered when butyrate production increases, this will affect the number of protons generated, thereby maintaining pH (Oba, 2011). When hexose is fermented to propionate and acetate, two protons (H+ ions) are produced (Oba, 2011). However, when a hexose is fermented to butyrate, only one proton is generated. A reduced number of protons improves ruminal pH stability, thus minimizing severe pH fluctuations (Oba, 2011). Maintaining a stable ruminal environment is crucial for preventing metabolic disorders which can otherwise impact milk yield, along with fat and protein production.

2.2.4 Microbial Protein Synthesis

Increasing dietary sugar content has been associated with enhanced ruminal microbial protein synthesis (Broderick and Radloff, 2004). Sugars such as beet pulp, molasses, and WP have a faster ruminal degradation rate (0.4) compared to starch (0.10-0.35), providing a more rapidly available energy source for microbial growth (Dong et al., 2021). Consequently, microorganisms fermenting sugars have been reported to yield approximately 18% more microbial protein than

those fermenting starch (Broderick and Radloff, 2004). Microbial protein synthesis is the primary AA source for ruminants, generating over 50% of the AAs required for maintenance and milk protein synthesis (Lima et al., 2023). The improved synchronization of rapidly fermentable carbohydrates with ruminal N availability may enhance the capture of NH_3, thereby reducing nitrogen losses in the form of NH_3, blood urea nitrogen (BUN), and milk urea nitrogen (MUN) (Shahzad et al., 2024).

2.2.5 Effects of WP on DMI

Feeding WP as a replacement for starch has been shown to increase DMI in lactating dairy cows through numerous mechanisms, including increased palatability, improved ruminal fermentation, and enhanced nutrient utilization (Schingoethe, 1976; Penner and Oba, 2009; Bernard et al., 2020). However, the inclusion level at which WP will become limiting remains unclear. The positive effects of feeding WP on DMI appears to be more pronounced during mid-lactation, potentially supporting the elevated energy requirements associated with peak milk production during this stage (Bernard et al., 2020). Ensuring adequate DMI is particularly important to avoid negative energy balance status, allowing an animal during early lactation to reach the feed intake needs of peak milk production (Fenwick et al., 2008; Bernard et al., 2020). While the mechanisms behind an alteration in DMI are not fully understood, hypotheses include increased diet palatability, enhanced ruminal pH stability, or shifts in fermentation patterns favouring beneficial microbial activity (Bernard et al., 2020). Additionally, WP has also been related to reduced feed sorting due to its binding and sticky properties (Ravelo et al., 2022a). Diets containing over 30% DM starch can reduce ruminal pH, impair buffering capacity of the rumen and negatively affect forage digestibility, increasing the risk of ruminal acidosis (Ravelo et al., 2022a). Replacing a portion of dietary starch with alternative carbohydrate sources such as

sugar can help mitigate these effects by altering fermentation end products, microbial populations and subsequently ruminal pH fluctuations, thereby supporting a more stable ruminal environment (Broderick and Radloff, 2004; DeFrain et al., 2004). Collectively these impacts highlight WP as a dietary strategy to support feed intake, ruminal health, and milk production.

2.3 pH regulation

The rumen is highly adaptable to pH fluctuations; as diet composition changes, populations of bacteria, protozoa, and fungi shift in response, altering the ruminal ecosystem and subsequently affecting pH (Petri et al., 2013). However, the adaptive capacity of the rumen can be overwhelmed when diets are abruptly or substantially shifted towards a diet high in starch. Research has shown that the optimal ruminal pH for cellulolytic bacteria is approximately 6.5 (Shi and Weimer, 1992; Plaizier et al., 2008). Low pH can result in damage and inflammation of ruminal epithelial cells and of the large intestine, negatively affecting an animal's absorptive capacity, fiber digestion, and ruminal papillae function (Plaizier et al., 2008). A decrease in pH can also have major effects on ruminal conditions by altering the types of ruminal bacteria present (Plaizier et al., 2008). Rumen buffering capacity is primarily influenced by dietary physically effective fiber (peNDF) composition, subsequently impacting microbial populations and saliva production (Plaizier et al., 2008). When multiplied by total dietary NDF content, peNDF is considered to be the fiber particles that are retained within the 8 to 19 mm screens in a Penn State Particle Separator (Plaizier et al., 2008). The ratio of peNDF to starch content should ideally remain above 1.45 to avoid impacting ruminal buffering capacity (Zebeli et al., 2010). When rumen buffering capacity is decreased, ruminal pH subsequently reduces, leading to the development of acidosis (Plaizier et al., 2008). The low ruminal pH often experienced when feeding a high starch diet is typically attributed to an accumulation of VFAs and lactic acid,

resulting in reduced rumen buffering (Plaizier et al., 2008). Lactic acid production is particularly prevalent when the ruminal pH drops below 6.0 (Hall, 2019; Strobel & Russell, 1986). Due to these concerns, identifying an alternative rapidly fermentable carbohydrate source is important to mitigate metabolic disorders while still maintaining milk production. DeFrain et al. (2004) included WP at inclusion rates of 14.3% for 21 d, and noted no negative impact on ruminal pH, demonstrating that sugars are a promising alternative for starch. Meanwhile, a study by Penner et al. (2009) found that substituting sugars for starches increased ruminal pH, hence providing evidence that partially replacing starch for sugars may be a viable method of maintaining or increasing ruminal pH.

2.4 Ruminal Acid-Base Balance

The fermentation rate of carbohydrates in the rumen depends on whether its form is structural or non-structural (Ravelo et al., 2022b). Lactose is a disaccharide composed of one molecule of galactose and one molecule of glucose (Ravelo et al., 2022b). Unlike starch, lactose fermentation tends to favor butyrate production, which generates fewer protons during fermentation which may contribute to a more stable ruminal pH, preventing a rapid pH drop and in turn preventing the onset of rumen acidosis (Oba, 2011; de Ondarza et al., 2020; Ravelo et al., 2022b). Consequently, considerable research has investigated WP as a potential replacement for starch in the diet. Studies have demonstrated that WP can maintain milk production while supporting favourable conditions compared with conventional high-starch diets (Schingoethe, 1976; Penner and Oba, 2009; Chibisa et al., 2015).

2.5 Subacute Ruminal Acidosis (SARA)

Subacute ruminal acidosis (SARA) occurs when the ruminal pH drops to below 5.6 for over 3 hours a day, or below a pH of 5.8 for over 5 hours a day (Kim et al., 2022). This disorder

occurs due to an accumulation of VFAs and lactic acid produced by ruminal microorganisms when an animal consumes a diet high in rapidly digestible starches (Keum et al., 2024). While it is natural for the rumen pH to fluctuate due to feed and water intake, a consistently low pH occurring frequently due to improperly balanced diets or health concerns can also lead to a myriad of metabolic disorders, including SARA, rumenitis, laminitis and liver abscesses if not dealt with promptly (Plaizier et al., 2008; Elmhadi et al., 2022). These metabolic disorders in turn decrease production by affecting milk production, fat yields, and protein yields, decreasing these measures by up to 2.7kg/day, 0.3% and 0.12% respectively (Plaizier et al., 2008).

2.6 Impacts of WP on Milk Production and Composition

2.6.1 Milk yield

Milk synthesis is primarily influenced by lactose and glucose. Lactose is the most common carbohydrate found in milk and is synthesized by enzymes located in the Golgi apparatus (Akers, 2002). As the secretory vesicle membranes within the mammary gland are impermeable to lactose, its accumulation creates an osmotic gradient, drawing water into the lumen, contributing to an increasing milk volume (Akers, 2002; Lakstina et al., 2020). Glucose is the primary precursor for lactose synthesis, and therefore largely contributes to regulating milk production (Akers, 2002; McCarthy et al., 2020). In lactating cows, approximately 90% of glucose is derived from endogenous synthesis, with only a small proportion (10%) supplied from the diet (Beckett et al., 2025). Propionate serves as the primary gluconeogenic precursor, contributing approximately 60-74% of the ruminal gluconeogenesis required to support lactation (Hall, 2023; Wang et al., 2023). Approximately 80% of endogenous glucose production occurs in the liver, with the other 20% occurring in the kidneys (Beckett et al., 2025). From this production of glucose through gluconeogenesis, a lactating cow requires an average of approximately 72

grams of glucose to produce 1 kg of milk (McCarthy et al., 2020). Consequently, alterations in ruminal fermentation affecting proportions of propionate produced may influence gluconeogenesis, and in turn, milk yield (Miettinen and Huhtanen, 1996). However, responses in milk yield to shifts in VFA production may not always be consistent due to other factors such as DMI and dietary net energy (Seymour et al., 2005). Research has shown that partially replacing starch with sugar tends to increase butyrate production, while decreasing or not affecting propionate concentration (Vallimont et al., 2005; de Ondarza et al., 2017; Ravelo et al., 2023). The impact of sugar supplementation on milk yield remains inconsistent across literature, with the majority of studies reporting no significant effect on milk yield when partially replacing starch with sugar (DeFrain et al., 2004; Penner and Oba, 2009; Chibisa et al., 2015). Collectively, these findings suggest that the reduction or lack of impact to propionate is not enough to significantly impact milk yield (DeFrain et al., 2004; Chibisa et al., 2015).

