

**Production and Physiological Effects of Including Soybean Meal or Canola Meal in Dairy
Cow Diets from the Close-Up Dry Period to Mid-Lactation**

By

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Dedication

This dissertation is dedicated first and foremost to my mother and father, Christine and Richard Kuehnl. Furthermore, I dedicate this dissertation to those I have lost over the years since starting college. Aunt Janet and grandpa Pete- you are gone, but never forgotten. Lastly, I dedicate this dissertation to all of my other grandparents, my late great-grandma Adeline, and her late husband Frank. Frank- we never met, but the more I learn about you, the more I appreciate your love of agriculture and how proud you were of your cows. Thank you all for passing along this love of agriculture and dairy science. It is truly an honor to carry on our family tradition.

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Table of Contents

Dedication	i
Acknowledgements	ii
Table of Contents	iv
List of Tables	ix
List of Figures	x
Abstract	xi
Chapter 1. Literature Review	1
Introduction.....	1
Canola Background Information.....	1
General Information.....	1
Anti-Nutritional Factors.....	2
Canola Processing.....	3
Solvent and Expeller-Extraction.....	3
Heat Treatment.....	4
Cold-Pressing.....	5
Moist Heat Pressure	7
Chemical Treatment.....	8
Lignosulfonates.....	9
Chemical Composition.....	9
Source of RUP	11
DMI and Milk Production Effects: Middle and Late Lactation.....	12
Canola Meal Studies: 1970s to 1980s.....	12
Canola Meal Studies: 1990s to 2010	14
Canola Meal Studies: Meta-Analyses.....	16
Canola Meal Studies: 2011 to Present-Day	17
Feed Efficiency	18
Milk Composition, Component Yields, MUN, and SCC	19
Physiological Effects	21
Plasma Amino Acids.....	21

Plasma Hormones and Metabolites.....	23
Diet and Nutrient Digestibility.....	26
Microbial Protein Synthesis.....	27
Methane Production.....	28
The Transition Period and Early Lactation.....	30
The Transition Period.....	30
Methionine Supplementation.....	31
Early Lactation Canola Meal Studies.....	33
Conclusions.....	35
References.....	37
Chapter 2. Production Effects of Soybean Meal versus Canola Meal Fed to Dairy Cows during the Close-Up Dry Period and Early Lactation.....	50
Abstract.....	50
Introduction.....	51
Materials and Methods.....	54
Cows, Facilities, and Diets.....	54
Sampling and Analysis.....	55
Statistical Analysis.....	59
Results.....	61
Nutrient Composition of Diets and Penn State Particle Separator.....	61
Body Weight and Body Condition Score.....	62
Dry Matter Intake, Milk Yields, and Feed Efficiency.....	62
Energy Balance.....	63
Milk Composition and Component Yields.....	63
Interaction between Prepartum and Postpartum Diet.....	63
Discussion.....	63
Body Weight, Body Weight Change, and Body Condition Score.....	64
Prepartum Dry Matter Intake.....	65
Postpartum Dry Matter Intake and Milk Yields.....	66
Feed Efficiency.....	68

Milk Composition and Component Yields	69
Milk Urea Nitrogen.....	70
Log ₁₀ Somatic Cell Count.....	70
Interaction between Prepartum and Postpartum Diet	71
Conclusions.....	71
Acknowledgements.....	72
References.....	73
Chapter 3. Temporal Plasma Hormone and Metabolite Effects of Soybean Meal versus Canola Meal Fed to Dairy Cows during the Close-Up Dry Period and Early Lactation.....	89
Abstract.....	89
Introduction.....	91
Materials and Methods.....	92
Cows, Facilities, and Diets.....	92
Sampling and Analysis	94
Statistical Analysis.....	97
Results.....	98
Nutrient Composition of Diets.....	98
Triiodothyronine and Thyroxine.....	99
Glucose and Insulin.....	99
Growth Hormone and Insulin-Like Growth Factor 1	99
Discussion.....	100
Dietary Effect: Soybean Meal versus Canola Meal.....	100
Triiodothyronine and Thyroxine.....	100
Glucose and Insulin.....	103
Growth Hormone and Insulin-Like Growth Factor 1	104
Temporal Fluctuations: Close-Up Dry Period and Early Lactation.....	106
Conclusions.....	108
Acknowledgements.....	109
References.....	110

Chapter 4. Temporal Diet Digestibility and Microbial Protein Synthesis Effects of Soybean Meal versus Canola Meal Fed to Dairy Cows during the Close-Up Dry Period and Early Lactation	121
Abstract	121
Introduction.....	123
Materials and Methods.....	124
Cows, Facilities, and Diets.....	124
Sampling and Analysis	126
Statistical Analysis.....	130
Results.....	131
Nutrient Composition of Diets.....	131
Diet Digestibility.....	132
Urinary Excretion.....	132
Discussion	133
Diet Digestibility: Dry Matter Intake.....	133
Diet Digestibility: Total Digestible Protein and Lignin Content of Canola Meal	134
Diet Digestibility: Results Comparison	135
Diet Digestibility: Effect of Dry Matter Intake in other Experiments	137
Diet Digestibility: Temporal Fluctuations	138
Microbial Protein Synthesis: Rumen Undegraded Protein	138
Urinary Excretion (Microbial Protein Synthesis): Results Comparison.....	139
Urinary Excretion (Nitrogen): Results Comparison	140
Microbial Protein Synthesis: Potential Relationship with Dry Matter Intake	141
Microbial Protein Synthesis: Temporal Fluctuations	142
Conclusions.....	142
Acknowledgements.....	143
References.....	144
Chapter 5. Production and Physiological Effects of Soybean Meal versus Canola Meal Fed to Dairy Cows with High or Low Residual Feed Intake	162
Abstract	162
Introduction.....	163

Materials and Methods.....	165
Residual Feed Intake Screening.....	165
Cows, Diets, and Facilities.....	167
Sampling and Analysis	168
Statistical Analysis.....	173
Results.....	176
Nutrient Composition of Diets and Penn State Particle Separator	176
Comparison of Residual Feed Intake Status between Periods.....	176
Body Weight and Body Condition Score.....	176
Dry Matter Intake, Milk Yields, and Feed Efficiency	177
Milk Composition and Component Yields	177
Plasma Hormones and Metabolites.....	178
Urinary Excretion.....	178
Discussion	178
Residual Feed Intake Repeatability across Diets and Lactation Stages.....	179
Body Weight and Body Condition Score.....	181
Dry Matter Intake.....	182
Milk Yields	183
Feed Efficiency	184
Milk Composition	185
Milk Component Yields.....	187
Milk Urea Nitrogen and Log ₁₀ Somatic Cell Count	188
Previous Experimental Designs and Diet × Week Interaction.....	189
Plasma Hormones and Metabolites.....	190
Urinary Excretion.....	193
Conclusions.....	195
Acknowledgements.....	196
References.....	197
General Conclusions	216

List of Tables

Table 2.1. Ingredient composition of the far-off, close-up, transition, and lactating diets	76
Table 2.2. Chemical composition of diets	77
Table 2.3. Chemical composition of diet ingredients	78
Table 2.4. Penn State Particle Separator descriptive analysis	79
Table 2.5. Body weight, BCS, DMI, milk yield, feed efficiency, and energy balance	80
Table 2.6. Milk composition.....	87
Table 3.1. Ingredient composition of the far-off, close-up, transition, and lactating diets.....	116
Table 3.2. Chemical composition of diets	117
Table 3.3. Chemical composition of diet ingredients	118
Table 3.4. Pre- and postpartum plasma triiodothyronine, thyroxine, glucose, insulin, growth hormone, and insulin-like growth factor 1 concentrations	119
Table 4.1. Ingredient composition of the far-off, close-up, transition, and lactating diets.....	149
Table 4.2. Chemical composition of diets	150
Table 4.3. Chemical composition of diet ingredients	151
Table 4.4. Pre- and postpartum diet dry matter, organic matter, and crude protein digestibility	152
Table 4.5. Prepartum urinary excretion of purine derivatives and urea.....	156
Table 4.6. Postpartum urinary excretion of purine derivatives and urea	157
Table 5.1. Ingredient composition of the lactating herd, SBM, and CM diets	203
Table 5.2. Chemical composition of diets	204
Table 5.3. Chemical composition of diet ingredients	205
Table 5.4. Penn State Particle Separator descriptive analysis	206
Table 5.5. Comparison of residual feed intake status during the screening period and during the 10-week experimental period.....	207
Table 5.6. Body weight, BCS, DMI, milk yield, and feed efficiency.....	208
Table 5.7. Milk composition.....	212
Table 5.8. Plasma hormone and metabolite concentrations.....	214
Table 5.9. Urinary excretion of purine derivatives and urea	215

List of Figures

Figure 2.1. Body weight LSM for each treatment group by week of lactation	81
Figure 2.2. Dry matter intake LSM for each treatment group by week relative to parturition.....	82
Figure 2.3. Milk yield LSM for each treatment group by week of lactation	83
Figure 2.4. Energy-corrected milk LSM for each treatment group by week of lactation.....	84
Figure 2.5. 4% fat-corrected milk LSM for each treatment group by week of lactation.....	85
Figure 2.6. Feed efficiency (ECM/DMI) LSM for each treatment group by week of lactation ...	86
Figure 2.7. Milk log ₁₀ SCC LSM for each treatment group by week of lactation.....	88
Figure 3.1. Pre- and postpartum plasma triiodothyronine, thyroxine, glucose, insulin, growth hormone, and insulin-like growth factor 1 LSM concentrations for each treatment group by week relative to parturition.....	120
Figure 4.1. Diet dry matter digestibility LSM for each treatment group by time.....	153
Figure 4.2. Diet organic matter digestibility LSM for each treatment group by time	154
Figure 4.3. Diet crude protein digestibility LSM for each treatment group by time	155
Figure 4.4. Urine volume LSM for each treatment group by time	158
Figure 4.5. Intestinal microbial N flow LSM for each treatment group by time	159
Figure 4.6. Urinary urea N LSM for each treatment group by time	160
Figure 4.7. Total urinary N LSM for each treatment group by time	161
Figure 5.1. Body weight LSM for each treatment group by week of experiment	209
Figure 5.2. Body condition score LSM for each treatment group by week of experiment	210
Figure 5.3. Dry matter intake LSM for each treatment group by week of experiment	211
Figure 5.4. Diet × week interactions for milk yield, feed efficiency (ECM/DMI), milk fat %, protein %, total solids %, milk protein yield, lactose yield, and MUN	213

Abstract

Protein-rich feedstuffs are used in dairy cow diets to increase overall dietary crude protein to maximize production. Traditionally, soybean meal (SBM) has been the primary protein source utilized in the United States. However, increasing knowledge of the positive production effects when substituting canola meal (CM) for other protein sources such as SBM is challenging this notion. While there is a long history of research demonstrating positive production effects during middle and late lactation, a scarcity of information exists for characterizing the effects of feeding CM during the close-up dry period and early lactation.

The primary objective of the first experiment was to determine the effect of feeding isonitrogenous diets with SBM or CM as the primary protein source during the close-up dry period and early lactation on production measures in Holstein dairy cows. Furthermore, we sought to characterize the effects on temporal plasma hormone and metabolite concentrations, diet digestibility, and urinary excretion of purine derivatives. Dry matter intake increased pre- and postpartum for cows fed the diets with CM compared to SBM. Additionally, cows fed the diets with CM tended to increase milk yield and decreased milk urea nitrogen compared to cows fed the diets with SBM postpartum. Plasma triiodothyronine concentrations decreased pre- and postpartum for cows fed the diets with CM compared to SBM, corresponding to increased milk yield. Diet digestibility decreased pre- and postpartum and intestinal microbial N flow decreased postpartum for cows fed the diets with CM compared to SBM.

The objective of the second experiment was to determine the effect of feeding isonitrogenous diets with SBM or CM as the primary protein source during mid-lactation on production and physiological measures in Holstein dairy cows determined to have high or low residual feed intake. Dry matter intake increased for cows fed the diet with CM compared to

SBM. A diet \times week interaction occurred for milk yield, with increased persistence for cows fed the diet with CM compared to SBM. Plasma triiodothyronine concentrations decreased and insulin concentrations increased for cows fed the diet with CM compared to SBM, corresponding to increased milk and milk protein yields.

Chapter 1. Literature Review

Introduction

Protein-rich feedstuffs are commonly used when formulating dairy cow diets to increase overall dietary crude protein (CP) concentration. This is due to forages and concentrates typically containing insufficient CP, in terms of amount and amino acid (AA) quality, for maximizing production. However, as protein-rich feedstuffs are expensive, determining which ones are advantageous in certain scenarios, i.e. when certain AA are limiting in the diet, is an important objective for dairy nutrition research.

Canola meal (CM) is one such option that has become more available to dairy producers in recent years due to increasing yields of canola seed. Traditionally, soybean meal (SBM) has been the primary protein source utilized in dairy cow diets in the United States for many years. However, increasing knowledge of the positive production effects when including CM in dairy cow diets, compared to other protein sources such as SBM, is challenging this notion. This literature review will explore published research pertaining to background information and processing methods for canola, which will be followed by production effects and physiological effects when comparing CM to other protein sources in dairy cow diets.

Canola Background Information

General Information

Canola is the second-most-traded protein feed worldwide and one of the most economically important crops for Canada. Western Canada serves as the primary production

region, yielding over 21 million metric tonnes of seed annually during the crop year 2017/2018. A portmanteau of “Canadian” and “oil,” canola is an offspring of the rapeseed species *Brassica napus* and *Brassica campestris/rapa*. Canola was bred to contain low concentrations of erucic acid (<2%) in the oil portion and glucosinolates (<30 $\mu\text{mol/g}$) in the meal portion (Bell, 1984; Canola Council of Canada, 2019), two anti-nutritional factors that were problematic in earlier versions of rapeseed. Prior to extraction, canola seeds contain approximately 44% oil. After extraction, the protein-rich byproduct, CM, is widely used in diets for livestock production (Canola Council of Canada, 2019). In other portions of the world, such as Europe, the term rapeseed is still commonly used in dairy nutrition, with “double-zero” (low erucic acid and low glucosinolate content) sometimes used as a distinguisher (Canola Council of Canada, 2019). When discussing processing, production, and physiological results later on, rapeseed and rapeseed meal (RSM) will refer to the “double-zero” version.

Anti-Nutritional Factors

Similar to other vegetable crops, rapeseed and canola contain various anti-nutritional factors for defense mechanisms. Before the discovery of a low-glucosinolate trait in the Polish rapeseed variety Bronowski in 1969, high glucosinolate content limited the use of RSM in dairy diets (Abadi and Leckband, 2011). Prior to genetic advances, rapeseed oil contained approximately 25-45% erucic acid and 50-100 μmoles of glucosinolates (Bell, 1993). Reducing glucosinolate content of rapeseed was critical, as glucosinolates yield breakdown products that are potentially goitrogenic, hepatotoxic, or pungent to ruminants (Fenwick, 1982). Goitrogenic compounds are of specific concern to dairy cows, as they reduce the availability of iodine, thus diminishing the synthesis of thyroxine, a hormone critical for the mechanisms of milk production

(Grenet and Journet, 1971). Furthermore, glucosinolates may negatively impact other processes in dairy cows such as reproduction (Mawson et al., 1994).

Beyond glucosinolates, three other anti-nutritional factors in rapeseed may have negatively affected its nutritional value. These compounds are sinapine, tannins, and phytic acid and comprised upwards of >10% of RSM on a dry matter (DM) basis (Bell, 1993). While still present in contemporary CM, these compounds typically comprise only 4.8-6.3% of the meal (Canola Council of Canada, 2019). Sinapine contains a flavor that is bitter to humans and may affect feed intake for livestock. However, Lee et al. (1984) found that glucosinolates have a more adverse effect on palatability and feed intake in pigs than sinapines. Tannins interfere with digestive enzymes, particularly those associated with protein hydrolysis. Despite this, no adverse effect of rapeseed tannins on alpha-amylase activity, an enzyme potentially inhibited by tannins, or other responses was found in broiler chicks (Yapar and Clandinin, 1972; Mitaru et al., 1982; Mitaru et al., 1983). Phytic acid is the main reservoir of phosphorus in canola seeds and can potentially bind essential minerals such as Mg, Ca, Mn, Zn, and Cu (Likuski and Forbes, 1965). Canola meal contains approximately 1.22% total P, with 0.53% of the total P being phytate-bound. In comparison, the corresponding values for SBM are approximately 0.66% and 0.38%, indicating that CM contains nearly 40% more phytate than SBM (Bell, 1993).

Canola Processing

Solvent and Expeller-Extraction

The overall goal of processing canola is to maximize the nutritional quality of the meal fed to livestock. In Canada, 13 canola processing plants currently exist, with 12 using solvent

extraction and 1 using expeller extraction, also known as “double pressing” (Broderick et al., 2016; Canola Council of Canada, 2019). The process of solvent extraction, also known as “prepress solvent extraction,” typically includes the following steps: “seed cleaning, seed preconditioning and flaking, seed cooking, pressing the flake to mechanically remove a portion of the oil, solvent extract of the press-cake to remove the remainder of the oil, desolventizing and toasting of the meal, and drying and cooling of the meal” (Canola Council of Canada, 2019). Conversely, expeller extraction follows a similar process up to the point of solvent extraction, after which the steps of solvent extraction, desolventization, and drying and cooling are excluded. Canola meal resulting from expeller extraction typically contains 8-12% oil, a 3 to 4-fold increase compared to solvent-extracted CM (Canola Council of Canada, 2019). While solvent and expeller extraction are the means of commercial CM production in Canada, other methods have been explored over the years, including cold-pressing, moist-heat pressure, chemical treatment, and lignosulfonate treatment. Given its integral role in the solvent extraction process, the nutritional effects of heat treatment on canola will be discussed first, followed by the aforementioned other methods.

Heat Treatment

Utilizing temperatures typically around 125-145°C for a duration of 10-30 minutes, heat treatment is an effective method for altering the nutritional composition of CM. (McKinnon et al., 1991; McKinnon et al., 1995; Jones et al., 2001). McKinnon et al. (1991) determined in situ DM and crude protein (CP) disappearance decreased after heating CM at 125 or 145°C for 10, 20, and 30 minutes compared to control, with the largest decreases at 30 minutes as expected. However, a marked increase occurred for acid detergent insoluble N (ADIN) at 145°C, indicating significant heat damage of protein. Furthermore, in situ 12- and 24-hour DM and CP

disappearance in both the intestines and total tract was shown to decrease for CM heated to 145 compared to 125°C for 10 to 30 minutes (McKinnon et al., 1995). Similar to McKinnon et al. (1991), a marked increase was observed for ADIN, suggesting extensive heat damage. After heating samples to 90°C for 20 minutes, Folawiyo et al. (1997) observed a moderate, irreversible change in the surface hydrophobicity of rapeseed albumin (napin), a protein that constitutes approximately 40% of the total soluble protein in rapeseed (Murphy et al., 1989). Jones et al. (2001) reported that heating canola press-cake and CM to 125°C for 20 minutes decreased DM and CP degradability, with increased milk yield for primiparous cows fed the heated presscake versus control. However, milk yield of multiparous cows was unaffected. Using Mehraban sheep, Eghbali et al. (2011) demonstrated heat treatment of CM at 125°C for 20 minutes decreased the protein A and B1 fractions, increased the protein B2 fraction, and did not change ADIN, neutral detergent insoluble N (NDIN), or apparent CP digestibility compared to unheated CM. These results indicate heat treatment shifted a portion of CP digestion postruminally. Comparing temperatures of 90, 110, 130, and 150°C for 60 minutes, Micek et al. (2020) reported a linear decrease for effective ruminal CP degradation of canola presscake. Furthermore, ADIN was only increased at 150°C. Collectively, these results demonstrate the effectiveness of heat treatment for increasing the rumen undegradable protein (RUP) content of CM. However, temperature and duration of heating are important factors to consider for mitigating ADIN and heat damage of protein.

Cold-Pressing

Differing from expeller extraction in that seeds are not cooked prior to pressing and less pressure is used to maintain temperatures of 50 to 70°C (Leming and Lember, 2005; Kaldmäe et al., 2010), cold-pressing is a relatively new alternative to solvent and expeller extraction. The

chemical compositions of expeller-extracted (95 to 105°C) and cold-pressed rapeseed cake were compared in Leming and Lember (2005). On a DM basis, cold-pressing increased crude fat (19.4 vs. 12.2%), due to the lower pressure applied, subsequently decreasing CP (30.6 vs. 36.1%) due to dilution. Supporting the results of Leming and Lember (2005), Kaldmäe et al. (2010) found increased crude fat (15.8 vs. 11.1%) and decreased CP (33.2 vs. 36.3%) for rapeseed cake that was cold-pressed (50 to 70°C) compared to expeller extracted (100°C). Over the course of three experiments, Australian researchers explored the effects of cold-pressing CM (Heim and Krebs, 2018a; Heim and Krebs, 2018b; Heim and Krebs, 2018c). Heating CM to 100 versus 20°C increased the A fraction and decreased the B1 fraction, with no difference for the B2, B3, or C fractions of protein, ADIN, NDIN, or effective CP degradability (Heim and Krebs, 2018a). Subsequent testing of temperatures ranging from 20 to 180°C revealed linear increases for the A and B2 fractions and RUP, a linear decrease for the B1 fraction, and no difference for the B3 or C fractions of protein or ADIN of CM (Heim and Krebs, 2018b). Interestingly, while not significant, intermediate Maillard reaction products numerically decreased when increasing temperature from 20 to 180°C, suggesting increased late Maillard reaction products and heat damage at higher temperatures (Heim and Krebs, 2018c). Given its infantile status in dairy nutrition, lack of widespread use, and commonly being assessed against ingredients not typically utilized in dairy diets, knowledge of the effects of cold-press processing on canola is scarce. When comparing cold-pressed canola with brewers grains or hominy meal (Moate et al., 2011) or cold-pressed rapeseed cake with full fat rapeseed (Johansson et al., 2015), milk production and methane emissions were unaffected.

Moist Heat Pressure

Incorporating steam with temperatures similar to heat treatment, moist heat pressure is another potential method for influencing the nutritional value of CM. After heating CM to 127°C with a steam pressure of 117 kPa for 0 to 90 minutes, Moshtaghi Nia and Ingalls (1992) observed decreased DM and N disappearance in the rumen, increased DM and N disappearance in the lower GI tract, and decreased DM and N disappearance in the total GI tract as the duration of heat treatment increased. Additionally, ADIN and NDIN increased, supporting the observation of decreased total tract disappearance. Further examination of CM heated to 127°C at 117 kPa for 15 or 45 minutes found decreased disappearance in the rumen, increased disappearance postminimally, and no difference for disappearance of essential (EAA) or nonessential (NEAA) AA along the total tract for CM heated 15 minutes compared to unheated CM. However, CM heated for 45 minutes decreased total tract disappearance of EAA and NEAA compared to CM heated for 15 minutes and unheated CM (Moshtaghi Nia and Ingalls, 1995), indicating negative effects of prolonged moist heat pressure. Interestingly, no difference for ruminal DM or CP disappearance or ADIN between untreated CM and CM moist heat-treated for 2 hours at 100°C was observed in Wright et al. (2005), suggesting heat treatment of 100°C was too low to elicit digestion effects. Along with evaluating the effects of cold-pressing versus higher temperatures on CM, the series of experiments by Heim and Krebs (2018a; 2018b; 2018c) also tested moisture heat pressure. Comparing durations of moist heat pressure (192 kPa, 120° C) lasting 0 to 12 minutes, linear increases for the A, B2, and B3 fractions of protein, NDIN, and effective CP degradability, a linear decrease for the B1 fraction, and no difference for the C fraction of protein or ADIN in CM was observed in Heim and Krebs (2018a). Furthermore, Heim and Krebs (2018b) reported increased RUP when applying moist heat pressure (192 kPa, 120°C) for 15

minutes. However, the decreased presence of intermediate Maillard reaction products in Heim and Krebs (2018c) suggests that the increased RUP is due to heat damage, rendering that protein unavailable.

Chemical Treatment

The effectiveness of treating CM with various chemicals prior to feeding has also been examined. Exploring formaldehyde treatment of CM, Rae et al. (1983) noted a 29% and 58% loss of the EAA lysine and tyrosine after treatment, with no difference for DM intake (DMI) or milk yield. Interestingly, post-experimental milk yield tended to decrease for cows fed treated CM compared to untreated CM, suggesting a delayed long-term negative effect. Going a step further by comparing formaldehyde treatment of RSM and SBM, apparent CP degradability decreased after treatment from 77 to 41% and 90 to 40% for RSM and SBM, respectively, indicating a marked decrease of nutritional value (Rooke et al., 1983). However, McKinnon et al. (1991) found no difference for apparent DM or CP disappearance or ADIN between untreated CM and CM treated with glacial acetic acid or formic acid. Conversely, ruminal and total tract degradability of DM and CP decreased for CM treated with 3% acetic acid compared to untreated CM (Khorasani et al., 1993). However, ADIN was unaffected by acid treatment. Supporting the results of McKinnon et al. (1991), substituting 0 to 100% of untreated CM with CM treated with acetic acid yielded no effect on DM, OM, NDF, ADF, starch, or CP digestion in either the rumen or total tract (Robinson et al., 1994). Along with evaluating heat effects, Folawiyo et al. (1997) observed extensive acid-induced structural modifications in rapeseed albumin (napin) after treatment with anilino-naphthalene-8-sulfonic acid, rendering a significant portion of the protein unavailable. Contrary to Rooke et al. (1983), Eghbali et al. (2011) reported increased CP digestibility for CM treated with formaldehyde compared to untreated CM, but no

effect of acetic acid treatment. Both formaldehyde and acetic acid treatment decreased the protein A and B1 fractions, increased the protein B2 fraction, and did not affect the B3 and C fractions of protein or ADIN and NDIN. While demonstrating the effectiveness of hydrogen peroxide bleaching for increasing the extractability of DM and total N in CM (El-Kadiri et al., 2013), consequences of this method on the functional properties and nutritional value are currently unknown.

Lignosulfonates

Lignosulfonates are byproducts of wood pulp production from the sulfite pulping process (Lebo et al., 2015). Based on the positive effects when treating SBM (Windschitl and Stern, 1988), lignosulfonate was hypothesized to decrease rumen degradability of CM. Compared to untreated CM, 5 and 10% lignosulfonate treatment combined with 60 or 120 minutes of 100° C heat increased NDIN and decreased effective rumen CP degradability. However, ADIN increased for CM heated for 2 hours and treated with 10% lignosulfonate, suggesting protein damage due to prolonged heat (McAllister et al., 1993). Furthermore, Wright et al. (2005) observed decreased ruminal DM and CP disappearance and increased ADIN for CM treated with 5% lignosulfonate and 100° C heat for 120 minutes compared to untreated CM and heated CM. Treatment of canola presscake with 5% lignosulfonate and 90, 110, 130, and 150°C heat for 60 minutes resulted in larger decreases for effective rumen degradation of CP compared to heating alone. However, ADIN was notably higher only at 150° C (Micek et al., 2020).

Chemical Composition

The composition of CM is influenced by multiple factors, including processing method, processing plant, and year of production. Mentioned previously, the majority of present-day CM

is produced via solvent extraction. However, nutritional values reported by the 2001 version of the Nutrient Requirements of Dairy Cattle (NRC, 2001) were for mechanically-extracted (expeller) CM rather than solvent-extracted. According to NRC (2001), on a DM basis (unless otherwise noted), CM contains 37.8% CP, 29.8% neutral detergent fiber (NDF), 20.5% acid detergent fiber (ADF), 9.5% lignin, 6.3% NDIN (% of CP), 2.4% ADIN (% of CP), 5.4% ether extract (EE), and 7.4% ash. For solvent-extracted CM, a 4-year survey (2011 to 2014) of 11 Canadian processing plants revealed ranges of 40.2 to 42.9% for CP, 3.0 to 4.3% for EE, and 7.1 to 7.9% for ash across plant and 40.6 to 43.7% CP, 2.8 to 4.0% for EE, and 7.2 to 8.0% for ash across year (Adewole et al., 2016). Similarly, Broderick et al. (2016) analyzed samples from 12 Canadian processing plants (11 solvent-, 1 expeller-extracted) during the same 4 years, reporting similar values for CP and ranges of 26.3 to 31.4% for NDF, 17.9 to 19.2% for ADF, 18.3 to 26.3% (% of CP) for NDIN, and 5.9 to 6.9% (% of CP) for ADIN across plant and 25.9 to 30.6% for NDF, 18.0 to 19.2% for ADF, 20.5 to 22.5% (% of CP) for NDIN, and 4.1 to 7.6% (% of CP) for ADIN. As expected, CP for CM samples from the plant using expeller-extraction were lower compared to solvent-extraction. The results from Adewole et al. (2016) and Broderick et al. (2016) demonstrated the effect of processing method, processing plant, and year of production on the nutritional value of CM. Confirming the low-glucosinolate content of CM, 10.06 and 3.57 μmol glucosinolates/g CM were found by Adewole et al. (2016) and Radfar et al. (2017), respectively. Adewole et al. (2016) found 10.06 μmol glucosinolates/g CM, whereas Radfar et al. (2017). In contrast to NRC (2001), the nutritional values reported for CM by the National Academies of Sciences, Engineering, and Medicine (NASEM, 2021) reflects that of solvent-extracted CM. According to NASEM (2021), on a DM basis (unless otherwise noted), CM

contains 41.5% CP, 29.0% NDF, 20.3% ADF, 8.51% lignin, 4.75% NDIN (% of CP), 2.50% ADIN (% of CP), 3.51% EE, and 7.9% ash.

Despite lower CP compared to other popular protein sources such as SBM, the increased methionine content of CM makes it an attractive option when formulating diets, as methionine is an essential amino acid (AA) generally recognized as first limiting for milk protein synthesis. According to NASEM (2021), CM contains 1.97% methionine (% of CP) compared to 1.38% for SBM. Conversely, SBM contains more lysine compared to CM (6.16 vs. 5.51%, % of CP), another AA regarded as first limiting for milk protein synthesis.

Source of RUP

Given the conduciveness of its AA profile for milk protein synthesis, maximizing RUP content, and therefore the proportion of protein reaching the intestines for degradation is desirable. In the 20th century, RSM/CM was considered a poor source of RUP for dairy cows. Data utilized by NRC (1989) suggested that RUP (% of CP) was merely 28% for RSM. Later on, Piepenbrink and Schingoethe (1998) revealed significantly higher RUP of 39.5% for CM. While this was a clear improvement, CM still contained the lowest RUP of the four protein sources tested (fish meal (FM) = 56.3%, corn gluten meal (CGM) = 81.3%, and blood meal (BM) = 91.9%). Several years after, NRC (2001) reported RUP values (DMI = 4% of body weight (BW), 50% diet DM as forage) of 35.7 and 42.6% for CM and 48% SBM, respectively. Subsequent results from Maxin et al. (2013a) suggested higher RUP for CM compared to SBM (52.5 vs. 41.5%). Additionally, RUP for wheat dried distiller's grains plus solubles (DDGS) and high protein DDGS were 39.2 and 63.6%, respectively. Analysis of CM samples collected from 12 Canadian processing plants from 2011 to 2014 observed a slightly lower average RUP value of 46.3% (Broderick et al., 2016), albeit still higher than values in NRC (2001). Surprisingly,

despite subsequent experiments suggesting vast improvements for RUP compared to values reported by NRC (2001), the RUP value for CM reported by NASEM (2021) is 32%. This value is similar to that reported for solvent-extracted SBM (33%) but less than that of DDGS (47%).

DMI and Milk Production Effects: Middle and Late Lactation

Given the extensive history of comparing production effects of including RSM and CM versus other protein sources in dairy cow diets, results will be discussed in several sections, including from the 1970s to 1980s, 1990s to 2010, and 2011 to present-day. The year 2011 is chosen as a cut-off point as two meta-analyses (Huhtanen et al., 2011; Martineau et al., 2013) were released around this time and summarized the production effects of RSM and CM versus other protein sources (SBM, DDGS, cottonseed meal (CSM), among others) up to that point. Results from the two meta-analyses will be discussed before progressing to the period of 2011 to present-day for comparison. While meta-analyses are helpful for characterizing the overall effect of RSM and CM versus other protein sources, the individual experiments help shed light on the variety of protein sources analyzed during the specific aforementioned time periods. It is important to note that the vast majority of experiments comparing the production effects of RSM and CM versus other protein sources in dairy cow diets pertains to middle and late lactation. Because of this, the few experiments focused on early lactation will be presented later on.

Canola Meal Studies: 1970s to 1980s

In the 1970s to 1980s, the majority of research focused on comparing the production effects of RSM or CM versus SBM or different varieties of RSM. In Waldern (1973), cows fed a diet with RSM concentrate decreased grain DMI, milk yield, and 4% fat-corrected milk (FCM)

yield compared to SBM with no effect on corn silage DMI. Despite decreased grain and hay DMI for cows fed diets with RSM concentrate compared to SBM, no difference was observed for silage or total DMI, milk yield, or FCM yield (Ingalls and Sharma, 1975). Likely due to using heat-damaged RSM as indicated by presence of a dark color, increasing RSM from 0 to 34% in the concentrate mixture decreased concentrate DMI and milk yield with no effect on silage DMI (Fisher and Walsh, 1976). No difference for DMI, milk yield, or FCM yield were found after feeding diets containing concentrate from one of two RSM varieties or SBM (Laarveld and Christensen, 1976). Furthermore, feeding diets containing concentrate from one of three RSM varieties or SBM resulted in no effect on grain, hay, silage, or total DMI, milk yield, or 4% FCM yield (Sharma et al., 1977). Reporting the findings of two trials, Papas et al. (1978) observed no difference for grain or hay DMI but increased milk yield for cows fed a diet with concentrate from one RSM variety compared to another RSM variety or SBM in trial 1 and no difference for grain DMI, hay DMI, or milk yield when comparing diets fed with concentrate from a RSM variety or SBM in trial 2. Further testing of cows fed diets with concentrate from one of two RSM varieties or SBM in Papas et al. (1979) yielded no effect on hay, silage, or grain mix DMI or milk yield. Comparing cows fed diets with 6, 13, or 19% of RSM versus SBM, there was no difference for DMI, milk yield, or FCM yield (Laarveld et al., 1981a). Adding CSM to the mix, no difference for milk yield or FCM yield between cows fed diets with CM, SBM, or CSM (Sanchez and Claypool, 1983) and no difference for milk yield or 4% FCM yield between cows fed diets with CM or CSM (DePeters and Bath, 1986) has been reported. Decreasing CM inclusion in the diet from 42.5 to 0% via sequential substitution with CGM resulted in no effect on DMI, milk yield, or 4% FCM yield (Robinson and Kennelly, 1988).

Canola Meal Studies: 1990s to 2010

Experiments conducted in the 1990s through 2010 broadened the research scope by also comparing RSM or CM to various other protein sources including DDGS, wet distillers grains plus solubles (WDGS), field peas, fava bean, and microalgae, among others. Still, many experiments focused on comparing RSM or CM to protein sources commonly used in diets, such as SBM. Huhtanen et al. (1991) observed no difference for silage or total DMI, milk yield, or FCM yield between cows fed diets with RSM or DDGS as concentrate. Feeding diets with CM or SBM in the concentrate yielded no effect on DMI, milk yield, or 3.5% FCM yield in McClean and Laarveld (1991). Comparing zero, medium (~1.25 kg/d), or high (~2.5 kg/d) RSM inclusion in concentrate, no difference was reported for concentrate and total DMI or milk yield between diets (Emanuelson et al., 1993). Despite no effect on concentrate DMI, increased silage and total DMI, milk yield, and FCM yield were observed for cows fed diets with RSM in the concentrate compared to control (Huhtanen et al., 1995). Mustafa et al. (1997) reported no effect for forage, concentrate, or total DMI, milk yield, or 3.5% FCM yield when including high-fiber CM, regular CM, or SBM in the concentrate. Feeding diets with CM or SBM did not affect DMI, milk yield, or 3.5% FCM in Piepenbrink et al. (1998). Compared to grass silage and barley alone, cows fed diets supplemented with RSM or rapeseed cake concentrate increased milk yield and energy-corrected milk (ECM) yield (Ahvenjärvi et al., 1999). No difference was reported for silage DMI, total DMI, or milk yield when feeding diets with RSM or a 1:3 mixture of FM:SBM as concentrate (Dewhurst et al., 1999). While feeding RSM in concentrate increased milk yield and ECM milk yield compared to the control diet, there was no added benefit for silage, concentrate, or total DMI, milk yield, or ECM when including crushed field pea in the concentrate (Khalili et al., 1999). Increasing the inclusion of RSM and rapeseed cake in concentrate from 0 to 3 kg/d

resulted in linear increases for silage and total DMI, milk yield, and ECM yield compared to feeding a control diet (Rinne et al., 1999).

Supplementing RSM as 16% of concentrate, Kokkonen et al. (2000) found increased silage and total DMI, milk yield, and ECM yield for cows fed diets with RSM as 16% of the concentrate compared to 0%. Cows fed a diet with rapeseed expeller in the concentrate increased silage and total DMI, milk yield, and ECM compared to a mixture of maize gluten and SBM (Khalili et al., 2001). Comparing wheat-gluten meal and RSM in concentrate, Shingfield et al. (2001) reported increased silage and total DMI, milk yield, and ECM for cows fed RSM. While concentrate DMI and milk yield were unaffected, replacing field pea with RSM in concentrate resulted in linear increases for silage DMI and ECM yield (Khalili et al., 2002). Supplementing grass silage with RSM increased silage and total DMI, milk yield, and FCM yield compared to grass silage alone (Ahvenjärvi et al., 2002). Despite no difference for silage DMI, total DMI, or ECM yield, incorporating RSM in concentrate increased milk yield compared to SBM (Shingfield et al., 2003). Compared to untreated CM, CM treated with lignosulfonate increased DMI, milk yield, and 4% FCM yield. However, heat-treated CM did not affect these production measures compared to untreated CM (Wright et al., 2005). In agreement with DePeters and Bath (1986), Maesoomi et al. (2006) reported that DMI, milk yield, and 4% FCM yield were not different between cows fed diets with CM or CSM. While DMI increased for cows fed a diet with CM compared to SBM or CSM, milk yield and 3.5% FCM yield were not different between diets (Brito and Broderick, 2007). Incremental replacement of DDGS with CM in the concentrate mix, resulting in diets with 100% DDGS, 2/3 DDGS and 1/3 CM, 1/3 DDGS and 2/3 CM, and 100% CM, did not affect DMI, milk yield, or ECM yield (Mulrooney et al., 2009). Furthermore, Christen et al. (2010) observed no difference for DMI, milk yield, or ECM yield were observed

between cows fed diets with CM, SBM, DDGS, or high protein DDGS (HPDDGS).

Additionally, DMI, milk yield, and 4% FCM yield were not different between cows fed diets with CM, SBM, triticale DDGS, or corn DDGS in Oba et al. (2010).

Canola Meal Studies: Meta-Analyses

While individual experiments are helpful in determining the effect of RSM and CM versus other protein sources when fed during a specific scenario, meta-analyses are useful for characterizing the overall effect of RSM and CM. Two meta-analyses (Huhtanen et al., 2011; Martineau et al., 2013) summarized and evaluated literature pertaining to RSM and CM inclusion in dairy cow diets through Oba et al. (2010). Huhtanen et al. (2011) was less stringent compared to Martineau et al. (2013), with data from 43 and 22 publications used in each, respectively. However, both meta-analyses presented the responses of production measures due to protein source as slopes and tested the significance of each. For Huhtanen et al. (2011), slope represented the effect of protein source increasing dietary CP concentration by 1 g per 1 kg of dietary DM, i.e. every 0.1% increase in dietary CP due to protein source, with a higher slope indicating a positive response. Conversely, Martineau et al. (2013) evaluated response based on 10% inclusion of CM as part of overall diet DM. In Huhtanen et al. (2011), the slope for untreated CM was higher compared to SBM regarding forage and total DMI, milk yield, and ECM yield. However, when comparing slopes for untreated CM and a mixture of SBM and FM, untreated CM was higher only for total DMI and ECM yield. Comparing untreated and heat-treated CM, only the slope for forage DMI was higher for heat-treated CM. In Martineau et al. (2013), the DMI, milk yield, 4% FCM yield, and ECM yield responses for CM were significant when comparing CM to all other protein sources (SBM, CGM, CSM, and DDGS). However, when comparing CM to only SBM, there was no response for 4% FCM or ECM.