2.6.2 Milk fat and fatty acids

Over half of the FAs in the milk of dairy cows are synthesized *de novo* from within the mammary gland, with the remainder originating from dietary sources, provided from absorption within the small intestine (Akers, 2002). These FAs can be broadly categorized based on their origin as preformed, *de novo*, or mixed (Akers, 2002). Preformed FAs originate from dietary sources and body fat, whereas *de novo* FAs are synthesized within the mammary gland from acetate and β-hydroxybutyrate, produced from ruminal fermentation (Benoit et al., 2024). Mixed FAs arise from a combination of these sources (Benoit et al., 2024). Milk contains over 400 major FAs, each with their own chemical and biological properties, and can be further classified into saturated, monounsaturated, and polyunsaturated FAs (Månsson, 2008; Djordjevic et al., 2019). Saturated FAs comprise the highest proportion of FAs in milk (approximately 60-70%),

followed by monounsaturated FAs (20-35%) (Djordjevic et al., 2019). These FAs can also be classified by chain length, with short chain fatty acids (SCFA, C4 to C14) synthesized *de novo* from mammary epithelial cells, mixed FAs (C16) originating from both *de novo* and sources circulating throughout the blood, and long chain FAs (≥ C18) derived from the bloodstream (Daley et al., 2022). The FA profile of milk is influenced by a variety of factors, including breed, parity, diet composition, farm management, stage of lactation, and dietary lipid composition (Hanuš et al., 2018). These variations are largely driven by shifts in *de novo* FA synthesis, biohydrogenation, and the mobilization of body fat reserves (Hanuš et al., 2018). Milk fat percentage has tended to increase with sugar supplementation across numerous studies, likely attributable to the associated increase in butyrate concentration due to its role as a precursor for *de novo* fatty acid synthesis (DeFrain et al., 2004; Broderick et al., 2008; Oba, 2011). Broderick et al. (2008) found that when supplementing sucrose at inclusion rates of 0%, 2.5%, 5%, and 7.5% that milk fat tended to linearly increase from 1.47 to 1.62 kg/d and milk fat percentage linearly increased from 3.81% to 4.16%. Sugar supplementation has also been found to alter fatty acid biohydrogenation pathways, shifting production away from the fatty acid C18:1 trans-10, which is associated with a depressed milk fat yield, towards cis-9, trans-10 promoting a normal biohydrogenation pathway (Oba, 2011; de Ondarza et al., 2017).

2.6.3 Effect of WP on other Milk Solids

Dietary N is necessary for animal maintenance and production and can be obtained through three sources: dietary intake, comprising rumen undegradable protein (RUP) and rumen degradable protein (RDP), microbial protein synthesis, and endogenous N (Kim and Lee, 2021). While RDP is degraded by ruminal microbes, RUP bypasses the rumen and is instead enzymatically digested in the abomasum and small intestine, supplying AA for absorption within

the blood (Kim and Lee, 2021). While rumen microbes that ferment sugar produce approximately 18% more microbial protein than those that ferment starch (Broderick and Radloff, 2004), this benefit is dependent on nitrogen availability as this becomes the limiting factor for microbial growth, potentially impacting microbial protein synthesis (Rosmalia et al., 2022). However, DeFrain et al (2004) found that lactose inclusion had no impact on milk protein when feeding lactose at 7.1% and 14.3% as a replacement for cornstarch. Chibisa et al. (2015) similarly reported no impact on milk protein when substituting corn or barley starch with lactose. These findings suggest that increased microbial protein synthesis does not necessarily translate to increased milk protein output, and that dietary nitrogen may not have been adjusted sufficiently to match the increasing fermentability of the sugar in the diet. Huhtanen and Hristov (2009) reported that crude protein (CP) digestibility is not a reliable predictor of milk protein yield, as competing metabolic demands and AA partitioning may influence the utilization of dietary N for milk protein synthesis. While Chibisa et al. (2015) found that milk protein tended to be higher when cows were fed with sugar replacing barley as opposed to a diet where sugar replaced corn, suggesting that the source of starch replacement may influence the fermentation environment and subsequent nitrogen use. Collectively, these findings suggest that sugar supplementation alone may not contribute to a sufficient increase in microbial protein supply to impact milk protein production. Therefore, changes to milk protein composition would likely be associated to differences in dietary N supply, rather than a direct effect of sugar supplementation (Huhtanen and Hristov, 2009).

2.7 Conclusions

Evaluating the effects of feeding WP provides insights into its potential as an alternative energy source to traditionally grown crops such as barley or corn. Understanding its impacts on

ruminal fermentation, nutrient digestibility, and lactation performance is essential to determining its suitability as a dietary energy source for lactating dairy cows. Identifying the differences between starch and sugar fermentation and how these influence VFA production and N utilization would provide insight into how this substitution affects animal health and production. In addition, reducing waste along with the associated disposal costs within the dairy and cheese industries presents an opportunity to valorize this byproduct while maximizing its nutritional and production benefits. While previous research has evaluated WP inclusion rates of up to 14.3%, caution has been recommended at inclusion rates above 7% due to concerns regarding decreased ruminal pH and metabolic health. Therefore, further investigation is required to determine the optimal inclusion rates of WP to clarify the mechanisms by which WP influences ruminal fermentation, nutrient utilization, milk production, and composition.

2.8 HYPOTHESIS AND OBJECTIVES

2.8.1 Hypothesis

The partial substitution of barley grain for dry whey permeate (DWP) will increase milk fat production and ruminal pH, while having no impact on milk components and yield, along with markers of metabolism such as milk urea nitrogen (MUN), milk β-hydroxybutyrate (BHB), ratios of VFAs, and blood BHB.

2.8.2 Objectives

Evaluate the impacts of partially substituting barley starch for whey permeate (WP) powder in isoenergetically and isonitrogenously formulated diets to quantify its impacts on:

i. Milk composition parameters
ii. Milk production
iii. Ruminal fermentation capabilities
iv. Total tract digestibility
v. Overall animal health

CHAPTER 3. The effect of feeding whey permeate on production and rumen conditions in lactating Holstein dairy cows

3.1 ABSTRACT

Feeding WP to dairy cows will reduce surpluses of solids non-fat in the Canadian dairy industry. Sourcing byproducts that can be used as a substitute for conventional crops, such as barley, will provide information on feed options when these conventional crops may become less available or accessible. The objective of this study was to evaluate the impacts of using dry WP as a replacement for starch in the diets of lactating Holstein cows. Dry whey permeate (DWP) was included in the diets of 12 lactating Holstein dairy cows at inclusion rates of 0, 4, 8 and 12% DM by replacing dietary barley starch in total mixed ration (TMR) diets. Body weights and body condition scores averaged 648kg ± 86.9kg and 2.97 ± 0.35 on a scale of 1 to 5. The TMR diets were iso-energetic and iso-nitrogenous and averaged 1.67 Mcal/kg NE_L and 15.5% CP (DM basis). Rumen fluid was collected by stomach tube prior to feed delivery and the reticulorumen pH was measured continuously using in-dwelling probes, summarized as average, minimum, and maximum ruminal pH. The inclusion of WP had no significant impact on DMI, milk yield, milk urea nitrogen (MUN), blood urea, milk protein, blood NEFA, and urine or fecal pH. There was also no noted impact on average, minimum, or maximum ruminal pH. Holstein cows fed DWP had a positive significant impact on milk fat %, increasing from 4.02% to 4.37%. Feeding DWP significantly increased butyrate in the rumen and beta hydroxybutyrate in the milk, increasing from 7.83 to 11.42% and 0.07 to 0.1 mmol/L respectively. This was also noted through the transport of beta hydroxybutyric acid in the blood, increasing from 0.72 to 1.38 mmol/L with increasing DWP supplementation. Furthermore, feeding DWP resulted in a significant linear decrease in blood glucose, quadratically decreasing from 3.54 to 3.13 mmol/L. The data from

this study demonstrates that DWP may be used in the diet of lactating Holstein cows reaching inclusion rates of up to 12% without showing negative impacts on milk production, ruminal health, and overall markers of metabolism, while providing beneficial impacts on milk fat production.

3.2 INTRODUCTION

Since 2014, Canada has seen cheese consumption increase by over 12% nationally (AAFC, 2025). This increase in consumption has resulted in an increase in byproducts being generated from the process of cheese manufacturing. For every kilogram of cheese produced, approximately nine kilograms of whey is generated (Parashar et al., 2016). In 2025, Canada produced over 500 tonnes of cheese, yielding over 4.5 billion kg of whey (GOC, 2026). At present, only about 50% of the WP produced by the dairy industry is being recycled and utilized by the industry, leaving a significant amount as a waste product (GOC, 2019). Sourcing uses for WP is essential in avoiding significant waste ending up in landfills and to contribute towards a more sustainable future (Das et al., 2016). The Canadian dairy industry has set a goal of achieving net-zero emissions by 2050, whereby the total greenhouse gas emissions produced will be equivalent to and hence offset by those removed from the atmosphere (DFC, 2023). Even more so, as Canada works towards the goal of net-zero emissions by 2050, continuing to contribute to these goals by reducing waste is crucial to meet the high standards of the Canadian dairy industry while maintaining a circular agri-food economy. Hence, it is vital for the industry to find a use for these byproducts to avoid excessive waste from being generated.

Carbohydrates are an important component of a ruminant diet as their digestion generates VFAs; however, protons are also produced during this process, and when accumulation occurs ruminal pH can drop into acidotic conditions (Oba, 2011). Barley is one of the most common sources of starch for cows in Western Canada due to the climates ideal growth conditions and high availability of the crop (Iqbal et al., 2012). While both WP and barley starch are forms of carbohydrates, their composition varies significantly. The chemical composition of WP is primarily lactose (>75%) and is highly fermentable within the rumen (Oba, 2011). On the other

hand, barley is a complex carbohydrate, meaning that it consists of many glucose molecules connected to one another, providing slower fermentation rates than WP, but faster than other sources of cereal grains, such as corn (Iqbal et al., 2012). In practice, WP is available in both solid and liquid forms. The liquid form of WP is most commonly used for large scale diet formulation to avoid the costs and labour associated with drying. However, this trial utilized dry WP to avoid potential variability in nutrient composition, often associated with liquid WP. With a large surplus of WP going unused in the dairy sector, this provides an opportunity to valorize WP and incorporate it back into the diets of lactating cows. Most studies at present investigate WP as a substitution for starch at inclusion at rates up to 6% (Chibisa et al., 2015), yielding a gap in the literature at inclusion rates above 6% on a DM basis. Moreover, findings from existing studies remain inconsistent, likely attributable to differences in WP inclusion rate, WP source, stage of lactation, lactation level, and dietary differences. One consistent finding across studies is the positive effect of sugar on ruminal butyrate production, which is often associated with increased milk fat synthesis (Chibisa et al., 2015; de Ondarza et al., 2017). In contrast, responses of other VFAs, such as acetate and propionate, appear to be more variable, with studies reporting inconsistent shifts in relation to butyrate. Characterizing how WP affects animal performance within parameters such as ruminal fermentation, milk production, and milk composition at incremental inclusion rates of up to 12% is required to contribute towards establishing clear inclusion guidelines. As such, the objective of this study was to evaluate the impact of partially substituting barley for dry WP in the diets of lactating Holstein cows at 0, 4, 8 and 12% on a DM basis. We hypothesized that partially substituting barley grain for DWP would improve milk fat production, while maintaining ruminal pH. We further hypothesized that WP would have no

impact on milk components and yield, along with markers of metabolism such as MUN, milk BHB, ratios of VFAs, and blood BHB.

3.3 MATERIALS AND METHODS

3.3.1 Experimental Design, Animal Husbandry, and Dietary Treatments

Experimental procedures for this study were approved by the Fort Garry Campus Animal Care Committee at the University of Manitoba (approval number F24-003). Eight primiparous and four multiparous lactating Holstein cows (body weight = 648 kg ± 86.9 kg) were used for this study. Cows were individually housed in tie stalls at the Glenlea Dairy Research Station at the University of Manitoba (Winnipeg, MB, Canada) with wood-shaving bedding and free access to water, in accordance with the guidelines set by the Canadian Council on Animal Care (CCAC, 2009). Cows were provided an opportunity for exercise three times per week for 1 h after morning milking and prior to feed delivery.

Cows were fed once daily at 0930 h, allowing for 5-10% refusals. Cows were randomly assigned to a treatment level in a 4 x 4 replicated Latin square design, and were blocked based on parity, days in milk (DIM), and milk production in the previous lactation. The experimental unit was each cow. The experimental periods each lasted 3 weeks, with sampling and data collection occurring during the third week. Treatments featured WP (sourced from Trouw Nutrition) replacing barley in the diet at increments of 0, 4, 8, and 12% on a DM basis (Table 1). Diets were formulated to be isoenergetic and isonitrogenous, with similar chemical compositions across treatments (Table 1). All cows completed all periods, however, one cow was excluded during the third and fourth period due to health complications associated with inflammation originating from the rumenocentesis site. Data from this cow was retained for periods 1 and 2.

Table 1. Ingredient and chemical composition (% DM) of the treatment groups

Item	**Treatment**			
	0%	**4%**	**8%**	**12%**
Ingredient composition				
Corn silage, %	34.0	34.0	34.0	34.0
Straw, %	0.53	0.53	0.53	0.53
Haylage, %	26.1	26.1	25.3	25.9
APF fat[1], %	1.7	1.6	1.7	1.7
Supplement[2], %	37.7	33.8	30.6	25.9
Whey permeate, %	0	4.0	8.0	12.0
Chemical composition				
DM, %	63.7	63.4	63.5	63.8
CP, % DM	16.0	16.2	16.8	15.8
ADF, % DM	32.1	34.7	30.3	29.5
NDF, % DM	34.5	33.6	33.2	30.8
Crude fat, % DM	4.8	4.4	4.5	4.3
NFC, % DM	38.0	38.3	39.0	40.7
Ash, % DM	0.02	0.02	0.02	0.02
Ca, ppm	81.3	89.8	92.7	95.9
P, ppm	33.7	37.2	38.9	40.5
Mg, ppm	34.4	38.1	36.2	35.7

Na, ppm	36.0	48.6	43.0	49.8
K, ppm	132.1	137.7	143.7	152.9
NE_L, Mcal/kg DM	1.57	1.58	1.59	1.58

[1]APF fat = Acidified palm fat

[2]Supplement: Barley (ground; 52.2%); canola meal (15.9%); corn distillers (13.6%); porcine meat meal (6.0%); GoldPass bypass soybean meal (3.45%); feather meal (3.0%); fat- Choice White- mix (1.59%), sodium bicarbonate (1.52%); salt (potash; 1.18%); Ground limestone (0.74%); magnesium oxide (0.47%); SG – MB Dairy Ultra Micro (36Grams; 0.38%); MHA methionine analogue – Novus (0.07%); Biopower SC (0.01%)

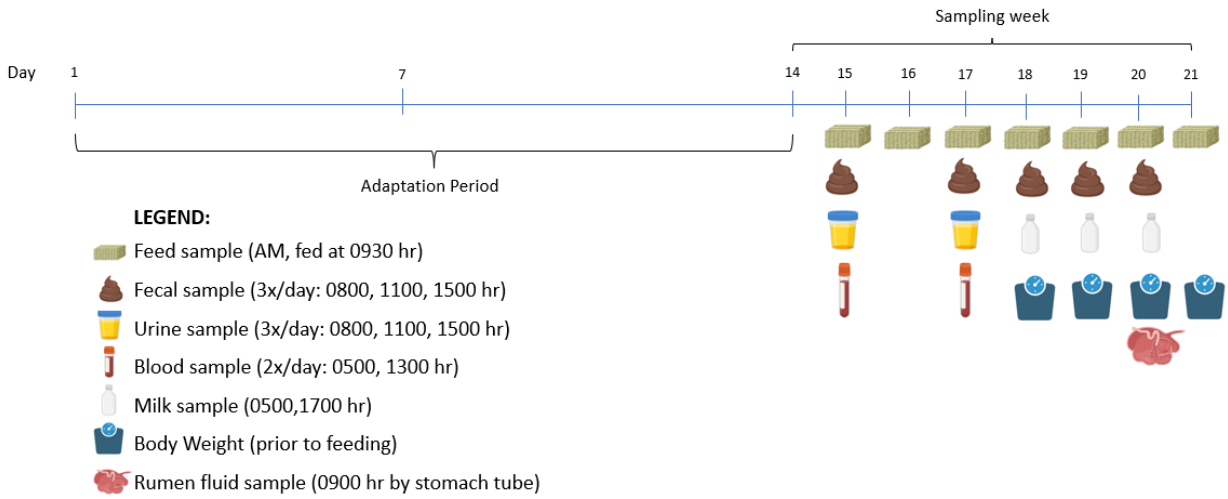

Figure 1. Study outline, indicating date and time during each period that samples were taken. Figure generated using BioRender.