Canola Meal Studies: 2011 to Present-Day

Experiments after Huhtanen et al. (2011) and Martineau et al. (2013) further examined RSM and CM versus other protein sources in dairy cow diets, with mixed results observed in subsequent experiments. Compared to the control diet with CM, incremental replacement of CM with DDGS resulted in linear increases for DMI and milk yield with no effect on ECM yield (Chibisa et al., 2012). Similar to Christen et al. (2010), Maxin et al. (2013b) found no difference for DMI, milk yield, or ECM yield between cows fed diets with CM, SBM, HPDDG, or wheat DDGS. While DMI was not affected, a quadratic response was reported for milk yield in Swanepoel et al. (2014), with cows fed a diet with 13.5% CM performing the best. Comparing diets with CM or HPDDG, Acharya et al. (2015) observed no effect for DMI, milk yield, ECM yield, or FCM yield. Broderick et al. (2015) determined that DMI, milk yield, and ECM yield increased for cows fed the diets with CM compared to SBM. While ECM was not different, cows fed diets with CM increased silage DMI, total DMI, and milk yield (Gidlund et al., 2015). Rinne et al. (2015) reported increased silage DMI and ECM for cows fed diets with rapeseed expeller compared to soybean expeller, with no effect on total DMI and milk yield. Comparing RSM and fava bean, Puhakka et al. (2016) observed linear increases for silage and total DMI, milk yield, and ECM when increasing RSM in the diet. Consistent with Maxin et al. (2013b), Mutsvangwa et al. (2016) observed no difference for DMI, milk yield, or 3.5% FCM between cows fed diets with CM or wheat DDGS. No difference for silage or total DMI, milk yield, or ECM yield was reported between cows fed diets with RSM or microalgae in Lamminen et al. (2017). Interestingly, Robinson and Swanepoel (2018) found no effect on DMI and a linear decrease for milk yield when increasing CM concentration in the diet from 5 to 17%. While silage DMI, total DMI, and milk yield were not affected, cows fed a diet with 14% rapeseed cake decreased ECM

compared to 0 and 20% (Trøan et al., 2018). Paula et al. (2018) observed no effect for DMI, milk yield, ECM yield, or 3.5% FCM between cows fed diets with CM, heat-treated CM, or SBM. Analyzing experiments through Trøan et al. (2018) that evaluated RSM or CM versus a blend of RSM or CM, a subsequent meta-analysis (Martineau et al., 2019) determined that blending CM with other protein sources does not outperform the beneficial production effects of using up to 1.55 kg CM per day alone. Despite decreased DMI, milk yield and ECM were not different between cows fed diets with RSM or fava beans (Lamminen et al., 2019). In Jersey cows, Reynolds et al. (2019) reported no effect for DMI, milk yield, or ECM yield between cows fed diets with CM, low-fat DDGS, or high-fat DDGS. In agreement with Paula et al. (2018), DMI, milk yield, 3.5% FCM, and ECM were not different between cows fed diets with CM or SBM in Paula et al. (2020). Contrary to Paula et al. (2020), Pereira et al. (2020) observed increased DMI, milk yield, 4% FCM, and ECM for cows fed diets with CM compared to SBM. Furthermore, linear increases for DMI, milk yield, ECM, and 4% FCM were found as CM inclusion increased from 0 to 24% of diet DM in Benchaar et al. (2021). While ECM was not affected, Lage et al. (2021) reported increased DMI and milk yield for cows fed a diet with CM compared to SBM. While silage and total DMI tended to increase for cows fed diets supplemented with RSM compared to fava bean or blue lupin, milk yield and ECM yield were not different (Kuoppala et al., 2021).

Feed Efficiency

The conversion of feed to milk is commonly reported multiple ways, including milk per unit DMI (milk/DMI) and ECM per unit DMI (ECM/DMI). Expressing feed conversion as ECM/DMI is more valuable as it factors in the energetic components of milk fat and protein. The meta-analysis from Martineau et al. (2013) found a marginal increase of +0.02 kg ECM/kg DMI

per 10% inclusion of CM in diet when compared to other protein sources such as SBM, CGM, BM, and DDGS. However, comparing CM to only SBM yielded no difference. No difference for ECM/DMI feed efficiency between diets with CM or SBM was reinforced by subsequent experiments (Broderick et al., 2015; Paula et al., 2018; Lage et al., 2021). Additionally, while not reported by Martineau et al. (2013), milk/DMI was not different between diets with CM or SBM in subsequent experiments (Broderick et al., 2015; Paula et al., 2018; Lage et al., 2021). Beyond SBM, no difference for ECM/DMI feed efficiency between diets with CM or DDGS (Mulrooney et al., 2009; Chibisa et al., 2012; Acharya et al., 2015) or between diets with CM or microalgae (Lamminen et al., 2017) has been observed.

Milk Composition, Component Yields, MUN, and SCC

For milk composition and component yields, i.e. fat, protein, and lactose, results from Huhtanen et al. (2011) and Martineau et al. (2013) will be discussed to encompass the results from individual experiments up to that point, with results from subsequent experiments after. As MUN and SCC were not analyzed by these two meta-analyses, results from recent experiments will be discussed. Mixed results for milk composition and component yields have been observed both in the meta-analyses and individual experiments thereafter. Huhtanen et al. (2011) determined that while there was no difference for milk fat percent, the slope for milk protein percent was lower for CM compared to a mixture of SBM and FM and for heat-treated CM compared to SBM or a mixture of SBM or FM, suggesting a negative incremental effect of CM on milk protein percent. However, Martineau et al. (2013) reported no response for milk fat percent, a positive response for milk protein percent, and a negative response for milk lactose percent when comparing CM to all other protein sources. When compared to only SBM, there was no response for milk protein or lactose percent. While milk fat and protein yield responded

positively to CM compared to all other protein sources, milk lactose yield was unaffected.

Comparing CM to only SBM, there was no response for milk fat yield while milk protein yield responded favorably to CM.

While subsequent experiments typically observed no difference for milk fat, protein, or lactose percent between cows fed diets with CM or other protein sources, milk protein (Rinne et al., 2015; Kuoppala et al., 2021) and lactose percent (Mutsvangwa et al., 2016; Paula et al., 2018; Pereira et al., 2020) are sometimes affected. In these few cases, milk protein percent increased (Rinne et al., 2015; Kuoppala et al., 2021), whereas milk lactose percent was shown to increase (Mutsvangwa et al., 2016; Paula et al., 2018) and decrease (Acharya et al., 2015; Pereira et al., 2020) when cows were fed diets with RSM or CM compared to other protein sources. Furthermore, given that milk composition is typically unaffected, milk fat, protein, and lactose yields tend to follow the same pattern as milk yield and ECM yield. However, increased milk yield with no difference for milk component yields (Lage et al., 2021) or increased milk yield with no difference for yields of one or two components of milk (Broderick et al., 2015; Gidlund et al., 2015; Paula et al., 2020) has also been reported. Milk lactose yield was unaffected in Broderick et al. (2015), whereas milk fat and lactose yields were not different in Gidlund et al. (2015) and Paula et al. (2020).

Milk urea nitrogen (MUN) is a proxy commonly used to evaluate nitrogen utilization due to its ease of measurement. When comparing isonitrogenous diets, lower values suggest more efficient utilization of nitrogen for milk protein synthesis. Decreased MUN for cows fed diets with RSM or CM compared to other protein sources such as SBM (Pereira et al., 2020; Benchaar et al., 2020; Lage et al., 2021), DDGS (Maxin et al., 2013b; Acharya et al., 2015), or fava bean (Puhakka et al., 2016) has been observed in the majority of recent experiments. However, no

difference for MUN between cows fed diets with RSM or CM and SBM (Brito and Broderick, 2007; Christen et al., 2010; Rinne et al., 2015), DDGS (Mulrooney et al., 2009; Christen et al., 2010; Mutsvangwa et al., 2016) or microalgae (Lamminen et al., 2017) has also been reported. Furthermore, increased MUN is uncommonly found for cows fed diets with CM compared to CSM (Brito and Broderick, 2007) or DDGS (Oba et al., 2010; Reynolds et al. 2019).

Somatic cell count (SCC) is an important indicator of mammary gland health and is also easily measured in milk, with lower concentrations suggesting better health. In the vast majority of recent experiments, no difference for SCC between cows fed diets with CM compared to SBM (Broderick et al., 2015; Pereira et al., 2020; Benchaar et al., 2021) or DDGS (Swanepoel et al., 2014; Acharya et al., 2015; Reynolds et al., 2019) has been observed. However, Robinson and Swanepoel (2018) found a linear decrease for SCC as CM replaced DDGS in the diet. It is important to note that raw SCC values were reported in all cases. Log-transformation of SCC is typically necessary to ensure normality due to the right-skewed distribution commonly observed.

Physiological Effects

Plasma Amino Acids

From a nutritional standpoint, the most attractive feature of feeding CM, compared to another protein source such as SBM, is its AA profile that is conducive for milk protein synthesis. This is especially true for methionine, one of the two essential AA generally recognized as first limiting for milk protein (NASEM, 2021). However, CM contains less lysine compared to SBM. According to NASEM (2021), lysine and methionine comprise 5.51 and 1.97% of the CP in CM. This results in a ratio of 2.80, closely matching the lysine to methionine

ratio of ~3 typically observed in cow milk protein (Posati and Orr, 1976; Rafiq et al., 2016). In comparison, SBM contains 6.16 and 1.38% lysine and methionine on a CP basis, which equates to a ratio of 4.46 (NASEM, 2021). Other EAA of particular interest are isoleucine, leucine, and valine, the three branched chain AA (BCAA) that can represent up to 50% of all milk EAA and strongly influence overall metabolism through ways such as anaplerosis and cellular signaling (Harper et al., 1984; Mackle et al., 1999a; Appuhamy et al., 2011). Concentrations of 3.93, 6.92, and 5.11% for CM and 4.54, 7.63, and 4.76% for SBM have been reported for isoleucine, leucine, and valine, respectively (NASEM, 2021). Given these differences for concentrations of EAA, plasma concentrations of EAA may be altered when cows are fed diets with CM or other protein sources such as SBM. In conjunction with their meta-analysis for production responses, a meta-analysis summarizing the effect of CM on plasma concentrations of AA was released shortly thereafter (Martineau et al., 2014). As it encompasses experiments through Oba et al. (2010), only this meta-analysis will be used to characterize results for plasma concentrations up to that point. According to Martineau et al. (2014), plasma concentrations of total EAA, BCAA, lysine, and methionine all responded positively to including CM in the diet compared to SBM, DDGS, or field peas. However, subsequent experiments yielded mixed results. Plasma concentrations of total EAA, total BCAA, lysine, methionine, isoleucine, leucine, and valine all increased for cows fed diets with RSM compared to fava bean in Puhakka et al. (2016) and Lamminen et al. (2019) and for cows fed a diet with CM compared to SBM in Pereira et al. (2020). Furthermore, Swanepoel et al. (2014) and Robinson and Swanepoel (2018) reported a linear increase for plasma concentrations of lysine, isoleucine, and valine as inclusion of CM in the diet linearly increased. Comparing diets with CM or DDGS, Acharya et al. (2015) found increased plasma concentrations of lysine, methionine, isoleucine, and valine for cows fed diets

with CM compared to DDGS. However, no difference for plasma concentrations of total EAA, BCAA, lysine, methionine, isoleucine, leucine, or valine have been reported between cows fed diets with CM or SBM (Maxin et al., 2013b; Gidlund et al., 2015; Lage et al., 2021), DDGS (Maxin et al., 2013b), or microalgae (Lamminen et al., 2017). Furthermore, decreased plasma concentrations of methionine (Swanepoel et al., 2014) and leucine (Swanepoel et al., 2014; Acharya et al., 2015; Robinson and Swanepoel, 2018) is sometimes observed.

Beyond just plasma concentrations, several experiments have also evaluated mammary extraction efficiency of AA using arteriovenous differences and yielded mixed results. Increased extraction efficiency of leucine and valine for cows fed a diet with CM compared to DDGS was observed in Mulrooney et al. (2009). Furthermore, increased extraction efficiency of total EAA, leucine, and valine was reported in Christen et al. (2010) for cows fed a diet with CM compared to SBM or HPDDG. However, no difference was found between cows fed a diet with CM or DDGS. Conversely, Acharya et al. (2015) noted a decrease for the extraction efficiency of lysine and isoleucine for cows fed a diet with CM compared to DDGS. Comparing diets with RSM or fava bean, Lamminen et al. (2019) determined there was no difference for plasma concentrations of total EAA, total BCAA, lysine, methionine, isoleucine, leucine, or valine.

Plasma Hormones and Metabolites

Plasma concentrations of various hormones and metabolites related to energy status and production have been analyzed for cows fed diets with CM compared to other protein sources. This section will focus on plasma triiodothyronine (T3), thyroxine (T4), glucose, insulin, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHBA), and urea nitrogen (PUN).

The two thyroid hormones, T3 and T4, are involved in numerous metabolic processes in dairy cows related to energy expenditure (Kaneko, 2008). As previously noted, nutritionists were hesitant to incorporate RSM into dairy cow diets for much of the 20th century as high concentrations of glucosinolates in RSM exerted a goitrogenic effect. This effect resulted in decreased plasma T4 concentrations for cows fed diets with RSM compared to SBM in most (Fisher and Walsh, 1976; Papas et al. 1979; Laarveld et al., 1981b) but not all (Papas et al., 1978) cases. Interestingly, concentrations of plasma T3, the more bioactive form of thyroid hormone (Gross and Pitt-Rivers, 1952; Premachandra et al., 1961; Sawin et al., 1977) were unaffected in all cases. However, McClean et al. (1991) and Maesoomi et al. (2006) later observed no difference for plasma T3 or T4 concentrations between cows fed diets with CM or SBM in the concentrate or between cows fed diets with CM or CSM, respectively. While plasma T3 concentrations were not different, plasma T4 concentrations tended to increase as rapeseed cake inclusion increased in the diet from 0 to 20%.

Glucose is an inherently important precursor for the synthesis of lactose, a disaccharide composed of one glucose and one galactose molecule. Milk yield is largely dependent on the osmoregulatory function of lactose (Bleck et al., 2009). In most cases, plasma glucose concentrations are not affected when feeding diets with RSM or CM compared to other protein sources such as SBM (Shingfield et al., 2003; Maxin et al., 2013b; Rinne et al., 2015), DDGS (Maxin et al., 2013b), wheat gluten meal (Shingfield et al., 2001), microalgae (Lamminen et al., 2017), or fava bean (Lamminen et al., 2019). However, Oba et al. (2010) found increased plasma glucose concentrations for cows fed diets with CM compared to DDGS, whereas Puhakka et al. (2016) noted a tendency for decreased plasma glucose concentrations for cows fed diets with RSM compared to fava bean.

While not needed by the mammary gland, insulin is necessary for glucose uptake in tissues such as adipose and skeletal muscle (Zhao, 2014). Furthermore, the influence of insulin on milk protein synthesis has long been recognized (Griinari et al., 1997; Mackle et al., 1999b). Similar to glucose, plasma insulin concentrations are typically unaffected when feeding diets with RSM or CM compared to other protein sources such as SBM (Shingfield et al., 2003; Oba et al., 2010; Rinne et al., 2015), DDGS (Oba et al., 2010), fava bean (Puhakka et al., 2016), or microalgae (Lamminen et al., 2017). However, plasma insulin concentrations decreased for cows fed diets with RSM compared to wheat gluten meal in Shingfield et al. (2001).

During periods of negative energy balance (NEB), dairy cows respond by increasing mobilization of energy reserves, i.e. adipose tissue. Plasma BHBA and NEFA are two biomarkers commonly measured to assess NEB, with increased concentrations associated with increased mobilization of adipose and increased incidence of clinical disorders such as ketosis, displaced abomasum, and metritis, among others (Duffield et al., 2009; Ospina et al., 2010; Raboisson et al., 2014). Shingfield et al. (2003) reported increased plasma NEFA concentrations for cows fed a diet with RSM compared to SBM. However, plasma BHBA and NEFA concentrations are typically not different between cows fed diets with RSM or CM compared to SBM (Rinne et al., 2015), wheat gluten meal (Shingfield et al., 2001), fava bean (Puhakka et al., 2016; Lamminen et al., 2019), or microalgae (Lamminen et al., 2017).

Similar to MUN, PUN is a proxy that is commonly measured to assess nitrogen utilization. Due to rapid equilibration between pools in the body (Broderick and Clayton, 1997), the results for PUN commonly reinforce those for MUN. According to the meta-analysis from Martineau et al. (2014), including RSM or CM in diets results in decreased PUN concentrations compared to other protein sources such as SBM, DDGS, or field peas. However, it is important

to note that this meta-analysis focused on the effect of feeding CM on plasma AA, and therefore previous experiments reporting PUN in the absence of plasma AA were not included. The results from individual experiments are mixed, with decreased PUN observed in some (Shingfield et al., 2001; Rinne et al., 2015) but not all (Brito and Broderick, 2007; Oba et al., 2010; Chibisa et al., 2012) instances between cows fed diets with RSM or CM compared to other protein sources. Interestingly, the cases of Oba et al. (2010) and Rinne et al. (2015), whereby increased MUN for cows fed a diet with CM compared to SBM or DDGS and no difference for MUN between cows fed a diet with RSM or SBM were observed, respectively, illustrate that differences between pools are sometimes observed.

Diet and Nutrient Digestibility

One of the key influencers of milk production, total tract diet digestibility is an important metric for determining how well cows perform on a given diet. Diet digestibility is affected by factors such as DMI, feed processing, and physical characteristics of the feed. Digestibility of specific nutrients, i.e. CP, NDF, ADF, and/or starch, is commonly reported along with DM and organic matter (OM). Mixed results have been observed for diet digestibility when comparing diets with CM or other protein sources. While increased (Maesoomi et al., 2006; Paula et al., 2018) and decreased (Lamminen et al., 2019; Pereira et al., 2020; Benchaar et al., 2021) dry matter digestibility (DMD) have been reported for cows fed diets with RSM or CM compared to other protein sources, no difference is typically observed (Gidlund et al., 2015; Lamminen et al., 2017; Lage et al., 2021). The meta-analysis from Huhtanen et al. (2011) found no difference between the slopes of OM digestibility (OMD) or CP digestibility (CPD) when comparing inclusion rates of CM, SBM, and a mixture of SBM and FM. Similar to DMD, while increased (Maesoomi et al., 2006; Paula et al., 2018) and decreased (Lamminen et al., 2019; Pereira et al.,

2020; Benchaar et al., 2021) OMD and CPD have been noted for cows fed diets with RSM or CM compared to other protein sources, no difference is typically found (Lamminen et al., 2017; Robinson and Swanepoel, 2018; Lage et al., 2021). Unfortunately, NDF (NDFD), ADF digestibility (ADFD), and starch digestibility (starchD) were not reported in Huhtanen et al (2011). However, NDFD follows a similar history as the other digestibility measures previously mentioned. Increased (Brito and Broderick, 2007; Paula et al., 2018; Lage et al., 2021) and decreased (Rinne et al., 2015; Pereira et al., 2020; Benchaar et al., 2021) NDFD has been observed in some cases for cows fed diets with RSM or CM compared to other protein sources, with no difference typically reported (Lamminen et al., 2017; Robinson et al., 2018; Lamminen et al., 2019). Contrary to NDFD, the results for ADFD are more consistent, with no difference typically found (Brito and Broderick, 2007; Chibisa et al., 2012; Lage et al., 2021) for cows fed diets with CM compared to other protein sources. However, Benchaar et al. (2021) observed a linear decrease for ADFD as CM inclusion increased in the diet. Similar to ADFD, starchD is typically unaffected between cows fed diets with RSM or CM compared to other protein sources (Oba et al., 2010; Pereira et al., 2020; Lage et al., 2021). While Lamminen et al. (2019) and Benchaar et al. (2021) reported decreased starchD and a linear increase for starchD for cows fed diets with RSM or fava bean or between cows fed diets with increasing CM inclusion, respectively, the largest difference between diets was 0.8%.

Microbial Protein Synthesis

Metabolizable protein utilized by dairy cows is comprised of 3 components: microbial protein synthesized in the rumen, RUP, and endogenous protein sources. Microbial protein synthesis (MPS) is of significant interest as microbial protein typically comprises over half of the metabolizable protein reaching the small intestine (Clark et al., 1992). Furthermore, the EAA

composition of microbial protein closely matches that of milk protein (Sok et al., 2017; NASEM, 2021), making it an ideal source of AA for milk protein synthesis. Consequently, maximizing MPS is desirable and may be achieved through utilization of different protein sources in the diet.

Measuring urinary excretion of allantoin and uric acid, the primary end points of purine catabolism is a relatively easy and non-invasive method commonly used to quantify MPS in dairy cows due to its strong relationship with the duodenal supply of microbial RNA (Chen et al., 1990; Balcells et al., 1991; Giesecke et al., 1994). In publications, MPS is typically represented as intestinal microbial N or CP flow. In the majority of recent experiments, no difference for MPS is observed between cows fed diets with RSM or CM compared to other protein sources such as SBM (Maxin et al., 2013b; Paula et al., 2018; Lage et al., 2021), DDGS (Maxin et al., 2013b), fava bean (Puhakka et al., 2016; Lamminen et al., 2019), or microalgae (Lamminen et al., 2017). However, Brito and Broderick (2007) reported a tendency for decreased MPS for cows fed a diet with CM compared to SBM or CSM, whereas Lamminen et al. (2017) observed increased MPS for cows fed a diet with RSM compared to microalgae.

Methane Production

Given its known atmospheric warming potential, enteric production of methane is a primary environmental concern in the dairy industry. However, there is conflicting information as to how big of a contributor agriculture truly is towards global greenhouse gas production. According to Knapp et al. (2014), agriculture accounts for 29% of global methane sources, of which 19% is attributed to enteric fermentation and manure. Conversely, other anthropogenic practices, including burning of fossil fuels and biomass, landfills, and wastewater, also contribute 29% to global methane sources. When comparing global anthropogenic sources of methane, carbon dioxide, and nitrous oxide, agriculture and land use contributed 22% compared to 51%

from industry, transportation, and energy supply. Regardless of what number is considered, given the known atmospheric potency of methane and the energetic loss in the rumen that it represents, reduction of enteric methane emissions is advantageous from multiple viewpoints.

Few experiments, both *in vitro* and *in vivo*, have analyzed the effect of CM on methane emissions. Using two *in vitro* systems, Paula et al. (2017) observed decreased methane concentrations (mM) and intensity (g/kg DM) in diets with CM compared to SBM. Interestingly, methane concentration and intensity was not affected by including CM with low (38%) or high (50%) RUP in the diet. Comparing three types of CM, Ramirez-Bribiesca (2018) evaluated the effect of different protein fractions on methane production. The variety of CM with the highest protein A and B1 fractions and lowest B2 fraction also had the highest methane concentrations (mL/g DM) at 12, 24, and 48 hours of incubation in rumen fluid.

The several *in vivo* experiments focused on the effect of feeding CM compared to other protein sources on methane have yielded mixed results. While methane concentrations expressed as g/d or g/kg DMI was not different, Gidlund et al. (2015) reported an interaction between protein source and linear inclusion of protein source. Although diets containing low or medium inclusions of SBM or CM produced similar methane concentrations expressed as g/kg ECM, the diet with high (15%) SBM increased methane expressed as g/kg ECM compared to the diet with high (21%) CM. Despite no difference for methane expressed as g/kg DMI or g/kg FCM, Moore et al. (2016) observed an interaction between diet CP concentration and protein source for methane expressed as g/d. Cows fed the high (18.1%) CP diet with CM decreased methane expressed as g/d compared to cows fed the low (16.1%) or high CP diet with SBM or low CP diet with CM. In Jersey cows, Reynolds et al. (2019) found no difference for methane expressed as L/d, L/DMI, or L/ECM between diets with CM or DDGS. However, methane concentration

expressed as g/d, g/kg milk yield, g/kg ECM, and g/kg FCM linearly decreased as CM inclusion in the diet increased from 0 to 24% in Benchaar et al. (2021). Furthermore, a quadratic effect occurred for methane expressed as g/kg DMI, with no further decrease after 16% inclusion of CM. While Lage et al. (2021) reported no difference for methane expressed as g/d or g/kg ECM, methane expressed as g/kg DMI decreased for cows fed a diet with CM compared to SBM.

Using cradle to farm-gate life cycle assessments, Holtshausen et al. (2021) determined that using CM in diets compared to SBM is a viable greenhouse gas mitigation strategy depending on where the CM is produced, with 6.6 and 3% reductions for CM produced in western and eastern Canada, respectively.

The Transition Period and Early Lactation

The Transition Period

The transition period is commonly referred to as the 3 weeks before and after parturition (Grummer, 1995). Along with parturition, this time is marked by significant events, such as a rapid increase for milk production accompanied by weeks of NEB. During NEB, dairy cows are unable to consume the necessary amount of nutrients for milk production via DMI, and therefore must mobilize the remainder from adipose and skeletal muscle reserves. The extent of tissue mobilization has been estimated previously, with adipose tissue mobilization between 50 and 70 kg during the first 5 to 12 weeks of lactation (Komaragiri et al., 1997) and skeletal tissue mobilization between 8 and 21 kg during the first 5 to 6 weeks of lactation (Komaragiri and Erdman, 1997; Komaragiri et al., 1998). Moreover, dairy cows must evade numerous metabolic disorders during this time, including ketosis, displaced abomasum, and milk fever. Knowing that

dairy cows are heavily stressed by NEB and metabolic disorders during the transition period, this begs the question of whether these stressors can be attenuated through nutritional intervention. One way this may be achieved is through methionine supplementation in the diet.

Methionine Supplementation

As mentioned previously, methionine is generally recognized as one of the EAA that is first limiting for milk protein synthesis. Since the primary advantage of utilizing CM in diets is due to its higher methionine content compared to other protein sources such as SBM, comparing the results of experiments focused on supplementing methionine during the transition period and early lactation may give some indication of the potential effects of including CM compared to SBM during this time. Several forms of methionine have been extensively researched, including methionine hydroxy analog (HMB), isopropyl ester of methionine hydroxy analog (HMBi; i.e. MetaSmart), and rumen-protected methionine (RPM; i.e. Mepron, Smartamine). Despite the form, the primary focus is maximizing bioavailability for milk production and health benefits. One key contrast between HMB/HMBi and RPM is the degree of bioavailability, with respective values of 40 to 58% (Robert et al., 2001; Schwab et al., 2001; Graulet et al., 2005) and 80% (Overton et al., 1996; Schwab, 2007) previously reported. Given the extensive history of studying methionine supplementation in dairy cows dating back to the late 1960s (Griel et al., 1968), during which wide ranges for both amount of supplemented methionine and time frame of supplementation have been analyzed, results from meta-analyses will be used to characterize the effects on milk production. While a handful of meta-analyses have been published, this section will focus on those that used a database evaluating only methionine supplemented via the diet, i.e. not via postruminal infusion (Patton, 2010; Wei et al., 2022), during the transition period and early lactation. Patton (2010) and Wei et al. (2022) utilized 36 experiments from 1984 to 2009

and 14 experiments from 2011 to 2022, respectively, thereby encompassing an expansive body of literature. Despite a marginal decrease for DMI (-0.04 kg/d), milk yield was not affected for cows fed diets supplemented with RPM in Patton (2010). However, true milk protein percent and yield exhibited larger magnitude responses to RPM supplementation, with increases of 0.07% and 0.027 kg/d detected, respectively. Milk fat percent and yield followed opposite trends, with a 0.01% decrease and 0.011 kg/d increase, respectively. In agreement, Wei et al. (2022) reported increased milk protein percent for cows fed diets supplemented with RPM. However, milk yield was unaffected by RPM supplementation, whereas milk fat percent increased. Furthermore, no difference was observed for milk lactose percent.

In addition to milk production effects, numerous health benefits may occur when supplementing diets with RPM during the transition period and early lactation. This is due not only to methionine's role as a methyl donor via synthesized S-adenosylmethionine (SAM), but also via synthesized products that include betaine, glutathione, homocysteine, and taurine. These products elicit a myriad of effects, including acting as antioxidants, anti-inflammatory agents, and by stimulating the immune system. In Osorio et al. (2013), a tendency for decreased incidence of ketosis was reported for cows fed diets supplemented with methionine compared to the control diet. Additionally, increased neutrophil phagocytic capacity was observed at 21 days in milk (DIM) for cows fed diets supplemented with methionine compared to the control diet (Osorio et al., 2013), suggesting improved immune system function during a time of immunosuppression. This finding was reinforced by Zhou et al. (2016a) and Batistel et al. (2018). Furthermore, Batistel et al. (2018) observed increased concentrations of albumin and paraoxonase for cows fed diets with RPM compared to a control diet, suggesting decreased inflammation. Additionally, Batistel et al. (2018) observed decreased concentrations of reactive

oxygen metabolites for cows fed diets with RPM compared to a control diet, suggesting reduced oxidative stress.

A common misconception in dairy nutrition is that supplementing diets with methionine decreases the incidence of fatty liver. Up to this point, all experiments reporting liver triglyceride content have found no reduction due to methionine supplementation (Zhou et al., 2016b; Batistel et al., 2017; Potts et al., 2020). However, using a composite index including albumin, cholesterol, and bilirubin as biomarkers, Zhou et al. (2017) observed a tendency for more cows to have a high liver functional index when fed diets supplemented with RPM compared to choline. Furthermore, Batistel et al. (2018) reported decreased concentrations of gamma glutamyl transferase, a biomarker associated with damage to the liver and bile ducts, for cows fed diets supplemented with RPM compared to a control diet.

Reproductive benefits due to methionine supplementation have also been observed. Alonso et al. (2008) reported increased follicular diameter and ovulation percentage after estrus synchronization for cows fed diets supplemented with RPM. Furthermore, Toledo et al. (2017) found decreased embryonic loss following first service in multiparous cows after feeding diets supplemented with RPM. Acosta et al. (2016) observed increased lipid content in embryos for cows fed diets supplemented with RPM, potentially facilitating the increased embryonic survival in Toledo et al. (2017).

Early Lactation Canola Meal Studies

Compared to the literature available for methionine supplementation, there is limited data evaluating the effect of including CM in dairy cow diets during early lactation. As CM contains an AA profile with increased methionine content compared to other protein sources such as SBM

(1.97 vs. 1.38%, CP basis; NASEM, 2021), substituting CM for SBM may elicit similar production effects as RPM supplementation.

To our knowledge, three experiments have evaluated the effects of CM in diets fed during early lactation (Moore et al., 2016; Gauthier et al., 2019; Swanepoel et al., 2020). While all three compared diets with CM and/or SBM, the experimental diets differed substantially. In Moore et al. (2016), cows were fed diets with low (16.2%) or high (18.1%) CP containing either CM or SBM. Cows were assigned to a diet at parturition and remained on the same diet through week 16 of lactation. Conversely, Gauthier et al. (2019) and Swanepoel et al. (2020) assigned diets to pens of cows starting at ~12-13 DIM, with pens switching diets every 3 or 4 to 5 weeks, respectively. Compared to Moore et al. (2016), this allowed for a drastically shorter evaluation of the protein sources. In Gauthier et al. (2020), CM incrementally substituted for SBM from 3.5 to 13.0% of diet DM. On the other hand, Swanepoel et al. (2020) compared diets with 14.3% CM and 0% SBM or 6.6% CM and 6.3% SBM. While DMI was unaffected in Gauthier et al. (2019) and Swanepoel et al. (2020), cows fed the diets with CM compared to SBM increased DMI by 0.80 kg/d in Moore et al. (2016). However, milk yield responded positively to CM inclusion in all 3 experiments, ranging from 1.76 kg/d (Swanepoel et al., 2020) to 4.45 kg/d (Moore et al., 2016). While ECM and FCM were not reported in the latter 2 experiments, Moore et al. (2016) reported increases of 4.02 and 3.92 kg/d, respectively, for cows fed the diets with CM compared to SBM. Due to the non-commensurate increase for DMI, compared to ECM, Moore et al. (2016) also observed increased ECM/DMI feed efficiency for cows fed the diets with CM compared to SBM. While milk composition was not affected by protein source in Moore et al. (2016), milk fat, protein, and lactose percent were highest for cows fed the diet with 13.0% CM in Gauthier et al. (2019). Furthermore, milk protein and lactose percent increased for cows fed

the diet with 14.3% CM compared to 6.6% CM and 6.3% SBM in Swanepoel et al. (2020). Due to increased milk yield, milk component yields also responded positively to CM inclusion in all 3 experiments. In Moore et al. (2016), MUN tended to decrease for cows fed the diets with CM compared to SBM, supporting results from mid-lactation experiments.

While a paucity of data currently exists for evaluating the effect of including CM in dairy cow diets during early lactation, to our knowledge, there is currently no data evaluating the effect of including CM in dairy cow diets during the close-up dry period.

Conclusions

Canola meal is a protein-rich feedstuff that is rapidly gaining popularity in dairy cow diets due to its consistent positive effects on production compared to other protein sources such as SBM, which is largely attributed to the increased methionine content of CM. While a long history of research demonstrates positive production effects when feeding CM during middle and late lactation, little research exists for early lactation and, to our knowledge, no information exists for the close-up dry period. Furthermore, increased knowledge of the physiological effects of feeding CM compared to other protein sources such as SBM will build on the existing framework of how and why CM increases production.

The objective of this research was to determine the effect of feeding isonitrogenous diets with SBM or CM as the primary protein source to dairy cows during the close-up dry period to mid-lactation. Experiment 1 focused on feeding diets with SBM or CM during the close-up dry period through early lactation, with data reported for production (Chapter 2), temporal plasma hormones and metabolites (Chapter 3), and temporal diet digestibility and urinary excretion

(Chapter 4). Experiment 2 focused on feeding diets with SBM or CM during mid-lactation to cows determined to have high or low residual feed intake, with data reported for production, plasma hormones and metabolites, and urinary excretion (Chapter 5).

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Chapter 2. Production effects of soybean meal versus canola meal fed to dairy cows during the close-up dry period and early lactation

Abstract

Canola meal (CM) is a protein-rich byproduct of canola oil production that is widely utilized in diets fed to dairy cows. Recent studies have shown positive production effects, including increased dry matter intake (DMI), milk yield, and feed efficiency, when CM is included in diets fed to early lactation dairy cows versus other protein sources such as soybean meal (SBM). However, the effect of feeding CM during the close-up dry period is uncharacterized. The primary objective of this experiment was to determine the effect of feeding isonitrogenous diets formulated with SBM or CM as the primary protein source during the close-up dry period and early lactation on production measures in Holstein dairy cows. In a 2×2 factorial randomized complete block design, eighty multiparous cows were assigned to one of four treatments, which were cows fed diets with: 1) SBM pre- and postpartum (SBM-SBM), 2) SBM pre- and CM postpartum (SBM-CM), 3) CM pre- and SBM postpartum (CM-SBM), and 4) CM pre- and postpartum (CM-CM). The close-up, transition, and lactating diets were fed for four weeks immediately before parturition, the first three weeks of lactation, and weeks four through sixteen of lactation, respectively. The close-up, transition, and lactating pairs of diets were formulated using most of the same ingredients, except SBM and soybean hulls were used only in the SBM-based diets and CM was used only in the CM-based diets. The close-up, transition, and lactating diets were formulated to contain 14.2 or 19.4%, 12.1 or 16.5%, and 9.9 or 13.5% SBM or CM, respectively, on a DM basis. The close-up, transition, and lactating SBM or CM diets were 14.3 or 14.5%, 17.6 or 17.4%, and 17.2 or 16.9% CP, respectively, on a DM basis. Pre- and postpartum data were analyzed separately using the MIXED procedure of SAS

with week as the repeated measure. There was no difference between diets for body weight (BW), most BW change measures, or body condition score (BCS). Maximum minus minimum weight increased for cows fed the CM diets compared to the SBM diets postpartum. Prepartum DMI increased for cows fed the CM diet compared to the SBM diet. Postpartum DMI increased for cows fed the CM diets compared to the SBM diets postpartum. Milk yield tended to increase for cows fed the CM diets compared to the SBM diets postpartum. An interaction between prepartum diet and week of lactation was observed for ECM and 4% FCM yields. An interaction between prepartum diet, postpartum diet, and week of lactation was found for feed efficiency. There was no difference between diets for milk component percentages or yields. Cows fed the CM diets postpartum decreased MUN compared to cows fed the SBM diets. A tendency for an interaction between prepartum diet, postpartum diet, and week of lactation was observed for $\log_{10}\text{SCC}$. The interaction between pre- and postpartum diet was not significant for any production measure. The absence of this interaction suggests that including CM as the primary protein source in diets fed during the close-up dry period and early lactation did not elicit even greater production responses compared to solely feeding CM during early lactation. In agreement with previous experiments, increased DMI, a tendency for increased milk yield, and decreased MUN were observed for cows fed diets formulated with CM compared to SBM during early lactation.

Introduction

The transition period, commonly regarded as the three weeks before and after parturition (Grummer, 1995), is the most challenging time period for a productive dairy cow from a metabolic standpoint. Immediately after parturition and lasting weeks, the energetic and nutrient

demands of milk production outpace that provided via DMI, resulting in negative energy balance (Bauman and Currie, 1980). In response, dairy cows have been shown to mobilize 50 to 70 kg of adipose tissue (Komaragiri and Erdman, 1997) and 8 to 21 kg of skeletal tissue (Komaragiri and Erdman, 1997; Komaragiri et al., 1998) during the first five to 12 or five to six weeks of lactation, respectively. One potential method of alleviating this burden may be through improved dietary formulations, which can be achieved by balancing dietary AA to better match the cow's requirements during this time.

While methionine is generally recognized as one of the EAA that is first limiting for milk protein synthesis (NASEM, 2021), supplementation of methionine in diets fed to dairy cows during the transition period and early lactation has yielded variable results for production. Despite a marginal decrease of -0.04 kg/d for DMI (Patton, 2010), two meta-analyses found no difference for milk yield between cows fed diets supplemented with methionine compared to control diets (Patton, 2010; Wei et al., 2022). However, cows fed diets with methionine increased milk protein percent in both cases, resulting in increased milk protein yield as well.

Another way of incorporating more methionine in diets is through utilization of different protein sources. Soybean meal (SBM) is traditionally regarded as the primary protein source utilized in dairy cow diets. However, the growing popularity of canola meal (CM) is challenging this long-held notion. While SBM contains more CP on a DM basis compared to CM (52.6 vs. 40.6-42.9%, respectively; Adewole et al., 2016; Broderick et al., 2016; NASEM, 2021), the increased methionine content of CM compared to SBM (1.97 vs. 1.38%; NASEM, 2021) makes it an attractive option in scenarios where methionine is limiting.

Two meta-analyses (Huhtanen et al., 2011; Martineau et al., 2013) explored the utilization of CM in diets fed to dairy cows. Both determined that DMI and milk yield increased

when including CM in diets compared to other protein sources. Furthermore, ECM yield responded positively to including CM in diets compared to SBM or SBM and fish meal in Huhtanen et al. (2011). However, while Martineau et al. (2013) found that ECM and FCM yields responded positively to the inclusion of CM in diets compared to all other protein sources, there was no effect when compared to only SBM. Importantly, these meta-analyses focused on experiments conducted during middle and late lactation due to a lack of information from early lactation. In agreement with Huhtanen et al. (2011), replacing SBM with CM during middle lactation in subsequent experiments further demonstrated positive effects on production (Broderick et al., 2015; Benchaar et al., 2021; Lage et al., 2021).

Experiments investigating CM during early lactation also observed positive responses for production measures such as DMI and milk yield for cows fed diets with CM compared to SBM (Moore and Kalscheur, 2016; Gauthier et al., 2019; Swanepoel et al., 2020). Furthermore, Moore and Kalscheur (2016) reported that cows fed the CM-based diets increased feed efficiency (ECM/DMI) compared to cows fed the SBM-based diets. In all cases, the increased methionine content of CM is hypothesized to be a main reason for the positive production responses. However, to our knowledge, the effect of including CM in diets fed during the dry period, specifically close-up, is unexplored. Additionally, it is unknown whether feeding CM during both the close-up dry period and early lactation has a synergistic effect on production measures.

The primary objective of this experiment was to determine the effect of feeding isonitrogenous diets formulated with SBM or CM as the primary protein source during the close-up dry period and early lactation on production measures in Holstein dairy cows. Furthermore, we attempted to establish whether a synergistic effect between feeding CM during both the close-up dry period and early lactation was present. To achieve this, it is necessary to determine

whether an interaction between pre- and postpartum diet was present for any production measure.

Materials and Methods

Cows, Facilities, and Diets

The procedures used in this experiment were approved by the University of Wisconsin-Madison IACUC protocol A006120. Eighty multiparous (2.72 ± 0.92 ; average parity \pm SD) Holstein cows enrolled in a randomized complete block design and were grouped into blocks of four cows by expected parturition date. Within block, cows were randomly assigned to one of four dietary treatments in a 2×2 factorial design. Experimental diets were fed from November 2019 through October 2020 and cows calved from December 2019 through June 2020. Cows were housed in individual tie-stalls at the USDA Dairy Forage Research Center farm in Prairie du Sac, WI. Diets were fed as a TMR once-daily for ad libitum intake with continuous access to water. Cows were milked $3 \times$ daily at approximately 0600, 1300, and 2100 in a double-8 herringbone parlor.

Diets were formulated using NRC (2001) software. Three isonitrogenous pairs of diets (close-up, transition, and lactating) were fed during the experiment and differed in the primary protein source utilized, which was SBM or CM. Prior to consuming the close-up diets, all cows were fed the common herd far-off dry cow diet. At 27.3 ± 1.3 (mean \pm SD) days before expected parturition date, cows began consuming the close-up SBM or CM diet and remained on the same diet until parturition. Of the 80 cows enrolled, half ($n = 40$) consumed the diet with SBM and the other half consumed the diet with CM during the close-up dry period. Cows consumed the close-

up SBM and CM diets for 27.0 ± 4.4 and 28.0 ± 4.7 (mean \pm SD) days before parturition, respectively. All cows consumed the close-up diet for at least 17 days before parturition. At parturition, half ($n = 20$) of the cows on each of the close-up diets switched to the transition diet containing the other protein source, while the other half consumed the transition diet with the same protein source. The transition and lactating diets were fed from parturition through week three and week four through 16 of lactation, respectively. The transition diets were formulated to contain more CP to supply more AA for the fresh cow to accommodate increasing milk production and less starch to minimize metabolic disorders. This design resulted in four treatment groups, which were cows fed diets with 1) SBM pre- and postpartum (SBM-SBM), 2) SBM pre- and CM postpartum (SBM-CM), 3) CM pre- and SBM postpartum (CM-SBM), and 4) CM pre- and postpartum (CM-CM).

Ingredient composition of the far-off, close-up, transition, and lactating diets are presented in Table 2.1. The forage:concentrate ratios of the far-off, close-up, transition, and lactating diets were 87.24:12.76, 70:30, 62:38, and 60:40, respectively. The far-off and close-up diets contained conventional corn silage, grass hay, and wheat straw, whereas the transition and lactating diets contained BMR corn silage and alfalfa silage as the forage sources. Two sources of high-moisture corn were used, with one used for the dry cow diets and the other used for the lactating cow diets.

Sampling and Analysis

Samples of all diets, silages, and high-moisture corn were collected daily and stored at -20°C in one week increments until dried. Samples of dry feeds were collected once weekly. All feed samples were dried in duplicate at 105°C for 24h for dry matter determination and at 55°C for 48h for nutrient analysis. All weekly composites of diets and forages (BMR corn silage,

conventional corn silage, alfalfa silage, grass hay, and wheat straw) were ground to pass through a 1-mm screen (Wiley mill, Arthur H. Thomas Co., Philadelphia, PA) and composited in eight week increments for nutrient analysis. All concentrate samples (SBM, soybean hulls, CM, corn gluten meal, and high-moisture corn) were ground to pass through a 1-mm screen (cross beater mill, Retsch GmbH and Co., Haan, Germany) and composited in eight week increments for nutrient analysis. All composited feed samples were analyzed by a commercial laboratory (Dairyland Laboratories, Inc., Arcadia, WI) using wet chemistry analysis. The far-off, close-up, transition, and lactating diets were analyzed for CP (method 990.03; AOAC International, 1996), NDF using alpha amylase and sodium sulfite (aNDF; method 2002.04; AOAC International, 2005), aNDF with ash correction (aNDFom), ether extract (EE) using diethyl ether (method 920.39; AOAC International, 1996), ash (method 942.05; AOAC International, 1996), and the following minerals: Ca, P, Mg, K, and S using a microwave and inductively coupled plasma spectroscopy (method 942.05; AOAC International, 1996). The corn silages, alfalfa silage, SBM, soybean hulls, CM, corn gluten meal, high-moisture corn, grass hay, and wheat straw were analyzed for CP, aNDF, aNDFom, ADF (method 973.18; AOAC International, 1996), NDICP and ADICP using fiber residuals from NDF and ADF determination (method 990.03; AOAC International, 1996), starch using a sodium acetate buffer method (Hall, 2009), EE, and ash. Non-fiber carbohydrates (NFC) was calculated as: $NFC = 100 - ((\%aNDFom - \%NDICP) + \%CP + \%EE + \%Ash)$, with NDICP expressed as % of DM. Nutrient analysis results for each composite were weighted relative to the corresponding number of days each diet and ingredient were fed, as a proportion of the overall days of cow enrollment, due to the unequal distribution of cows consuming each diet and ingredient during each week of the experiment. The nutrient compositions of the diets (CP, NDICP, ADICP, aNDF, aNDFom, ADF, NFC, starch, EE, ash,

and NE_L) were calculated using the analyzed composition of the individual feed ingredients and their inclusion rate in the TMR.

The BMR corn silage, alfalfa silage, transition diets, and lactating diets were analyzed using the Penn State Particle Separator (PSPS) for each week of the experiment that they were utilized. A five-pan system was used, including 19, 8, 4, and 1.18 mm screens and a bottom pan. The results for each week's silages and diets were weighted relative to the corresponding number of days each silage and diet were fed, as a proportion of the overall days of cow enrollment, due to the unequal distribution of cows consuming each silage and diet during each week of the experiment.

Body weight (BW) measurements were collected for each cow immediately after parturition (fresh BW) and twice weekly on two consecutive days from week one through 16 of lactation and averaged for each week. Body weight change was characterized four ways, including the change between fresh to minimum weight, minimum to end weight, fresh to end weight, and maximum to minimum weight. Body condition score (BCS) was measured for each cow on a scale of one to five (Elanco Animal Health) during weeks one, four, eight, 12, and 16 of lactation by three trained observers and averaged by cow for each time point. Feed intake (as-is) was recorded daily for each cow by a Rissler TMR Mixer (Fritsch Equipment Corporation, De Pere, WI) equipped with FeedWatch (Valley Agricultural Software, Tulare, CA). The feeder recorded individual weigh-back and drop weights by hand daily to correct weight discrepancies that were electronically recorded. Dry matter intake (DMI) was calculated daily as the amount of TMR fed to the cow minus the weigh-back, multiplied by the weekly diet DM corresponding to the day, and averaged for each week of the experiment for each cow from three weeks prepartum through week 16 of lactation. Milk yield was recorded daily for each cow and averaged for each

week of the experiment for each cow from week one through 16 of lactation. Energy-corrected milk (ECM) was calculated (Tyrrell and Reid, 1965) daily using the formula: $ECM = (0.327 \times \text{Milk Yield (kg/d)}) + (12.95 \times \text{Milk Fat Yield (kg/d)}) + (7.20 \times \text{Milk Protein Yield (kg/d)})$ and averaged for each week of the experiment for each cow from week one through 16 of lactation. Fat-corrected milk (4% FCM) was calculated (Gaines, 1928) daily using the formula: $4\% \text{ FCM} = (0.4 \times \text{Milk Yield (kg/d)}) + (15 \times \text{Milk Fat Yield (kg/d)})$ and averaged for each week of the experiment for each cow from week one through 16 of lactation. Feed efficiency was calculated daily as ECM divided by DMI and averaged for each week of the experiment for each cow from week one through 16 of lactation. Milk samples were collected at each milking over two consecutive days from each cow from week one through 16 of lactation. Milk samples were collected in bottles with a bronopol tablet used as a preservative and analyzed within one week of collection by a commercial laboratory (AgSource Laboratories, Menomonie, WI) by mid-infrared analysis using a Foss MilkoScan FT6000 for fat, true protein, lactose, SNF, SCC, and MUN. Total solids were calculated as the sum of milk fat and SNF. Daily milk component percentages were weighted according to the milk yield at each milking for each of the consecutive sampling days and the two daily values were averaged by cow for each week of lactation. Values for SCC were \log_{10} -transformed for normality before analysis. Milk component yields were calculated as the weekly average value of each milk component multiplied by the weekly average value of milk yield for each cow during each week of lactation.

NE_L intake was calculated as diet NE_L (Mcal/kg) \times DMI (kg/d). NE_L milk was calculated as milk yield (kg/d) \times [(0.0929 \times fat %) + (0.0563 \times protein %) + (0.0395 \times lactose %)]. NE_L maintenance was calculated as $0.10 \times BW^{0.75}$. Energy equations were based on NASEM (2021).

Energy balance was calculated as $NE_{L(Intake)} - (NE_{L(Milk)} + NE_{L(Maintenance)})$. Energy efficiency was defined as $(NE_{L(Milk)} / NE_{L(Intake)}) * 100$.

Cows were monitored by the farm staff for clinical metabolic disorders, including mastitis, pneumonia, milk fever, ketosis, displaced abomasum, metritis, and retained placenta. Clinical mastitis was recorded 20 times in 18 different cows throughout the experiment. Pneumonia was recorded nine times in six different cows. A total of five cases of milk fever, three cases of ketosis, two cases of displaced abomasum, two cases of metritis, and one case of retained placenta were recorded. Displaced abomasum and ketosis were recorded concurrently in one cow. All other cases of milk fever, ketosis, displaced abomasum, metritis, and pneumonia occurred in separate cows.

Statistical Analysis

Statistical analysis was performed on weekly averages of BW, pre- and postpartum DMI, milk yield, ECM yield, 4% FCM yield, feed efficiency, energy balance measures, milk component percentages, and milk component yields and on average BCS every four weeks. Overall magnitude of change was analyzed for the BW change measures of fresh to minimum weight (FreshToMin), minimum to end weight (MinToEnd), fresh to end weight (FreshToEnd), and maximum to minimum weight (MaxToMin).

Prepartum DMI was analyzed using the MIXED procedure of SAS (version 9.4, SAS Institute, Inc., Cary, NC) with week as the repeated measure. Body weight and BCS before cows began consuming the close-up diet and ME305 for the previous lactation were tested as covariates and retained in the model if significant ($P \leq 0.10$). The covariance structure with the lowest AIC was used. The prepartum statistical model is:

$$Y_{ijk} = \mu + \text{Covariate} + \text{Treatment}_i + \text{Week}_j + (\text{Treatment}_i \times \text{Week}_j) + \text{Block}_k + \varepsilon_{ijk},$$

where Y is the response variable; μ is the overall mean; covariate is the BW, BCS, or ME305 measure (if significant); treatment is the fixed effect of prepartum diet ($i = \text{SBM or CM}$); week is the fixed effect of week relative to parturition ($j = -3 \text{ to } -1$); treatment \times week is the fixed effect 2-way interaction of diet and week; block is the random effect of block ($k = 1 \text{ to } 20$); and ε is the residual error.

Postpartum data was analyzed using the MIXED procedure of SAS with week as the repeated measure. Body weight and BCS at parturition and ME305 for the previous lactation were tested as covariates and retained in the model if significant ($P \leq 0.10$). The covariance structure with the lowest AIC was used. The postpartum statistical model is:

$$Y_{ijkl} = \mu + \text{Covariate} + \text{Prepartum}_i + \text{Postpartum}_j + (\text{Prepartum}_i \times \text{Postpartum}_j) + \text{Week}_k + (\text{Prepartum}_i \times \text{Week}_k) + (\text{Postpartum}_j \times \text{Week}_k) + (\text{Prepartum}_i \times \text{Postpartum}_j \times \text{Week}_k) + \text{Block}_l + \varepsilon_{ijkl},$$

where Y is the response variable; μ is the overall mean; covariate is the BW, BCS, or ME305 measure (if significant); prepartum is the fixed effect of the prepartum diet ($i = \text{SBM or CM}$); postpartum is the fixed effect of the postpartum diet ($j = \text{SBM or CM}$); prepartum \times postpartum is the fixed effect 2-way interaction of the prepartum and postpartum diets; week is the fixed effect of week of lactation ($k = 1 \text{ to } 16$); prepartum \times week is the fixed effect 2-way interaction of the prepartum diet and week; postpartum \times week is the fixed effect 2-way interaction of the postpartum diet and week; prepartum \times postpartum \times week is the fixed effect 3-way interaction of the prepartum diet, postpartum diet, and week; block is the random effect of block ($l = 1 \text{ to } 20$); and ε is the residual error.

Overall magnitude of change for BW change measures was analyzed using the MIXED procedure of SAS. Body weight and BCS at parturition and ME305 for the previous lactation were tested as covariates and retained in the model if significant ($P \leq 0.10$). The statistical model for BW change measures is:

$$Y_{ijk} = \mu + \text{Covariate} + \text{Prepartum}_i + \text{Postpartum}_j + (\text{Prepartum}_i \times \text{Postpartum}_j) + \text{Block}_k + \varepsilon_{ijk}$$

where Y is the response variable; μ is the overall mean; covariate is the BW, BCS, or ME305 measure (if significant); prepartum is the fixed effect of the prepartum diet ($i = \text{SBM or CM}$); postpartum is the fixed effect of the postpartum diet ($j = \text{SBM or CM}$); prepartum \times postpartum is the fixed effect 2-way interaction of the prepartum and postpartum diets; block is the random effect of block ($k = 1 \text{ to } 20$); and ε is the residual error. Significance was declared when $P \leq 0.05$ and tendency was declared when $0.05 < P \leq 0.10$. The Tukey-Kramer adjustment was performed for pairwise comparisons of LSM.

Results

Nutrient Composition of Diets and Penn State Particle Separator

The chemical composition of the diets and diet ingredients are presented in Tables 2.2 and 2.3, respectively. Each pair of diets (close-up, transition, and lactating) was within 0.3% CP. As expected, the CP content of SBM was higher (51.4 vs. 41.4%) and the aNDFom content of SBM was lower (8.66 vs. 23.1%) than CM. However, the high aNDFom content of soybean hulls (67.7%) compensated for this difference in aNDFom content in the SBM-based diets. The PSPS analysis is presented in Table 2.4. For both the transition and lactating pairs of diets, particle size was similar between diets for the 19, 8, and 4 mm sieves. However, the CM diets were

noticeably lower compared to the SBM diets for the 1.18 mm sieve (18.1 vs. 23.6% and 18.0 vs. 22.6%, respectively), whereas the CM diets were noticeably higher compared to the SBM diets for the bottom pan (17.1 vs. 10.8% and 15.2 vs. 10.7%, respectively).

Body Weight and Body Condition Score

Body weight, BW change, and BCS data are presented in Table 2.5. Body weight LSM for each treatment group by week of lactation are presented in Figure 2.1. There was no difference for BW between treatment groups ($P > 0.10$). Additionally, the BW change measures of FreshToMin, MinToEnd, and FreshToEnd were not different between treatment groups ($P > 0.10$). However, MaxToMin BW change was affected by postpartum diet ($P = 0.04$), with a larger decrease observed for cows fed the CM diets compared to the SBM diets. There was no difference for BCS between treatment groups ($P > 0.10$).

Dry Matter Intake, Milk Yields, and Feed Efficiency

Pre- and postpartum DMI, milk yield, ECM yield, 4% FCM yield, and feed efficiency data are presented in Table 2.5. Dry matter intake, milk yield, ECM, 4% FCM, and feed efficiency LSM for each treatment group by week relative to parturition are presented in Figures 2.2 to 2.6. Prepartum DMI increased for cows fed the CM diet compared to the SBM diet ($P = 0.04$). Additionally, postpartum DMI increased for cows fed the CM diets compared to the SBM diets postpartum ($P = 0.02$). Milk yield tended to increase for cows fed the CM diets compared to the SBM diets postpartum ($P = 0.09$). There was an interaction between prepartum diet and week of lactation for ECM ($P = 0.02$) and 4% FCM yields ($P = 0.05$). An interaction between prepartum diet, postpartum diet, and week of lactation occurred for feed efficiency ($P = 0.01$).

Energy Balance

Energy balance data are presented in Table 2.5. Intake of NE_L tended to increase for cows fed the CM diets compared to the SBM diets postpartum ($P = 0.09$). There was no difference for NEL maintenance, NEL milk, energy balance, or energy efficiency between treatment groups ($P > 0.01$).

Milk Composition and Component Yields

Milk composition data are presented in Table 2.6. Milk $\log_{10}SCC$ LSM for each treatment group by week of lactation are presented in Figure 2.7. There was no difference for milk fat, protein, lactose, or total solids percentage or yield between treatment groups ($P > 0.10$). Milk urea nitrogen decreased for cows fed the CM diets compared to the SBM diets postpartum ($P = 0.01$). A tendency for an interaction between prepartum diet, postpartum diet, and week of lactation was observed for $\log_{10}SCC$ ($P = 0.10$).

Interaction between Prepartum and Postpartum Diet

The interaction between prepartum diet and postpartum diet was not significant for any production measure ($P > 0.10$).

Discussion

To our knowledge, this is the first experiment analyzing the production effects of feeding diets formulated with SBM or CM as the primary protein source during the close-up dry period in cows. Furthermore, this experiment adds to the limited data available when comparing the production effects for cows fed diets formulated with SBM or CM as the primary protein source

during early lactation. Additionally, as the two protein sources were utilized in diets fed during both periods in a 2×2 factorial arrangement of treatments, this is the first experiment able to determine whether a synergistic effect between feeding CM during both the close-up dry period and early lactation may be present.

Body Weight and Body Condition Score

No difference between treatment groups for most measures related to BW and BCS indicate that the tendency for increased milk yield for cows fed the CM diets postpartum is likely not explained by body tissue mobilization and utilization. The greater difference in maximum and minimum weight suggests that the cows fed the CM diets postpartum experienced a greater overall fluctuation in BW compared to cows fed the SBM diets. However, the metabolic impact of this result is likely negligible, as the incidence of disorders such as ketosis and displaced abomasum during the experiment was minimal. Agreeing with the present experiment, Moore and Kalscheur (2016) observed no difference for BW or BCS between cows fed low (16.2%) or high (18.1%) CP diets with SBM or CM as the primary protein source from parturition through week 16 of lactation. However, a linear increase was observed for BCS and BCS change when CM inclusion increased from 3.5 to 13.0% in diets fed to cows from approximately 12 through 160 DIM (Gauthier et al., 2019). Interestingly, BCS change was positive only for the cows fed the diet with 13.0% CM, suggesting better energy balance for those cows. Consistent with the present experiment, Swanepoel et al. (2020) found no difference for BCS between cows fed diets with 14.3% CM or 6.6% CM and 6.3% SBM from approximately 13 through 160 DIM. Contrary to Gauthier et al. (2019), no difference was observed between diets for BCS change. Furthermore, there was no difference for BCS between cows fed the diet with 6.6% CM and 6.3% SBM or with 6.6% CM, 6.3% SBM, and 7.9 g rumen-

protected methionine (RPM)/day, suggesting either that methionine was not a limiting factor or the amount of supplemented methionine was not sufficient to elicit a response. Similarly, no difference for BW or BCS between cows fed diets with or without methionine during the transition period and early lactation is commonly reported (Zhou et al., 2016; Batistel et al., 2017; Cardoso et al., 2021).

Prepartum Dry Matter Intake

In mature close-up dry cows, three routes of nutrient utilization may help explain the increase for prepartum DMI for cows fed the CM diet in the present experiment. Fetal development adheres to an exponential curve, with approximately 60% occurring during the last 2 months of pregnancy (Bauman and Currie, 1980). Although neither fetal development nor calf birth weights were measured in this experiment, the difference in prepartum DMI could be partially utilized by this route. While calf birth weight was not affected by three weeks of RPM supplementation before parturition in Jacometo et al. (2016), Alharthi et al. (2018) observed increased calf birth weight for dams fed a diet with RPM during the last four weeks of pregnancy compared to dams fed a control diet. Furthermore, while not different in Jacometo et al. (2016), ADG during the first eight weeks of life was higher for calves from dams fed a diet with RPM in Alharthi et al. (2018), suggesting a potential fetal programming effect due to the methionine fed during late gestation. Interestingly, Peñagaricano et al. (2013) observed 276 differentially expressed genes (DEGs) in preimplantation embryos between Holstein cows fed a control diet or a diet with RPM for the first 10 weeks of lactation. Furthermore, some of the most significant DEGs were related to embryonic development, providing evidence that RPM supplementation may influence growth of the calf during pregnancy. Additionally, the increase for prepartum DMI could be partitioned to mammary development and deposition of parenchymal tissue in the

udder. Capuco et al. (1997) observed a 230 g/d increase in parenchymal tissue for udder halves extracted from dry Holstein cows from day -20 to -7 relative to parturition, demonstrating rapid growth during this time. However, this scenario appears unlikely in the present experiment as prepartum diet did not affect milk yield. Increasing body reserves, i.e. adipose and skeletal muscle, to prepare for lactation is another potential expenditure of the extra prepartum DMI. This scenario also appears unlikely in the present experiment as BW and BCS were not different during week one of lactation. Mixed results have been reported for prepartum DMI when comparing diets with or without methionine. In some cases, cows fed diets supplemented with methionine increased prepartum DMI compared to cows fed the control diet (Zhou et al., 2016; Batistel et al., 2017; Cardoso et al., 2021). However, there was no difference in other scenarios (Ordway et al., 2009; Osorio et al., 2013; Potts et al., 2020).

Postpartum Dry Matter Intake and Milk Yields

Agreeing with the present experiment, Moore and Kalscheur (2016) observed a tendency for increased postpartum DMI for cows fed diets with CM as the primary protein source compared to SBM from parturition through week 16 of lactation. However, the increase for DMI was numerically less than the increase observed for the present experiment (0.8 vs. 1.2 kg/d). Conversely, Gauthier et al. (2019) and Swanepoel et al. (2020) found no difference for postpartum DMI between diets. Since BW, most BW change measures, and BCS were not different between treatment groups, the increased postpartum DMI for cows fed the CM diets compared to the SBM diets postpartum in the present experiment is likely explained by the tendency for increased milk yield of the cows fed the CM diets. According to Huhtanen et al. (2011), the more balanced supply of AA for milk production in the postpartum CM diets, due to the greater supply of methionine in CM compared to SBM, may have resulted in a “pull effect,”

whereby increased milk yield causes increased energetic demand that must be fulfilled via increased DMI. While the present experiment observed a larger increase for postpartum DMI compared to Moore and Kalscheur (2016), the 1.8 kg/d increase for milk yield in the present experiment was numerically less than the 4.5 kg/d increase of Moore and Kalscheur (2016). A quadratic response was observed in Gauthier et al. (2019), as cows fed the diets with 8.2% or 13.0% CM increased milk yield compared to the diet with 3.5% CM. However, milk yield was not different between the cows fed the diets with 8.2% or 13.0% CM. Furthermore, Swanepoel et al. (2020) reported increased milk yield for cows fed the diet with 14.3% CM compared to the diet with 6.6% CM and 6.3% SBM. Interestingly, milk yield was not different between cows fed the diet with 6.6% CM and 6.3% SBM compared to the diet with 6.6% CM, 6.3% SBM, and 7.9 g RPM/d diet. This result suggests that either the amount of RPM supplemented was not enough to make up the difference in methionine supply between CM and SBM or another factor intrinsic to CM was responsible for the increased milk yield in their experiment.

While milk yield tended to increase for cows fed the CM diets compared to the SBM diets postpartum, there was no postpartum diet effect for ECM or 4% FCM yields. As ECM and 4% FCM yields are derived from equations that incorporate milk yield along with milk fat and protein yield or just milk fat yield, respectively, it is not surprising that the SEM for ECM and 4% FCM yields were higher than for milk yield (1.31 and 1.30 vs. 1.04 kg/d). Increased variability for ECM and 4% FCM yields coupled with a decreased effect size compared to milk yield (1.57 and 1.38 vs. 1.81 kg/d) resulted in no difference. Interestingly, there was an interaction between prepartum diet and week of lactation for both ECM and 4% FCM yields. For ECM yield, cows in the SBM-CM group were the highest numerically during weeks one through six. Cows in the SBM-SBM group ranged from the lowest to the second highest during this time.

However, cows in the CM-SBM group were second-highest during week one and then lowest during weeks three through six. Cows in the CM-CM group were either second lowest or second highest during this time. During weeks seven through 16, cows in either the SBM-CM or CM-CM group were the highest numerically. Conversely, cows in either the SBM-SBM or CM-SBM groups were lowest. Since ECM yield differs from milk yield by also factoring in milk fat and protein yields, fluctuations in temporal ranking of ECM yield between treatment groups may be attributed in part to variability for milk component percentages from week to week. Given that milk protein yield is not used to calculate 4% FCM and milk protein yield was not different between treatment groups, it is not surprising that the same temporal ranking of groups occurred for 4% FCM yield. The results for ECM and 4% FCM yields in the present experiment contrast with Moore and Kalscheur (2016), whereby increases of 4.02 and 3.92 kg/d were observed for ECM and 4% FCM yields, respectively, for cows fed the CM-based diets compared to the SBM-based diets.

Feed Efficiency

The 3-way interaction observed for feed efficiency (ECM/DMI) is likely due to differences occurring during the first seven weeks of lactation. During week one, the CM-SBM group had higher feed efficiency than the other three groups which were not different. During week two, the SBM-SBM group increased feed efficiency to be higher than the other three groups, which all decreased. From weeks three through five, the difference between treatment groups decreased and then increased again through week seven. From week eight through 16, the difference between the treatment groups remained small. In Moore and Kalscheur (2016), cows fed the CM-based diets tended to increase feed efficiency compared to cows fed the SBM-based diets. While Gauthier et al. (2019) and Swanepoel et al. (2020) did not directly report feed

efficiency, increased milk yield for cows fed diets with higher inclusion rates of CM compared to SBM coupled with no difference for DMI in both experiments suggests feed efficiency potentially increased for the cows fed the diets with higher inclusion rates of CM.

Milk Composition and Component Yields

Substituting CM for SBM in diets fed during early lactation has yielded mixed results for milk composition. In agreement with the present experiment, Moore and Kalscheur (2016) observed no differences for milk component percentages between cows fed diets with SBM or CM as the primary protein source during early lactation. However, Gauthier et al. (2019) noted a tendency for a linear effect on milk fat and a quadratic effect for milk protein and lactose percentages, with cows fed the diet with 13.0% CM producing the highest concentrations of each. While milk fat percent was not different, Swanepoel et al. (2020) reported a tendency for increased milk protein percent and increased milk lactose percent for cows fed the diet with 14.3% CM compared to the diet with 6.6% CM and 6.3% SBM.

Despite a tendency for increased milk yield, milk component yields were not affected by postpartum diet in the present experiment. This is in contrast to the results of Moore and Kalscheur (2016), Gauthier et al. (2019), and Swanepoel et al. (2020), whereby milk fat, protein, lactose, and total solids yields all concomitantly increased with milk yield. No difference for milk fat yield may be attributed to a slight “dilution effect” of milk fat percent caused by increased milk yield as previously observed (Couvreur et al., 2007; Bondan et al., 2019). Although postpartum diet did not significantly alter milk fat percent, it was numerically lower for the SBM-CM and CM-CM groups compared to the SBM-SBM and CM-SBM groups, potentially nullifying a difference for milk fat yield that may have occurred if milk fat percent was more similar between groups. For milk protein, lactose, and total solids yields, no effect of

postpartum diet for the percentage of each component combined with inherent variability for measuring the components and calculating component yields on a weekly basis may have erased the expected increases due to increased milk yield.

Milk Urea Nitrogen

Ammonia is a toxic byproduct of proteolysis in the rumen that must be converted to urea in the liver before excretion or recycling (Getahun et al., 2019). Once released into the bloodstream, urea rapidly equilibrates between bodily fluids such as blood and milk (Broderick and Clayton, 1997). Unsurprisingly, a high correlation exists between MUN and dietary nitrogen (Nousiainen et al., 2004), making MUN a useful tool for evaluating nitrogen balance in the dairy cow. As this source of nitrogen is lost from the cow once milk is removed from the mammary gland, lower concentrations of MUN suggest more efficient utilization of dietary nitrogen due to the dietary AA profile being more conducive to milk protein synthesis. In the case of methionine as the first limiting AA for milk protein production, substituting CM for SBM may achieve lower MUN. Similar to the present experiment, Moore and Kalscheur (2016) found a tendency for decreased MUN for cows fed the CM-based diets compared to the SBM-based diets during early lactation.

Log₁₀Somatic Cell Count

Somatic cell count is commonly measured in milk and used as a proxy for mammary gland health, with decreased concentrations suggesting better mammary gland health. Given its right skewed distribution, log-transformation is sometimes utilized before SCC is statistically analyzed to adhere to a normal distribution. The tendency for a 3-way interaction for log₁₀SCC is likely attributed to the lack of difference between the treatment groups observed during week

one, after which differences became more apparent. Between weeks two and 12, the hierarchy of treatment groups for $\log_{10}\text{SCC}$ changed nearly every week. By week 13 through 16, the CM-SBM group increased above the other three groups that stayed relatively constant. In Gauthier et al. (2019) and Swanepoel et al. (2020), no difference was observed for SCC between diets.

Interaction between Prepartum and Postpartum Diet

The absence of an interaction between the pre- and postpartum diets for all postpartum production measures indicates that there was no added benefit of including CM as the primary protein source in diets fed during both the close-up dry period and early lactation compared to only during early lactation. As previously discussed, the prepartum DMI increase for cows fed the CM diet was potentially utilized for fetal development rather than tissue deposition and development of mammary gland parenchyma, as BW, BCS, and milk yield were not different between treatment groups during week one of lactation. In Fehlberg et al. (2020), there was no interaction between pre- and postpartum diet on milk yield for cows fed diets with or without rumen-protected lysine during the four weeks before and after parturition. Analogous to the present experiment, cows consumed the diet either with or without rumen-protected lysine before parturition and, at parturition, either continued to consume the same diet (with or without lysine) or switched to the other diet.

Conclusions

In conclusion, substituting CM for SBM in diets fed to dairy cows during the close-up dry period and early lactation improves various production measures. Cows fed the CM diet during the close-up dry period increased prepartum DMI compared to cows fed the SBM diet,

with the extra supplied nutrients potentially used for fetal development. The interaction between pre- and postpartum diet was not significant for any production measure, indicating no synergistic effect between including CM as the primary protein source in diets fed during both the close-up dry period and early lactation in this experiment. The increased postpartum DMI, tendency for increased milk yield, and decreased MUN for cows fed the CM diets compared to the SBM diets postpartum in the present experiment agrees with the results of previous experiments comparing inclusion of CM or SBM in diets fed during early lactation.

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Tables and Figures

Table 2.1. Ingredient composition of the far-off, close-up, transition, and lactating diets.

Ingredient (% DM)	Far-Off ¹	Close-Up ²		Transition ³		Lactating ⁴	
		SBM	CM	SBM	CM	SBM	CM
Corn Silage ⁵	53.3	44.9	44.9	36.0	36.0	30.0	30.0
Alfalfa Silage	-	-	-	26.0	26.0	30.0	30.0
Soybean Meal	-	14.2	-	12.1	-	9.90	-
Soybean Hulls	-	5.20	-	4.40	-	3.60	-
Canola Meal	2.73	-	19.4	-	16.5	-	13.5
Corn Gluten Meal	-	-	-	2.00	2.00	2.00	2.00
Roasted Soybeans	7.27	-	-	-	-	-	-
High-Moisture Corn ⁶	-	8.00	8.00	15.0	15.0	20.0	20.0
Grass Hay	14.5	10.8	10.8	-	-	-	-
Wheat Straw	19.4	14.3	14.3	-	-	-	-
Rumen Inert Fat ⁷	-	-	-	2.00	2.00	2.00	2.00
Mineral/Vitamin Mix ^{8,9}	2.47	1.90	1.90	2.50	2.50	2.50	2.50
Reashure ¹⁰	-	0.45	0.45	-	-	-	-
Urea	0.29	0.25	0.25	-	-	-	-

¹Far-off diet fed during the dry period until cows assigned to close-up diet.

²Close-up diet fed from approximately four weeks prepartum through parturition.

³Transition diet fed from parturition through week three of lactation.

⁴Lactating diet fed from week four of lactation through week 16 of lactation.

⁵Conventional corn silage used for the far-off and close-up diets; BMR corn silage used for the transition and lactating diets.

⁶Different sources of high-moisture corn used for diets fed to the dry versus lactating cows.

⁷Energy Booster 100 (Milk Specialties, Dundee, IL).

⁸Dry cow mineral contained (on a DM basis): 18.6% Ca, 9.6% salt, 7.8% Mg, 9.9% S, 22.9 ppm Se, 549,100 IU vitamin A/kg, 163,800 IU vitamin D3/kg, 8,780 IU vitamin E/kg, and 1.10 g Monensin/kg (Vita Plus Corporation, Madison, WI).

⁹Lactating cow mineral contained (on a DM basis): 16.7% Ca, 10.5% salt, 14.4% Na, 5.6% Mg, 0.3% K, 0.6 ppm Se, 274,500 IU vitamin A/kg, 54,900 IU vitamin D3/kg, 1,250 IU vitamin E/kg, and 0.46 g Monensin/kg (Vita Plus Corporation, Madison, WI).

¹⁰Precision Release Choline (Balchem Corporation).

Table 2.2. Chemical composition of diets.

Item (%DM, unless noted)	Far-Off	Close-Up		Transition		Lactating	
		SBM ¹	CM ¹	SBM	CM	SBM	CM
DM, %AF	45.4	48.8	49.6	44.2	45.1	44.9	45.3
CP ²	12.0	14.3	14.5	17.6	17.4	17.2	16.9
NDICP, ² %CP	-	16.1	16.0	8.60	8.57	8.34	8.32
ADICP, ² %CP	-	9.62	10.1	5.10	5.49	5.06	5.38
aNDF ²	43.4	40.7	40.9	28.7	28.8	27.8	27.9
aNDFom ²	42.5	39.6	39.3	27.8	27.6	27.0	26.8
ADF ²	-	27.1	27.5	20.5	20.9	20.3	20.6
NFC ^{2,3}	35.2	38.3	38.1	42.2	42.0	43.4	43.2
Starch ²	-	23.8	23.9	26.7	26.8	28.3	28.4
EE ²	4.34	2.29	2.77	5.38	5.78	5.45	5.78
Ash ²	7.77	5.17	5.48	5.49	5.76	5.59	5.81
Ca	0.77	0.64	0.71	0.93	0.97	0.94	0.99
P	0.27	0.28	0.43	0.32	0.45	0.32	0.43
Mg	0.44	0.35	0.43	0.37	0.45	0.39	0.44
K	1.35	1.33	1.13	1.61	1.42	1.65	1.51
S	0.39	0.33	0.42	0.22	0.30	0.22	0.30
RDP ⁴	7.89	9.35	9.82	11.0	11.2	10.6	10.8
RUP ⁴	4.11	4.95	4.68	6.59	6.19	6.62	6.09
Dig Lys, ^{4,5} g/d	68	95	96	139	135	180	171
Dig Met, ^{4,5} g/d	19	26	28	41	43	54	55
RDP ⁶	8.32	9.89	10.1	12.0	11.9	11.7	11.6
RUP ⁶	3.68	4.41	4.40	5.62	5.49	5.47	5.32
Metab Lys, ^{6,7} g/d	85	110	110	148	146	185	181
Metab Met, ^{6,7} g/d	25	31	33	45	48	57	60
NE _L , ^{2,8} Mcal/kg DM	1.47	1.53	1.50	1.60	1.57	1.60	1.58
NE _L , ⁹ Mcal/kg DM	1.57	1.56	1.59	1.68	1.65	1.61	1.60
NE _L , ¹⁰ Mcal/kg DM	1.66	1.70	1.64	1.84	1.79	1.82	1.78

¹SBM = soybean meal; CM = canola meal.

²Calculated using nutrient analysis of individual feed ingredients.

³NFC = 100 - ((%aNDFom - %NDICP) + %CP + %EE + %Ash); NDICP expressed as % DM.

⁴Estimated based on NRC (2001) using average DMI and BW of cows during the experiment.

⁵Dig Lys = digestible lysine; Dig Met = digestible methionine.

⁶Estimated based on NASEM (2021) using average DMI and BW of cows during the experiment.

⁷Metab Lys = metabolizable lysine; Metab Met = metabolizable methionine.

⁸NE_L = net energy for lactation, calculated using nutrient analysis of individual feed ingredients.

⁹Estimated based on NRC (2001) using average DMI and BW of cows during the experiment.

¹⁰Estimated based on NASEM (2021) using average DMI and BW of cows during the experiment.

Table 2.3. Chemical composition of diet ingredients.

Item (%DM, unless noted)	Corn Silage		AS ¹	SBM ¹	SH ¹	CM ¹	CGM ¹	HMC ¹		GH ¹	WS ¹
	Conv ¹	BMR ¹						Dry ²	Lact ²		
DM, %AF	42.4	36.0	33.9	87.5	89.7	87.8	92.5	71.6	71.2	87.0	88.0
CP	7.20	7.65	21.8	51.4	11.4	41.4	69.6	7.59	7.43	8.84	3.93
NDICP, %CP	13.4	11.2	7.57	1.28	29.1	8.54	3.78	7.98	7.46	27.3	33.3
ADICP, %CP	7.79	5.85	6.40	0.83	11.7	6.13	2.94	3.29	3.34	13.1	26.1
aNDF	36.6	34.9	42.6	8.87	69.4	25.8	3.60	5.25	5.52	68.3	81.3
aNDFom	35.9	34.1	41.0	8.66	67.7	23.1	3.47	4.69	5.37	66.1	78.3
ADF	23.2	20.5	37.2	6.58	50.1	20.2	1.34	1.83	1.88	45.8	56.9
NFC ³	51.2	51.8	23.4	31.4	17.8	26.5	23.9	83.0	82.8	17.9	9.72
Starch	39.2	39.2	0.81	0.75	0.77	1.27	15.0	73.3	79.6	0.64	0.49
EE	2.88	3.60	4.57	2.27	1.85	4.62	1.35	3.83	3.38	1.59	0.70
Ash	3.83	3.69	10.9	6.95	4.59	7.94	3.09	1.48	1.52	7.98	8.68

¹Conv = conventional corn silage; BMR = brown midrib; AS = alfalfa silage; SBM = soybean meal; SH = soybean hulls; CM = canola meal; CGM = corn gluten meal; HMC = high-moisture corn; GH = grass hay; WS = wheat straw.

²Dry = used for the close-up diets; Lact = used for the transition and lactating diets.

³NFC = 100 - ((%aNDFom - %NDICP) + %CP + %EE + %Ash); NDICP expressed as % DM.

Table 2.4. Penn State Particle Separator descriptive analysis.

Item	>19 mm	8 to 19 mm	4 to 8 mm	1.18 to 4 mm	Pan
BMR Corn Silage	6.77 ¹	64.0	17.1	11.6	0.54
Alfalfa Silage	29.6	49.3	12.4	7.06	1.69
Transition SBM ²	7.96	43.3	14.4	23.6	10.8
Transition CM ²	7.35	44.0	13.5	18.1	17.1
Lactating SBM	8.75	43.2	14.7	22.6	10.7
Lactating CM	8.79	44.1	14.0	18.0	15.2

¹Proportion remaining on each sieve and pan for each ingredient and diet.

²SBM = soybean meal; CM = canola meal.

Table 2.5. Body weight, BCS, DMI, milk yield, feed efficiency, and energy balance.