3.3.2 DMI and Feed Chemical Composition Analyses

Samples of individual diets and orts were taken daily (Figure 1) throughout sampling days to be analyzed by wet chemistry. To calculate DMI, the DM concentration of the corn silage was determined twice weekly by drying the samples in a microwave for 4 minutes, then weighing the samples and drying at reduced intervals thereafter until the final sample weight did not change. Feed samples of individual diets and pooled diet samples were taken daily, along with each animal's orts. Samples were stored in a freezer at -20 ºC, later dried in a forced-air oven at 60 ºC for 72 h, then ground in a Wiley mill with a 1-mm screen (Thomas-Wiley, Philadelphia, PA). Animal DMI was calculated using wet and dry weights of orts and diet samples. Pooled diet samples were later analyzed for CP, acid insoluble ash (AIA), non digestible fiber (NDF), and acid detergent fiber (ADF). Samples were analyzed for DM and CP using AOAC method 990.03, as outlined by Horwitz and AOAC International (2006). The AIA was analyzed according to AOAC method 923.03 (AOAC 2005). NDF was analyzed using a neutral detergent solution, with α-amylase (Sigma No. A3306; Sigma Chemical Co., St. Louis, MO, USA) and sodium sulfite included in the procedure; while ADF was analyzed using AOAC method 973.18 (AOAC 1990). Both ADF and NDF were analyzed using the same Ankom 200 Fiber Analyzer. The macrominerals Ca, P, Mg, Na, and K were analyzed using inductively coupled plasma emission spectroscopy (AOAC method 968.08; AOAC 1990) and a Plasma Spectrometer (Thermo Jarrell Ash Corp., Grand Junction, CO, USA) following acid digestion. Net energy for lactation (NE_L) was calculated following NASEM, (2021) recommendations for dairy cows.

3.3.3 Milk Yields and Milk Analyses

Cows were milked twice daily at 0500 and 1700 h, with milk yield measured using a BouMatic milking system. Composite milk samples were pooled on a yield to weight ratio from the evening milking of d-17 to the morning milking of d-20 and placed into 50 mL vials containing bronopol preservative tablets. Milk samples were stored at 4 °C until all six milkings had been completed. Samples were later warmed to room temperature, vortexed, and aliquots were made based on a total daily milk yield proportion into 50 mL vials and stored at 4 °C. Samples were later analyzed by Horizon Laboratories (Winnipeg, MB, Canada) using a Milk-o-Scan 303AB mid-infrared analyzer (Foss Electric, Hillerød, Denmark) to quantify milk fat, protein, lactose, SCC, MUN, and BHB.

Milk fat was extracted and FAs were methylated using the method described by Rico et al. (2021). FA composition was analyzed with a gas chromatograph (7890A GC; Agilent Technologies Canada Inc., Mississauga, ON) using a 100-m CP-Sil 88 capillary column (0.25mm i.d., 0.20-um film thickness; Agilent Technologies Canada Inc.) and a flame ionization detector. The inlet and detector temperatures were 220°C and 230°C, respectively. The split ratio was 100:1, and the flow rate for hydrogen carrier gas was 1 mL/min. At the time of injection, the oven temperature was set to 100 °C and was held for 1 min, then increased by 5 °C per min to 220 °C, where it was held for 20 min. Most FA peaks were identified and quantified using either a quantitative mixture or pure methyl ester standards (Larodan Fine Chemicals, Malmö, Sweden; Sigma-Aldrich, Oakville, ON, Canada; Matreya LLC, Pleasant Gap, PA, USA; and Nu Chek Prep, Elysian, MN, USA). FAs for which standards were not available commercially were identified by order of elution according to Kramer et al. (2008). Energy corrected milk (ECM) was assessed using the following equation (Hall, 2023):

ECM (kg/d) = (0.327 × milk yield (kg/d)) + (12.95 × fat yield (kg/d)) + (7.2 × protein yield (kg/d))

3.3.4 Apparent total tract digestibility

The AIA, CP, and NDF concentrations of pooled diets and individual fecal samples were used to determine dry matter digestibility (DMD) and the apparent total tract digestibility coefficient (ADC) of CP and NDF. These were used as a marker for assessing digestibility using methods described by Mc Geough et al. (2010) and Van Keulen and Young, (1977).

The measures for DMD and ADC were assessed using the following equations:

$$DMD = 1 - \left(\frac{AIA\%\ DM\ in\ diet}{AIA\ \%\ DM\ in\ feces}\right)$$

$$ADC\ CP = 1 - \left[\left(\frac{CP\ \%\ DM\ in\ feces}{CP\ \%\ DM\ in\ diet}\right) \times \left(\frac{AIA\ \%\ DM\ in\ diet}{AIA\ \%\ DM\ in\ feces}\right)\right]$$

$$ADC\ NDF = 1 - \left[\left(\frac{NDF\ \%\ DM\ in\ feces}{NDF\ \%\ DM\ in\ diet}\right) \times \left(\frac{AIA\ \%\ DM\ in\ diet}{AIA\ \%\ DM\ in\ feces}\right)\right]$$

3.3.5 Rumen pH, Rumen Fluid Analysis, and Rumination time

Rumen pH and rumination time were continuously recorded at 10-minute intervals on a 24-hr basis using a MoonSyst pH bolus inserted into the reticulum. This data was summarized as average, minimum, and maximum pH. Subacute ruminal acidosis was classified as having >3 hrs/d spent below a pH value of 5.6, or > 5 hrs/d below a pH value of 5.8; as defined by Gozho et al. (2006). Rumen pH was measured by stomach tube at 0900 h on d-19. The pH was measured using a Fisher Scientific Accumet AB15 pH probe, calibrated using pH 4.0, pH 7.0, and pH 10.0 buffer solutions. Animals were contained using headlocks, then sampled using a stomach tube,

inserted orally with a speculum. Samples were pumped through the tube, then strained through three layers of sterile cheesecloth. Lastly, the pH was taken immediately following sampling. The first 250ml of the sample was discarded, with the following 50 mL being kept for VFA analysis. Additionally, 2 mL of rumen fluid was pipetted into tubes containing either 0.4 mL of 25% metaphosphoric acid or 0.4 mL of sulphuric acid (H_2SO_4) and kept at -20 °C for the future analysis of VFA and NH_3, respectively. The analysis of NH_3 was conducted using a Bio-rad model 3550 microphone reader spectrophotometer with methods described Novamsky et al. (1974). Volatile fatty acid (VFA) analysis was conducted using a 436 Scion gas chromatograph, with methods described by Erwin et al. (1961).

3.3.6 Urine and Fecal Sample Collection and Analyses

Urine was collected through perirenal stimulation on d-15 and 17. The pH values of these samples was taken immediately using a Fisher Scientific Accumet AB15 pH probe, calibrated using pH 4.0, pH 7.0, and pH 10.0 buffer solutions. Approximately 80mL of sample was taken from middle phase of urination, then stored at -20 °C until later analyzed for urea. Urine urea nitrogen (UUN) analysis was completed by the Veterinary Diagnostic Services Laboratory (Winnipeg, MB, Canada) using the Cobas C 311 analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

Fecal grab samples were collected from the rectum using hand palpation methods during d-15, 17, 18, 19, and 20 at 0800, 1100, and 1500 hrs. The pH values of these samples were taken immediately, and measured using methods as mentioned above, then frozen at -20 °C to maintain integrity. These samples were later dried in an oven at 60 ºC for 6 consecutive days and broken up with a mallet to increase surface area on day 3. Samples were then weighed and later further broken down with a mortar and pestle. Next, samples were pooled by animal and period, and

lastly, ground using a fecal grinder. These samples were analyzed for crude protein using AOAC method 990.03, as outlined by Horwitz and AOAC International (2006). The AIA was analyzed according to AOAC method 923.03 (AOAC 2005), and NDF was analyzed using α-amylase (Sigma No. A3306; Sigma Chemical Co., St. Louis, MO, USA) using an ANKOM 200 Fiber analyzer.