Item	Treatment ¹				SEM	CM-CM	CM-SBM	Pre	Post	Pre×Post	Week	P-value ²		
	SBM-SBM	SBM-CM	CM-CM	CM-SBM								Pre×Week	Post×Week	Pre×Post×Week
Body Weight, kg	661	657	658	660	9.15	0.98	0.96	0.74	<0.01	0.85	0.52	0.48		
Body Weight Change														
FreshToMin ³ , kg Δ	-69.3	-69.5	-64.0	-73.4	5.71	0.90	0.40	0.41	-	-	-	-		
MinToEnd ³ , kg Δ	46.2	45.8	42.5	34.0	5.26	0.13	0.39	0.42	-	-	-	-		
FreshToEnd ³ , kg Δ	-23.5	-22.9	-21.8	-39.3	7.45	0.31	0.25	0.22	-	-	-	-		
MaxToMin ³ , kg Δ	-74.1	-82.6	-71.2	-82.2	4.75	0.73	0.04	0.78	-	-	-	-		
BCS, units	3.05	3.04	3.08	3.04	0.03	0.60	0.46	0.71	<0.01	0.97	0.46	0.16		
Prepartum DMI, kg/d	14.5	14.5	15.3	15.3	0.26	0.04	-	-	<0.01	0.80	-	-		
Postpartum DMI, kg/d	24.9	26.5	25.1	26.0	0.52	0.75	0.02	0.47	<0.01	0.61	0.82	0.30		
Milk Yield, kg/d	51.2	52.7	50.7	52.8	1.04	0.82	0.09	0.74	<0.01	0.31	0.45	0.95		
ECM Yield ⁴ , kg/d	53.4	55.2	53.4	54.8	1.31	0.89	0.24	0.85	<0.01	0.02	0.62	0.62		
4% FCM Yield ⁵ , kg/d	50.7	52.2	50.8	52.1	1.30	0.99	0.29	0.95	<0.01	0.05	0.42	0.64		
Feed Efficiency ⁶	2.18	2.11	2.17	2.15	0.05	0.76	0.34	0.66	<0.01	0.59	0.30	0.01		
NE _l Intake, ⁷ Mcal/d	39.9	41.9	40.4	41.1	0.82	0.89	0.09	0.44	<0.01	0.59	0.95	0.34		
NE _l Maintenance, ⁸ Mcal/d	12.9	13.1	12.9	13.1	0.11	0.89	0.20	0.92	<0.01	0.78	0.71	0.24		
NE _l Milk, ⁹ Mcal/d	36.7	37.8	36.9	37.6	0.95	0.96	0.34	0.82	<0.01	0.44	0.76	0.73		
Energy Balance, ¹⁰ Mcal/d	-9.78	-9.04	-9.53	-9.51	0.78	0.88	0.62	0.63	<0.01	0.92	0.64	0.35		
Energy Efficiency, ¹¹ %	93.7	91.0	93.4	92.5	1.91	0.75	0.33	0.60	<0.01	0.96	0.50	0.28		

¹SBM-SBM = cows fed diets with SBM pre- and postpartum; SBM-CM = cows fed diets with SBM prepartum and CM postpartum; CM-SBM = cows fed diets with CM pre- and postpartum; and SBM postpartum; CM-CM = cows fed diets with CM pre- and postpartum.

²Pre = effect of prepartum diet; Post = effect of postpartum diet; Week = effect of week of lactation.

³FreshToMin = fresh weight minus minimum weight during the experiment; MinToEnd = minimum weight during the experiment minus weight at end of the experiment; FreshToEnd = fresh weight minus weight at end of the experiment; MaxToMin = maximum weight during the experiment minus minimum weight during the experiment.

⁴ECM = $0.327 \times \text{Milk Yield (kg/d)} + 12.95 \times \text{Milk Fat Yield (kg/d)} + 7.20 \times \text{Milk Protein Yield (kg/d)}$.

⁵4% FCM = $0.4 \times \text{Milk Yield (kg/d)} + 15 \times \text{Milk Fat Yield (kg/d)}$.

⁶Feed Efficiency = ECM/DMI.

⁷NE_l Intake = Diet NE_l (Mcal/kg) × DMI (kg/d).

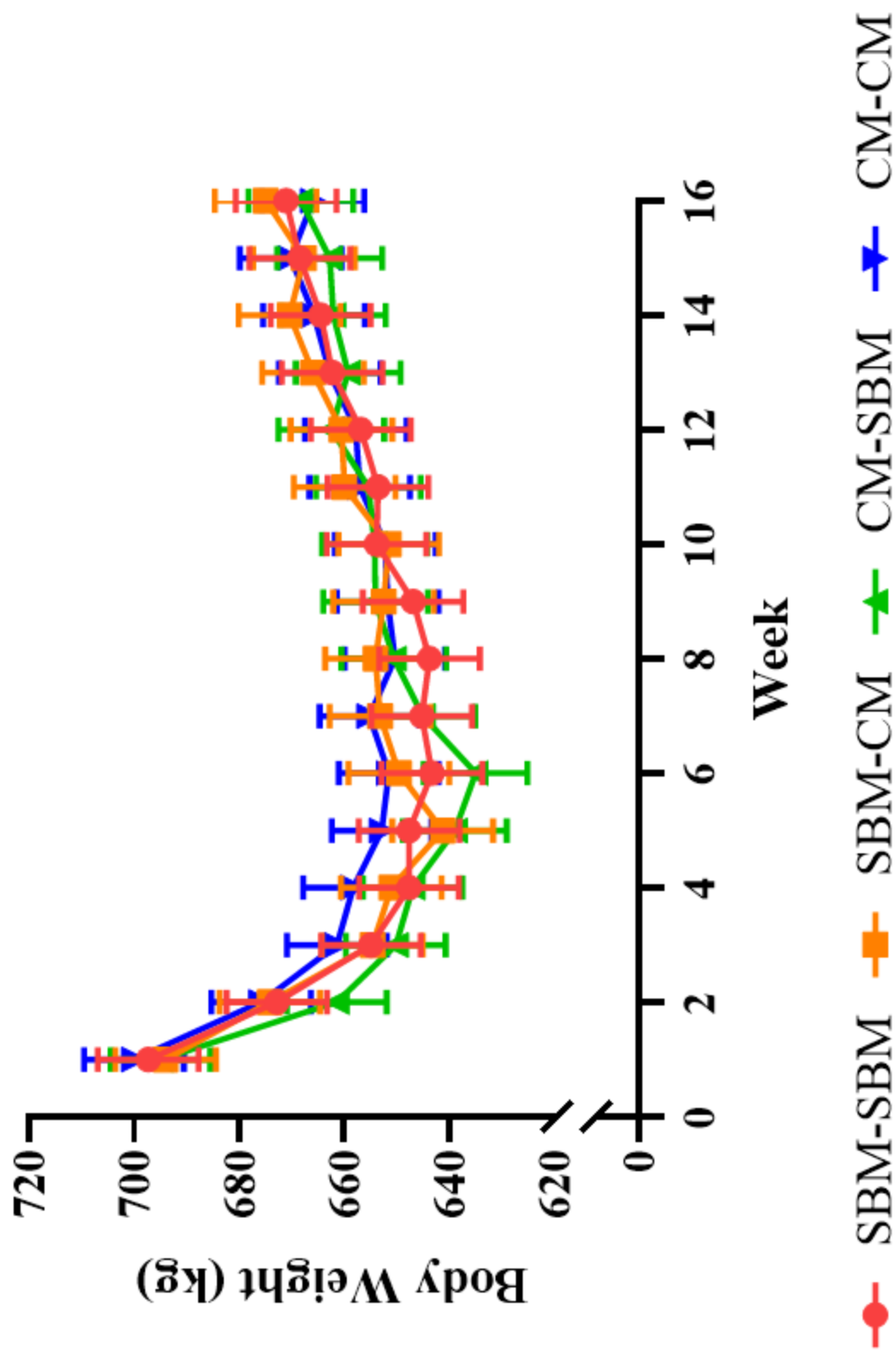
⁸NE_l Maintenance = $0.10 \times \text{BW}^{0.75}$; NASEM, 2021.

⁹NE_l Milk = Milk Yield (kg/d) × [(0.0929 × milk fat %) + (0.0585 × milk protein %) + (0.0395 × milk lactose %)]; NASEM, 2021.

¹⁰Energy Balance = NE_l intake - (NE_l milk + NE_l maintenance).

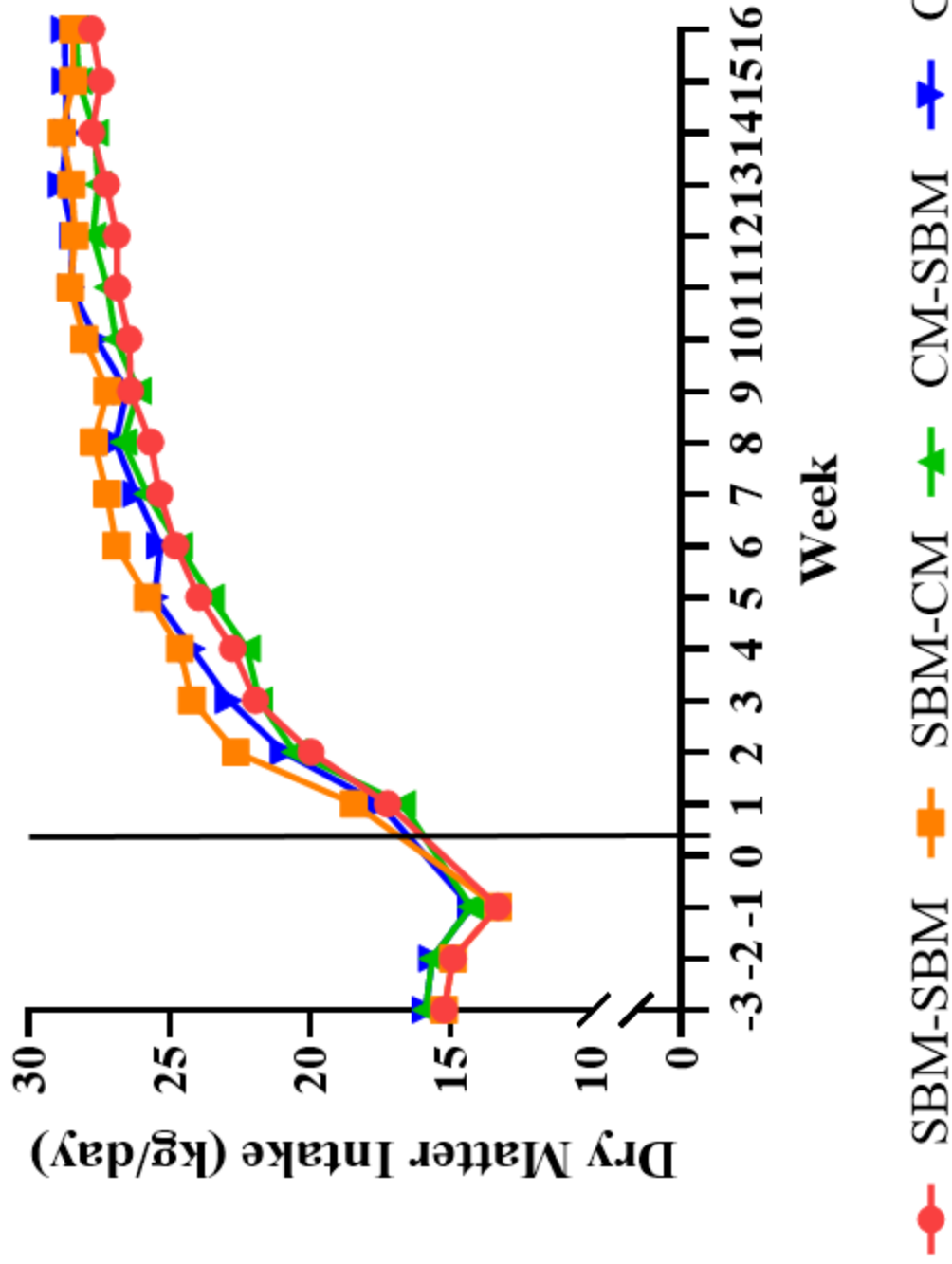
¹¹Energy Efficiency = NE_l milk/NE_l intake.

Figure 2.1. Body weight LSM for each treatment group by week of lactation.



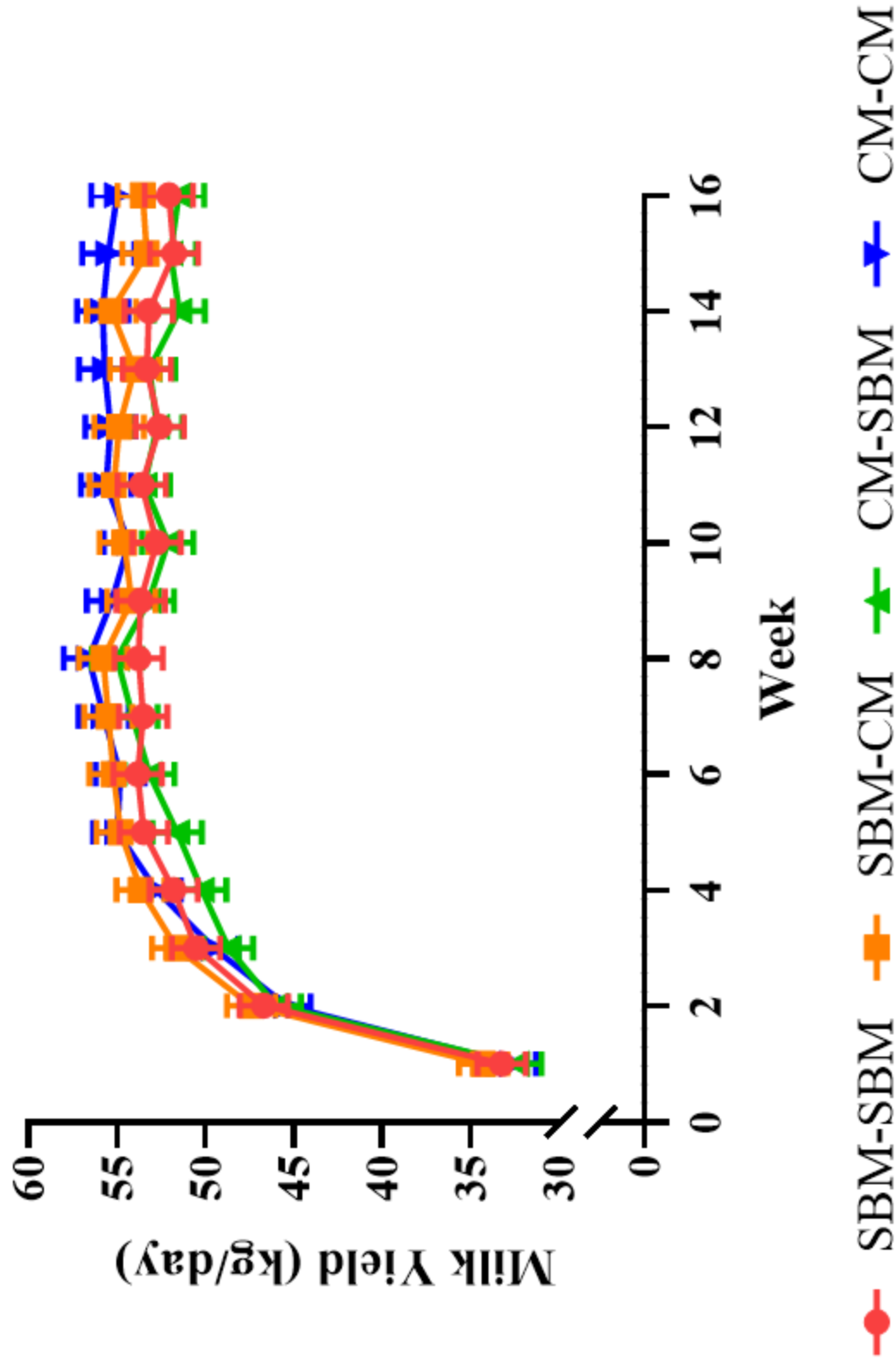
Post: $P = 0.96$.

Figure 2.2. Dry matter intake LSM for each treatment group by week relative to parturition.



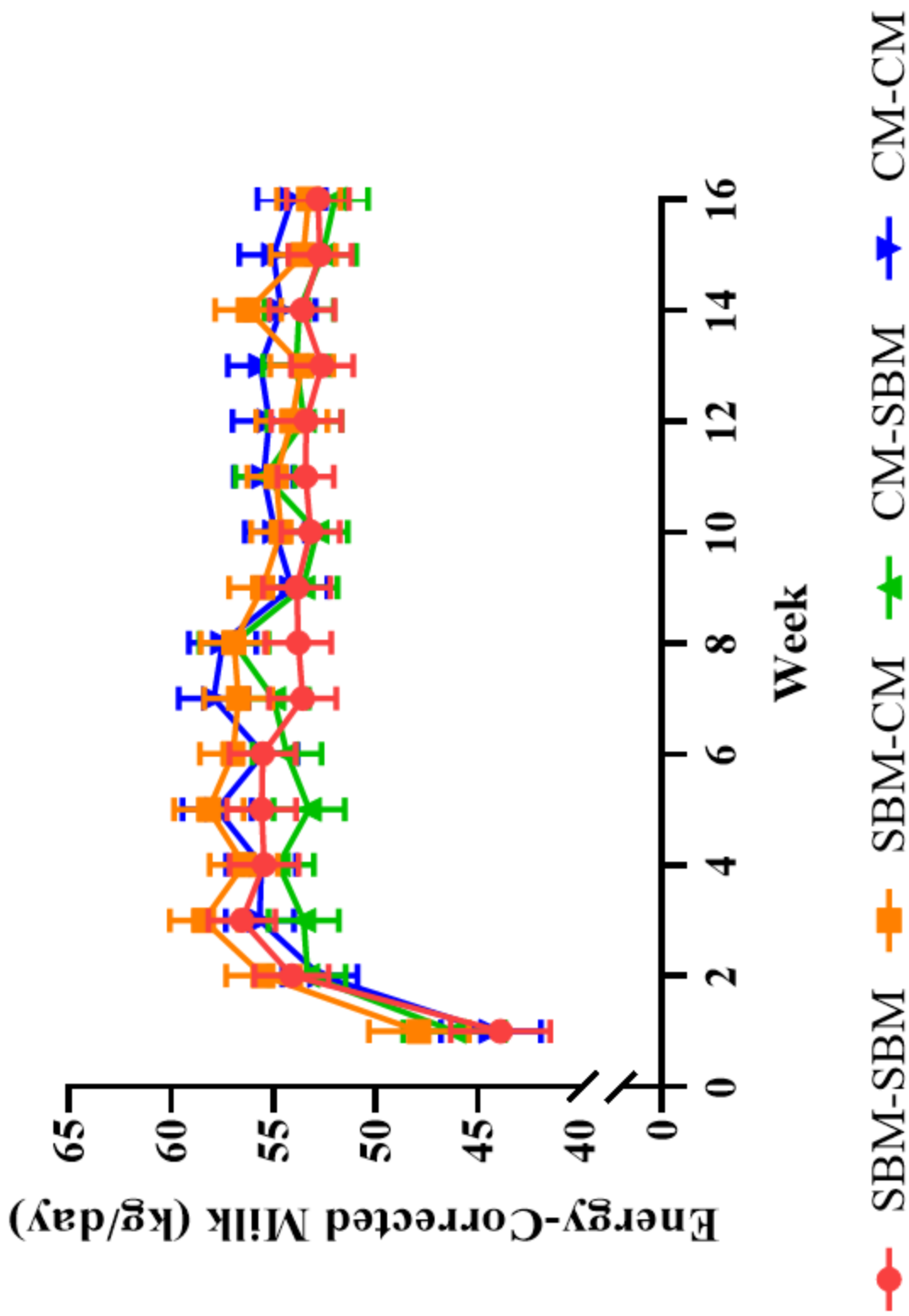
The vertical line at X = 0 denotes parturition.
 Prepartum (SBM or CM): $P = 0.04$; Postpartum - Post (SBM or CM): $P = 0.02$.

Figure 2.3. Milk yield LSM for each treatment group by week of lactation.



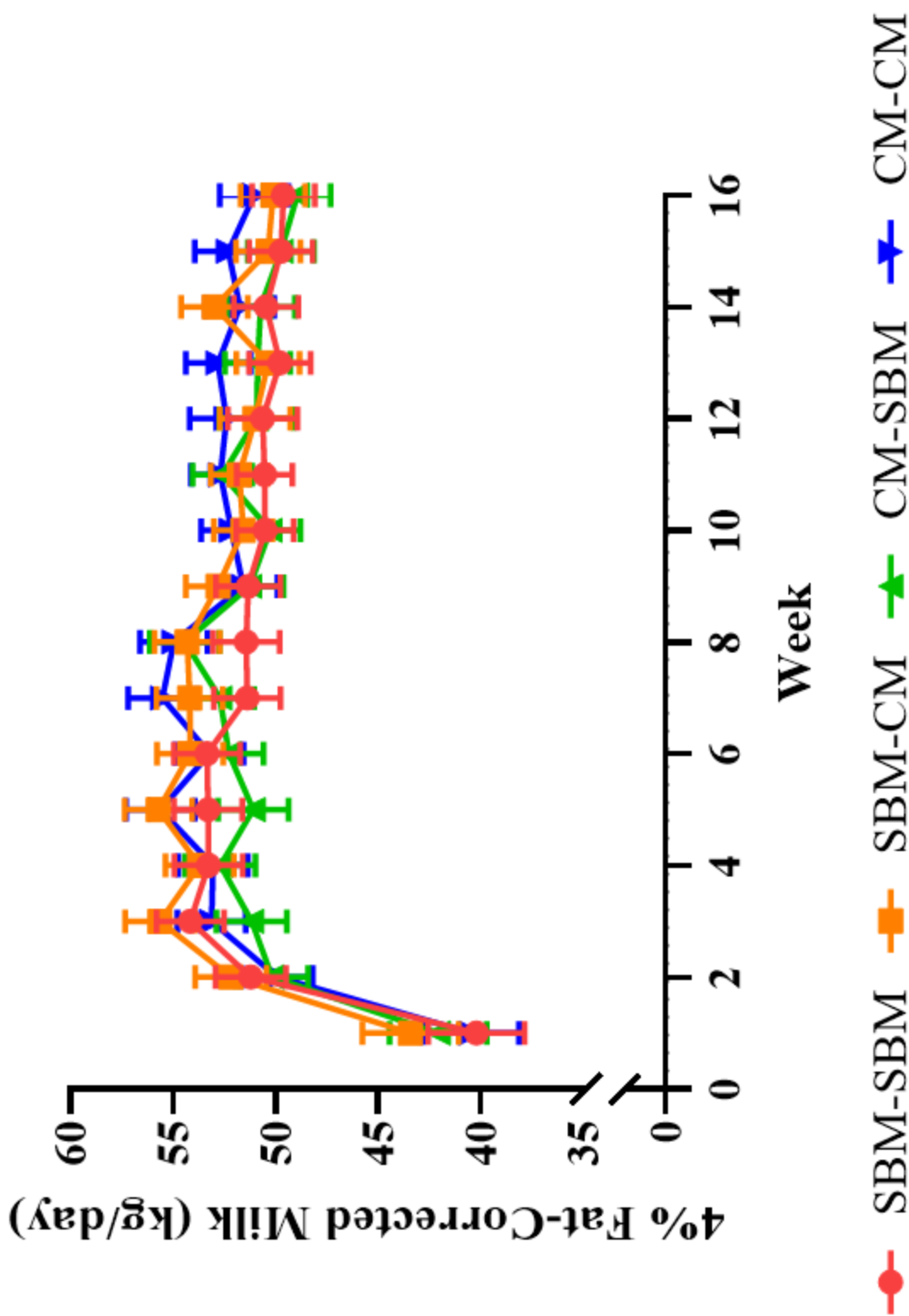
Post (SBM or CM): $P = 0.09$.

Figure 2.4. Energy-corrected milk LSM for each treatment group by week of lactation.



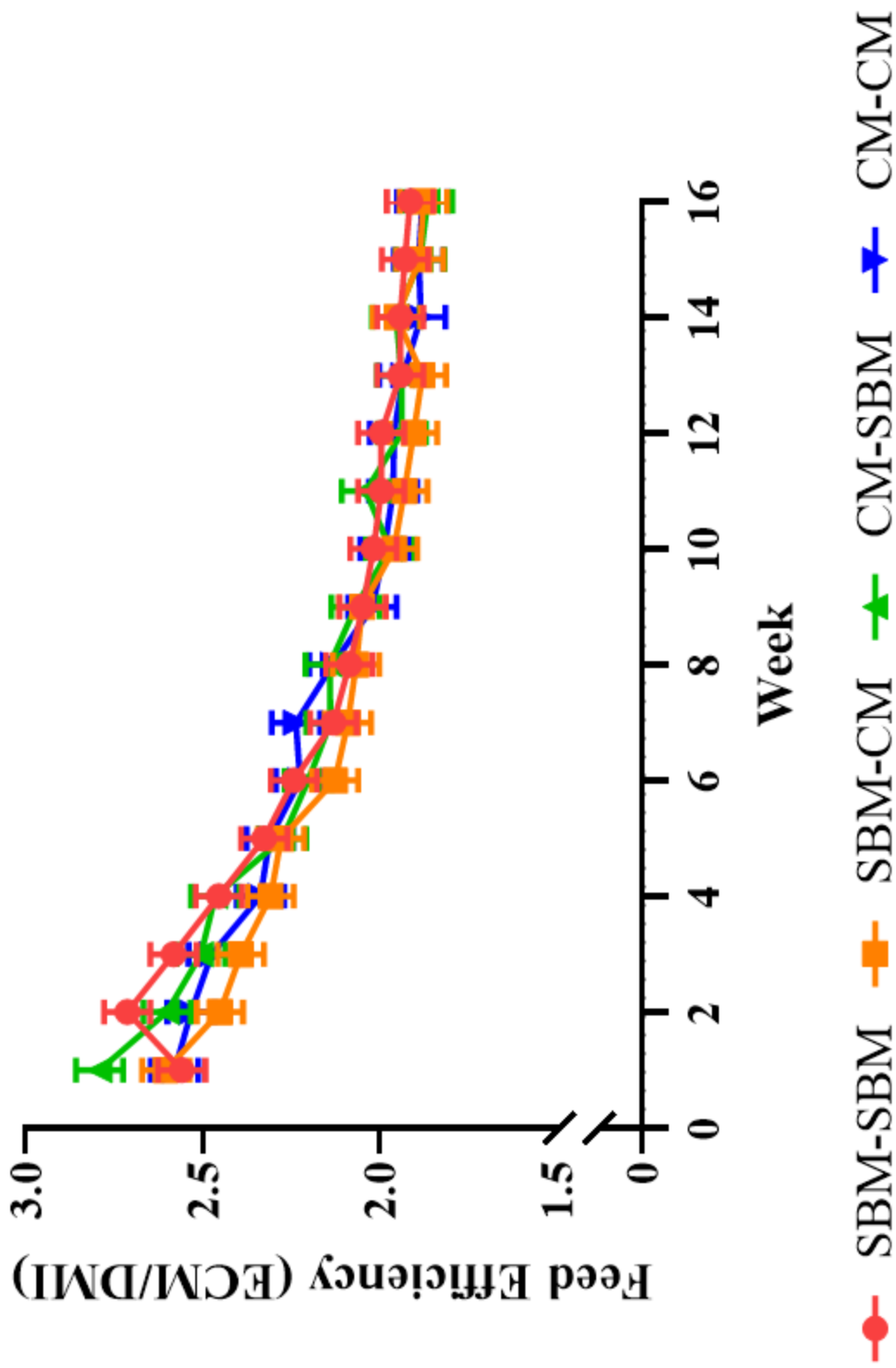
Pre \times Week: $P = 0.02$.

Figure 2.5. 4% fat-corrected milk LSM for each treatment group by week of lactation.



Pre×Week: $P = 0.05$.

Figure 2.6. Feed efficiency (ECM/DMI) LSM for each treatment group by week of lactation.



Pre×Post×Week: $P = 0.01$.

Table 2.6. Milk composition.

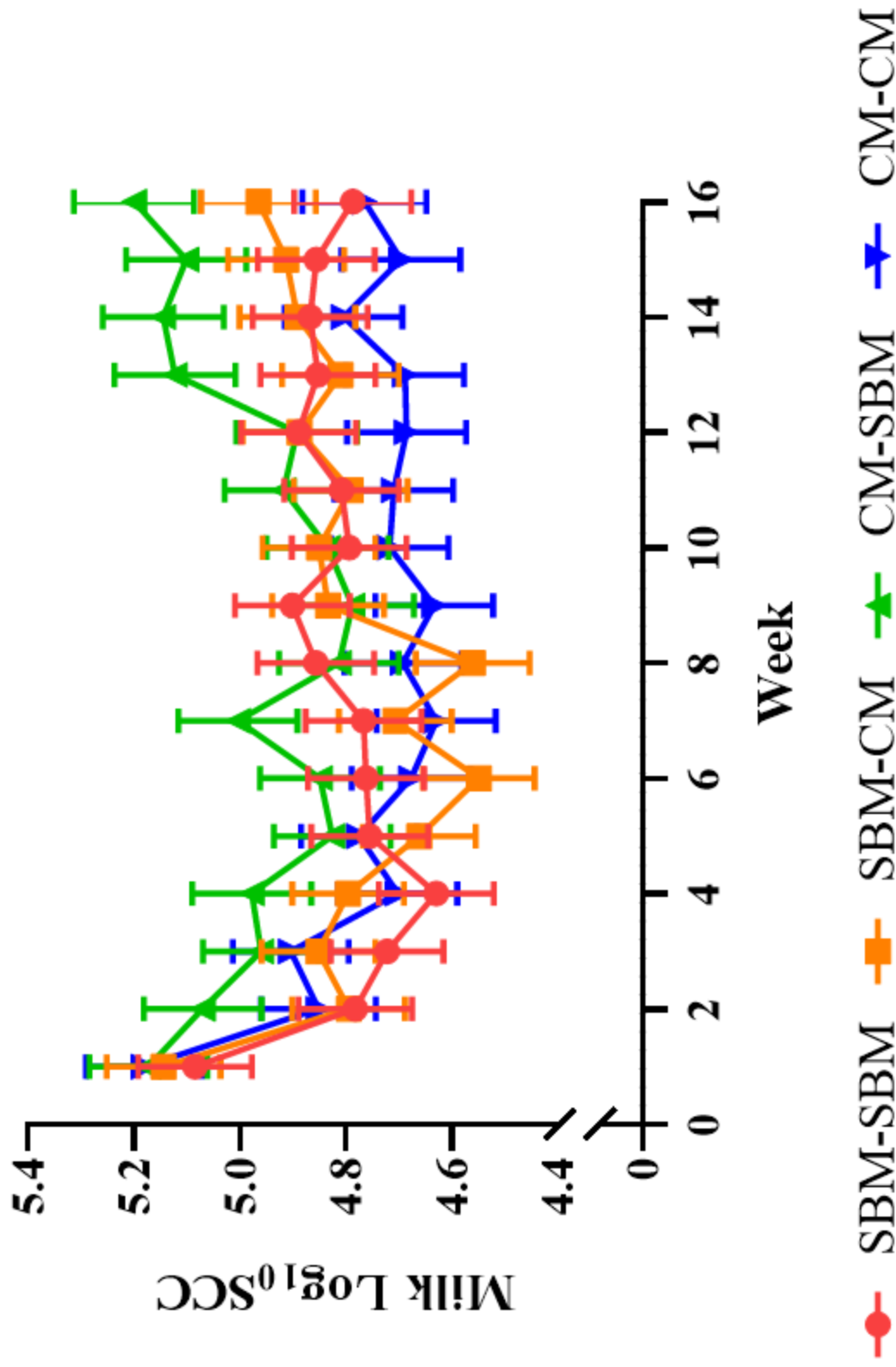
Item	Treatment ¹				P-value ²							
	SBM-SBM	SBM-CM	CM-SBM	CM-CM	SEM	Pre	Post	Pre×Post	Week	Pre×Week	Post×Week	Pre×Post×Week
Fat, %	3.95	3.91	4.00	3.89	0.07	0.80	0.33	0.58	<0.01	0.75	0.15	0.81
Fat, kg/d	2.02	2.04	2.04	2.04	0.06	0.83	0.87	0.83	<0.01	0.36	0.26	0.72
Protein, %	2.89	2.96	2.89	2.89	0.03	0.18	0.13	0.19	<0.01	0.28	0.31	0.73
Protein, kg/d	1.47	1.53	1.47	1.49	0.03	0.47	0.15	0.52	<0.01	0.34	0.72	0.73
Lactose, %	4.69	4.72	4.71	4.76	0.03	0.36	0.30	0.72	<0.01	0.15	0.99	0.93
Lactose, kg/d	2.46	2.42	2.46	2.48	0.05	0.60	0.91	0.53	<0.01	0.33	0.90	0.58
Total Solids ³ , %	12.6	12.7	12.7	12.7	0.09	0.87	0.95	0.53	<0.01	0.39	0.14	0.84
Total Solids, kg/d	6.47	6.62	6.49	6.62	0.15	0.95	0.36	0.93	<0.01	0.22	0.81	0.71
MUN, mg/dL	13.7	13.1	13.6	12.6	0.32	0.37	0.01	0.53	<0.01	0.47	0.39	0.83
Log ₁₀ SCC	4.82	4.81	4.98	4.76	0.08	0.48	0.14	0.15	<0.01	0.47	0.46	0.10

¹SBM-SBM = cows fed diets with SBM pre- and postpartum; SBM-CM = cows fed diets with SBM prepartum and CM postpartum; CM-SBM = cows fed diets with SBM prepartum and CM postpartum; CM-CM = cows fed diets with CM prepartum and SBM postpartum; CM-CM = cows fed diets with CM pre- and postpartum.

²Pre = effect of prepartum diet; Post = effect of postpartum diet; Week = effect of week of lactation.

³Total Solids = Fat + Protein + Lactose + Ash.

Figure 2.7. Milk \log_{10} SCC L-SM for each treatment group by week of lactation.



Pre×Post×Week: $P = 0.10$.

Chapter 3. Temporal plasma hormone and metabolite effects of soybean meal versus canola meal fed to dairy cows during the close-up dry period and early lactation

Abstract

In a companion paper, substituting canola meal (CM) for soybean meal (SBM) in diets fed during the close-up dry period and early lactation increased dry matter intake (DMI) and tended to increase milk yield. While including CM in diets fed during early lactation is known to improve production, the effect on temporal plasma hormone and metabolite concentrations is largely uncharacterized. Furthermore, the effect of feeding CM on temporal plasma hormone and metabolite concentrations during the close-up dry period is unknown. The objective of this experiment was to determine the effect of feeding isonitrogenous diets formulated with SBM or CM as the primary protein source during the close-up dry period and early lactation on temporal plasma hormone and metabolite concentrations in Holstein dairy cows to provide a physiological framework for the production differences. In a 2×2 factorial randomized complete block design, eighty multiparous cows were assigned to one of four treatments, which were cows fed diets with: 1) SBM pre- and postpartum (SBM-SBM), 2) SBM pre- and CM postpartum (SBM-CM), 3) CM pre- and SBM postpartum (CM-SBM), and 4) CM pre- and postpartum (CM-CM). The close-up, transition, and lactating diets were fed for four weeks immediately before parturition, the first three weeks of lactation, and weeks four through eight of lactation, respectively. The close-up, transition, and lactating pairs of diets were formulated using most of the same ingredients, except SBM and soybean hulls were used only in the SBM-based diets and CM was used only in the CM-based diets. The close-up, transition, and lactating diets were formulated to contain 14.2 or 19.4%, 12.1 or 16.5%, and 9.9 or 13.5% SBM or CM, respectively, on a DM basis. The close-up, transition, and lactating SBM or CM diets were 14.3 versus 14.5%, 17.6

versus 17.4%, and 17.2 versus 16.9% CP, respectively, on a DM basis. Plasma samples were collected three hours after TMR was fed twice weekly from each cow during every week from -3 through +8 relative to parturition. Samples were analyzed for triiodothyronine (T3), thyroxine (T4), insulin, growth hormone (GH), and insulin-like growth factor 1 (IGF-1) using ELISA and for glucose using colorimetry. Pre- and postpartum temporal plasma hormone and metabolite data were analyzed separately using the MIXED procedure of SAS with week as the repeated measure. Prepartum plasma T3 concentration decreased for cows fed the CM diet compared to the SBM diet. Postpartum plasma T3 concentration decreased for cows fed the CM diets compared to the SBM diets postpartum. There was no difference between diets for prepartum plasma T4 concentration. A tendency for an interaction between prepartum diet and week of lactation was observed for postpartum plasma T4 concentration. Prepartum plasma glucose concentration tended to increase for cows fed the CM diet compared to the SBM diet. There was no difference between diets for postpartum plasma glucose concentration. There was no difference between diets for pre- or postpartum plasma insulin concentration. Prepartum plasma GH concentration decreased for cows fed the CM diet compared to the SBM diet. An interaction between prepartum diet and week of lactation and a tendency for an interaction between postpartum diet and week of lactation was observed for postpartum GH concentration. There was no difference between diets for pre- or postpartum plasma IGF-1 concentration. Prepartum plasma T4, insulin, and GH concentrations as well as postpartum plasma T3, glucose, and insulin concentrations were affected by week relative to parturition. The interaction between pre- and postpartum diet was not significant for any plasma hormone or metabolite. These data suggest that utilizing CM as the primary protein source in diets fed to dairy cows during the close-up dry

period and early lactation alters plasma concentrations of hormones and metabolites related to production, such as T3, and may help explain observed production responses.

Introduction

Soybean meal (SBM) is traditionally considered to be the primary protein source used to formulate dairy cow diets. However, the positive effects of replacing SBM with canola meal (CM) during middle and late lactation on production measures such as DMI and milk yield has been demonstrated over a long history of research that includes a meta-analysis (Huhtanen et al., 2011) and subsequent experiments (Broderick et al., 2015; Benchaar et al., 2021; Lage et al., 2021). Furthermore, positive production effects have also been observed during early lactation when substituting CM for SBM (Moore and Kalscheur, 2016; Gauthier et al., 2019; Swanepoel et al., 2020). While CM contains less CP on a DM basis compared to SBM (40.6-42.9 vs. 52.6%; Adewole et al., 2016; Broderick et al., 2016; NASEM, 2021), the increased methionine content of CM (1.97 vs. 1.38%; NASEM, 2021) makes it a valuable protein source in situations where methionine is limiting.

In a companion paper (Kuehnl and Kalscheur, 2021), milk yield tended to increase for cows fed diets formulated with CM compared to SBM as the primary protein source during the first 16 weeks of lactation. Furthermore, cows fed the diets with CM increased pre- and postpartum DMI compared to cows fed the diets with SBM. Increased milk yield and DMI during both periods raises the question of whether plasma concentrations of hormones and metabolites related to production were altered and can help explain the observed production differences.

Limited data is currently available for characterizing the effect of feeding diets with SBM or CM during early lactation on the temporal plasma profile of hormones and metabolites related to production (Moore and Kalscheur, 2017; Kuehnl et al., 2021). Furthermore, to our knowledge, the effect of feeding diets with SBM or CM as the primary protein source on the temporal plasma profile of hormones and metabolites related to production during the close-up dry period is unknown.

The objective of this experiment was to determine the effect of feeding isonitrogenous diets formulated with SBM or CM as the primary protein source during the close-up dry period and early lactation on temporal plasma hormone and metabolite concentrations related to production in Holstein dairy cows. The plasma hormones and metabolites that were analyzed include triiodothyronine (T3), thyroxine (T4), glucose, insulin, growth hormone (GH), and insulin-like growth factor 1 (IGF-1). Since replacing SBM with CM in diets fed during the close-up dry period and early lactation increased milk yield and DMI, plasma concentrations of these hormones and metabolites may be altered and can help explain the observed increases.

Materials and Methods

Cows, Facilities, and Diets

The procedures used in this experiment were approved by the University of Wisconsin-Madison IACUC protocol A006120. Eighty multiparous (2.72 ± 0.92 ; average parity \pm SD) Holstein cows enrolled in a randomized complete block design and were grouped into blocks of four cows by expected parturition date. Within block, cows were randomly assigned to one of four dietary treatments in a 2×2 factorial design. Experimental diets were fed from November

2019 through August 2020 and cows calved from December 2019 through June 2020. Cows were housed in individual tie-stalls at the USDA Dairy Forage Research Center farm in Prairie du Sac, WI. Diets were fed as a TMR once-daily for ad libitum intake with continuous access to water. Cows were milked 3× daily at approximately 0600, 1300, and 2100 in a double-8 herringbone parlor.

Diets were formulated using NRC (2001) software. Three isonitrogenous pairs of diets (close-up, transition, and lactating) were fed during the experiment and differed in the primary protein source utilized, which was SBM or CM. Prior to consuming the close-up diet, all cows were fed the common herd far-off dry cow diet. At 27.3 ± 1.3 (mean \pm SD) days before expected parturition date, cows began consuming the close-up SBM or CM diet and remained on the same diet until parturition. Of the 80 cows enrolled, half ($n = 40$) consumed the diet with SBM and the other half consumed the diet with CM during the close-up dry period. Cows consumed the close-up SBM and CM diets for 27.0 ± 4.4 and 28.0 ± 4.7 (mean \pm SD) days before parturition, respectively. All cows consumed the close-up diet for at least 17 days before parturition. At parturition, half ($n = 20$) of the cows on each of the close-up diets switched to the transition diet containing the other protein source, while the other half consumed the transition diet with the same protein source. The transition and lactating diets were fed from parturition through week three and week four through eight of lactation, respectively. The transition diets were formulated to contain more CP to supply more AA for the fresh cow to accommodate increasing milk production and less starch to minimize metabolic disorders. This design resulted in four treatment groups, which were cows fed diets with 1) SBM pre- and postpartum (SBM-SBM), 2) SBM pre- and CM postpartum (SBM-CM), 3) CM pre- and SBM postpartum (CM-SBM), and 4) CM pre- and postpartum (CM-CM).