3.3.7 Blood Collection

Approximately 10mL of blood was taken into a vacutainer tube from the tail coccygeal vein on d-15 and 17 at 0500 and 1300 hr both days. Samples of serum were left to sit for 30-60 minutes to allow for clot formation before centrifugation at 3000 RPM for 15 minutes. Plasma was centrifuged immediately following collection at 3000 RPM for 10 minutes. Supernatant was collected proceeding centrifugation and stored at -20°C until the time of analysis. Serum sample analysis was performed by the Veterinary Diagnostic Services Laboratory (Winnipeg, MB, Canada). Concentrations of BHBA and NEFA were analyzed using the RX Monda analyzer (Randox Laboratories Ltd, Crumlin, UK). Additionally, concentrations of glucose and BUN were analyzed using the Cobas C 311 analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

3.3.8 Statistical Analysis

The statistical analyses were carried out using R version 4.4.2 (R Core Team, 2024). A 4 × 4 Latin square model with 3 replicates was used to analyze all variables with the following model:

$$Y_{kijl} = \mu + B_k + C_{i(k)} + P_j + T_l + e_{kijl}$$

where Y_{kijl} = observed response variable, μ = overall mean, B_k = effect of the replicate, $C_{i(k)}$ = fixed effect of cow nested within replicate for response variables without repeated measures or a

random effect of cow nested within replicate for response variables with repeated measures, P_j = fixed effect of period, T_l = fixed effect of treatment, e_{kijl} = residual error.

Mixed-effects models were fit using the *nlme* package in R and analyzed the variables with repeated measures (milk composition parameters, milk yield, and DMI; R Core Team, 2024). A linear model was used for variables without repeated measures (ruminal pH, urine pH, fecal pH, blood parameters, and VFAs). For variables with repeated measures, the autocorrelation structures compound symmetry and auto regressive of order 1 (AR1) were evaluated, and the structure that resulted in the lowest AIC value was selected. Statistical significance of treatment was declared at $\alpha < 0.05$, in which case linear and quadratic trends were assessed ($\alpha < 0.05$). Ruminal pH was log-transformed to address residual non-normality, and urine pH, fecal pH, and the ADC of DM and NDF were transformed using Box-Cox to address residual non-normality. For urine pH, DMI, and the ADC of CP, a mixed-effect gamma generalized linear model was used to analyze the data using the *glmmTMB* package in R, as the residual normality assumption was not observed with either log or Box-Cox transformations.

3.4 RESULTS

3.4.1 Dry matter intake and milk production

The impacts of dry WP supplementation on DMI and milk production parameters are depicted in Table 2. There was no significant impact ($P > 0.05$) of WP supplementation on DMI as well as milk yield (kg/d) and milk fat yield (kg/d). There was a positive linear effect of WP inclusion on percentage of milk fat ($P < 0.001$) and BHB ($P < 0.001$). On the other hand, a negative linear effect of WP inclusion was found for lactose ($P < 0.001$) and LOS ($P < 0.001$). Treatment had no influence on ECM, milk protein content, or MUN (Table 2).

Increasing WP inclusion increased the proportion of C16:0 FA by 7.9% from 37.5 to 40.5 g/d (Table 3). Individual *de novo* FAs C6:0, C8:0, C10:0, C10:1, C11:0, C12:0, C14:1 cis-11 exhibited a quadratic increase with increasing WP inclusion ($P < 0.05$). However, total *de novo* FAs were not impacted ($P > 0.05$). As the inclusion of WP increased, there was a linear increase ($P < 0.05$) in milk FA proportions of C10:1, C14:1 cis-9, C16 :1 cis-9, C16:1 cis-13.

Table 2. The effect of feeding whey permeate on dry matter intake, milk yield, and milk composition in lactating Holstein dairy cows

Variable[1]	Treatment				Significance	Contrasts[2]	
	0%	4%	8%	12%		Linear	Quadratic
DMI, kg/d	25.0±0.63	26.2±0.68	25.6±0.67	26.3±0.68	0.08	---	---
Yield, kg/d	41.2±2.28	43.8±2.4	43.4±2.4	37.5±2.3	0.18	---	---
Fat, %	4.02±0.13	4.22±0.14	4.23±0.14	4.37±0.14	0.01	0.002	0.72
Fat, kg/d	1.66±0.33	1.73±0.28	1.71±0.28	1.71±0.24	0.48	---	---
Protein, %	3.44±0.05	3.44±0.05	3.40±0.05	3.45±0.05	0.08	---	---
Lactose, %	4.68±0.04	4.68±0.04	4.66±0.04	4.64±0.04	<0.01	<0.001	0.14
SCC, 1000/ml	82.6±26.4	90.8±27.2	71.6±27.2	59.3±27.2	0.59	---	---
MUN, mg/dL	11.9±0.52	12.1±0.53	12±0.53	12.8±0.53	0.24	---	---
BHB, mmol/L	0.07±0.01	0.08±0.01	0.09±0.01	0.10±0.01	<0.01	<0.001	0.77
LOS, %	5.81±0.04	5.81±0.04	5.79±0.04	5.77±0.04	0.001	<0.001	0.16
ECM, kg/d	45.4±1.46	46.8±1.5	46.0±1.5	45.5±1.5	0.74	---	---

[1]LOS = Lactose and other milk solids; ECM = Energy corrected milk

[2]Linear and quadratic were preplanned contrasts testing the effect of increasing WP inclusion.

Table 3. Effect of WP inclusion in lactating dairy cow diets on proportions of *de novo* and C16 milk fatty acids (g/100g of FA)

Fatty Acid	Treatment				Contrasts[2]		
	0%	4%	8%	12%	SEM[1]	Linear	Quadratic
C4:0	3.320	3.184	3.277	3.500	0.137	0.280	0.176
C6:0	2.051	1.872	1.913	2.150	0.101	0.451	0.047
C8:0	1.159	1.035	1.049	1.223	0.070	0.501	0.038
C10:0	2.573	2.293	2.261	2.738	0.172	0.533	0.030
C10:1	0.237	0.227	0.234	0.276	0.024	0.036	0.048
C11:0	0.064	0.053	0.051	0.061	0.008	0.608	0.023
C12:0	2.961	2.743	2.666	3.228	0.186	0.358	0.034
C14:0	9.815	9.770	9.586	10.363	0.284	0.172	0.091
C14:1 cis-9	0.801	0.874	0.867	0.934	0.102	0.000	0.901
C14:1 cis-11	0.042	0.039	0.038	0.047	0.006	0.236	0.047
Total *de novo*	19.716	18.920	18.678	21.043	0.831	0.261	0.041
C16:0	37.547	38.814	39.544	40.496	0.365	0.000	0.521
C16:1 trans-9	0.039	0.036	0.037	0.042	0.005	0.578	0.418
C16:1 cis-9	1.439	1.612	1.684	1.680	0.088	0.010	0.185
C16:1 cis-11	0.029	0.031	0.034	0.031	0.002	0.255	0.119
C16:1 cis-13	0.104	0.107	0.104	0.129	0.015	0.050	0.183

[1]SEM = standard error of mean

[2]Linear and quadratic were preplanned contrasts testing the effect of increasing WP inclusion.

3.4.2 Apparent total-tract digestibility of nutrients

The inclusion of WP had no impact on the ADC of CP or NDF (Table 4; $P > 0.05$). Increasing WP inclusion linearly increased the ADC of DM from 69.8% to 74.5% ($P = 0.004$).

Table 4. Effect of WP inclusion in lactating dairy cow diets on apparent total tract digestibility coefficients of nutrients

ADC	Treatment				Significance	Contrasts[1]	
	0%	4%	8%	12%		Linear	Quadratic
DMD, %	69.8±0.01	71.9±0.01	72.8±0.01	74.5±0.01	0.04	0.004	0.84
CP, %	70.4±0.05	71.8±0.06	71.3±0.06	62.5±0.05	0.83	--	--
NDF, %	52.1±0.02	53.6±0.02	56.4±0.02	59.6±0.02	0.11	--	--

[1]: Linear and quadratic were preplanned contrasts testing the effect of increasing WP inclusion.

3.4.3 Rumen fermentation and pH

There was no significant impact of treatment on minimum, maximum, or mean ruminal pH ($P > 0.05$; Table 5). There was also no influence of treatment on total VFA concentration, acetate, isobutyrate, or valerate molar proportions ($P > 0.05$). Increasing WP supplementation linearly decreased propionate by 23.7% ($P = 0.006$). This was also reflected in the acetate: propionate ratio linearly decreased ($P < 0.001$). However, butyrate increased from 7.83 to 11.42 mmol/L with increasing WP supplementation ($P = 0.003$). Treatment caused a linear decrease in NH_3, decreasing 33.5% from 4.99 to 3.32 mg/dL ($P = 0.012$) and a negative linear effect on isovalerate ($P = 0.05$).

Table 5. Influence of dry WP supplementation on ruminal fermentation products and pH

Variable	Treatment				Significance	Contrasts[1]	
	0%	4%	8%	12%		Linear	Quadratic
Total VFA, mmol/L	72.4±3.84	79.5±4.44	72.9±4.21	76.0±4.21	0.61	---	---
Acetate, mmol/L	46.7±2.57	52±2.98	47.9±2.82	50.1±2.82	0.55	---	---
Propionate, mmol/L	15.2±0.76	15.1±0.88	12.3±0.84	11.6±0.84	0.006	<0.001	0.74
Isobutyrate, mmol/L	0.69±0.03	0.71±0.04	0.64±0.03	0.62±0.03	0.20	---	---
Butyrate, mmol/L	7.83±0.73	9.45±0.84	10.01±0.8	11.42±0.8	0.02	0.003	0.89
Isovalerate, mmol/L	0.883±0.05	0.92±0.05	0.787±0.05	0.732±0.05	0.05	0.013	0.36
Valerate, mmol/L	1.18±0.06	1.32±0.07	1.29±0.07	1.42±0.07	0.11	---	---
Acetate : Propionate	3.17±0.14	3.61±3.92	3.92±0.15	4.25±0.15	<0.001	<0.001	0.72
NH_3, mg/dL	4.99±0.38	3.14±0.42	3.59±0.42	3.32±0.42	0.012	0.017	0.06
Mean pH	6.41±0.02	6.41±0.02	6.40±0.02	6.41±0.02	0.96	---	---
Min pH	5.99±0.03	6.05±0.03	6.05±0.03	6.09±0.03	0.18	---	---
Max pH	6.81±0.02	6.76±0.02	6.78±0.02	6.75±0.02	0.41	---	---

[1]: Linear and quadratic contrasts were preplanned contrasts testing the effect of increasing WP inclusion

3.4.4 Blood metabolites, fecal and urine samples

Within the blood, (Table 6) WP had a quadratic effect on glucose (P = 0.009) with concentrations increasing to a maximum at 4% WP inclusion (3.54 mmol/L) before decreasing at higher inclusion levels (3.13 mmol/L). BHBA increased linearly (P = <0.001) from 0.72 to 1.39. mmol/L. Urea and NEFA were not influenced by WP inclusion in the diet (p > 0.05). WP inclusion had no significant impact on urine or fecal pH (P > 0.05; Table 7). Urine urea was impacted by increasing WP inclusion, decreasing linearly (P = <0.001) from 237 to 169 g/L (Table 7).

Table 6. Effects of dry WP supplementation on blood serum concentrations of metabolites

Variable[1]	**Treatment**				**Significance**	**Contrasts**[2]	
	0%	**4%**	**8%**	**12%**		**Linear**	**Quadratic**
Glucose, mmol/L	3.54±0.06	3.58±0.07	3.48±0.07	3.13±0.06	<0.001	<0.001	0.009
BHBA, mmol/L	0.72±0.11	0.79±0.11	0.88±0.11	1.39±0.11	<0.001	<0.001	0.13
Urea, mmol/L	4.39±0.11	4.4±0.12	4.65±0.12	4.73±0.11	0.098	---	---
NEFA, mmol/L	0.08±0.004	0.074±0.004	0.069±0.004	0.069±0.004	0.15	---	---

1: BHBA: beta hydroxy-butyric acid; NEFA = nonesterified fatty acid.

2: Linear and quadratic were preplanned contrasts testing the effect of increasing WP inclusion

Table 7. Influence of dry WP supplementation on urine and fecal parameters

Variable	Treatment				Significance	Contrasts[1]	
	0%	4%	8%	12%		Linear	Quadratic
Urine urea, g/L	237±8.87	198±9.51	186±9.51	169±8.87	<0.001	<0.001	0.28
Urine pH	8.08±0.01	8.08±0.01	8.08±0.01	8.06±0.01	0.36	---	---
Fecal pH	7.02±0.02	7.07±0.02	7.00±0.02	7.01±0.02	0.02	0.076	0.43

[1]: Linear and quadratic were preplanned contrasts testing the effect of increasing WP inclusion.

3.5 DISCUSSION

This study was conducted to evaluate the effects of substituting barley starch for DWP at inclusion rates of 0, 4, 8, and 12% (DM basis) on ruminal health, total tract digestibility, milk composition, and production. The inclusion of dry WP did not adversely affect ruminal pH, DMI, or milk production. Alterations in VFA profiles favoured butyrate production, resulting in an increased milk fat percentage. Additionally, total tract digestibility increased while ruminal NH_3 concentrations and urea excretion decreased, suggesting improved utilization of dietary nutrients. These results imply that feeding WP as a replacement for barley starch can yield positive outcomes without compromising production performance or rumen function.

3.5.1 Effects on Rumen Fermentation

The inclusion of WP had no impact on mean, minimum, or maximum ruminal pH in our study. While other studies (Penner and Oba, 2009; Penner et al., 2009) noted a positive impact of sugar inclusion in the form of sucrose, leading to increased ruminal pH, other studies also reported no impact on pH when feeding molasses (Broderick and Radloff, 2004; Hoover et al., 2006) or lactose (DeFrain et al., 2004; Chibisa et al., 2015). Similar to this study, Chibisa et al. (2015) found that the inclusion of 6% WP did not alter ruminal pH when directly replacing corn and barley starch. While within the range of this study at comparable inclusion levels, this supports the notion that WP may only impact ruminal pH at higher inclusion levels or under different dietary contexts. Both WP and starch provide energy to microbes; however, their digestion and absorption rates vary significantly (Sniffen et al., 1992; Hua et al., 2022). The digestion site and rate of degradation of starch can vary significantly depending on source, diet inclusion rates, and processing mechanisms, yielding digestion rates of 2-50% per hr (Sniffen et al., 1992). However, lactose is fermented much quicker at around 300% per hr (Sniffen et al., 1992). Due to this rapid

digestion rate, digestion site is often in the rumen, with minimal lactose exiting into the small intestine (Sniffen et al., 1992). In addition, the upregulation of VFA transport mechanisms modulated by butyrate facilitates absorption, preventing excessive proton accumulation and aiding in pH regulation (Penner, 2015). Collectively, ruminal pH remained stable across all treatment levels (0, 4, 8, and 12%), despite increasing diet fermentability, indicating that WP can replace barley starch at up to 12% of dietary DM without adversely affecting ruminal pH.

When protein is degraded within the rumen, NH_3 is a product that can be used by microbes alongside a fermentable carbohydrate source for microbial protein synthesis (Bach et al., 2005). However, in situations when digestible carbohydrate supply is limiting relative to nitrogen, an accumulation of NH_3 can occur within the rumen (Bach et al., 2005). Excess NH_3 is then absorbed and converted to urea in the liver for excretion (Weiner et al., 2015). This mechanism was observed in this study, as ruminal NH_3 decreased with increasing WP inclusion, UUN followed this trend and decreased with increasing treatment inclusion. These impacts may be related to the positive effect of WP inclusion on DMD, increasing 6.7%, which could increase the availability of fermentable energy for rumen microbes (Chibisa et al., 2015). The observed linear decrease in ruminal NH_3 and UUN, despite similar dietary CP across treatments, suggests improved utilization of ruminal NH_3. This may reflect improved synchronization of nitrogen and fermentable energy availability within the rumen. Increasing the fermentable carbohydrate supply may have supported microbial assimilation of NH_3, reducing its accumulation within the rumen and subsequent urea excretion (Broderick et al., 2008). If this NH_3 were to be used for microbial protein, it would thus be subjected to digestion in the small intestine, allowing for AAs to be available for absorption (Bach et al., 2005). Ondarza et al. (2020) suggested that dietary sugar improves nitrogen utilization by enhancing energy and nitrogen availability, hence

promoting microbial growth and protein synthesis. In contrast, Chibisa et al. (2015) described no effect of sugar inclusion when feeding a diet containing 6% WP on urine urea. Sugar fermenting organisms will provide 18% more microbial protein than starch fermenting organisms, yielding less NH_3 and urea produced from the process of deamination (Broderick and Radloff, 2004).