Ingredient composition of the far-off, close-up, transition, and lactating diets are presented in Table 3.1. The forage:concentrate ratios of the far-off, close-up, transition, and lactating diets were 87.24:12.76, 70:30, 62:38, and 60:40, respectively. The far-off and close-up diets contained conventional corn silage, grass hay, and wheat straw, whereas the transition and lactating diets contained BMR corn silage and alfalfa silage as the forage sources. Two sources of high-moisture corn were used, with one used for the dry cow diets and the other used for the lactating cow diets.

Sampling and Analysis

Samples of all diets, silages, and high-moisture corn were collected daily and stored at -20°C in one week increments until dried. Samples of dry feeds were collected once weekly. All feed samples were dried in duplicate at 105°C for 24h for dry matter determination and at 55°C for 48h for nutrient analysis. All weekly composites of diets and forages (BMR corn silage, conventional corn silage, alfalfa silage, grass hay, and wheat straw) were ground to pass through a 1-mm screen (Wiley mill, Arthur H. Thomas Co., Philadelphia, PA) and composited in eight week increments for nutrient analysis. All concentrate samples (SBM, soybean hulls, CM, corn gluten meal, and high moisture corn) were ground to pass through a 1-mm screen (cross beater mill, Retsch GmbH and Co., Haan, Germany) and composited in eight week increments for nutrient analysis. All composited feed samples were analyzed by a commercial laboratory (Dairyland Laboratories, Inc., Arcadia, WI) using wet chemistry analysis. The far-off, close-up, transition, and lactating diets were analyzed for CP (method 990.03; AOAC International, 1996), NDF using alpha amylase and sodium sulfite (aNDF; method 2002.04; AOAC International, 2005), aNDF with ash correction (aNDFom), ether extract (EE) using diethyl ether (method 920.39; AOAC International, 1996), ash (method 942.05; AOAC International, 1996), and the

following minerals: Ca, P, Mg, K, and S using a microwave and inductively coupled plasma spectroscopy (method 942.05; AOAC International, 1996). The corn silages, alfalfa silage, SBM, soybean hulls, CM, corn gluten meal, high-moisture corn, grass hay, and wheat straw were analyzed for CP, aNDF, aNDFom, ADF (method 973.18; AOAC International, 1996), NDICP and ADICP using fiber residuals from NDF and ADF determination (method 990.03; AOAC International, 1996), starch using a sodium acetate buffer method (Hall, 2009), EE, and ash. Non-fiber carbohydrates (NFC) was calculated as: $NFC = 100 - ((\%aNDFom - \%NDICP) + \%CP + \%EE + \%Ash)$, with NDICP expressed as % of DM. Nutrient analysis results for each composite were weighted relative to the corresponding number of days each diet and ingredient were fed, as a proportion of the overall days of cow enrollment, due to the unequal distribution of cows consuming each diet and ingredient during each week of the experiment. The nutrient compositions of the diets (CP, NDICP, ADICP, aNDF, aNDFom, ADF, NFC, starch, EE, ash, and NE_L) were calculated using the analyzed composition of the individual feed ingredients and their inclusion rate in the TMR.

Blood samples were collected three hours after TMR was fed twice weekly on Tuesdays and Saturdays from each cow during every week from -3 through +8 relative to parturition. Samples were collected into 10 mL vacutainers from the coccygeal artery or vein using vessel puncture with a multi-sample needle (EXELINT International Co., reference # 26501). Blood collected from the coccygeal artery or vein were considered the same supply due to minimal extraction by the tail. Three different vacutainers (BD Vacutainer, Franklin Lakes, NJ) were used for every blood draw, and each contained a different anticoagulant. The anticoagulants included 18 mg of K_2 -EDTA (reference # 366643), 158 USP units of sodium heparin (reference # 367874), and 100 mg of sodium fluoride with 20 mg of potassium oxalate (reference # 367001).

Samples were placed in ice until they were centrifuged (20 minutes, 2500 RPM, 4°C). After centrifugation, plasma was aliquoted into 1.7 mL microcentrifuge tubes (VWR International, reference # C-3260-01) in triplicate and stored at -80°C. The two weekly plasma samples from each cow were thawed and composited on an equal volume basis for each type of anticoagulant to represent a weekly average from each week relative to parturition.

Plasma samples were analyzed in duplicate for triiodothyronine (T3), thyroxine (T4), glucose, insulin, growth hormone (GH), and insulin-like growth factor 1 (IGF-1). Plasma samples from vacutainers with K₂-EDTA anticoagulant were used for T3, T4, and insulin determination. Plasma samples from vacutainers with sodium heparin anticoagulant were used for GH and IGF-1 determination. Plasma samples from vacutainers with sodium fluoride and potassium oxalate anticoagulant were used for glucose determination. Triiodothyronine and thyroxine were analyzed using ELISA kits (Monobind, Inc., Lake Forest, CA; reference # 125-300 and 225-300, respectively). The intra-assay CVs for T3 and T4 were 4.82 and 3.49%, respectively. The inter-assay CVs for T3 and T4 were 10.06 and 4.64%, respectively. Glucose was analyzed using glucose oxidase-peroxidase enzymatic colorimetry. The intra- and inter-assay CVs for glucose were 1.21 and 2.07%, respectively. Insulin was analyzed using an ELISA kit (Merckodia, Uppsala, Sweden; reference # 10-1201-01). The intra- and inter-assay CVs for insulin were 2.81 and 8.36%, respectively. Growth hormone and IGF-1 were analyzed using ELISA kits (ABclonal Technology, Woburn, MA; reference # RK00459 and RK00469, respectively). The intra-assay CVs for GH and IGF-1 were 4.68 and 7.36%, respectively. The inter-assay CVs for GH and IGF-1 were 10.26 and 9.90%, respectively.

Statistical Analysis

Statistical analysis was performed on weekly averages of pre- and postpartum plasma T3, T4, glucose, insulin, GH, and IGF-1 concentrations. Prepartum data included weekly averages for each cow from -3 through -1 weeks relative to parturition. Postpartum data included weekly averages for each cow from +1 through +8 weeks relative to parturition.

Prepartum data was analyzed using the MIXED procedure of SAS (version 9.4, SAS Institute, Inc.) with week as the repeated measure. Body weight and BCS before cows began consuming the close-up diet and ME305 for the previous lactation were tested as covariates and retained in the model if significant ($P \leq 0.10$). The covariance structure with the lowest AIC was used. The prepartum statistical model is:

$$Y_{ijk} = \mu + \text{Covariate} + \text{Treatment}_i + \text{Week}_j + (\text{Treatment}_i \times \text{Week}_j) + \text{Block}_k + \varepsilon_{ijk},$$

where Y is the response variable; μ is the overall mean; covariate is the BW, BCS, or ME305 measure (if significant); treatment is the fixed effect of the prepartum diet ($i = \text{SBM or CM}$); week is the fixed effect of week relative to parturition ($j = -3 \text{ to } -1$); treatment \times week is the fixed effect 2-way interaction of diet and week; block is the random effect of block ($k = 1 \text{ to } 20$); and ε is the residual error.

Postpartum data was analyzed using the MIXED procedure of SAS with week as the repeated measure. Body weight and BCS at parturition and ME305 for the previous lactation were tested as covariates and retained in the model if significant ($P \leq 0.10$). The covariance structure with the lowest AIC was used. The postpartum statistical model is:

$$Y_{ijkl} = \mu + \text{Covariate} + \text{Prepartum}_i + \text{Postpartum}_j + (\text{Prepartum}_i \times \text{Postpartum}_j) + \text{Week}_k + (\text{Prepartum}_i \times \text{Week}_k) + (\text{Postpartum}_j \times \text{Week}_k) + (\text{Prepartum}_i \times \text{Postpartum}_j \times \text{Week}_k) + \text{Block}_l + \varepsilon_{ijkl},$$

where Y is the response variable; μ is the overall mean; covariate is the BW, BCS, or ME305 measure (if significant); prepartum is the fixed effect of the prepartum diet ($i = \text{SBM or CM}$); postpartum is the fixed effect of the postpartum diet ($j = \text{SBM or CM}$); prepartum \times postpartum is the fixed effect 2-way interaction of the prepartum and postpartum diets; week is the fixed effect of week of lactation ($k = 1 \text{ to } 8$); prepartum \times week is the fixed effect 2-way interaction of the prepartum diet and week; postpartum \times week is the fixed effect 2-way interaction of the postpartum diet and week; prepartum \times postpartum \times week is the fixed effect 3-way interaction of the prepartum diet, postpartum diet, and week; block is the random effect of block ($l = 1 \text{ to } 20$); and ε is the residual error. Significance was declared when $P \leq 0.05$ and tendency was declared when $0.05 < P \leq 0.10$. The Tukey-Kramer adjustment was performed for pairwise comparisons of LSM.

Results

Nutrient Composition of Diets

The chemical composition of the diets and diet ingredients are presented in Tables 3.2 and 3.3, respectively. Each pair of diets (close-up, transition, and lactating) was within 0.3% CP. As expected, the CP content of SBM was higher (51.5 vs. 41.1%) and the aNDFom content of SBM was lower (8.56 vs. 23.3%) than CM. However, the higher aNDFom content of soybean hulls (67.6%) compensated for this difference in aNDFom content in the SBM diets.

Triiodothyronine and Thyroxine

Pre- and postpartum plasma T3 and T4 concentrations are presented in Table 3.4. Temporal pre- and postpartum plasma T3 and T4 concentrations are presented in Figure 3.1. Prepartum plasma T3 concentration decreased for cows fed the CM diet compared to the SBM diet ($P < 0.01$) but was not affected by week ($P > 0.10$). Postpartum plasma T3 concentration decreased for cows fed the CM diets compared to the SBM diets postpartum ($P < 0.01$) and was affected by week ($P < 0.01$). Prepartum plasma T4 concentration was not affected by diet ($P > 0.10$) but was affected by week ($P < 0.01$). A tendency for an interaction between prepartum diet and week was observed for postpartum plasma T4 concentration ($P = 0.06$).

Glucose and Insulin

Pre- and postpartum plasma glucose and insulin concentrations are presented in Table 3.4. Temporal pre- and postpartum plasma glucose and insulin concentrations are presented in Figure 3.1. Prepartum plasma glucose concentration tended to increase for cows fed the CM diet compared to the SBM diet ($P = 0.10$) but was not affected by week ($P > 0.10$). Postpartum plasma glucose concentration was not affected by diet ($P > 0.10$) but was affected by week ($P < 0.01$). Pre- and postpartum plasma insulin concentration was not affected by diet ($P > 0.10$) but were both affected by week ($P < 0.01$).

Growth Hormone and Insulin-Like Growth Factor 1

Pre- and postpartum plasma GH and IGF-1 concentrations are presented in Table 3.4. Temporal pre- and postpartum plasma GH and IGF-1 concentrations are presented in Figure 3.1. Prepartum plasma GH concentration decreased for cows fed the CM diet compared to the SBM diet ($P = 0.03$) and was affected by week ($P < 0.01$). An interaction between prepartum diet and

week ($P = 0.01$) and a tendency for an interaction between postpartum diet and week ($P = 0.08$) was observed for postpartum plasma GH concentration. Pre- and postpartum plasma IGF-1 concentration was not affected by diet ($P > 0.10$) or week ($P > 0.10$).

Discussion

To our knowledge, this is the first experiment analyzing the effects of feeding diets formulated with SBM or CM as the primary protein source during the close-up dry period on temporal plasma concentrations of hormones and metabolites related to production. Furthermore, this experiment adds to the limited data available for temporal plasma concentrations of hormones and metabolites related to production for cows fed diets formulated with SBM or CM as the primary protein source during early lactation.

Dietary Effect: Soybean Meal versus Canola Meal

Triiodothyronine and Thyroxine

The thyroid hormones T3 and T4 are critical for numerous metabolic processes related to energy expenditure in dairy cows, including carbohydrate, lipid, and protein metabolism (Kaneko, 2008). While both molecules are biologically active, T3 possesses a higher potency than T4 in numerous species including dairy cows (Gross and Pitt-Rivers, 1952; Premachandra et al., 1961; Sawin et al., 1977). Plasma concentrations of these hormones are negatively correlated with milk production (Blum et al., 1983; Tiirats, 1997; Steinhoff et al., 2019). This is potentially due to overall energy metabolism and decreased utilization of energetic substrates in tissues peripheral to the mammary gland to conserve energy for milk production (Shetty, 1990; Vernon and Pond, 1997; Vernon et al., 2002). As milk production is increased for cows fed diets

formulated with CM as the primary protein source compared to SBM during early lactation (Moore and Kalscheur, 2016; Swanepoel et al., 2020; Kuehnl and Kalscheur, 2021), decreased concentrations of thyroid hormones may be expected in these cows.

In the present experiment, a strong and consistent diet effect was observed for plasma T3 concentration pre- and postpartum, with decreased plasma T3 concentration for cows fed the diets with CM compared to the diets with SBM. Furthermore, when cows switched from the SBM diet prepartum to the CM diet postpartum, and vice versa, plasma T3 concentrations immediately shifted accordingly. Decreased plasma T3 concentrations pre- and postpartum for cows fed the CM diets compared to the SBM diets suggests differential energy partitioning between treatment groups. During the prepartum period, this could be attributed to fetal development, body reserves, or mammary development for mature cows. As BW, BCS, and milk yield did not differ during week one of lactation (Kuehnl and Kalscheur, 2021), fetal development appears the most plausible explanation. During early lactation, the tendency for increased milk yield for cows fed the CM diets compared to the SBM diets postpartum is the most reasonable explanation. Consistent with the negative relationship between plasma T3 concentrations and milk yield, cows fed the CM diets tended to increase milk yield compared to cows fed the SBM diets postpartum (Kuehnl and Kalscheur, 2021). Contrary to the results of this experiment, Kuehnl et al. (2021) observed no difference in plasma T3 concentrations for cows fed diets formulated with SBM or CM as the primary protein source during early lactation. This result is unexpected as milk yield increased to a larger degree for cows fed the CM diets compared to the SBM diets in the previous experiment (Moore and Kalscheur, 2016) as compared to the present experiment.

While the results for plasma T3 concentration were steady in the present experiment, plasma T4 concentration was variable between treatment groups. Although there was no difference for plasma T4 concentration during the prepartum period, a tendency for an interaction between prepartum diet and week of lactation was present for postpartum concentrations. Fluctuations in plasma T4 concentrations between treatment groups during weeks one through four appear to contribute to this interaction, with cows in the CM-SBM and CM-CM groups experiencing more volatility compared to cows in the SBM-SBM and SBM-CM groups. However, little difference for plasma T4 concentration between treatment groups was present from weeks five through eight of lactation. In Kuehnl et al. (2021), an interaction between protein source and week of lactation was observed for plasma T4 concentration. During weeks two through four of lactation, plasma T4 concentration remained constant for cows fed the low-protein CM diet (LoCM), whereas plasma T4 concentration decreased for cows fed the low-protein SBM diet (LoSBM), high-protein SBM diet (HiSBM), or the high-protein CM diet (HiCM). During weeks six and eight of lactation, plasma T4 concentration decreased for cows in the LoCM group, whereas plasma T4 concentration increased for cows in the LoSBM and HiSBM groups or decreased then increased for cows in the HiCM group.

Rapeseed is the genetic precursor to canola, with the latter bred to contain low concentrations of glucosinolates (<30 $\mu\text{mol/g}$) in the meal portion (Bell, 1984). Earlier in the 20th century, nutritionists were hesitant to incorporate rapeseed meal (RSM) in diets fed to dairy cows due to the high content of glucosinolates, compounds that yield breakdown products which are goitrogenic to ruminants (Fenwick, 1982). Goitrogenic compounds are of specific concern to dairy cows, as they reduce the availability of iodine, which exerts a negative effect on the synthesis of T4, a hormone critical for lactation (Grenet and Journet, 1971). While decreased

plasma T4 concentration was observed for cows fed diets with RSM compared to SBM in most (Fisher and Walsh, 1976; Papas et al., 1979; Laarveld et al. 1981), but not all (Papas et al., 1978), early experiments, plasma T3 concentration was unaffected in all cases. Considered together, improved production performance appears the most likely explanation for the strong and consistent plasma T3 response observed herein.

Glucose and Insulin

Lactose, the major osmoregulator of milk production (Bleck et al., 2009), is a disaccharide comprised of one glucose and one galactose molecule. During the first few days after parturition, glucose demand increases by one kg/d to support milk production (Bertics et al., 1992; Reynolds et al., 2003). While the lactating mammary gland utilizes insulin-independent GLUT transporters (Zhao et al., 1993; Zhao et al., 2004; Miller et al., 2005), peripheral tissues such as adipose and skeletal muscle depend on insulin for glucose uptake (Zhao, 2014).

In the present experiment, prepartum plasma glucose concentration tended to increase for cows fed the CM diet compared to the SBM diet, suggesting either increased glucose availability or decreased glucose uptake from circulation. This result disagrees with most previous experiments that observed no effect of supplementing methionine in diets fed to dairy cows during the close-up dry period on plasma glucose concentration (Sun et al., 2016; Batistel et al., 2017; Potts et al., 2020), similar to including CM to increase dietary methionine. However, Ardalan et al (2011) observed an interaction between methionine and time for plasma glucose concentration during the close-up dry period, with plasma glucose concentration increased at parturition for cows fed a diet with methionine compared to control, whereas no difference was observed 15 days before parturition. Agreeing with the present experiment, no difference was reported for plasma glucose concentration in most previous experiments that compared the effect

of including SBM versus CM as the primary protein source (Moore and Kalscheur, 2017) or supplementing methionine versus no supplementation (Batistel et al., 2017; Potts et al., 2020; Cardoso et al., 2021) in diets fed during early lactation. However, Sun et al. (2016) observed increased plasma glucose concentration during the first 3 weeks of lactation for cows fed diets supplemented with methionine compared to the control. Additionally, an interaction between methionine and time was present for postpartum plasma glucose concentration in Ardalan et al. (2011), whereby plasma glucose concentration was not different between cows fed the diet with or without methionine at 7 and 30 DIM but differed at 15 and 60 DIM, with cows fed the control diet higher at 15 DIM and cows fed the diet with methionine higher at 60 DIM.

Agreeing with the present experiment, Osorio et al (2013) and Zhou et al. (2016) observed no effect of methionine supplementation in diets fed to dairy cows during the close-up dry period on plasma insulin concentration. Conversely, Cardoso et al. (2021) found increased plasma insulin concentration for cows fed the high protein diet supplemented with methionine compared to cows fed the high protein diet at 8 days before parturition. Consistent with the present experiment, no difference has been observed for plasma insulin concentration during early lactation between cows fed diets with SBM or CM (Kuehnl et al., 2021) or diets supplemented with RPM versus not (Osorio et al., 2013; Zhou et al., 2016; Cardoso et al., 2021). Collectively, these results suggest that it is difficult to perturb plasma glucose and insulin concentrations pre- and postpartum via dietary methionine concentration.

Growth Hormone and Insulin-Like Growth Factor 1

In ruminants, GH is widely accepted as the hormone responsible for galactopoiesis (Tucker, 2000). However, while mRNA for the GH receptor is present, a lack of binding between GH and receptors in the bovine mammary gland has been observed (Keys and Djiane,

1988), suggesting that the galactopoietic effect of GH is fulfilled outside of the mammary gland. This is partially achieved through the increased partitioning of nutrients toward the mammary gland for milk synthesis (Peel and Bauman, 1987; Bauman et al., 1989). Additionally, IGF-1 has been hypothesized to help mediate the galactopoietic effect of GH. Growth hormone is known to bind to receptors on hepatocytes, stimulating the secretion of IGF-1. From there, IGF-1 can potentially bind to receptors that have been observed on mammary epithelial cells (Gluckman et al., 1987) and exert its mitogenic effect. While definitive evidence of IGF-1 exhibiting an indirect galactopoietic effect was not demonstrated using the *in vitro* bovine mammary epithelial cell model (Shamay et al., 1988), another experiment observed increased milk secretion in goats within two to four hours after arterial injection of IGF-1 near the mammary gland (Prosser et al., 1990). However, this acute response for milk production suggests that an IGF-1-mediated effect unrelated to mitogenesis may be the reason in this scenario. Nevertheless, increased cell renewal rate, preventing the decline of cell numbers, and a corresponding increase in secretory activity possibly explains the long-term mitogenic effects of IGF-1 and its mediation of GH's galactopoietic effects (Capuco et al., 2001).

In the present experiment, plasma GH concentration was hypothesized to increase for cows fed the CM diets compared to the SBM diets postpartum to help explain the tendency for increased milk yield in the cows fed the CM diets (Kuehnl and Kalscheur, 2021). Concurrent increases for plasma GH concentration and milk yield have been observed when analyzing the effects of methionine supplementation during the transition period (Osorio et al., 2013). In Osorio et al. (2013), increased plasma GH concentration was only found during the first three weeks of lactation and not during the close-up dry period for cows fed the diets with methionine compared to the control. Interestingly, the results of Osorio et al. (2013) conflict with the present

experiment, whereby plasma GH concentration decreased during the close-up dry period for cows fed the CM diet compared to the SBM diet. This may be due to a need for increased blood flow and nutrient partitioning towards fetal development for cows fed the SBM diet compared to the CM diet to compensate for lower DMI (Kuehnl and Kalscheur, 2021). This potential compensation is supported by no difference for BW, BCS, or milk yield during week one of lactation. Additionally, an interaction between prepartum diet and week of lactation was present for postpartum plasma GH concentration. This interaction appears to be due to decreasing differences for plasma GH concentration between cows fed the SBM or CM diet prepartum as lactation advanced from week one through eight. Furthermore, there was a tendency for an interaction between postpartum diet and week of lactation on postpartum plasma GH concentration, which may be due to inconsistent fluctuations of plasma GH concentration between the postpartum diets from week one through eight of lactation. Consistent with the present experiment, Osorio et al. (2013) found no differences for plasma IGF-1 concentration during the transition period between cows fed diets with or without methionine.

Temporal Fluctuations: Close-Up Dry Period and Early Lactation

Temporal fluctuations of plasma hormone and metabolite concentrations related to production in mature dairy cows during the close-up dry period and early lactation can largely be attributed to physiological events that must be adapted to during this time. Specifically, events such as the end of gestation, commencement of lactation, and increasing milk yield until peak lactation all require a concerted effort by hormones and metabolites to achieve.

Since plasma T3 and T4 concentrations are negatively correlated with milk production (Blum et al., 1983; Tiirats, 1997; Steinhoff et al., 2019), it could be hypothesized that concentrations of these hormones would steadily decrease as cows progressed towards peak

lactation. In the present experiment, postpartum plasma T3 and T4 concentrations decreased after week one of lactation and remained lower than prepartum concentrations through week eight of lactation. The decrease from week one to two of lactation and sustained decrease thereafter may have occurred to decrease the utilization of nutrients in tissues peripheral to the mammary gland and conserve them for milk production (Shetty, 1990; Vernon and Pond, 1997; Vernon et al., 2002). Compared to the sustained decrease herein, a transient decrease has been observed for plasma T3 and/or T4 concentrations during the periparturient period through the first few weeks of lactation (Meikle et al., 2004; Steinhoff et al., 2019). Similar to the present experiment, Steinhoff et al. (2019) reported no effect of time on serum T3 concentrations but decreasing serum T4 concentrations during the close-up dry period.

Decreased plasma insulin concentration during the transition period is a consistent and important response in periparturient dairy cows as they enter a state of insulin resistance to conserve circulating glucose for milk lactose production in the mammary gland (Bell, 1995). In agreement with the present experiment, decreasing plasma insulin concentrations from the close-up dry period through the first few weeks of lactation is routinely observed (Park et al., 2010; Osorio et al., 2013; Zhou et al., 2016). Similar to the present experiment, no effect of time on plasma glucose concentration during the close-up dry period has been reported (Park et al., 2010; Sun et al., 2016; Potts et al., 2020). However, when analyzing the entire transition period, plasma glucose concentration has been shown to decrease around the time of parturition (Osorio et al., 2013; Zhou et al., 2016) as observed in the present experiment.

In the present experiment, decreasing plasma insulin concentrations during the close-up dry period coincided with decreasing plasma GH concentrations. This is potentially due to hypoinsulinemia, which has been shown to reduce the number of hepatic GH receptors (Kim et

al., 2004; Rhoads et al., 2004), potentially acting as feedback to the anterior pituitary to produce less GH. Interestingly, Osorio et al. (2013) did not observe a time effect for plasma GH concentration during the close-up dry period. Furthermore, downregulation of hepatic GH receptors should reduce hepatic IGF-1 production, with a decrease of approximately 70% previously reported around parturition (Kobayashi et al., 1999; Block et al., 2001). While this decrease occurred in Osorio et al. (2013), it did not in the present experiment. Conversely, synchronous increases for both plasma insulin and IGF-1 concentrations have also been observed during early lactation, further providing evidence of their association (Taylor et al., 2004; Osorio et al., 2013; Beltman et al., 2020). Indeed, Rhoads et al. (2004) showed that plasma IGF-1 concentration increased within 16 hours of using a hyperinsulinemic-euglycemic clamp in dairy cows during both late pregnancy and early lactation.

Conclusions

In conclusion, replacement of SBM with CM in diets fed to dairy cows during the close-up dry period and early lactation alters plasma concentrations of hormones and metabolites related to production. Prepartum plasma T3 and GH concentrations decreased for cows fed the CM diet compared to the SBM diet, which may be related to nutrient partitioning for fetal development. Postpartum plasma T3 concentration decreased for cows fed the CM diets compared to the SBM diets postpartum, which corresponded to a tendency for increased milk yield for cows fed the CM diets.

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Tables and Figures

Table 3.1. Ingredient composition of the far-off, close-up, transition, and lactating diets.

Ingredient (% DM)	Far-Off ¹	Close-Up ²		Transition ³		Lactating ⁴	
		SBM	CM	SBM	CM	SBM	CM
Corn Silage ⁵	53.3	44.9	44.9	36.0	36.0	30.0	30.0
Alfalfa Silage	-	-	-	26.0	26.0	30.0	30.0
Soybean Meal	-	14.2	-	12.1	-	9.90	-
Soybean Hulls	-	5.20	-	4.40	-	3.60	-
Canola Meal	2.73	-	19.4	-	16.5	-	13.5
Corn Gluten Meal	-	-	-	2.00	2.00	2.00	2.00
Roasted Soybeans	7.27	-	-	-	-	-	-
High-Moisture Corn ⁶	-	8.00	8.00	15.0	15.0	20.0	20.0
Grass Hay	14.5	10.8	10.8	-	-	-	-
Wheat Straw	19.4	14.3	14.3	-	-	-	-
Rumen Inert Fat ⁷	-	-	-	2.00	2.00	2.00	2.00
Mineral/Vitamin Mix ^{8,9}	2.47	1.90	1.90	2.50	2.50	2.50	2.50
Reashure ¹⁰	-	0.45	0.45	-	-	-	-
Urea	0.29	0.25	0.25	-	-	-	-

¹Far-off diet fed during the dry period until cows assigned to close-up diet.

²Close-up diet fed from approximately four weeks prepartum through parturition.

³Transition diet fed from parturition through week three of lactation.

⁴Lactating diet fed from week four of lactation through week eight of lactation.

⁵Conventional corn silage used for the far-off and close-up diets; BMR corn silage used for the transition and lactating diets.

⁶Different sources of high-moisture corn used for diets fed to the dry versus lactating cows.

⁷Energy Booster 100 (Milk Specialties, Dundee, IL).

⁸Dry cow mineral contained (on a DM basis): 18.6% Ca, 9.6% salt, 7.8% Mg, 9.9% S, 22.9 ppm Se, 549,100 IU vitamin A/kg, 163,800 IU vitamin D3/kg, 8,780 IU vitamin E/kg, and 1.10 g Monensin/kg (Vita Plus Corporation, Madison, WI).

⁹Lactating cow mineral contained (on a DM basis): 16.7% Ca, 10.5% salt, 14.4% Na, 5.6% Mg, 0.3% K, 0.6 ppm Se, 274,500 IU vitamin A/kg, 54,900 IU vitamin D3/kg, 1,250 IU vitamin E/kg, and 0.46 g Monensin/kg (Vita Plus Corporation, Madison, WI).

¹⁰Precision Release Choline (Balchem Corporation).

Table 3.2. Chemical composition of diets.

Item (%DM, unless noted)	Far-Off	Close-Up		Transition		Lactating	
		SBM ¹	CM ¹	SBM	CM	SBM	CM
DM, %AF	45.4	48.8	49.6	44.2	45.1	44.9	45.3
CP ²	12.0	14.3	14.5	17.6	17.4	17.2	16.9
NDICP, ² %CP	-	16.1	16.0	8.62	8.62	8.39	8.39
ADICP, ² %CP	-	9.57	10.1	5.04	5.48	5.01	5.37
aNDF ²	43.4	40.7	40.9	28.9	29.0	28.1	28.2
aNDFom ²	42.5	39.5	39.3	28.0	27.9	27.2	27.1
ADF ²	-	27.2	27.5	20.8	21.1	20.6	20.9
NFC ^{2,3}	35.2	38.3	38.1	42.1	41.9	43.2	43.0
Starch ²	-	23.8	23.9	26.8	26.9	28.5	28.6
EE ²	4.34	2.29	2.76	5.37	5.77	5.44	5.77
Ash ²	7.77	5.17	5.49	5.47	5.74	5.56	5.78
Ca	0.77	0.64	0.71	0.93	0.97	0.95	1.00
P	0.27	0.28	0.43	0.32	0.45	0.32	0.44
Mg	0.44	0.35	0.43	0.37	0.45	0.39	0.45
K	1.35	1.33	1.13	1.61	1.42	1.65	1.51
S	0.39	0.33	0.42	0.22	0.30	0.22	0.30
RDP ⁴	7.89	9.35	9.82	11.0	11.2	10.6	10.8
RUP ⁴	4.11	4.95	4.68	6.59	6.19	6.62	6.09
Dig Lys, ^{4,5} g/d	68	95	96	139	135	180	171
Dig Met, ^{4,5} g/d	19	26	28	41	43	54	55
RDP ⁶	8.32	9.89	10.1	12.0	11.9	11.7	11.6
RUP ⁶	3.68	4.41	4.40	5.62	5.49	5.47	5.32
Metab Lys, ^{6,7} g/d	85	110	110	148	146	185	181
Metab Met, ^{6,7} g/d	25	31	33	45	48	57	60
NE _L , ^{2,8} Mcal/kg DM	1.47	1.53	1.50	1.60	1.57	1.60	1.58
NE _L , ⁹ Mcal/kg DM	1.57	1.56	1.59	1.68	1.65	1.61	1.60
NE _L , ¹⁰ Mcal/kg DM	1.66	1.70	1.64	1.84	1.79	1.82	1.78

¹SBM = soybean meal; CM = canola meal.

²Calculated using nutrient analysis of individual feed ingredients.

³NFC = 100 - ((%aNDFom - %NDICP) + %CP + %EE + %Ash); NDICP expressed as % DM.

⁴Estimated based on NRC (2001) using average DMI and BW of cows during the experiment.

⁵Dig Lys = digestible lysine; Dig Met = digestible methionine.

⁶Estimated based on NASEM (2021) using average DMI and BW of cows during the experiment.

⁷Metab Lys = metabolizable lysine; Metab Met = metabolizable methionine.

⁸NE_L = net energy for lactation, calculated using nutrient analysis of individual feed ingredients.

⁹Estimated based on NRC (2001) using average DMI and BW of cows during the experiment.

¹⁰Estimated based on NASEM (2021) using average DMI and BW of cows during the experiment.

Table 3.3. Chemical composition of diet ingredients.

Item (%DM, unless noted)	Corn Silage		AS ¹	SBM ¹	SH ¹	CM ¹	CGM ¹	HMC ¹		GH ¹	WS ¹
	Conv ¹	BMR ¹						Dry ²	Lact ²		
DM, %AF	42.4	36.0	33.9	87.5	89.7	87.8	92.5	71.6	71.2	87.0	88.0
CP	7.20	7.67	21.8	51.5	11.4	41.0	69.6	7.59	7.24	8.84	3.93
NDICP, %CP	13.4	11.2	7.64	1.26	28.9	8.59	1.74	7.98	7.77	27.3	33.3
ADICP, %CP	7.79	5.81	6.38	0.84	10.7	6.15	10.2	3.29	3.38	13.1	26.1
aNDF	36.6	34.7	43.6	8.81	69.1	26.0	3.66	5.25	5.75	68.3	81.3
aNDFom	35.9	33.9	42.0	8.56	67.6	23.3	3.48	4.69	5.61	66.1	78.3
ADF	23.1	20.5	38.3	6.67	50.2	20.3	1.34	1.83	1.90	45.8	56.9
NFC ³	51.2	52.0	22.6	31.4	17.8	26.6	23.7	83.0	82.8	17.9	9.72
Starch	39.2	39.4	0.71	0.77	0.76	1.28	15.0	73.3	80.4	0.64	0.49
EE	2.88	3.63	4.51	2.26	1.86	4.59	1.35	3.83	3.39	1.59	0.69
Ash	3.83	3.73	10.8	6.95	4.60	7.98	3.09	1.48	1.50	7.98	8.68

¹Conv = conventional corn silage; BMR = brown midrib; AS = alfalfa silage; SBM = soybean meal; SH = soybean hulls; CM = canola meal; CGM = corn gluten meal; HMC = high-moisture corn; GH = grass hay; WS = wheat straw.

²Dry = used for the close-up diets; Lact = used for the transition and lactating diets.

³NFC = 100 - ((%aNDFom - %NDICP) + %CP + %EE + %Ash); NDICP expressed as % DM.

Table 3.4. Pre- and postpartum plasma triiodothyronine, thyroxine, growth hormone, insulin, glucose, and insulin-like growth factor 1 concentrations.

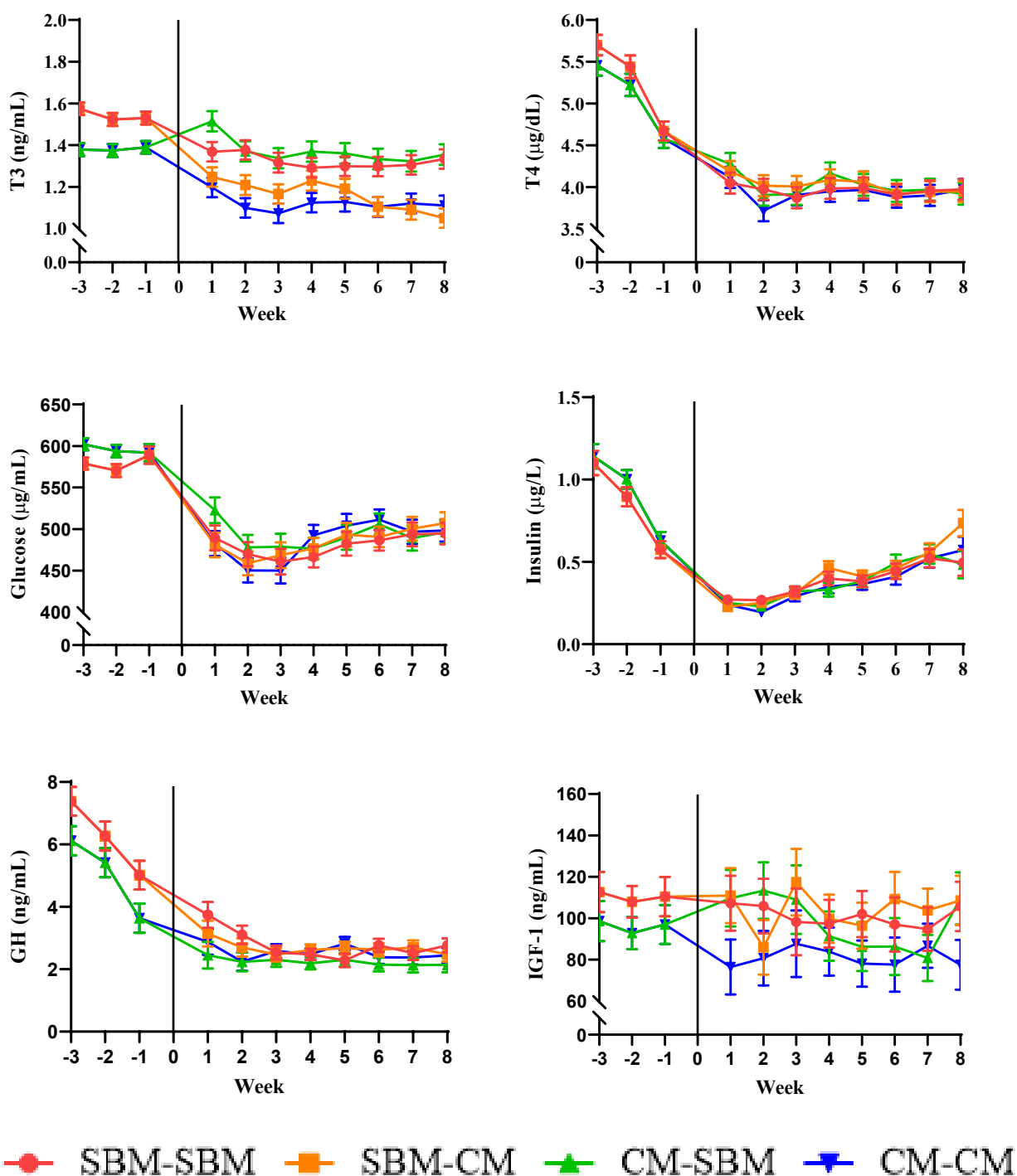
Item	Treatment ¹						P-value ²						
	SBM-SBM	SBM-CM	CM-SBM	CM-CM	SEM		Pre	Post	Pre×Post	Week	Pre×Week	Post×Week	Pre×Post×Week
Pre T ₃ ³ , ng/mL	1.54	1.54	1.38	1.38	0.03	<0.01	-	-	-	0.28	0.39	-	-
Post T ₃ , ng/mL	1.32	1.16	1.37	1.12	0.04	0.94	<0.01	0.27	0.27	<0.01	0.13	0.30	0.13
Pre T ₄ ³ , µg/dL	5.27	5.27	5.09	5.09	0.11	0.23	-	-	-	<0.01	0.58	-	-
Post T ₄ , µg/dL	3.96	4.02	4.02	3.93	0.11	0.85	0.88	0.50	0.50	<0.01	0.06	0.61	0.65
Pre Glucose, µg/mL	579	579	596	596	6.96	0.10	-	-	-	0.19	0.28	-	-
Post Glucose, µg/mL	480	484	492	486	9.16	0.47	0.90	0.56	0.56	<0.01	0.43	0.20	0.60
Pre Insulin, µg/L	0.86	0.86	0.92	0.92	0.05	0.37	-	-	-	<0.01	0.69	-	-
Post Insulin, µg/L	0.39	0.43	0.38	0.37	0.03	0.26	0.65	0.39	0.39	<0.01	0.28	0.18	0.91
Pre GH ³ , ng/mL	6.22	6.22	5.06	5.06	0.38	0.03	-	-	-	<0.01	0.59	-	-
Post GH, ng/mL	2.77	2.68	2.24	2.53	0.20	0.08	0.62	0.31	0.31	0.06	0.01	0.08	0.74
Pre IGF-1 ³ , ng/mL	110	110	96.2	96.2	6.54	0.13	-	-	-	0.70	0.99	-	-
Post IGF-1, ng/mL	101	104	98.3	81.1	9.77	0.18	0.46	0.29	0.29	0.36	0.81	0.13	0.38

¹SBM-SBM = cows fed diets with SBM pre- and postpartum; SBM-CM = cows fed diets with SBM prepartum and CM postpartum; CM-SBM = cows fed diets with CM prepartum and SBM postpartum; CM-CM = cows fed diets with CM pre- and postpartum.

²Pre = effect of prepartum diet; Post = effect of postpartum diet; Week = effect of week of lactation.

³T₃ = triiodothyronine, T₄ = thyroxine, GH = growth hormone, IGF-1 = insulin-like growth factor 1.

Figure 3.1. Pre- and postpartum plasma triiodothyronine, thyroxine, glucose, insulin, growth hormone, and insulin-like growth factor 1 concentrations for each treatment group by week relative to parturition.