The rapid digestion rate of sugar fermentation increases the availability of fermentable energy, thereby enhancing its utilization by ruminal microbes (de Ondarza et al., 2020). Notably, a limitation exists in this study as total urine output was not measured to compare urea yield between treatment groups. Therefore, incorporating total urea output to accurately capture nitrogen utilization and excretion in relation to urea production would provide a clearer understanding of how WP impacts nitrogen utilization.

3.5.2 Effects on DMI and Milk Composition

The increasing inclusion of WP had no impact on DMI and consistently, milk yield also remained unaffected. The lack of effect of WP inclusion on DMI is reported by Chibisa et al. (2015); however, Defrain et al. (2004) report an increase in DMI. Sugars are a highly palatable feed ingredient (Penner, 2015); therefore, the lack of an effect of WP inclusion on DMI was not anticipated. The lack of a response in DMI is not readily apparent in these studies, and thus, the underlying mechanisms remain unclear. However, studies feeding alternative sugar sources, such as sucrose or molasses noted an increase in DMI (Broderick and Radloff, 2004; Penner and Oba, 2009), hence sugar composition may be factor contributing to DMI.

It was demonstrated that the inclusion of WP selectively increased molar proportions of butyrate, while decreasing propionate and having no impact on acetate. Although an increase in butyrate was expected to reduce the proportions of other VFAs, the observed decrease in propionate

production had no impact on milk yield. In support of this finding, Hoover et al. (2006) reported that replacing starch with sugar increases the molar proportion of butyrate while decreasing propionate - a shift consistent with the present findings. In conjunction with increasing butyrate concentrations, milk fat percentage was observed to increase linearly with increasing WP inclusion. Butyrate is a substrate for *de novo* FA synthesis, and as butyrate is catabolized to BHB by the ruminal epithelium, it is transported by the portal blood system to be used by peripheral tissues to provide energy for lipid synthesis (Wattiaux et al., 1997). Chibisa et al. (2015) and DeFrain et al. (2004) suggested that alterations to ruminal fermentation and microbial populations are the primary factors causing change in blood metabolites and milk composition, ultimately impacting milk fat synthesis. Butyrate has been shown to enhance ruminal papillae growth and development, thereby increasing epithelial surface area and absorptive capacity (DeFrain et al., 2006). In addition to these structural adaptations, butyrate also influences rumen epithelial function, moderating acid and glucose transport, and contributing to maintaining a stable ruminal pH (Oba et al., 2015; de Ondarza et al., 2020). The absence of a treatment effect on DMI, despite increased milk fat %, suggests that the butyrate produced to replace other VFAs may have improved nutrient utilization, supporting ruminal fermentation efficiency (DeFrain et al., 2006).

Blood markers of lipid metabolism, such as BHBA, were elevated (1.32 mmol/L) in the 12% WP inclusion treatment group, exceeding the threshold (1.2 mmol/L) of hyperketonemia (Rico and Barrientos-Blanco, 2024). Hyperketonemia is a metabolic disorder identified in cows exhibiting a negative energy balance and oftentimes leads to a decreased milk production (Duffield et al., 2009). However, Rico & Barrientos-Blanco (2024) suggested that it is important to consider factors other than BHBA to assess energy status, due to the many factors influencing animal

health and metabolism throughout their productive lifespan. These can include blood glucose and NEFA. Given the decreased blood glucose concentrations observed with increasing WP inclusion, and propionate being the primary driver of gluconeogenesis (Wang et al., 2023), the linear decrease in ruminal propionate production likely reduced gluconeogenic substrate availability, consequently decreasing blood glucose. Furthermore, as NEFA concentrations increase during negative energy balance (Adewuyi et al., 2005), and NEFA was not impacted by treatment in this study, this finding suggests that the cows' energy status was not impacted by WP inclusion, instead reflecting increased BHBA transportation due to additional BHB being produced in the rumen.

In this study, the inclusion of WP resulted in a positive quadratic trend on *de novo* SCFAs (C4 – C14) synthesis and an increased production of mixed FAs (C:16), suggesting an underlying optimal inclusion rate when using WP as a replacement for barley starch to improve milk fat %. Conversely, preformed FAs (> C18) showed a linearly decreased effect, indicating that there may have been a redistribution of the FA composition, favoring *de novo* FAs with increasing WP inclusion as blood NEFA concentration and milk fat (kg/d) were not affected. This shift in FA profile may reflect a greater reliance on mammary FA synthesis, rather than a reliance on circulating lipids. These findings, paired with WP causing no effect on ruminal pH, further imply that the altered FA profile when replacing starch for WP did not result in negative metabolic energy balance. Lastly, treatment diets were formulated isoenergetically with equivalent fat content, eliminating dietary fat supply as a confounding explanation for the observed milk FA changes.

Including additional analyses, such as total urine output would provide additional insights into whether the observed decrease in urine urea output reflected decreased ureagenesis or was

altered due to differences in urine volume. Furthermore, as VFAs were noted to change significantly, 16S RNA sequencing to provide additional insight into the impact of WP replacement on microbial populations, which may have influenced fermentation capacity and efficiency.

3.6 CONCLUSION

The inclusion of DWP as a replacement for barley starch at inclusion rates of up to 12% (DM basis) resulted in a linear increase in milk fat percentage, while having no significant effect on ruminal pH or DMI. The observed improvements in milk fat percentage can be attributed to the altered VFA proportions within the rumen, favouring butyrate. Elevated butyrate concentrations facilitated increased BHBA being transported throughout the blood, providing more energy to the liver to improve milk fat production. Milk FA profile accounted for a shift toward more mixed FAs when WP inclusion increased in the diet. As hypothesized, treatment had no impact on MUN or blood urea, and decreased rumen NH_3. Although microbial populations were not assessed, ruminal pH and production responses suggest that overall rumen function was not adversely affected. Increasing WP yielded an increased BHBA content within the blood, hence ketosis should be monitored when feeding WP. Our results suggest that WP can be utilized as a replacement for barley starch in the diets of lactating Holstein cows at inclusion rates of up to 12% without impacting milk yield, milk protein content, or ruminal pH.

CHAPTER 4. GENERAL DISCUSSION

4.1 Overview

Exploring WP as a replacement for barley in the diets of lactating Holstein cows provides an opportunity to reduce the waste generated by the dairy industry while sourcing a product to improve animal health and production. Sources of sugar and starch are vital for microbial function and proliferation within the rumen, providing glucose as the primary source of energy for microbes (Hoover et al., 2006). These microbes provide energy and the building blocks for numerous bodily functions, while digesting a variety of feedstuffs (Bickhart and Weimer, 2018). There are numerous sources of sugar available for feeding dairy cows to support these microbes, including molasses, WP, sucrose, or dextrose (Hoover et al., 2006). Given the surplus availability of WP within the industry (Besediuk et al., 2024), this byproduct was selected as an isoenergetic and isonitrogenous replacement for barley starch based on their comparable fermentable carbohydrate fractions and digestibility characteristics (Chibisa et al., 2015). This study aimed to analyze the impact of feeding WP as a replacement for barley starch in the diets of lactating Holstein cows at inclusion rates of 0, 4, 8, and 12% on a DM basis.

4.2 Implications of using WP on gut health

Utilizing WP as a replacement for traditionally grown crops, such as barley, has previously been shown to offer several benefits for feeding dairy cows, including increased palatability, improved milk fat production, and maintaining ruminal pH (Penner and Oba, 2009). Preventing health incidents, such as ruminal acidosis while maintaining milk production can prevent a plethora of associated health disorders from occurring, affecting the productive performance of cattle (Kim et al., 2022). Maintaining a balanced or increased ruminal pH is crucial in maintaining production and health (Plaizier et al., 2008). This study concluded that rumen pH was not

impacted by WP supplementation, even at the highest inclusion rate of 12%, and the minimum pH did not drop into ranges suggesting acidotic conditions (<5.6; Plaizier et al., 2008). While the reason lactose, or more specifically WP, had no impact on pH has not been confirmed, Ondarza et al. (2020) hypothesize that pH is not affected by the rapid digestibility for microbes, but instead that lactose is more likely to be converted into a form for energy storage, slowing ruminal fermentation and microbial use and preventing sudden pH fluctuations.