T3 = triiodothyronine, T4 = thyroxine, GH = growth hormone, IGF-1 = insulin-like growth factor 1.

The vertical line at X = 0 denotes parturition.

Pre T3: $P < 0.01$; Post T3 (Post): $P < 0.01$; Post T4 – Pre \times Week: $P = 0.06$; Pre Glucose: $P = 0.10$; Pre GH: $P = 0.03$; Post GH – Pre \times Week: $P = 0.01$ and Post \times Week: $P = 0.08$.

Chapter 4. Temporal diet digestibility and microbial protein synthesis effects of soybean meal versus canola meal fed to dairy cows during the close-up dry period and early lactation

Abstract

In a companion paper, replacing soybean meal (SBM) with canola meal (CM) in diets fed during the close-up dry period and early lactation increased dry matter intake (DMI) and tended to increase milk yield. While including CM in diets fed during early lactation is known to improve production, the effect on temporal diet digestibility and microbial protein synthesis (MPS) is largely uncharacterized. Furthermore, the effect of feeding CM on diet digestibility and MPS during the close-up dry period is unknown. The objective of this experiment was to determine the effects of feeding isonitrogenous diets formulated with SBM or CM as the primary protein source during the close-up dry period and early lactation on temporal diet digestibility and MPS to expand the physiological framework for the production differences. In a 2×2 factorial randomized complete block design, eighty multiparous cows were assigned to one of four treatments, which were cows fed diets with: 1) SBM pre- and postpartum (SBM-SBM), 2) SBM pre- and CM postpartum (SBM-CM), 3) CM pre- and SBM postpartum (CM-SBM), and 4) CM pre- and postpartum (CM-CM). The close-up, transition, and lactating diets were fed for four weeks immediately before parturition, the first three weeks of lactation, and weeks four through sixteen of lactation, respectively. The close-up, transition, and lactating pairs of diets were formulated using most of the same ingredients, except SBM and soybean hulls were used only in the SBM-based diets and CM was used only in the CM-based diets. The close-up, transition, and lactating diets were formulated to contain 14.2 or 19.4%, 12.1 or 16.5%, and 9.9 or 13.5% SBM and CM, respectively, on a DM basis. The close-up, transition, and lactating

SBM or CM diets were 14.3 versus 14.5%, 17.6 versus 17.4%, and 17.2 versus 16.9% CP, respectively, on a DM basis. Fecal and urine samples were collected every four weeks of the experiment from every cow consuming an experimental diet, with the goal of collecting from every cow once during the close-up dry period and four times throughout early lactation. Samples were collected at six time points over two days representing a 24-hour cycle to account for diurnal variation. Diet digestibility was determined using iNDF as the internal marker after TMR and fecal samples were ruminally incubated for 288 hours. Prepartum and temporal postpartum diet digestibility and urinary data were analyzed separately using the MIXED procedure of SAS with week as the repeated measure. Dry matter, organic matter, and crude protein digestibility all decreased both pre- and postpartum for cows fed the CM diets compared to the SBM diets. While prepartum uric acid excretion decreased for cows fed the CM diets compared to the SBM diets, diet did not affect any other prepartum urinary excretion measure. Postpartum excretion of allantoin, uric acid, purine derivatives, intestinal microbial N flow, urea, urinary urea N, and total urinary N decreased for cows fed the CM diets compared to the SBM diets postpartum. The interaction between pre- and postpartum diet was not significant for any diet digestibility or urinary excretion measure. Given that diet digestibility and intestinal microbial N flow decreased for cows fed the CM diets compared to the SBM diets postpartum, increased milk yield suggests that the positive effect of utilizing CM compared to SBM occurred postruminally and that the quality of CM protein outweighed the quantity of protein digested in the total tract.

Introduction

Soybean meal (SBM) is typically considered to be the primary protein source utilized in dairy cow diets. However, the positive effects of replacing SBM with canola meal (CM) during middle and late lactation on production measures such as DMI and milk yield has been demonstrated over a long history of research that includes a meta-analysis (Huhtanen et al., 2011) and subsequent experiments (Broderick et al., 2015; Benchaar et al., 2021; Lage et al., 2021). Furthermore, positive production effects have also been observed during early lactation when substituting CM for SBM (Moore and Kalscheur, 2016; Gauthier et al., 2019; Swanepoel et al., 2020). While CM contains less CP on a DM basis compared to SBM (40.6-42.9 vs. 52.6%; Adewole et al., 2016; Broderick et al., 2016; NASEM, 2021), the increased methionine content of CM (1.97 vs. 1.38%; NASEM, 2021) gives it additional value in scenarios where methionine is limiting.

In a companion paper (Kuehnl and Kalscheur, 2021), milk yield tended to increase for cows fed diets formulated with CM compared to SBM as the primary protein source during the first 16 weeks of lactation. Furthermore, cows fed the diets with CM increased pre- and postpartum DMI compared to cows fed the diets with SBM. Increased DMI during both periods begs the question of its potential effects on diet digestibility due to increased passage rate. Additionally, characterizing the effect of feeding diets with SBM or CM on microbial protein synthesis (MPS) is of utmost interest due to microbial protein constituting more than 50% of duodenal CP flow (Clark et al., 1992) and its similarities with the AA profile of milk (Sok et al., 2017; NASEM, 2021).

Diet digestibility can be evaluated using a marker that is measured in both TMR and feces. A commonly used internal marker, iNDF is the fraction of NDF remaining after 288-hours

of ruminal incubation that is considered to be completely unavailable to microbes (Huhtanen et al., 2006) due to cross linkages between lignin and hemicellulose in the cell wall (Van Soest, 1994). Quantifying the flow of urinary purine derivatives (allantoin and uric acid) is a relatively easy and noninvasive technique that is utilized as a proxy to evaluate MPS (Valadares et al., 1999) due to its established relationship with the duodenal supply of microbial RNA (Chen et al., 1990; Balcells et al., 1991; Giesecke et al., 1994). Using equations from Chen and Gomes (1992), MPS will be reported herein as intestinal microbial N flow.

The objective of this experiment was to determine the effect of feeding isonitrogenous diets formulated with SBM or CM as the primary protein source during the close-up dry period and early lactation on prepartum and temporal postpartum diet digestibility and MPS. Since replacing SBM with CM in diets fed during the close-up dry period and early lactation increased milk yield and DMI, diet digestibility and MPS may be altered and can help explain the observed increases.

Materials and Methods

Cows, Facilities, and Diets

The procedures used in this experiment were approved by the University of Wisconsin-Madison IACUC protocol A006120. Eighty multiparous (2.72 ± 0.92 ; average parity \pm SD) Holstein cows enrolled in a randomized complete block design and were grouped into blocks of four cows by expected parturition date. Within block, cows were randomly assigned to one of four dietary treatments in a 2×2 factorial design. Experimental diets were fed from November 2019 through October 2020 and cows calved from December 2019 through June 2020. Cows

were housed in individual tie-stalls at the USDA Dairy Forage Research Center farm in Prairie du Sac, WI. Diets were fed as a TMR once-daily for ad libitum intake with continuous access to water. Cows were milked 3× daily at approximately 0600, 1300, and 2100 in a double-8 herringbone parlor.

Diets were formulated using NRC (2001) software. Three isonitrogenous pairs of diets (close-up, transition, and lactating) were fed during the experiment and differed in the primary protein source utilized, which was SBM or CM. Prior to consuming the close-up diets, all cows were fed the common herd far-off dry cow diet. At 27.3 ± 1.3 (mean \pm SD) days before expected parturition date, cows began consuming the close-up SBM or CM diet and remained on the same diet until parturition. Of the 80 cows enrolled, half ($n = 40$) consumed the diet with SBM and the other half consumed the diet with CM during the close-up dry period. Cows consumed the close-up SBM and CM diets for 27.0 ± 4.4 and 28.0 ± 4.7 (mean \pm SD) days before parturition, respectively. All cows consumed the close-up diet for at least 17 days before parturition. At parturition, half ($n = 20$) of the cows on each of the close-up diets switched to the transition diet containing the other protein source, while the other half consumed the transition diet with the same protein source. The transition and lactating diets were fed from parturition through week three and week four through 16 of lactation, respectively. The transition diets were formulated to contain more CP to supply more AA for the fresh cow to accommodate increasing milk production and less starch to minimize metabolic disorders. This design resulted in four treatment groups, which were cows fed diets with 1) SBM pre- and postpartum (SBM-SBM), 2) SBM pre- and CM postpartum (SBM-CM), 3) CM pre- and SBM postpartum (CM-SBM), and 4) CM pre- and postpartum (CM-CM).

Ingredient composition of the far-off, close-up, transition, and lactating diets are presented in Table 4.1. The forage:concentrate ratios of the far-off, close-up, transition, and lactating diets were 87.24:12.76, 70:30, 62:38, and 60:40, respectively. The far-off and close-up diets contained conventional corn silage, grass hay, and wheat straw, whereas the transition and lactating diets contained BMR corn silage and alfalfa silage as the forage sources. Two sources of high-moisture corn were used, with one used for the dry cow diets and the other used for the lactating cow diets.

Sampling and Analysis

Samples of all diets, silages, and high-moisture corn were collected daily and stored at -20°C in one week increments until dried. Samples of dry feeds were collected once weekly. All feed samples were dried in duplicate at 105°C for 24h for dry matter determination and at 55°C for 48h for nutrient analysis. All weekly composites of diets and forages (BMR corn silage, conventional corn silage, alfalfa silage, grass hay, and wheat straw) were ground to pass through a 1-mm screen (Wiley mill, Arthur H. Thomas Co., Philadelphia, PA) and composited in eight week increments for nutrient analysis. All concentrate samples (SBM, soybean hulls, CM, corn gluten meal, and high-moisture corn) were ground to pass through a 1-mm screen (cross beater mill, Retsch GmbH and Co., Haan, Germany) and composited in eight week increments for nutrient analysis. All composited feed samples were analyzed by a commercial laboratory (Dairyland Laboratories, Inc., Arcadia, WI) using wet chemistry analysis. The far-off, close-up, transition, and lactating diets were analyzed for CP (method 990.03; AOAC International, 1996), NDF using alpha amylase and sodium sulfite (aNDF; method 2002.04; AOAC International, 2005), aNDF with ash correction (aNDFom), ether extract (EE) using diethyl ether (method 920.39; AOAC International, 1996), ash (method 942.05; AOAC International, 1996), and the

following minerals: Ca, P, Mg, K, and S using a microwave and inductively coupled plasma spectroscopy (method 942.05; AOAC International, 1996). The corn silages, alfalfa silage, SBM, soybean hulls, CM, corn gluten meal, high-moisture corn, grass hay, and wheat straw were analyzed for CP, aNDF, aNDFom, ADF (method 973.18; AOAC International, 1996), NDICP and ADICP using fiber residuals from NDF and ADF determination (method 990.03; AOAC International, 1996), starch using a sodium acetate buffer method (Hall, 2009), EE, and ash. Non-fiber carbohydrates (NFC) was calculated as: $NFC = 100 - ((\%aNDFom - \%NDICP) + \%CP + \%EE + \%Ash)$, with NDICP expressed as % of DM. Nutrient analysis results for each composite were weighted relative to the corresponding number of days each diet and ingredient were fed, as a proportion of the overall days of cow enrollment, due to the unequal distribution of cows consuming each diet and ingredient during each week of the experiment. The nutrient compositions of the diets (CP, NDICP, ADICP, aNDF, aNDFom, ADF, NFC, starch, EE, ash, and NE_L) were calculated using the analyzed composition of the individual feed ingredients and their inclusion rate in the TMR.

Equally spaced every four hours over a 24-hour cycle to account for diurnal variation, six spot fecal and urine samples were collected over two days from every cow consuming an experimental diet every four weeks of the experiment. There were 11 collection periods, with the first during the second week of December 2019 and the last during the third week of September 2020. The intention of this schedule was to collect samples from every cow once during the four weeks before and once every four weeks after parturition for a total of five collections per cow while consuming an experimental diet. Due to early parturition, 73 out of 80 cows were collected prepartum, with samples missed from four or three cows assigned to the SBM or CM diet, respectively.

Fecal samples were dried at 55°C for one week, ground to pass through a 1-mm screen (cross beater mill, Retsch GmbH and Co., Haan, Germany) and composited on an equal mass basis for each cow. Fecal composites and TMR samples corresponding to collection periods were weighed (0.5 g) into filter bags with a pore size of 25 µm (F57, Ankom Technology Corp., Macedon, NY). Four and three replicates were analyzed for each fecal composite and TMR sample, respectively. The filter bags were placed into mesh laundry bags with weights and placed directly into the rumen of four cannulated cows for 288 hours, with approximately half of the total filter bags placed into each pair of cows. After incubation, filter bags were rinsed in a washing machine until the effluent water was visually clear. From there, filter bags underwent digestion in an Ankom 200 Fiber Analyzer using neutral detergent solution with heat-stable α -amylase and sodium sulfite following the batch procedures outlined by the manufacturer (Ankom Technology Corp., Macedon, NY). NDF residues were corrected for residual ash via combustion in a muffle furnace for 6 hours at 500°C, with the remaining material considered the indigestible internal marker iNDF used to calculate diet digestibility (Huhtanen et al., 1994). Nitrogen % of TMR and fecal samples was determined using a N analyzer (Leco FP-2000 N Analyzer; Leco Instruments, Inc.). The internal marker iNDF and analysis of DM, OM, and CP in TMR and fecal samples were utilized in the following equation to determine digestibility: apparent total tract nutrient digestibility (%) = $100 - [(TMR \text{ marker concentration}/\text{fecal marker concentration}) \times (\text{fecal nutrient concentration}/\text{TMR nutrient concentration})]$.

After collection, 15 mL of urine was pipetted into a 120 mL cup containing 60 mL of 0.144 N diluted H₂SO₄. From there, the acidified and diluted urine samples were stored at -20°C until they were composited. To composite, each individual sample for each cow was thawed and vortexed to ensure adequate distribution of deposited crystals throughout the sample and 5 mL

was pipetted into a 50 mL conical tube to create a 30 mL volume composite sample for each cow. The composite sample was frozen at -20°C until it was thawed at room temperature the day of analysis. Each composite sample was used to determine allantoin, uric acid, creatinine, urea, and total N %. Allantoin concentration was evaluated using a phenylhydrazine colorimetric method adapted to a 96-well plate reader (Vogels and van der Drift, 1970). The intra- and inter-assay CVs for allantoin were 5.35 and 6.81%, respectively. Uric acid concentration was evaluated using an uricase colorimetry method with a commercial reagent and standard solution adapted to a 96-well plate reader (Stanbio Laboratory, Boerne, TX; reference # 1045-225). The intra- and inter-assay CVs for uric acid were 6.41 and 5.10%, respectively. Additionally, the sum of allantoin and uric acid were used to represent urinary excretion of purine derivatives (PD) from each cow. Creatinine and urea were evaluated using a picric acid method and a urease colorimetric method, respectively, adapted to flow injection analysis (Lachat Quik-Chem 8500; Lachat Instruments). Total urinary N % was evaluated using a N analyzer (Leco FP-2000 N Analyzer; Leco Instruments, Inc.). Urine volume was calculated using average body weight (BW) during the collection week, the composite creatinine concentration, and an excretion rate of 29 mg creatinine/kg BW/d as determined by Valadares et al. (1999). Daily excretions of allantoin, uric acid, PD, and urea were calculated by multiplying urine volume and the concentration of each. Purine derivative absorption and intestinal microbial N flow were determined using equations from Chen and Gomes (1992). Excretion of urinary urea N (UUN) and total urinary N (TUN) were calculated using urine volume and urea concentration or total N %, respectively. Urinary urea N, as a % of TUN, was determined via dividing UUN by TUN and multiplying by 100.

Statistical Analysis

Statistical analysis was performed on pre- and temporal postpartum diet DM, OM, and CP digestibility, daily urine volume, daily urinary excretions of creatinine, allantoin, uric acid, PD, urea, urea N, and total N, urinary urea N (% of TUN), purine absorption, and intestinal microbial N flow. Prepartum data included one measurement from each cow in the 28 days before parturition and postpartum data included four measurements from each cow, with one measurement from each 4-week time period including weeks 1 to 4, 5 to 8, 9 to 12, and 13 to 16 of lactation.

Prepartum data was analyzed using the MIXED procedure of SAS (version 9.4, SAS Institute, Inc.). Body weight and BCS before cows began consuming the close-up diet and ME305 for the previous lactation was tested as covariates and retained in the model if significant ($P \leq 0.10$). The covariance structure with the lowest AIC was used. The prepartum model is:

$$Y_{ijk} = \mu + \text{Covariate} + \text{CollPeriod}_i + \text{Treatment}_j + \text{Block}_k + \varepsilon_{ijk},$$

where Y is the response variable; μ is the overall mean; covariate is the body weight (BW), BCS, or ME305 measure (if significant); collperiod is the fixed effect of the collection period ($i = 1$ to 7); treatment is the fixed effect of the prepartum diet ($j = \text{SBM or CM}$); block is the random effect of block ($k = 1$ to 20); and ε is the residual error.

Postpartum data was analyzed using the MIXED procedure of SAS with week as the repeated measure. Body weight and BCS at parturition and ME305 for the previous lactation were tested as covariates and retained in the model if significant ($P \leq 0.10$). The covariance structure with the lowest AIC was used. The postpartum statistical model is:

$$Y_{ijklm} = \mu + \text{Covariate} + \text{CollPeriod}_i + \text{Prepartum}_j + \text{Postpartum}_k + (\text{Prepartum}_j \times \text{Postpartum}_k) + \text{Time}_l + (\text{Prepartum}_j \times \text{Time}_l) + (\text{Postpartum}_k \times \text{Time}_l) + (\text{Prepartum}_j \times \text{Postpartum}_k \times \text{Time}_l) + \text{Block}_m + \varepsilon_{ijklm},$$

where Y is the response variable; μ is the overall mean; covariate is the BW, BCS, or ME305 measure (if significant); collperiod is the fixed effect of the collection period ($i = 2$ to 11); prepartum is the fixed effect of the prepartum diet ($j = \text{SBM}$ or CM); postpartum is the fixed effect of the postpartum diet ($k = \text{SBM}$ or CM); prepartum \times postpartum is the fixed effect 2-way interaction of the prepartum and postpartum diets; time is the fixed effect of the four 4-week time periods ($l = 1$ to 4); prepartum \times time is the fixed effect 2-way interaction of the prepartum diet and time; postpartum \times time is the fixed effect 2-way interaction of the postpartum diet and time; prepartum \times postpartum \times time is the fixed effect 3-way interaction of the prepartum diet, postpartum diet, and time; block is the random effect of block ($m = 1$ to 20); and ε is the residual error. Significance was declared when $P \leq 0.05$ and tendency was declared when $0.05 < P \leq 0.10$. The Tukey-Kramer adjustment was performed for pairwise comparisons of LSM.

Results

Nutrient Composition of Diets

The chemical composition of the diets and diet ingredients are presented in Tables 4.2 and 4.3, respectively. Each pair of diets (close-up, transition, and lactating) was within 0.3% CP. As expected, the CP content of SBM was higher (51.4 vs. 41.4%) and the aNDFom content of SBM was lower (8.66 vs. 23.1%) than CM. However, the high aNDFom content of soybean hulls (67.7%) compensated for this difference in aNDFom content in the SBM-based diets.

Diet Digestibility

Pre- and postpartum diet DM, OM, and CP digestibility data are presented in Table 4.5. Pre- and postpartum diet DM, OM, and CP digestibility LSM for each treatment group by time are presented in Figures 4.1 to 4.3. Prepartum DM, OM, and CP digestibility decreased for cows fed the CM diet compared to the SBM diet ($P < 0.01$). Furthermore, postpartum DM, OM, and CP digestibility decreased for cows fed the CM diets compared to the SBM diets postpartum ($P < 0.01$). While postpartum DM and OM digestibility were unaffected ($P > 0.10$), postpartum CP digestibility was affected by time ($P = 0.01$). There were no interactions between diet and time during the experiment ($P > 0.10$).

Urinary Excretion

Prepartum urinary excretion data are presented in Table 4.6. Urinary excretion of uric acid decreased for cows fed the CM diet compared to the SBM diet ($P = 0.01$). Urinary excretion of creatinine, allantoin, PD, urea, UUN, and TUN, urine volume, PD absorption, intestinal microbial N flow, and UUN (% of TUN) were not affected by diet ($P > 0.10$).

Postpartum urinary excretion data are presented in Table 4.7. Pre- and postpartum urinary excretion data for urine volume, intestinal microbial N flow, urea N, and total N are presented in Figures 4.4 to 4.7. Urinary excretion of allantoin, uric acid, PD, urea, UUN, and TUN, PD absorption, and intestinal microbial N flow decreased for cows fed the CM diets compared to the SBM diets postpartum. Creatinine, urine volume, and UUN (% of TUN) were not affected ($P > 0.10$) by postpartum diet. Except for UUN (% of TUN), all urinary excretion measures were affected by time ($P < 0.01$). There were no interactions between diet and time during the experiment ($P > 0.10$).

Discussion

To our knowledge, this is the first experiment analyzing the temporal diet digestibility and MPS effects of feeding diets formulated with SBM or CM as the primary protein source during the close-up dry period and early lactation in cows. Differences observed for diet digestibility and MPS are potentially explained by production differences, physical properties, and chemical properties of the protein sources. The production measure of interest in the present experiment is DMI, as it is inherently interwoven with retention time and passage rate and therefore diet digestibility and potentially MPS. Furthermore, the chemical and physical properties of total digestible protein (TDP), lignin content, and RDP discussed herein may help to explain differences observed for diet digestibility and MPS as indirectly measured via urinary excretion of PD.

Diet Digestibility: Dry Matter Intake

Reported in a companion paper (Kuehnl and Kalscheur, 2021), DMI increased pre- and postpartum for cows fed the CM diets compared to the SBM diets. These values represent an increase for pre- and postpartum DMI of 5.5 and 4.9%, respectively. The corresponding decreases for pre- and postpartum diet DM, OM, and CP digestibility (DMD, OMD, and CPD) for cows fed the CM diets compared to the SBM diets were 7.8, 5.0, and 4.8% and 3.7, 4.2, and 1.6%, respectively. Given the finite volume of the gastrointestinal tract, the decreases for DMD and OMD may be due in large part to increased DMI and passage rate and therefore decreased retention time and degradation of dietary components such as NDF. Increased DMI in the present experiment was potentially caused by a “pull effect,” whereby the CM offered a more balanced AA supply for milk production compared to SBM, due to higher methionine content, which increased milk production and therefore energy demand (Huhtanen et al., 2011). Given

that BW and BCS were not different, increased DMI is realistically the avenue that fulfilled the increased demand for energy in the present experiment. No difference for BW, BCS, and milk yield during week one of lactation (Kuehnl and Kalscheur, 2021) is potentially explained by a similar percentage decrease of prepartum OMD, compared to the increase for prepartum DMI, for cows fed the CM diet compared to the SBM diet, negating potential positive effects of the extra nutrients consumed. However, the increased methionine supply of the CM diet, coupled with a similar percentage decrease for prepartum CPD compared to the increase for prepartum DMI, for cows fed the CM diet compared to the SBM diet, suggests extra methionine was available to be potentially utilized by the fetus for growth (Alharthi et al., 2018) and programming (Peñagaricano et al., 2013).

Diet Digestibility: Total Digestible Protein and Lignin Content of Canola Meal

While DMI may help to explain decreased DMD for cows fed the CM diets compared to the SBM diets, dissimilar percentage decreases between DMD, OMD, and CPD suggests that specific dietary components were differentially influenced. Along with DMI and passage rate, the smaller decrease for postpartum CPD compared to postpartum DMD and OMD could be due to decreased TDP, i.e. the sum of RUP and protein digested in the intestines, for CM compared to SBM. Previous reports for TDP values of CM and SBM range between 85.1 to 93.5 and 98.2 to 99.3%, respectively (Jayasinghe et al., 2014; Paz et al., 2014), indicating that, while the majority of protein in both sources is digestible in the total tract, a significant portion of CP in CM remains unavailable potentially due to heat damage. In the present experiment, the higher NDICP (8.54 and 1.28%, % of CP, respectively) and ADICP (6.13 vs. 0.83%, % of CP, respectively) content of CM compared to SBM supports the lower TDP values typically observed for CM. The NDICP and ADICP fractions of CP represent the portion bound to NDF

and slowly degraded in the rumen or unavailable due to heat damage, respectively (Sniffen et al., 1992; Van Soest, 1994). Despite not being measured herein, one inherent component of DMD that potentially contributed to the larger decrease for postpartum DMD, compared to postpartum CPD, is NDF digestibility (NDFD). Although non-cell wall components are unaffected, a negative correlation between digestibility of cell wall components, i.e. NDF, and lignin content is well-established (Dehority et al., 1962; Jung and Vogel, 1986; Raffrenato et al., 2017). While CM samples were not analyzed for lignin in the present experiment, results from Dairy One Forage Lab (2022) indicate that lignin content of CM produced during the last five crop years ranged between averages of 8.93 to 9.85% (% of DM). During the same five years, lignin content of SBM and soybean hulls ranged between averages of 1.08 to 1.89% and 2.52 to 3.37% (% of DM), respectively. These values equate to 30.2 to 31.8%, 8.29 to 12.8%, and 3.84 to 5.28% for CM, SBM, and soybean hulls, when expressed as % of NDF, emphasizing the stark differences between lignin content and degree of lignification in these feedstuffs. Utilizing 120 or 288 hour ruminal incubations, respectively, Cotanch et al. (2014) and Paula et al. (2017) determined potentially digestible NDF values of 68.0 and 80.2% for CM, with lower values undoubtedly expected for normal total tract degradation, highlighting the drastic effects of lignin on NDFD of CM.

Diet Digestibility: Results Comparison

A dearth of information exists regarding diet digestibility for cows fed diets with CM or SBM during early lactation. A quadratic effect of CM inclusion on OMD, CPD, and aNDFD was observed in Gauthier et al. (2019), as cows fed a diet with 8.2% CM increased all three measures compared to cows fed the diets with 3.5 and 13.0% CM during early lactation. While Swanepoel et al. (2020) reported decreased OMD and CPD for cows fed a diet with 14.3% CM compared to

a diet with 6.6% CM and 6.3% SBM during early lactation, similar to the present experiment, there was no difference for aNDFomD or aNDFD. However, the effect of CM lignin on NDFD in Swanepoel et al. (2020) was likely confounded by other dietary components, as inclusion rate of ingredients such as alfalfa silage and wheat silage differed by 4.5 and 2.6%, respectively. Summarizing data from mid- and late lactation, a meta-analysis from Huhtanen et al. (2011) found no difference between the slopes of OMD and CPD, when comparing inclusion rates of SBM and CM in diets. Due to limited data for SBM, Huhtanen et al. (2011) did not report NDFD. More recent experiments comparing digestibility between diets formulated with CM or SBM during mid-lactation have yielded mixed results. No difference for DMD or OMD between cows fed diets with CM or SBM has been observed previously (Oba et al., 2010; Gidlund et al., 2015; Lage et al., 2021). Paula et al. (2018) noted increased DMD and OMD for cows fed a diet with CM compared to SBM. In contrast, Pereira et al. (2020) found decreased DMD and OMD for cows fed a diet with CM compared to SBM. Furthermore, a linear decrease for DMD and OMD was observed by Benchaar et al. (2021) as the inclusion rate of CM increased in the diet at the expense of SBM. Following a similar trend as DMD and OMD, CPD was not different in Lage et al. (2021), increased in Paula et al. (2018), and decreased in Pereira et al. (2020) for cows fed the diets with CM compared to SBM. Additionally, Benchaar et al. (2021) reported a linear decrease for CPD as CM inclusion in the diet increased at the expense of SBM. While NDFD performed similarly to CPD for Paula et al. (2018) and Pereira et al. (2020), Lage et al. (2021) observed increased NDFD for cows fed a diet with CM compared to SBM. However, Benchaar et al. (2021) noted a linear decrease for NDFD as CM inclusion in the diet increased at the expense of SBM, with a more drastic decrease occurring relative to CPD as hypothesized for

the present experiment. The more drastic decrease for NDFD compared to CPD was attributed to the nearly 10-fold higher lignin content (% of NDF) of CM compared to SBM and soybean hulls.

Diet Digestibility: Effect of Dry Matter Intake in other Experiments

While a negative relationship between DMI and diet digestibility potentially happened in the present experiment, mixed results occurred in other instances. In agreement with the present experiment, Pereira et al. (2020) observed increased DMI with decreased diet digestibility for cows fed a diet with CM compared to SBM and Benchaar et al. (2021) noted a linear increase for DMI with a linear decrease for diet digestibility as CM inclusion in the diet increased at the expense of SBM. However, despite increased DMI for cows fed diets with CM compared to SBM, the slopes for diet digestibility of CM and SBM were not different in Huhtanen et al. (2011), suggesting no effect of inclusion level. Furthermore, although Gidlund et al. (2015) reported a tendency for increased DMI for cows fed diets with CM compared to SBM, diet digestibility was unaffected. In this case, the slight increase for DMI may not have been substantial enough to elicit decreased digestibility. While Brito and Broderick (2007) and Lage et al. (2021) found increased DMI for cows fed diets with CM compared to SBM, most diet digestibility parameters were not affected. Interestingly, NDFD increased for cows fed the CM diet in both experiments, in contrast to the negative effect of CM lignification on NDFD hypothesized by Benchaar et al. (2021) and the present experiment. On the other hand, increased diet digestibility for cows fed a diet with CM compared to SBM in Paula et al. (2018) was accompanied by no difference for DMI.

Diet Digestibility: Temporal Fluctuations

As fecal samples are typically collected at one time point during an experiment to evaluate treatment differences for diet digestibility, a scarcity of data exists regarding temporal fluctuations. While postpartum DMD and OMD were unaffected, time was significant for postpartum CPD in the present experiment. However, despite a numerical decrease for postpartum CPD during period two (29-56 DIM) compared to all other periods, there was no difference between periods after Tukey-Kramer adjustment. Despite analyzing a less expansive data set collected only at 82 and 109 DIM, Swanepoel et al. (2020) observed decreased CPD at 109 DIM compared to 82 DIM. However, OMD, aNDFomD, and aNDFD were not affected by DIM. Similarly, the diet×time interaction was not significant for Swanepoel et al. (2020), suggesting that time affected cows fed the SBM or CM diets to the same extent. In de Souza et al. (2019), whereby fecal samples were collected multiple times during a fresh (5, 12, and 19 DIM) and a peak period (33, 47, and 61 DIM), DMD decreased over time during the fresh period, potentially due to increasing DMI and a concomitant increase for passage rate. However, DMD in the peak period and NDFD in both periods were unaffected by DIM.

Microbial Protein Synthesis: Rumen Undegraded Protein

Traditionally, CM and its genetic precursor, rapeseed meal (RSM), were regarded as a poorer source of RUP compared to SBM, with values as low as 28% (% of CP) according to data utilized by NRC (1989). Piepenbrink and Schingoethe (1998) challenged this belief by demonstrating increased RUP values of 39.5% for CM. Afterwards, NRC (2001) released an RUP value of 35.7% for CM (DMI = 4% of BW, forage = 50% of DMI), still notably less than the 42.6% for 48% SBM. Later on, Maxin et al. (2013a) revealed RUP values of 52.5 and 41.5%, corrected for small particle loss, for CM and SBM, respectively. Supporting the observations of

Maxin et al. (2013a), Broderick et al. (2016) observed differing RUP values of 41.0 to 51.0% for CM and 30.5 or 34.9% for solvent SBM based on 2 versions of the Michaelis-Menten inhibitor in vitro method. It is important to note that Broderick et al. (2016) collected CM samples from 12 Canadian processing plants over four consecutive years (2011-2014) and observed an interaction between production year and processing plant for RUP, demonstrating variability of RUP across time and location. Despite these results, NASEM (2021) reported RUP values of 32 and 33% for CM and SBM, respectively. The potentially higher RUP content of CM compared to SBM may help explain the decreased intestinal microbial N flow for cows fed the CM diets during early lactation, as higher RUP suggests that CM protein is degraded to a lesser extent over time compared to SBM protein, decreasing the availability of substrate for incorporation into microbial protein.

Urinary Excretion (Microbial Protein Synthesis): Results Comparison

Similar to diet digestibility, knowledge of the effect of feeding diets with SBM or CM to cows during early lactation on MPS is limited. Gauthier et al. (2019) observed no effect of CM inclusion during early lactation on urinary allantoin or intestinal microbial CP flow. Contrary to the present experiment, Swanepoel et al. (2020) reported increased urinary allantoin and intestinal microbial CP flow for cows fed a diet with 14.3% CM compared to 6.6% CM and 6.3% SBM during early lactation. During mid-lactation, no effect of including SBM or CM in the diet on allantoin, uric acid, PD, or intestinal microbial N flow is typically observed (Maxin et al., 2013b; Paula et al., 2018; Lage et al., 2021). Interestingly, RUP content of SBM and CM was not different in Paula et al. (2018), whereas it was 14% higher (% of CP) for CM in Lage et al. (2021), suggesting that less synchrony between protein and carbohydrate sources in the diet may have exerted a larger effect on MPS rather than the amount of available protein substrate in Lage

et al. (2021). Furthermore, increasing CM inclusion in the diet from 0 to 24% at the expense of SBM did not affect intestinal microbial N flow in Benchaar et al. (2021).

Urinary Excretion (Nitrogen): Results Comparison

While Gauthier et al. (2019) and Swanepoel et al. (2020) did not report measures relating to urinary nitrogen excretion, the results for mid-lactation are mixed. In agreement with the present experiment, Broderick et al. (2015), Pereira et al. (2020), and Lage et al. (2021) observed decreased UUN for cows fed diets with CM compared to SBM. Furthermore, Benchaar et al. (2021) noted a linear decrease for UUN as CM inclusion in the diet increased at the expense of SBM. As observed in the present experiment (Kuehnl and Kalscheur, 2021), MUN also decreased in all previously noted instances of decreased UUN for cows fed diets with CM compared to SBM, supporting the notion that urea rapidly equilibrates between pools of bodily fluids (Broderick and Clayton, 1997). However, Brito and Broderick (2007) and Paula et al. (2018) found no difference for UUN between cows fed diets with CM compared to SBM. Conflicting results for MUN are reported by Brito and Broderick (2007) and Paula et al. (2018), with no difference in the former and decreased MUN for cows fed the diets with CM compared to SBM reported by the latter. Similar to the present experiment, TUN also decreased and linearly decreased in Broderick et al. (2015) and Benchaar et al. (2021), respectively. Conversely, despite a difference for UUN, no difference was observed for TUN in Pereira et al. (2020) or Lage et al. (2021). Additionally, Brito and Broderick (2007), Maxin et al. (2013b), and Paula et al. (2018) reported no difference for TUN. Agreeing with the present experiment, Brito and Broderick (2007) found no effect of diet on UUN as a % of TUN. However, most other experiments observed decreased UUN as a % of TUN (Broderick et al., 2015; Paula et al., 2018; Pereira et al., 2020). Concomitant to the linear decrease for TUN, Benchaar et al. (2021)

observed a linear increase for fecal N as CM inclusion in the diet increased at the expense of SBM, demonstrating a shift of nitrogen excretion to a route where NH_3 and N_2O are less susceptible to volatilization (Dijkstra et al., 2011, 2013; Hristov et al., 2013). Despite decreased UUN for cows fed the CM diets compared to the SBM diets postpartum in the present experiment, there was no difference for postpartum urine volume. Decreased urine volume is commonly observed when UUN is decreased (Broderick et al., 2015; Pereira et al., 2020; Lage et al., 2021). Given that urine osmolality remains constant (Bannink et al., 1999), Lage et al. (2021) hypothesized that increased urine volume for cows fed the SBM diet compared to the CM diet is due to higher osmotic pressure related to increased UUN excretion.

Microbial Protein Synthesis: Potential Relationship with Dry Matter Intake

While Clark et al. (1992) postulated that increased feed intake within the same diet increases microbial N flowing to the small intestine, the effect of DMI on microbial N when comparing diets formulated with SBM or CM is unclear. In agreement with the present experiment, Brito and Broderick (2007) and Lage et al. (2021) reported increased DMI for cows fed diets with CM compared to SBM. On the other hand, Maxin et al. (2013b) and Paula et al. (2018) observed no difference for DMI between cows fed diets with CM compared to SBM. However, in all previous cases, microbial N/CP flow was not different between protein sources, in contrast to the decreased microbial N flow for cows fed the CM diets compared to the SBM diets in the present experiment. Although the results of Maxin et al. (2013b) and Paula et al. (2018) suggest similar DMI may have limited substrate availability for MPS, increased DMI for cows fed the CM diets compared to the SBM diets in Brito and Broderick (2007) and Lage et al. (2021) suggest substrate availability was not the issue. Results from the present experiment suggest that factors such as RUP and particle size potentially play a role in MPS, with the overall

goal of increasing synchrony between dietary ingredients in the rumen. Given the difficulty of evaluating synchrony in this limited context, further assessment on the effects of different inclusion rates of SBM and CM across different inclusion rates of other dietary ingredients, including forages and concentrates, is needed to more adequately quantify the effects of synchrony on MPS.

Microbial Protein Synthesis: Temporal Fluctuations

As is the case with diet digestibility, urine samples are typically collected at only one time point of an experiment, consequently resulting in little data describing temporal fluctuations of urinary excretion measures. In agreement with the present experiment, Swanepoel et al. (2020) reported a DIM effect for urine volume, allantoin, and intestinal microbial CP flow between samples collected at 51 and 109 DIM, potentially related to DMI and passage rate. Furthermore, no interaction between diet and DIM was observed for any measure, suggesting that cows fed the SBM or CM diets were affected to the same extent over time as observed in the present experiment.

Conclusions

In conclusion, substitution of CM for SBM in diets fed to dairy cows during the close-up dry period and early lactation affects diet digestibility, MPS, and urinary nitrogen excretion. Despite decreased diet digestibility and intestinal microbial N flow for cows fed the CM diets compared to the SBM diets postpartum, milk yield increased for cows fed the CM diets with no effect on BW, BCS, ECM, 4% FCM, milk component percentages, or milk component yields. Collectively, these data suggest that the positive effect of feeding diets formulated with CM

compared to SBM in the present experiment occurred postruminally, with the quality of CM protein outweighing the overall quantity of protein digested and utilized for milk production.

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Tables and Figures

Table 4.1. Ingredient composition of the far-off, close-up, transition, and lactating diets.

Ingredient (% DM)	Far-Off ¹	Close-Up ²		Transition ³		Lactating ⁴	
		SBM	CM	SBM	CM	SBM	CM
Corn Silage ⁵	53.3	44.9	44.9	36.0	36.0	30.0	30.0
Alfalfa Silage	-	-	-	26.0	26.0	30.0	30.0
Soybean Meal	-	14.2	-	12.1	-	9.90	-
Soybean Hulls	-	5.20	-	4.40	-	3.60	-
Canola Meal	2.73	-	19.4	-	16.5	-	13.5
Corn Gluten Meal	-	-	-	2.00	2.00	2.00	2.00
Roasted Soybeans	7.27	-	-	-	-	-	-
High-Moisture Corn ⁶	-	8.00	8.00	15.0	15.0	20.0	20.0
Grass Hay	14.5	10.8	10.8	-	-	-	-
Wheat Straw	19.4	14.3	14.3	-	-	-	-
Rumen Inert Fat ⁷	-	-	-	2.00	2.00	2.00	2.00
Mineral/Vitamin Mix ^{8,9}	2.47	1.90	1.90	2.50	2.50	2.50	2.50
Reashure ¹⁰	-	0.45	0.45	-	-	-	-
Urea	0.29	0.25	0.25	-	-	-	-

¹Far-off diet fed during the dry period until cows assigned to close-up diet.

²Close-up diet fed from approximately four weeks prepartum through parturition.

³Transition diet fed from parturition through week three of lactation.

⁴Lactating diet fed from week four of lactation through week 16 of lactation.

⁵Conventional corn silage used for the far-off and close-up diets; BMR corn silage used for the transition and lactating diets.

⁶Different sources of high-moisture corn used for diets fed to the dry versus lactating cows.

⁷Energy Booster 100 (Milk Specialties, Dundee, IL).

⁸Dry cow mineral contained (on a DM basis): 18.6% Ca, 9.6% salt, 7.8% Mg, 9.9% S, 22.9 ppm Se, 549,100 IU vitamin A/kg, 163,800 IU vitamin D3/kg, 8,780 IU vitamin E/kg, and 1.10 g Monensin/kg (Vita Plus Corporation, Madison, WI).