We observed that the inclusion of WP linearly increased butyrate production within the rumen, and hence BHBA transportation throughout the blood, leading to increased milk fat %. This might be attributed to the increased DM digestibility, making nutrients more readily available for microbes to digest. Chibisa et al. (2015) reported similar findings, noting that when using whey permeate as a partial replacement for barley or corn starch (6% DM basis), dry matter digestibility tended to increase linearly in both cases, along with an increase in milk fat %. In support, Daley et al. (2022) identified that the FA C16:0 is one of the primary drivers of milk fat production, which was noted to increase with increasing WP supplementation in this study.

4.3 Limitations and future directions

This study aimed to broaden the range of viable WP inclusion rates as a replacement for starch while maintaining animal health and production. It is important to note that animals in this study only received each treatment group for 3 weeks at a time. Thus, further research into the long-term impacts of feeding higher inclusion rates is warranted to ensure that health and production are maintained. Alongside this, identifying the impacts of feeding WP at other stages of production may provide interesting information about long-term use. Defrain et al. (2004) demonstrated that lactose at inclusion rates of 15.7 % (DM basis) as a replacement for corn grain did not depress the DMI in transition animals pre- or post-calving. Given that the present study

similarly observed no adverse effects on DMI or ruminal pH when increasing WP inclusion rates in lactating cows, future research is warranted to evaluate WP as a replacement for starch sources in the diets of dairy cows within other production stages to identify how this feedstuff supports energy balance and subsequent lactation performance.

Further research is warranted to identify the impacts of feeding WP in a liquid form, including how the associated storage methods impact quality and palatability, compared to feeding dry WP. The solids non-fat content of liquid WP requires frequent testing to ensure consistency and accurate diet formulation may be achieved before inclusion in the diet. Depending on accessibility, identifying practical solutions for both feeding strategies is essential in accommodating to the diverse production systems within the dairy industry, while establishing a framework for practical uses of WP. While these operational and financial hurdles can be justified, they are important factors to take into consideration when deciding to incorporate WP into the diets of lactating Holstein cows.

The results from this study provide a basis for future investigations regarding the impact of WP on ruminal epithelial health, along with the coincidental impacts of the VFAs produced due to the altered fermentation profile. Studying the permeability and resilience of ruminal epithelia may aid in identifying the impacts of increasing rates of lactose supplementation.

4.4 Industry Applications

With cheese demand and production projected to steadily increase (GOC, 2025), sourcing uses for WP is imperative to ensure that minimal waste is generated. Producing an increased milk fat percentage, without negatively impacting milk yield and DMI is commercially relevant within the dairy sector as milk fat % directly impacts production quota and producer compensation.

Collectively, the results of this study demonstrate that feeding WP as a substitute for barley starch can offer a nutritionally and commercially viable strategy for valorizing surplus WP generated by the cheese making sector while contributing towards an increasingly circular economy within the dairy industry.

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SUPPLEMENTARY TABLES

Supplementary Table 1. Effects of increasing WP inclusion in lactating dairy cow diets on proportions of saturated and monounsaturated long chain fatty acids (MUFA; g/100 g of FA)

Fatty Acid	Treatment					Contrasts	
	0%	4%	8%	12%	SEM[1]	Linear	Quadratic
C18:0	9.057	8.392	8.059	7.400	0.281	0.000	0.987
C20:0	0.168	0.156	0.143	0.134	0.006	0.000	0.748
C22:0	0.045	0.045	0.043	0.040	0.002	0.020	0.387
C24:0	0.027	0.026	0.024	0.023	0.002	0.070	0.997
C18:1 trans-4	0.027	0.024	0.022	0.020	0.002	0.005	0.757
C18:1 trans-5	0.024	0.023	0.021	0.020	0.002	0.030	0.868
C18:1 trans-6-8	0.267	0.261	0.248	0.204	0.015	0.003	0.192
C18:1 trans-9	0.249	0.251	0.242	0.206	0.013	0.018	0.142
C18:1 trans-10	0.391	0.452	0.479	0.344	0.057	0.627	0.073
C18:1 trans-11	0.860	0.772	0.686	0.590	0.066	0.004	0.949
C18:1 trans-12	0.356	0.355	0.327	0.263	0.022	0.002	0.142
C18:1 trans-15	0.632	0.619	0.649	0.547	0.053	0.313	0.375
C18:1 trans-16	0.236	0.226	0.218	0.196	0.007	0.000	0.320
C18:1 cis-9	17.257	17.347	17.321	15.524	0.566	0.035	0.087
C18:1 cis-11	0.925	0.956	0.958	0.836	0.054	0.191	0.098
C18:1 cis-12	0.375	0.389	0.380	0.320	0.022	0.074	0.088
C18:1 cis-13	0.066	0.063	0.069	0.055	0.005	0.180	0.283
C18:1 cis-15	0.049	0.049	0.046	0.039	0.004	0.039	0.270

20:1 cis-9	0.135	0.137	0.126	0.111	0.004	0.000	0.052
20:1 cis-11	0.057	0.059	0.057	0.046	0.003	0.013	0.023
22:1 cis-13	0.010	0.010	0.009	0.007	0.001	0.004	0.189
24:1	0.007	0.007	0.007	0.007	0.000	0.392	0.861
Sum iso FA	0.736	0.742	0.728	0.687	0.019	0.067	0.232
Sum anteiso FA	0.784	0.807	0.763	0.709	0.026	0.016	0.114
Sum odd FA	1.364	1.352	1.302	1.238	0.058	0.033	0.550
Summ OBCFA	2.884	2.902	2.793	2.634	0.075	0.000	0.081

[1]: SEM = Standard error mean

Supplementary Table 2. Effects of increasing WP inclusion in lactating dairy cow diets on proportions of polyunsaturated long chain fatty acids (PUFA; g/100 g of FA)

Fatty Acid	Treatment					Contrasts	
	0%	4%	8%	12%	SEM[1]	Linear	Quadratic
C18:2 trans-9, trans-12	0.017	0.015	0.015	0.013	0.002	0.086	0.897
C18:2 trans-9, cis-13 + 18:2 trans-8, cis-12	0.179	0.195	0.187	0.150	0.015	0.171	0.092
C18:2 cis-13, trans-8	0.094	0.105	0.098	0.082	0.006	0.106	0.027
C18:2 cis-9, trans-12	0.032	0.034	0.030	0.029	0.002	0.133	0.432
C18:2 cis-12, trans-9	0.031	0.033	0.029	0.027	0.002	0.056	0.214
C18:2 cis-9, trans-11	0.399	0.387	0.347	0.292	0.040	0.051	0.586
C18:2 cis-15, trans-11	0.045	0.046	0.040	0.035	0.004	0.045	0.461
C18:2 cis-12, trans-10	0.010	0.011	0.010	0.009	0.001	0.257	0.213
C18:2 cis-6, cis-9	0.056	0.054	0.049	0.050	0.003	0.032	0.593
C18:2 cis-9, cis-12	1.908	1.948	1.967	1.880	0.076	0.796	0.278
C18:3 n-3	0.232	0.266	0.267	0.268	0.010	0.003	0.040
C18:3 n-6	0.039	0.035	0.035	0.037	0.002	0.276	0.050
C18:3 cis-9, trans-11, cis-15	0.014	0.014	0.014	0.015	0.001	0.279	0.841
C18:4 n-3	0.008	0.010	0.009	0.009	0.001	0.485	0.192
C20:2 n-6	0.036	0.036	0.033	0.035	0.002	0.352	0.561
C20:3 n-9	0.007	0.007	0.007	0.007	0.001	0.680	0.553
C20:3 n-6	0.102	0.102	0.099	0.100	0.007	0.674	0.867
C20:3 n-3	0.008	0.009	0.008	0.008	0.001	0.195	0.557

C20:4 n-3	0.015	0.018	0.015	0.017	0.001	0.897	0.538
C20:4 n-6	0.126	0.123	0.125	0.128	0.005	0.664	0.543
C20:5 n-3	0.027	0.026	0.024	0.024	0.002	0.143	0.705
C22:2 n-6	0.008	0.007	0.008	0.007	0.001	0.279	0.442
C22:3 n-3	0.007	0.006	0.006	0.007	0.000	0.279	0.252
C22:4 n-6	0.025	0.024	0.023	0.023	0.002	0.462	0.721
C22:5 n-3	0.039	0.038	0.041	0.040	0.003	0.553	0.930
C22:6 n-3	0.007	0.008	0.008	0.008	0.001	0.698	0.417

[1]: SEM = Standard error mean