⁹Lactating cow mineral contained (on a DM basis): 16.7% Ca, 10.5% salt, 14.4% Na, 5.6% Mg, 0.3% K, 0.6 ppm Se, 274,500 IU vitamin A/kg, 54,900 IU vitamin D3/kg, 1,250 IU vitamin E/kg, and 0.46 g Monensin/kg (Vita Plus Corporation, Madison, WI).

¹⁰Precision Release Choline (Balchem Corporation).

Table 4.2. Chemical composition of diets.

Item (%DM, unless noted)	Far-Off	Close-Up		Transition		Lactating	
		SBM ¹	CM ¹	SBM	CM	SBM	CM
DM, %AF	45.4	48.8	49.6	44.2	45.1	44.9	45.3
CP ²	12.0	14.3	14.5	17.6	17.4	17.2	16.9
NDICP, ² %CP	-	16.1	16.0	8.60	8.57	8.34	8.32
ADICP, ² %CP	-	9.62	10.1	5.10	5.49	5.06	5.38
aNDF ²	43.4	40.7	40.9	28.7	28.8	27.8	27.9
aNDFom ²	42.5	39.6	39.3	27.8	27.6	27.0	26.8
ADF ²	-	27.1	27.5	20.5	20.9	20.3	20.6
NFC ^{2,3}	35.2	38.3	38.1	42.2	42.0	43.4	43.2
Starch ²	-	23.8	23.9	26.7	26.8	28.3	28.4
EE ²	4.34	2.29	2.77	5.38	5.78	5.45	5.78
Ash ²	7.77	5.17	5.48	5.49	5.76	5.59	5.81
Ca	0.77	0.64	0.71	0.93	0.97	0.94	0.99
P	0.27	0.28	0.43	0.32	0.45	0.32	0.43
Mg	0.44	0.35	0.43	0.37	0.45	0.39	0.44
K	1.35	1.33	1.13	1.61	1.42	1.65	1.51
S	0.39	0.33	0.42	0.22	0.30	0.22	0.30
RDP ⁴	7.89	9.35	9.82	11.0	11.2	10.6	10.8
RUP ⁴	4.11	4.95	4.68	6.59	6.19	6.62	6.09
Dig Lys, ^{4,5} g/d	68	95	96	139	135	180	171
Dig Met, ^{4,5} g/d	19	26	28	41	43	54	55
RDP ⁶	8.32	9.89	10.1	12.0	11.9	11.7	11.6
RUP ⁶	3.68	4.41	4.40	5.62	5.49	5.47	5.32
Metab Lys, ^{6,7} g/d	85	110	110	148	146	185	181
Metab Met, ^{6,7} g/d	25	31	33	45	48	57	60
NE _L , ^{2,8} Mcal/kg DM	1.47	1.53	1.50	1.60	1.57	1.60	1.58
NE _L , ⁹ Mcal/kg DM	1.57	1.56	1.59	1.68	1.65	1.61	1.60
NE _L , ¹⁰ Mcal/kg DM	1.66	1.70	1.64	1.84	1.79	1.82	1.78

¹SBM = soybean meal; CM = canola meal.

²Calculated using nutrient analysis of individual feed ingredients.

³NFC = 100 - ((%aNDFom - %NDICP) + %CP + %EE + %Ash); NDICP expressed as % DM.

⁴Estimated based on NRC (2001) using average DMI and BW of cows during the experiment.

⁵Dig Lys = digestible lysine; Dig Met = digestible methionine.

⁶Estimated based on NASEM (2021) using average DMI and BW of cows during the experiment.

⁷Metab Lys = metabolizable lysine; Metab Met = metabolizable methionine.

⁸NE_L = net energy for lactation, calculated using nutrient analysis of individual feed ingredients.

⁹Estimated based on NRC (2001) using average DMI and BW of cows during the experiment.

¹⁰Estimated based on NASEM (2021) using average DMI and BW of cows during the experiment.

Table 4.3. Chemical composition of diet ingredients.

Item (%DM, unless noted)	Corn Silage		AS ¹	SBM ¹	SH ¹	CM ¹	CGM ¹	HMC ¹		GH ¹	WS ¹
	Conv ¹	BMR ¹						Dry ²	Lact ²		
DM, %AF	42.4	36.0	33.9	87.5	89.7	87.8	92.5	71.6	71.2	87.0	88.0
CP	7.20	7.65	21.8	51.4	11.4	41.4	69.6	7.59	7.43	8.84	3.93
NDICP, %CP	13.4	11.2	7.57	1.28	29.1	8.54	3.78	7.98	7.46	27.3	33.3
ADICP, %CP	7.79	5.85	6.40	0.83	11.7	6.13	2.94	3.29	3.34	13.1	26.1
aNDF	36.6	34.9	42.6	8.87	69.4	25.8	3.60	5.25	5.52	68.3	81.3
aNDFom	35.9	34.1	41.0	8.66	67.7	23.1	3.47	4.69	5.37	66.1	78.3
ADF	23.2	20.5	37.2	6.58	50.1	20.2	1.34	1.83	1.88	45.8	56.9
NFC ³	51.2	51.8	23.4	31.4	17.8	26.5	23.9	83.0	82.8	17.9	9.72
Starch	39.2	39.2	0.81	0.75	0.77	1.27	15.0	73.3	79.6	0.64	0.49
EE	2.88	3.60	4.57	2.27	1.85	4.62	1.35	3.83	3.38	1.59	0.70
Ash	3.83	3.69	10.9	6.95	4.59	7.94	3.09	1.48	1.52	7.98	8.68

¹Conv = conventional corn silage; BMR = brown midrib; AS = alfalfa silage; SBM = soybean meal; SH = soybean hulls; CM = canola meal; CGM = corn gluten meal; HMC = high-moisture corn; GH = grass hay; WS = wheat straw.

²Dry = used for the close-up diets; Lact = used for the transition and lactating diets.

³NFC = 100 - ((%aNDFom - %NDICP) + %CP + %EE + %Ash); NDICP expressed as % DM.

Table 4.4. Pre- and postpartum diet dry matter, organic matter, and crude protein digestibility.

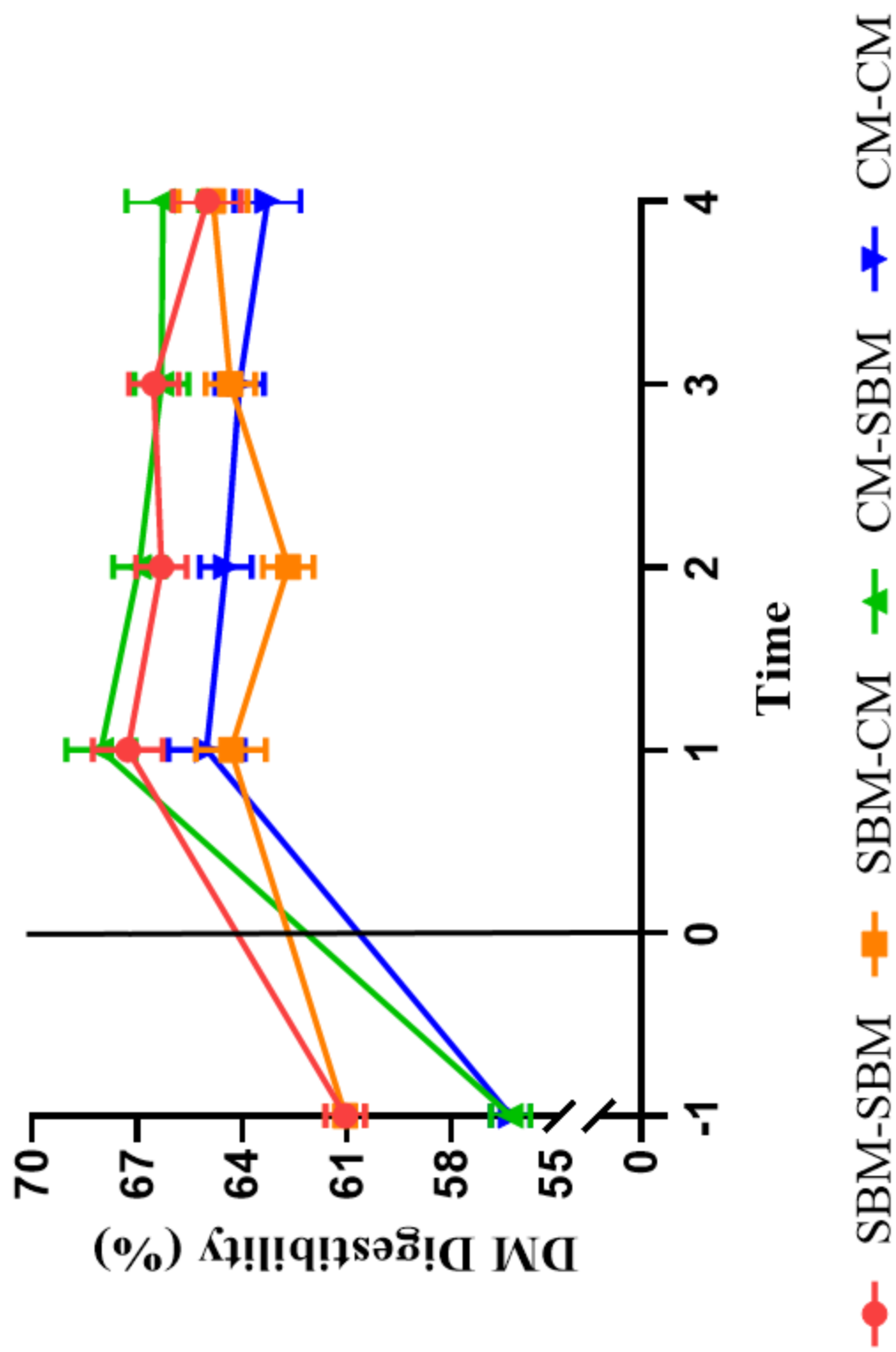
Item	Treatment ¹						P-value ²						
	SBM-SBM	SBM-CM	CM-SBM	CM-CM	SEM		Pre	Post	Pre×Post	Time	Pre×Time	Post×Time	Pre×Post×Time
Pre DMD ³	61.1	61.1	56.3	56.3	0.57		<0.01	-	-	-	-	-	-
Pre OMD ³	63.7	63.7	60.5	60.5	0.54		<0.01	-	-	-	-	-	-
Pre CPD ³	69.5	69.5	66.3	66.3	0.75		<0.01	-	-	-	-	-	-
Post DMD	66.3	64.0	66.9	64.2	0.37		0.24	<0.01	0.51	0.41	0.34	0.36	0.20
Post OMD	70.3	67.4	70.6	67.6	0.37		0.36	<0.01	0.88	0.63	0.39	0.20	0.15
Post CPD	67.8	66.9	68.3	67.1	0.45		0.35	<0.01	0.63	0.01	0.65	0.44	0.38

¹SBM-SBM = cows fed diets with SBM pre- and postpartum; SBM-CM = cows fed diets with SBM pre- and postpartum; CM-SBM = cows fed diets with CM pre- and postpartum; CM-CM = cows fed diets with CM pre- and postpartum.

²Pre = effect of prepartum diet; Post = effect of postpartum diet; Time = effect of time.

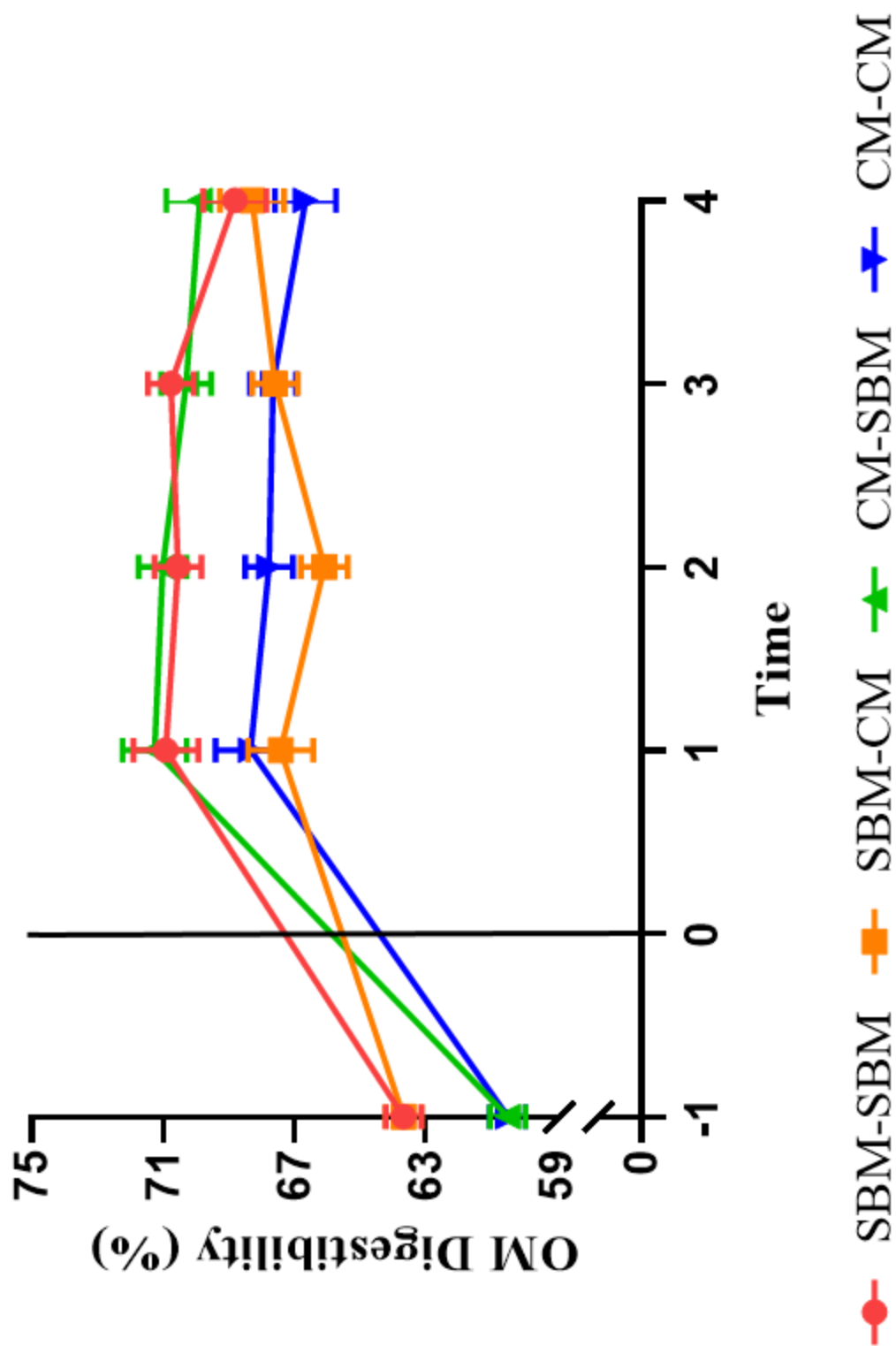
³DMD = dry matter digestibility, OMD = organic matter digestibility, CPD = crude protein digestibility.

Figure 4.1. Diet dry matter digestibility LSM for each treatment group by time.



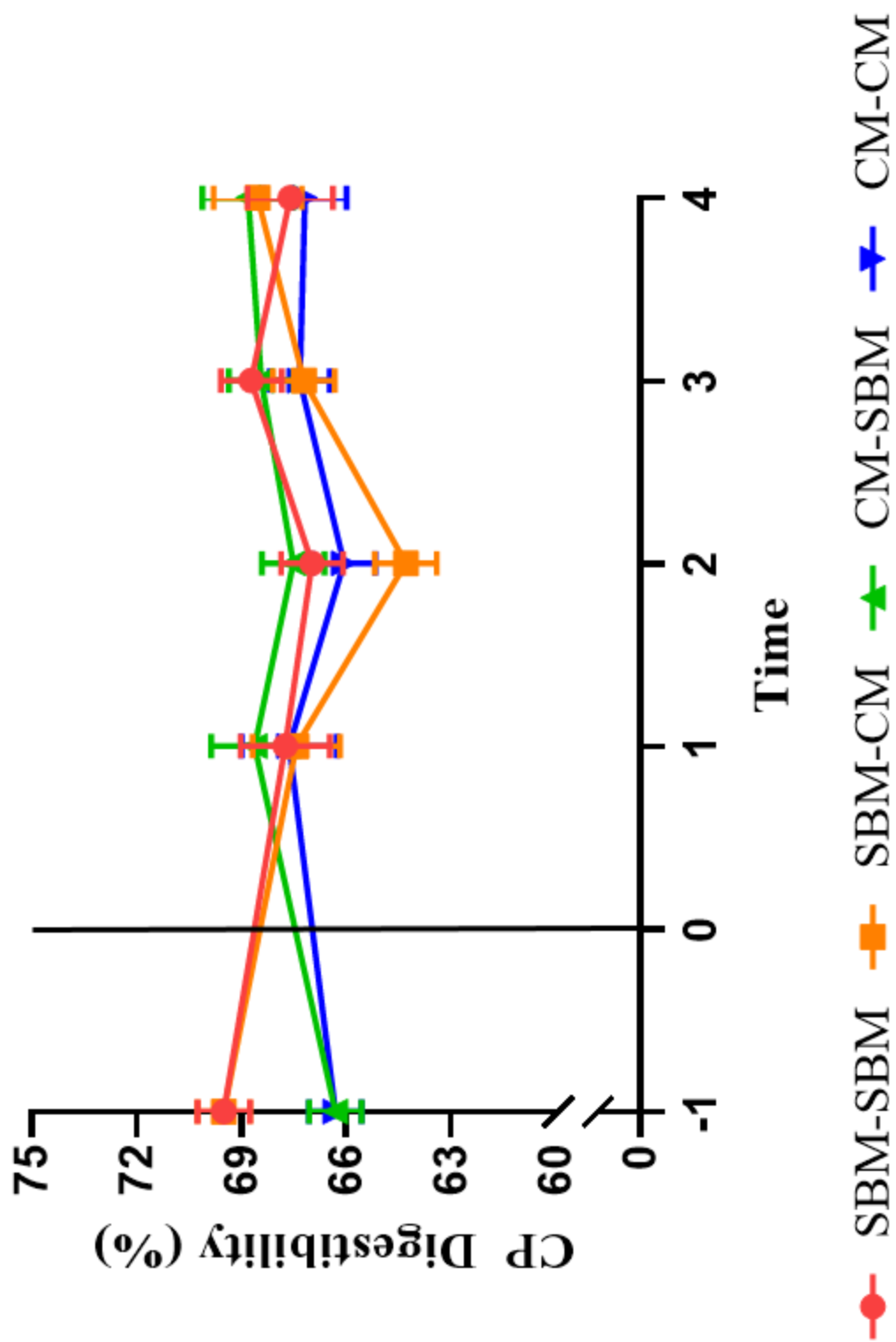
Time: -1 = prepartum; 0 = parturition; 1 = 1 to 28 DIM; 2 = 29 to 56 DIM; 3 = 57 to 84 DIM; 4 = 85 to 112 DIM.
 Prepartum (SBM vs. CM): $P < 0.01$; Postpartum - Post (SBM vs. CM): $P < 0.01$; Time (Postpartum): $P = 0.41$.

Figure 4.2. Diet organic matter digestibility LSM for each treatment group by time.



Time: -1 = prepartum; 0 = parturition; 1 = 1 to 28 DIM; 2 = 29 to 56 DIM; 3 = 57 to 84 DIM; 4 = 85 to 112 DIM.
 Prepartum (SBM vs. CM): $P < 0.01$; Postpartum - Post (SBM vs. CM): $P < 0.01$; Time (Postpartum): $P = 0.63$.

Figure 4.3. Diet crude protein digestibility LSM for each treatment group by time.



Time: -1 = prepartum; 0 = parturition; 1 = 1 to 28 DIM; 2 = 29 to 56 DIM; 3 = 57 to 84 DIM; 4 = 85 to 112 DIM.
 Prepartum (SBM vs. CM): $P < 0.01$; Postpartum - Post (SBM vs. CM): $P < 0.01$; Time (Postpartum): $P = 0.01$.

Table 4.5. Prepartum urinary excretion of purine derivatives and urea.

Item	Diet		SEM	<i>P</i> -value
	SBM ¹	CM ¹		
Creatinine, mmol/d	189	190	3.20	0.84
Urine Volume, L/d	14.0	13.2	0.35	0.11
Allantoin, mmol/d	326	307	13.4	0.31
Uric Acid, mmol/d	25.0	20.7	1.16	0.01
PD ² , mmol/d	351	328	14.0	0.23
PD Absorption ³ , mmol/d	349	321	16.3	0.23
Intestinal Microbial N flow ³ , g/d	254	233	11.9	0.23
Urea, g/d	358	365	12.2	0.68
UUN ² , g/d	167	170	5.67	0.68
TUN ² , g/d	193	197	5.91	0.65
UUN, % of TUN	87.6	86.7	1.49	0.67

¹SBM = soybean meal; CM = canola meal.

²PD = purine derivatives (allantoin + uric acid); UUN = urinary urea-N; TUN = total urinary N.

³Estimated using equations from Chen and Gomes (1992).

Table 4.6. Postpartum urinary excretion of purine derivatives and urea.

Item	Treatment ¹						P-value ²						
	SBM-SBM	SBM-CM	CM-SBM	CM-CM	SEM		Pre	Post	Pre×Post	Time	Pre×Time	Post×Time	Pre×Post×Time
Creatinine, mmol/d	171	166	169	169	3.42		0.95	0.38	0.91	<0.01	0.29	0.19	0.53
Urine Volume, L/d	31.8	31.0	31.9	28.9	1.27		0.43	0.14	0.39	<0.01	0.95	0.81	0.49
Allantoin, mmol/d	566	510	556	526	17.1		0.86	<0.01	0.41	<0.01	0.89	0.64	0.42
Uric Acid, mmol/d	43.2	41.0	47.6	41.1	2.10		0.26	0.03	0.30	<0.01	0.36	0.28	0.51
PD ³ , mmol/d	610	551	604	567	17.8		0.78	<0.01	0.51	<0.01	0.94	0.60	0.40
PD Abs ^{3,4} , mmol/d	658	591	651	608	20.8		0.79	<0.01	0.53	<0.01	0.93	0.61	0.40
Int Micr N Flow ^{3,4} , g/d	478	429	473	442	15.1		0.79	<0.01	0.53	<0.01	0.93	0.61	0.40
Urea, g/d	366	333	371	351	11.8		0.29	0.02	0.53	<0.01	0.90	0.15	0.54
UUN ³ , g/d	171	155	173	164	5.50		0.29	0.02	0.53	<0.01	0.90	0.15	0.54
TUN ³ , g/d	250	232	259	246	6.55		0.07	0.02	0.67	<0.01	0.74	0.63	0.45
UUN, % of TUN	69.5	68.1	68.8	67.4	1.26		0.54	0.24	0.98	0.54	0.66	0.55	0.80

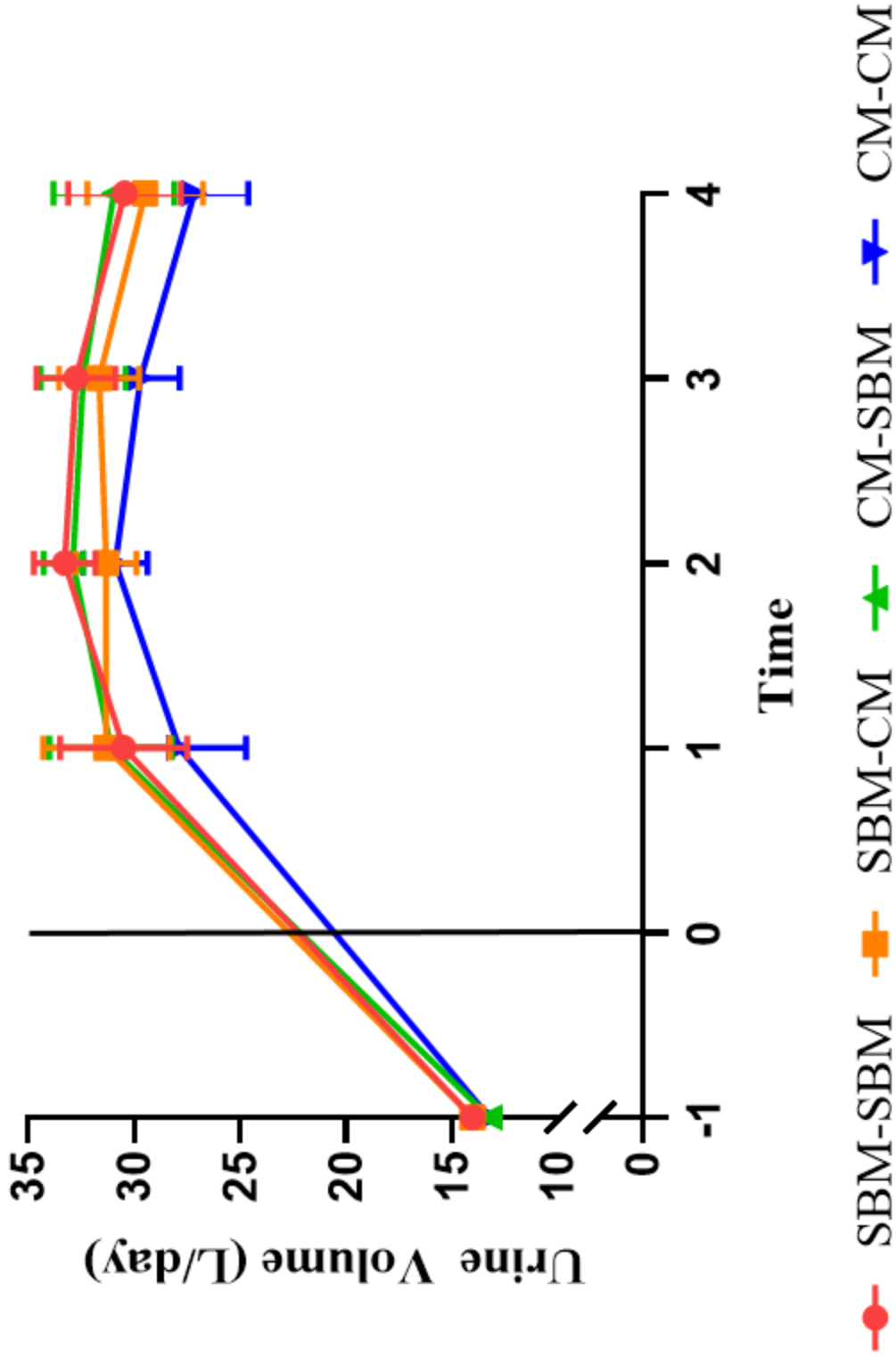
¹SBM-SBM = cows fed diets with SBM pre- and postpartum; SBM-CM = cows fed diets with SBM prepartum and CM postpartum; CM-SBM = cows fed diets with SBM prepartum and CM postpartum; CM-CM = cows fed diets with CM pre- and postpartum.

²Pre = effect of prepartum diet; Post = effect of postpartum diet; Time = effect of time.

³PD = purine derivatives (allantoin + uric acid); PD Abs = purine derivative absorption; Int Micr N Flow = intestinal microbial N flow; UUN = urinary urea-N; TUN = total urinary N.

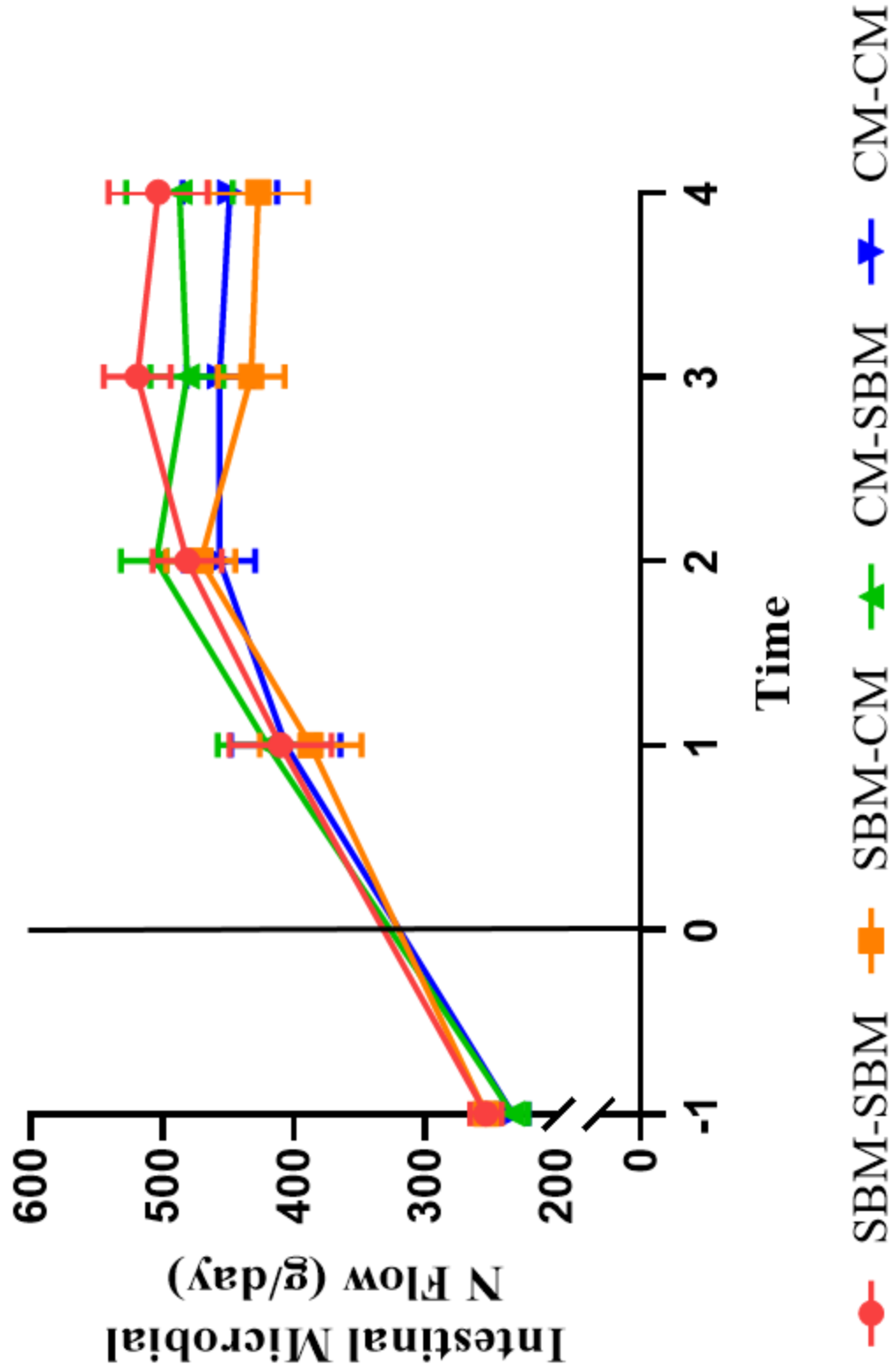
⁴Estimated using equations from Chen and Gomes (1992).

Figure 4.4. Urine volume LSM for each treatment group by time.



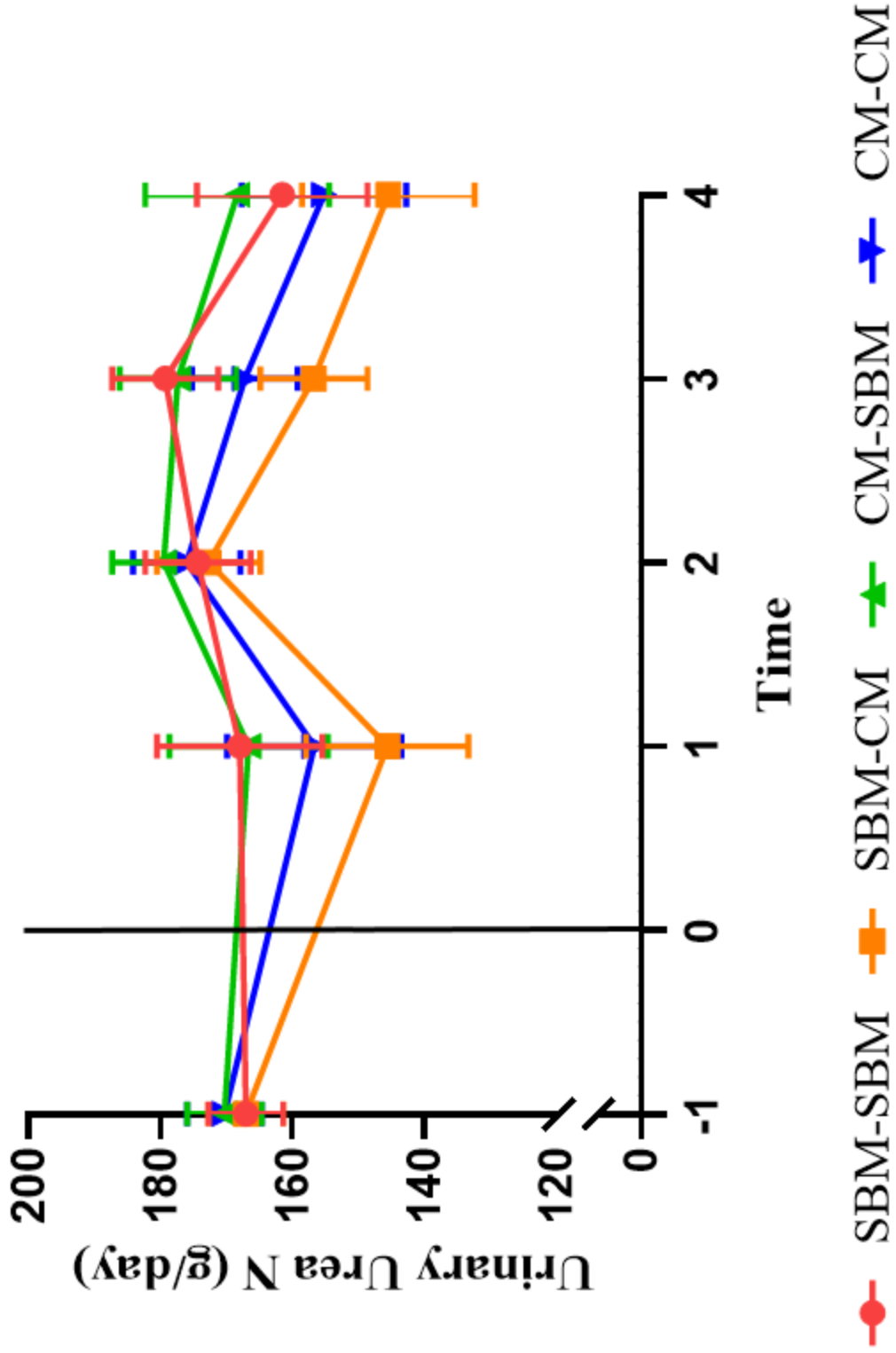
Time: -1 = prepartum; 0 denotes parturition; 1 = 1 to 28 DIM; 2 = 29 to 56 DIM; 3 = 57 to 84 DIM; 4 = 85 to 112 DIM.
 Prepartum (SBM vs. CM): $P = 0.11$; Postpartum - Post (SBM vs. CM): $P = 0.14$; Time (Postpartum): $P < 0.01$.

Figure 4.5. Intestinal microbial N flow LSM for each treatment group by time.



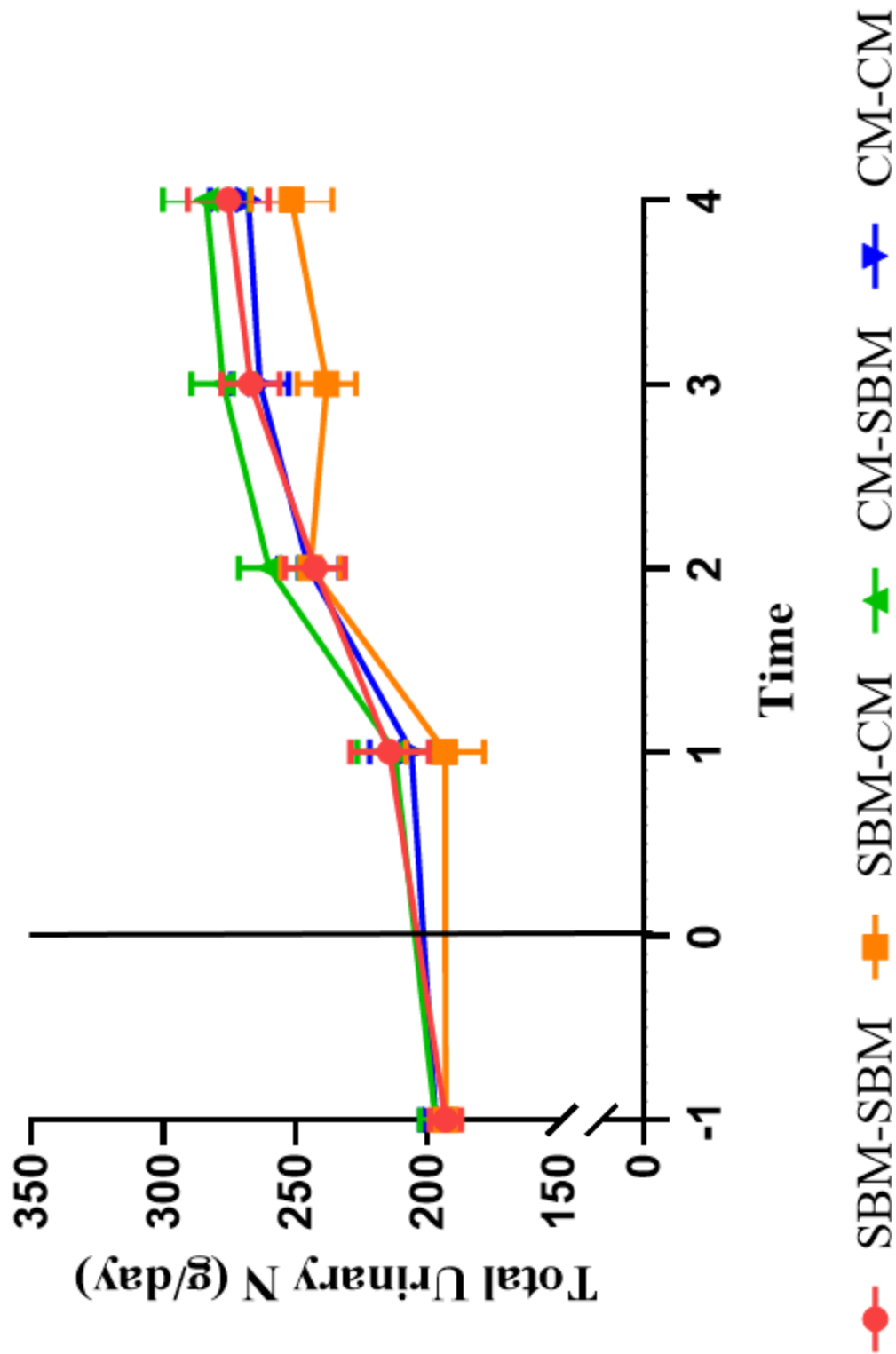
Time: -1 = prepartum; 0 denotes parturition; 1 = 1 to 28 DIM; 2 = 29 to 56 DIM; 3 = 57 to 84 DIM; 4 = 85 to 112 DIM.
 Prepartum (SBM vs. CM): $P = 0.23$; Postpartum - Post (SBM vs. CM): $P < 0.01$; Time (Postpartum): $P < 0.01$.

Figure 4.6. Urinary urea N LSM for each treatment group by time.



Time: -1 = prepartum; 0 denotes parturition; 1 = 1 to 28 DIM; 2 = 29 to 56 DIM; 3 = 57 to 84 DIM; 4 = 85 to 112 DIM.
 Prepartum (SBM vs. CM): $P = 0.68$; Postpartum - Post (SBM vs. CM): $P = 0.02$; Time (Postpartum): $P < 0.01$.

Figure 4.7. Total urinary N LSM for each treatment group by time.



Time: -1 = prepartum; 0 denotes parturition; 1 = 1 to 28 DIM; 2 = 29 to 56 DIM; 3 = 57 to 84 DIM; 4 = 85 to 112 DIM.
 Prepartum (SBM vs. CM): $P = 0.65$; Postpartum - Post (SBM vs. CM): $P = 0.02$; Time (Postpartum): $P < 0.01$.

Chapter 5. Production and physiological effects of soybean meal versus canola meal fed to dairy cows with high or low residual feed intake

Abstract

Canola meal (CM) is a protein-rich byproduct of canola oil production that is routinely incorporated in dairy cow diets. Recent meta-analyses have shown positive production effects, including increased dry matter intake (DMI), milk yield, and feed efficiency, when CM is included in diets fed to dairy cows during mid-lactation compared to other protein sources such as soybean meal (SBM). However, the effect of feeding CM to cows determined to have high or low residual feed intake (RFI) is uncharacterized. The objective of this experiment was to determine the effect of feeding diets formulated with SBM or CM as the primary protein source during mid-lactation on production measures, plasma hormone and metabolite concentrations, and urinary excretion measures in Holstein dairy cows determined to have high or low RFI. One hundred and fifteen Holstein cows were screened for four weeks during early lactation to determine RFI. Dry matter intake, milk yield, milk composition, body weight (BW), and body condition score (BCS) data were collected on each cow during the screening period. These data were used to determine the energy losses of milk energy output, metabolic body weight, and body energy change. Along with parity, these three energy losses were regressed against DMI to determine RFI. Cows enrolled in the experiment if RFI were ≥ 0.5 or ≤ -0.5 standard deviations from expected intake. Seventy-two Holstein cows enrolled in a 2×2 factorial randomized complete block design, with high or low RFI cows assigned to either the SBM or CM diet. This resulted in four treatment groups, which were 1) high RFI cows fed the SBM diet (HiSBM), 2) high RFI cows fed the CM diet (HiCM), 3) low RFI cows fed the SBM diet (LoSBM), and 4) low RFI cows fed the CM diet (LoCM). Cows were blocked by parity and days in milk (DIM)

and consumed their diet for 10 weeks beginning at 86.5 ± 6.7 DIM. The SBM and CM diets contained 11.7% SBM or 15.5% CM, respectively, on a DM basis. Data were analyzed using the MIXED procedure of SAS with week as the repeated measure. While BW increased for cows fed the CM diet, diet did not affect BW change. Increased DMI was observed for cows fed the CM diet and for high RFI cows. A diet \times week interaction occurred for milk yield, with cows fed the CM diet persisting better compared to the SBM diet as the experiment progressed. Cows fed the CM diet tended to increase ECM yield compared to the SBM diet. Low RFI cows tended to increase feed efficiency compared to high RFI cows. A diet \times week interaction was observed for milk fat percent, protein percent and yield, lactose yield, and total solids percent, which is largely attributed to the increased persistency for cows fed the CM diet. Milk total solids yield increased for cows fed the CM diet compared to the SBM diet. Plasma triiodothyronine and insulin concentrations increased and decreased, respectively, for cows fed the SBM diet compared to the CM diet, corresponding to increases for milk yield and milk protein yield, respectively. Plasma thyroxine, glucose, growth hormone, and insulin-like growth factor-1 concentrations were not affected by diet. Diet did not affect intestinal microbial N flow, suggesting that MPS in the rumen was not affected. Collectively, these results suggest that substituting CM for SBM increases production measures during mid-lactation, with milk yield responding more positively to long-term feeding of CM. Furthermore, the results for MPS suggest that this effect is mediated postruminally, potentially due to the increased methionine content of CM.

Introduction

Soybean meal (SBM) and canola meal (CM) are two protein rich byproducts that are commonly used in diets fed to dairy cows. Historically, SBM has been utilized to a greater extent

than CM, largely due to the lower CP content of CM (52.6 vs. 40.6-42.9; Adewole et al., 2016; Broderick et al., 2016; NASEM, 2021). However, when examining the AA profile of both protein sources, the higher methionine content of CM, compared to SBM (1.97 vs. 1.38%, DM basis; NASEM, 2021), makes it a valuable protein source in situations where methionine is limiting.

The effectiveness of substituting CM for other protein sources, such as SBM, in diets fed to dairy cows has been explored extensively. A meta-analysis (Huhtanen et al., 2011) observed positive production responses, including increased DMI, milk yield, and ECM yield, when CM replaced SBM in diets fed during middle and late lactation. In agreement with Huhtanen et al. (2011), subsequent experiments reinforced positive effects on production when substituting CM for SBM in diets fed during middle lactation (Broderick et al., 2015; Benchaar et al., 2021; Lage et al., 2021).

While a plethora of data exists examining the production and physiological effects of including SBM versus CM in diets fed to dairy cows, to our knowledge, the effect of feeding these two protein sources to cows determined to have high or low residual feed intake (RFI) is unexplored. Residual feed intake is a feed efficiency metric first described by Koch et al. (1963) and is expressed as the difference between observed and expected feed (energy) intake. As RFI measurements are cohort-based, positive and negative values correspond to cows that consumed more or less feed than expected (less or more feed efficient, respectively) relative to the average cow in that cohort. Since low RFI cows are more feed efficient than expected for a given production level, feeding a diet formulated with a protein source more conducive for milk production, such as CM, may be advantageous for milk production and nutrient utilization.

The objective of this experiment was to determine the effect of feeding isonitrogenous diets formulated with SBM or CM as the primary protein source during mid-lactation on production measures in Holstein dairy cows determined to have high or low RFI. Furthermore, we sought to quantify the effects on plasma hormones and metabolites related to production and urinary excretion measures. We hypothesized that cows determined to have low RFI (higher feed efficiency) may better utilize the diet formulated with CM, due to higher methionine content and therefore more conducive to milk protein, for milk production and measures related to physiology compared to cows determined to have high RFI (lower feed efficiency).

Materials and Methods

Residual Feed Intake Screening

The procedures used in this experiment were approved by the University of Wisconsin-Madison IACUC protocol A006120. Over a period of four consecutive weeks beginning at 35.4 ± 10.0 (mean \pm SD) DIM, 115 Holstein cows (40 primiparous and 75 multiparous) were screened using a common herd diet to assess RFI. To achieve this, the 3 energy losses of milk energy output (MEO), metabolic body weight (MBW), and body energy change (BEC) were determined using equations derived from NRC (2001) for each cow during the screening period (Potts et al., 2015). After determining the amount of energy (Mcal/d) partitioned towards each energy loss, average DMI was regressed against these 3 energy losses and parity to determine RFI using Proc Reg of SAS (version 9.4, SAS Institute, Inc.). Cows were housed in individual tie-stalls at the USDA Dairy Forage Research Center farm in Prairie du Sac, WI. The screening

diet was fed as a TMR once-daily for ad libitum intake with continuous access to water. Cows were milked 3× daily at approximately 0600, 1300, and 2100 in a double-8 herringbone parlor.

The model used to determine RFI, equations to calculate each energy loss, and the collection of each measurement used for each energy loss are detailed below.

$$\text{DMI}_{ijkl} = \mu + \text{MEO}_i + \text{MBW}_j + \text{BEC}_k + \text{Parity}_l + \varepsilon_{ijkl}$$

$$\text{MEO, Mcal/d} = (9.29 * \text{Average Milk Fat Yield (kg/d)}) + (5.63 * \text{Average Milk Protein Yield (kg/d)}) + (3.95 * \text{Average Milk Lactose Yield (kg/d)})$$

$$\text{MBW, Mcal/d} = 0.08 * (\text{Average BW} ^ 0.75)$$

$$\text{BEC, Mcal/d} = (2.88 + 1.036 * \text{Average BCS}) * \text{Daily BW Change (kg/d)}$$

Feed intake (as-fed) was recorded daily for each cow by a Rissler TMR Mixer (Fritsch Equipment Corporation, De Pere, WI) equipped with FeedWatch (Valley Agricultural Software, Tulare, CA). The feeder recorded individual weigh-back and drop weights by hand daily to correct weight discrepancies that were electronically recorded. Dry matter intake was calculated daily as the amount of diet fed to the cow minus the weigh-back and multiplied by the weekly diet DM corresponding to the day. Daily DMI data for each cow was inspected to confirm that values were realistic and not artificially inflated due to feed stealing by other cows. Daily DMI was averaged over the 4-week screening period to determine average DMI. Milk yield was determined daily for each cow and averaged for each week. Milk samples were collected each week at every milking over two consecutive days and weighted for milk composition. Milk fat, protein, and lactose yields were determined each week by multiplying the average weekly milk yield by the milk component percent. Average milk fat, protein, and lactose yields were determined by averaging each milk component yield over the 4-week screening period. Body

weight was measured each week on two consecutive days and averaged over the 4-week screening period to determine average BW for each cow. Body condition score was measured on a scale of one to five (Elanco Animal Health) at the beginning and end of the screening period by three trained observers and averaged over the two time points to determine average BCS for each cow. Daily BW change was determined using simple linear regression of the four average weekly BW measurements for each cow. Cows were automatically excluded from the feeding experiment if a clinical disorder occurred during the 4-week screening period. After determining RFI, cows were utilized in the feeding experiment if their RFI was ≥ 0.5 or ≤ -0.5 SD from expected intake. Values that were ≥ 0.5 or ≤ -0.5 SD from expected intake corresponded to cows that were determined to have high or low RFI, respectively, which corresponds to low or high feed efficiency, respectively, relative to the average cow in the cohort.

Cows, Facilities, and Diets

Seventy-two (24 primiparous and 48 multiparous) Holstein cows enrolled in a randomized complete block design beginning at 86.5 ± 6.7 (average \pm SD) DIM and were grouped into blocks of four cows by parity and DIM. Each block contained two cows determined to have high RFI and two cows determined to have low RFI. Diets were formulated using NRC (2001) software. One of the cows in each RFI pair was randomly assigned to one of two isonitrogenous diets formulated with either soybean meal (SBM) or canola meal (CM) as the primary protein source and remained on the same diet for 10 weeks. This resulted in four treatment groups, which were 1) high RFI cows fed the SBM diet (HiSBM), 2) high RFI cows fed the CM diet (HiCM), 3) low RFI cows fed the SBM diet (LoSBM), and 4) low RFI cows fed the CM diet (LoCM). Experimental diets were fed from October 2021 through April 2022. Cows were housed in individual tie-stalls at the USDA Dairy Forage Research Center farm in Prairie

du Sac, WI. Diets were fed as a TMR once-daily for ad libitum intake with continuous access to water. Cows were milked 3× daily at approximately 0600, 1300, and 2100 in a double-8 herringbone parlor.

Ingredient composition of the herd, SBM, and CM diets are presented in Table 5.1. In the SBM and CM diets, all ingredients were used at the same inclusion rate except for CM, SBM, and soybean hulls. The diets contained a forage:concentrate ratio of 60:40, with 75% of the forage from BMR corn silage and 25% from alfalfa silage.

Sampling and Analysis

Samples of all diets, silages, and high moisture corn were collected daily and stored at -20°C in one week increments until dried. Samples of dry feeds were collected once weekly. All feed samples were dried in triplicate at 105°C for 24h for dry matter determination and at 55°C for 48h for nutrient analysis. All weekly composites of diets and forages were ground to pass through a 1-mm screen (Wiley mill, Arthur H. Thomas Co., Philadelphia, PA) and composited in six week increments for nutrient analysis. All concentrate samples were ground to pass through a 1-mm screen (cross beater mill, Retsch GmbH and Co., Haan, Germany) and composited in eight week increments for nutrient analysis. All composited feed samples were analyzed by a commercial laboratory (Dairyland Laboratories, Inc., Arcadia, WI) using wet chemistry analysis. The herd, SBM, and CM diets were analyzed for CP (method 990.03; AOAC International, 1996), NDF using alpha amylase and sodium sulfite (aNDF; method 2002.04; AOAC International, 2005), aNDF with ash correction (aNDFom), ether extract (EE) using diethyl ether (method 920.39; AOAC International, 1996), ash (method 942.05; AOAC International, 1996), and the following minerals: Ca, P, Mg, K, and S using a microwave and inductively coupled plasma spectroscopy (method 942.05; AOAC International, 1996). The BMR corn silage, alfalfa

silage, high-moisture corn, SBM, soybean hulls, CM, and corn gluten meal were analyzed for CP, aNDF, aNDFom, ADF (method 973.18; AOAC International, 1996), NDICP and ADICP using fiber residuals from NDF and ADF determination (method 990.03; AOAC International, 1996), starch using a sodium acetate buffer method (Hall, 2009), EE, and ash. Non-fiber carbohydrates (NFC) was calculated as: $NFC = 100 - ((\%aNDFom - \%NDICP) + \%CP + \%EE + \%Ash)$, with NDICP expressed as % of DM. Nutrient analysis results for each composite were weighted relative to the corresponding number of days each diet and ingredient were fed, as a proportion of the overall days of cow enrollment, due to the unequal distribution of cows consuming each diet and ingredient during each week of the experiment. The nutrient compositions of the diets (CP, NDICP, ADICP, aNDF, aNDFom, ADF, NFC, starch, EE, ash, and NE_L) were calculated using the analyzed composition of the individual feed ingredients and their inclusion rate in the TMR.

The BMR corn silage, alfalfa silage, and SBM and CM diets were analyzed using the Penn State Particle Separator on both an as-fed and DM basis for each week of the experiment that they were utilized. A five-pan system was used, including 19, 8, 4, and 1.18 mm screens and a bottom pan. The results for each week's silages and diets were weighted relative to the corresponding number of days each silage and diet were fed, as a proportion of the overall days of cow enrollment, due to the unequal distribution of cows consuming each silage and diet during each week of the experiment.

Body weight measurements were collected for each cow twice weekly on two consecutive days for each week of the experiment and averaged for each week. Body weight change (kg/week) was determined via simple linear regression using all the weekly average BW for each cow over the entirety of the experiment. Body condition score was measured for each

cow on a scale of one to five (Elanco Animal Health) during weeks one, four, and eight of the experiment by three trained observers and averaged by cow for each time point. Body condition score change (units/week) was determined via simple linear regression using all the average BCS for each cow over the three time points. Feed intake and weigh-backs were recorded daily for each cow. Dry matter intake was calculated daily as the amount of diet fed to the cow minus the weigh-back and multiplied by the weekly diet DM corresponding to the day and averaged for each week of the experiment for each cow. Milk yield was recorded daily for each cow and averaged for each week of the experiment for each cow. Energy-corrected milk (ECM) was calculated (Tyrrell and Reid, 1965) daily using the formula: $ECM = (0.327 \times \text{Milk Yield (kg/d)}) + (12.95 \times \text{Milk Fat Yield (kg/d)}) + (7.20 \times \text{Milk Protein Yield (kg/d)})$ and averaged for each week of the experiment for each cow. Fat-corrected milk (4% FCM) was calculated (Gaines, 1928) daily using the formula: $4\% \text{ FCM} = (0.4 \times \text{Milk Yield (kg/d)}) + (15 \times \text{Milk Fat Yield (kg/d)})$ and averaged for each week of the experiment for each cow. Feed efficiency was calculated daily as ECM divided by DMI and averaged for each week of the experiment for each cow. Milk samples were collected from each cow at every milking over two consecutive days for each week of the experiment. Milk samples were collected in bottles with a bronopol tablet used as a preservative and analyzed within one week of collection by a commercial laboratory (AgSource Laboratories, Menomonie, WI) by mid-infrared analysis using a Foss MilkoScan FT6000 for fat, true protein, lactose, SNF, SCC, and MUN. Total solids were calculated as the sum of milk fat and SNF. Daily milk component percentages were weighted according to the milk yield at each milking for each of the consecutive sampling days and the two daily values were averaged by cow for each week of the experiment. Values for SCC were \log_{10} -transformed for normality before analysis. Milk component yields were calculated as the weekly average

value of each milk component multiplied by the weekly average value of milk yield for each cow during each week of the experiment.

Blood samples were collected three hours after TMR was fed twice weekly on Tuesdays and Fridays from each cow during week eight of the experiment. Samples were collected into 10 mL vacutainers from the coccygeal artery or vein using vessel puncture with a multi-sample needle (EXELINT International Co., reference # 26501). Blood collected from the coccygeal artery or vein were considered the same supply due to minimal extraction by the tail. Three different vacutainers (BD Vacutainer, Franklin Lakes, NJ) were used for every blood draw, and each contained a different anticoagulant. The anticoagulants included 18 mg of K₂-EDTA (reference # 366643), 158 USP units of sodium heparin (reference # 367874), and 100 mg of sodium fluoride with 20 mg of potassium oxalate (reference # 367001). Samples were placed in ice until they were centrifuged (20 minutes, 2500 RPM, 4°C). After centrifugation, plasma was aliquoted into 1.7 mL microcentrifuge tubes (VWR International, reference # C-3260-01) in triplicate and stored at -80°C). The two weekly plasma samples from each cow were thawed and composited on an equal volume basis for each type of anticoagulant to represent an average value for week eight.

Plasma samples were analyzed in duplicate for triiodothyronine (T3), thyroxine (T4), glucose, insulin, growth hormone (GH), and insulin-like growth factor (IGF-1). Plasma samples from tubes with K₂-EDTA anticoagulant were used for T3, T4, and insulin determination. Plasma samples from tubes with sodium heparin anticoagulant were used for GH and IGF-1 determination. Plasma samples from tubes with sodium fluoride and potassium oxalate anticoagulant were used for glucose determination. Triiodothyronine and thyroxine were analyzed using ELISA kits (Monobind, Inc., Lake Forest, CA; reference # 125-300 and 225-300,

respectively). The intra-assay CVs for T3 and T4 were 6.34 and 4.63%, respectively. The inter-assay CVs for T3 and T4 were 7.54 and 6.65%, respectively. Glucose was analyzed using glucose oxidase-peroxidase enzymatic colorimetry. The intra- and inter-assay CVs for glucose were 1.20 and 0.67%, respectively. Insulin was analyzed using an ELISA kit (Merckodia, Uppsala, Sweden; reference # 10-1201-01). The intra- and inter-assay CVs for insulin were 3.12 and 2.91%, respectively. Growth hormone and insulin-like growth factor-1 were analyzed using ELISA kits (Abnova, Taipei, Taiwan; reference # KA2279 and LifeSpan Biosciences, Inc., Seattle, WA; reference # LS-F32450). The intra-assay CVs for GH and IGF-1 were 4.58 and 9.94%, respectively. The inter-assay CVs for GH and IGF-1 were 6.67 and 10.89%, respectively.

Spot urine samples were collected from each cow during week eight of the experiment at six time points over four days, with each time point spaced four hours apart relative to a 24-hour cycle to account for diurnal variation. After collection, 7.5 mL of urine was pipetted into a 50 mL conical tube containing 30 mL of 0.144 N diluted H₂SO₄. From there, the acidified and diluted urine samples were stored at -20°C until they were composited. To composite, each individual sample for each cow was thawed and vortexed to ensure adequate distribution of deposited crystals throughout the sample and 5 mL was pipetted into a 50 mL conical tube to create a 30 mL volume composite sample for each cow. The composite sample was frozen at -20°C until it was thawed at room temperature the day of analysis. The composite sample for each cow was used to determine allantoin, uric acid, creatinine, urea, and total N %. Allantoin concentration was evaluated using a phenylhydrazine colorimetric method adapted to a 96-well plate reader (Vogels and van der Drift, 1970). The intra- and inter-assay CVs for allantoin were 5.16 and 5.00%, respectively. Uric acid concentration was evaluated using a uricase colorimetry method with a commercial reagent and standard solution adapted to a 96-well plate reader

(Stanbio Laboratory, Boerne, TX; reference # 1045-225). The intra- and inter-assay CVs for uric acid were 5.87 and 7.09%, respectively. Additionally, the sum of allantoin and uric acid were used to represent purine derivatives (PD). Creatinine and urea were evaluated using a picric acid method and a urease colorimetric method, respectively, adapted to flow-injection analysis (Lachat Quik-Chem 8500; Lachat Instruments). Total N % was evaluated using a N analyzer (Leco FP-2000 N Analyzer; Leco Instruments, Inc.). Urine volume was calculated using average BW during the collection week, the composite creatinine concentration, and an excretion rate of 29 mg creatinine/kg BW/d as determined by Valadares et al. (1999). Daily excretions of allantoin, uric acid, PD, and urea were calculated by multiplying urine volume and the concentration of each. Purine derivative absorption and intestinal microbial N flow were determined using equations from Chen and Gomes (1992). Excretion of urinary urea N (UUN) and total urinary N (TUN) were calculated using urine volume and urea concentration or total N %, respectively. Urinary urea N, as a % of TUN, was determined via dividing UUN by TUN and multiplying by 100.

Statistical Analysis

The RFI status of each cow during the experiment was analyzed by regressing DMI against MEO, MBW, BEC, and parity using Proc Reg of SAS (version 9.4, SAS Institute, Inc.). The DMI value for each cow was the average during the entire 10-week experiment and values for MEO, MBW, and BEC were calculated from averages of the components of each respective equation during the entire 10-week experiment. Comparing the screening period and the 10-week experimental period, the number and proportion of cows that stayed either high or low RFI, switched from high or low RFI to medium RFI, or switched from high to low or low to high RFI were assessed. While the thresholds for high or low RFI status remained at ≥ 0.5 or ≤ -0.5 SD

from expected intake for the 10-week experimental period, respectively, medium RFI status during the entire 10-week experimental period was assigned to cows that were > -0.5 and < 0.5 SD from expected intake.

Statistical analysis was performed on weekly averages of BW, DMI, milk yield, ECM yield, 4% FCM yield, feed efficiency, milk component percentages and yields, on average BCS every 4 weeks, and on BW change, BCS change, plasma hormone and metabolite concentrations, and urinary excretion measures during the experiment.

Weekly data were analyzed using the MIXED procedure of SAS with week as the repeated measure. Body weight and BCS at the start of the experiment were tested as covariates and retained in the model if significant ($P \leq 0.10$). The covariance structure with the lowest AIC was used. The statistical model is:

$$Y_{ijklm} = \mu + \text{Covariate} + \text{RFI}_i + \text{Diet}_j + (\text{RFI}_i \times \text{Diet}_j) + \text{Week}_k + (\text{RFI}_i \times \text{Week}_k) + (\text{Diet}_j \times \text{Week}_k) + \text{Parity}_l + (\text{RFI}_i \times \text{Parity}_l) + (\text{Diet}_j \times \text{Parity}_l) + (\text{Week}_k \times \text{Parity}_l) + (\text{RFI}_i \times \text{Diet}_j \times \text{Week}_k) + (\text{RFI}_i \times \text{Diet}_j \times \text{Parity}_l) + (\text{RFI}_i \times \text{Week}_k \times \text{Parity}_l) + (\text{Diet}_j \times \text{Week}_k \times \text{Parity}_l) + (\text{RFI}_i \times \text{Diet}_j \times \text{Week}_k \times \text{Parity}_l) + \text{Cow}_m(\text{RFI}_i \times \text{Diet}_j \times \text{Parity}_l) + \varepsilon_{ijklm},$$

where Y is the response variable; μ is the overall mean; covariate is the BW or BCS measure (if significant); RFI is the fixed effect RFI ($i = \text{high or low}$); diet is the fixed effect of diet ($j = \text{SBM or CM}$); RFI \times diet is the fixed effect 2-way interaction of RFI and diet; week is the fixed effect of week during the experiment ($k = 1 \text{ to } 10$); RFI \times week is the fixed effect 2-way interaction of RFI and week; diet \times week is the fixed effect 2-way interaction of diet and week; parity is the fixed effect of parity ($l = \text{primiparous or multiparous}$); RFI \times parity is the fixed effect 2-way interaction of RFI and parity; diet \times parity is the fixed effect 2-way interaction of diet and parity;

week \times parity is the fixed effect 2-way interaction of week and parity; RFI \times diet \times week is the fixed effect 3-way interaction of RFI, diet, and week; RFI \times diet \times parity is the fixed effect 3-way interaction of RFI, diet, and parity; RFI \times week \times parity is the fixed effect 3-way interaction of RFI, week, and parity; diet \times week \times parity is the fixed effect 3-way interaction of diet, week, and parity; RFI \times diet \times week \times parity is the fixed effect 4-way interaction of RFI, diet, week, and parity; cow(RFI \times diet \times parity) is the random effect of cow within RFI, diet, and parity; and ε is the residual error.

Data for BW change, BCS change, plasma hormone and metabolite concentrations, and urinary excretion measures were analyzed using the MIXED procedure of SAS. Body weight and BCS at the start of the experiment were tested as covariates and retained in the model if significant ($P \leq 0.10$). The statistical model is:

$$Y_{ijkl} = \mu + \text{Covariate} + \text{RFI}_i + \text{Diet}_j + (\text{RFI}_i \times \text{Diet}_j) + \text{Parity}_k + (\text{RFI}_i \times \text{Parity}_k) + (\text{Diet}_j \times \text{Parity}_k) + (\text{RFI}_i \times \text{Diet}_j \times \text{Parity}_k) + \text{Cow}_l(\text{RFI}_i \times \text{Diet}_j \times \text{Parity}_k) + \varepsilon_{ijkl},$$

where Y is the response variable; μ is the overall mean; covariate is the BW or BCS measure (if significant); RFI is the fixed effect RFI ($i = \text{high or low}$); diet is the fixed effect of diet ($j = \text{SBM or CM}$); RFI \times diet is the fixed effect 2-way interaction of RFI and diet; parity is the fixed effect of parity ($k = \text{primiparous or multiparous}$); RFI \times parity is the fixed effect 2-way interaction of RFI and parity; diet \times parity is the fixed effect 2-way interaction of diet and parity; RFI \times diet \times parity is the fixed effect 3-way interaction of RFI, diet, and parity; cow(RFI \times diet \times parity) is the random effect of cow within RFI, diet, and parity; and ε is the residual error. Significance was declared when $P \leq 0.05$ and tendency was declared when $0.05 < P \leq 0.10$. The main effect of parity and interactions including parity are reported in tables when $P \leq 0.10$. The Tukey-Kramer adjustment was performed for pairwise comparisons of LSM.

Results

Nutrient Composition of Diets and Penn State Particle Separator

The chemical composition of the diets and diet ingredients are presented in Tables 5.2 and 5.3. As expected, the CP content of SBM was higher (52.0 vs. 45.8%) and the aNDFom content of SBM was lower (9.77 vs. 22.6%) than CM. However, the high aNDFom content of soybean hulls (61.9%) compensated for this difference in aNDFom content in the SBM diet. The PSPS analysis is presented in Table 5.4. Particle size was similar between diets for the 19, 8, and 4 mm sieves. However, the CM diet was noticeably lower compared to the SBM diet for the 1.18 mm sieve (17.8 vs. 21.8% as-fed (AF) basis, respectively), whereas the CM diet was noticeably higher compared to the SBM diet for the bottom pan (13.2 vs. 11.0% AF, respectively).

Comparison of Residual Feed Intake Status between Periods

A comparison of RFI status during the screening period and the 10-week experimental period is presented in Table 5.5. The proportion of cows that were determined to have either high or low RFI during the screening period and stayed high or low RFI during the 10-week experimental period was 0.49. The proportion of cows that changed from either high or low RFI during the screening period to medium RFI during the 10-week experimental period was 0.33. The proportion of cows that switched from either high or low RFI during the screening period to low or high RFI during the 10-week experimental period was 0.18.

Body Weight and Body Condition Score

Body weight, BW change, BCS, and BCS change data are presented in Table 5.6. Body weight and BCS LSM for each treatment group by week of experiment are presented in Figures 5.1 and 5.2. Cows fed the CM diet increased BW compared to cows fed the SBM diet ($P = 0.03$).

However, diet did not affect BW change ($P > 0.10$). There was a tendency for an interaction between diet, RFI, and week for BCS ($P = 0.09$) and an interaction between diet and RFI for BCS change ($P < 0.01$).

Dry Matter Intake, Milk Yields, and Feed Efficiency

Dry matter intake, milk yield, ECM yield, 4% FCM yield, and feed efficiency data are presented in Table 5.6. Dry matter intake LSM for each treatment group by week of experiment are presented in Figure 5.3. Cows fed the CM diet increased DMI compared to cows fed the SBM diet ($P < 0.01$). Furthermore, low RFI cows decreased DMI compared to high RFI cows ($P < 0.01$). An interaction between diet and week was observed for milk yield ($P < 0.01$; Figure 5.4). Cows fed the CM diet tended to increase ECM yield compared to cows fed SBM diet ($P = 0.06$). Diet did not affect 4% FCM yield ($P > 0.10$). There was an interaction between diet and week for feed efficiency ($P = 0.03$; Figure 5.4). Additionally, low RFI cows tended to increase feed efficiency compared to high RFI cows ($P = 0.08$).

Milk Composition and Component Yields

Milk composition data are presented in Table 5.7. There was an interaction between diet and week for milk fat %, milk protein %, milk total solids %, milk protein yield, and milk lactose yield ($P \leq 0.03$; Figure 5.4). Low RFI cows tended to decrease milk protein yield compared to high RFI cows ($P = 0.08$). Cows fed the CM diet increased milk total solids yield compared to cows fed the SBM diet ($P = 0.02$). There was a tendency for an interaction between diet and week for MUN ($P = 0.10$; Figure 5.4). Diet did not affect $\log_{10}\text{SCC}$ ($P > 0.10$).

Plasma Hormones and Metabolites

Plasma hormone and metabolite data are presented in Table 5.8. Cows fed the CM diet decreased plasma T3 concentration ($P < 0.01$) and increased plasma insulin concentration ($P = 0.02$) compared to cows fed the SBM diet. Plasma T4, glucose, GH, and IGF-1 concentrations were not affected by RFI or diet ($P > 0.10$).

Urinary Excretion

Urinary excretion data are presented in Table 5.9. Cows fed the CM diet tended to decrease urine volume ($P = 0.07$) and uric acid excretion ($P = 0.09$) compared to cows fed the SBM diet. Low RFI cows decreased excretion of allantoin, uric acid, and PD and PD absorption ($P \leq 0.03$) and tended to decrease excretion of urea and urinary urea-N ($P \leq 0.09$) compared to high RFI cows. Furthermore, low RFI cows decreased intestinal microbial N flow compared to high RFI cows ($P = 0.03$). There was an interaction between RFI and diet for total urinary N ($P = 0.04$) and tendency for an interaction between RFI and diet for UUN, % of TUN ($P = 0.09$).

Discussion

To our knowledge, this is the first experiment analyzing the production and physiological effects of feeding diets formulated with SBM or CM as the primary protein source to cows determined to have high or low RFI. As this experiment utilized a 2×2 factorial arrangement, we were able to investigate whether cows determined to have low RFI (more feed efficient) performed better than cows determined to have high RFI (less feed efficient) when fed a diet with a nutrient profile more conducive to milk production (increased methionine content of CM compared to SBM).

Residual Feed Intake Repeatability across Diets and Lactation Stages

Some of the biggest concerns when conducting research with dairy cows determined to have high or low RFI is the repeatability of RFI status across a diet change and lactation stages. Cows were fed a herd diet consisting of BMR corn silage, alfalfa silage, high-moisture corn, CM, roasted soybeans, and mineral/vitamin mix during the screening period in early lactation. Many of these ingredients were utilized in the SBM and CM diets as well, albeit in different proportions, with the largest discrepancies being the corn silage to alfalfa silage ratios and the addition of SBM, soybean hulls, corn gluten meal and energy booster and deletion of roasted soybeans in the experimental diets. When comparing the chemical composition of the screening diet to the experimental diets, CP, EE, and ash were higher, NFC was lower, and aNDFom was similar. The mineral composition of the diets was similar as well, with the exception of higher K in the screening diet.

In the present experiment, thresholds of ± 0.5 SD were used to determine low and high RFI cows. Comparison of RFI status from when cows consumed the screening diet to the experimental diet suggest that 35 of 72 cows remained either low or high RFI using ± 0.5 SD as a threshold. When using a threshold of zero SD, 50 of 72 cows remained either low or high RFI. However, it is important to note that a limitation of calculating and comparing RFI is that it is a cohort-based measurement. Therefore, directly comparing RFI values for a subset of cows to their RFI values when a part of a larger cohort is likely not appropriate. However, previous experiments have determined the change, or lack thereof, of RFI status for the same cohort of cows across a diet switch. Liu and VandeHaar (2020) determined that RFI ranking was moderately repeatable ($r = 0.59$) when switching between a low (14%) and high (18%) CP diet around peak lactation. This discrepancy in diet CP was much higher than the present experiment

(17.8 vs. 16.4 vs. 16.7%; Herd vs. SBM vs. CM TMR, respectively). Furthermore, Potts et al. (2015) observed a relatively high ($r = 0.73$) correlation between RFI when cows were fed a high starch (30% starch, 26% NDF) and a low starch (14% starch, 40% NDF) diet during mid-lactation, indicating reliable repeatability of RFI status over wider dietary nutrient alterations. Importantly, only 4 of 109 cows in their experiment switched from low to high RFI (or vice versa), after a dietary change when using ± 0.5 SD as a threshold. Additionally, while Fischer et al. (2021) noted that RFI was less reproducible when changing starch and NDF concentrations in the diet (22.6% and 35.1% vs. 18.3% and 42.0%) during early to mid-lactation compared to within each diet over different lactation stages, all three comparisons yielded moderate to highly repeatable correlations ($r = 0.64$ vs. 0.72 vs. 0.85). Carrasquillo-Mangual et al. (2016) determined that switching between diets with 19% starch and 36% NDF or 32% starch and 26% NDF during mid-lactation yielded only moderate repeatability ($r = 0.44$) of RFI ranking. However, similar to Potts et al. (2015), Carrasquillo-Mangual et al. (2016) observed that only 5% of the cows changed from low to high RFI or vice versa when using ± 0.5 SD as a threshold.

According to Connor et al. (2019), the highest correlation coefficient ($r = 0.83$ to 0.86) between RFI measured during a 28-day window within lactation and from 10 to 305 DIM (i.e. full lactation data) occurred between 132 and 232 DIM. Moreover, using 182 DIM as a midpoint, Connor et al. (2019) determined that, as expected, the correlation coefficient between RFI data collected during a portion of lactation and a full lactation increased as the test duration increased, with greatly diminished returns ($r \geq 0.90$) occurring after 64 days of data collection. While a period of greater than 2 months during mid-late lactation appears to be the gold standard for determining RFI in dairy cows, the potential for sufficiently determining RFI earlier in lactation remains. Data from Mäntysaari et al. (2012) and Hurley et al. (2018) suggest that this may be

difficult to achieve, with weak correlations ($r = 0.12$ to 0.47) observed between RFI estimates collected for a minimum of 63 days during early lactation and later lactation stages. However, Connor et al. (2013; 2019) demonstrated higher correlations ($r = 0.65$ to 0.67) between RFI estimates collected for a period as short as four weeks beginning during the first month of early lactation and either the entirety of early lactation (i.e. through 98 DIM) or a full 305-day lactation, suggesting that this technique may be feasible.

Body Weight and Body Condition Score

In the present experiment, cows fed the CM diet increased BW compared to cows fed the SBM diet, suggesting better energy balance for cows fed the CM diet. However, no effect of diet on BW change was observed. The statistical analysis for BW included initial BW as a covariate, strengthening the argument that cows fed the CM diet experienced better energy balance during the experiment compared to cows fed the SBM diet. Interestingly, initial BW was not significant for BW change, suggesting that BW change was independent of initial BW. In agreement, Paula et al. (2020) observed increased BW change for cows fed diets with CM compared to SBM during mid-lactation. Furthermore, Benchaar et al. (2021) noted a tendency for a linear increase for BW change as inclusion of CM in the diet increased from 0% to 24% at the expense of SBM during mid-lactation. However, contrary to the present experiment, no difference is typically reported for BW (Christen et al., 2010; Gidlund et al., 2015; Lage et al., 2021) or BW change (Brito and Broderick, 2007; Broderick et al., 2015; Paula et al., 2018) between cows fed diets with CM or SBM during mid-lactation.

While BW was affected by diet, BCS and BCS change were affected by more complex factors in the present experiment. The tendency for a 3-way interaction between diet, RFI, and week for BCS resulted from high RFI cows on the CM diet and low RFI cows on the SBM diet

increasing BCS from week one to eight of the experiment to a greater extent than low RFI cows on the SBM diet and high RFI cows on the CM diet. This 3-way interaction for BCS was reflected in the 2-way interaction between diet and RFI for BCS change as well, with high RFI cows on the CM diet and low RFI cows on the SBM diet having greater BCS change compared to high RFI cows on the SBM diet and low RFI cows on the CM diet.

Dry Matter Intake

In the present experiment, the interaction between RFI status and diet was not significant for DMI. However, the main effect of diet was significant, with cows fed the CM diet increasing DMI compared to cows fed the SBM diet. Collectively, these results suggest that cows the CM diet increased DMI independently of RFI status. While the interaction between RFI status and diet was not significant in the present experiment, the main effects of RFI status and diet were both significant, with low RFI cows decreasing DMI and cows fed The effect of substituting CM for other protein sources, such as SBM, in diets fed to mid-lactation dairy cows on DMI has been widely examined. Two meta-analyses (Huhtanen et al., 2011; Martineau et al., 2013) evaluated data from 122 and 27 experiments, respectively, during middle and late lactation to determine the incremental effect of including CM in diets. Huhtanen et al. (2011) determined that CM increased DMI by 0.026 kg for every 1 g dietary CP/kg DM increase (i.e. 0.1% CP increase), which was higher than the 0.011 kg increase for SBM. Furthermore, Martineau et al. (2013) observed a positive response for DMI in 35 of 49 comparisons of CM to a different protein source (SBM, cottonseed meal (CSM), etc.) that averaged out to a marginal increase of 0.24 kg DMI/cow/d per 10% inclusion of CM in the diet. However, a comparison of CM to only SBM was not reported. In the present experiment, DMI increased by 1.5 kg/cow/d when CM was included at 15.5% of diet DM, higher than the 0.37 kg/cow/d increase suggested by Martineau et

al. (2013) but similar to the 1.5 kg/cow/d increase suggested by Huhtanen et al. (2011) based on the present inclusion rates of CM and SBM and differences of their respective slopes for total DMI. Additionally, more recent experiments generally support the notion of increased DMI for cows fed diets with CM compared to SBM during mid-lactation (Broderick et al., 2015; Gidlund et al., 2015; Benchaar et al., 2021; Lage et al., 2021). However, this is not always the case (Paula et al., 2018; Paula et al., 2020). The increased DMI typically observed for cows fed diets with CM compared to SBM or other protein sources during middle and late lactation may be due to a “pull effect,” whereby the more balanced supply of amino acids from CM (i.e. increased methionine content) for milk production leads to increased energetic demand and a compensatory increase in DMI (Huhtanen et al., 2011).

Milk Yields

Similar to DMI, the interaction between RFI status and diet was not significant for milk, ECM, or 4% FCM yields. Interestingly, a diet \times week interaction was observed for milk yield in the present experiment, with the difference between cows fed the CM or SBM diet increasing as the experiment progressed. The smallest and largest increases for cows fed the CM diet occurred during weeks two and 10, respectively (Figure 5.4). Collectively, this suggests that lactation persistency was higher for cows fed the CM diet compared to the SBM diet, given that cows enrolled around peak milk production and remained on the same diet for 10 weeks during mid-lactation. The effect of substituting CM for other protein sources, such as SBM, in diets fed to dairy cows on milk yield was also analyzed by Huhtanen et al. (2011) and Martineau et al. (2013). Huhtanen et al. (2011) determined a marginal increase for milk yield of 3.4 kg/d per kg increase of CP intake/d for CM, higher than the 2.4 kg/d increase for SBM. Furthermore, Martineau et al. (2013) observed a 0.62 kg/d marginal increase for milk yield per 10% inclusion

of CM in the diet. However, as was the case for DMI, Martineau et al. (2013) compared CM to all other protein sources and did not report CM compared to only SBM. Similar to the results for DMI, more recent experiments generally support the notion of increased milk yield for cows fed diets with CM compared to SBM during mid-lactation (Paula et al., 2020; Benchaar et al., 2021; Lage et al., 2021). However, this is not always the case (Paula et al., 2018).

While ECM yield tended to increase for cows fed the CM diet compared to the SBM diet in the present experiment, 4% FCM yield was not affected by diet. Similarly, the marginal increase for ECM yield was higher per unit increase of CP intake for CM (3.71 kg/kg) compared to SBM (2.18 kg/kg) in Huhtanen et al. (2011). In contrast, Martineau et al. (2013) reported no difference for ECM yield when comparing cows fed diets with CM or SBM. However, Martineau et al. (2013) also observed no difference for 4% FCM, which is in agreement with the present experiment. In recent experiments, the effect of CM versus SBM in diets fed to dairy cows during mid-lactation is mixed, with increased ECM yield reported for cows fed diets with CM compared to SBM in some (Broderick et al., 2015; Benchaar et al., 2021) but not all cases (Paula et al., 2018; Paula et al., 2020; Lage et al., 2021). Moreover, the results for FCM yield are mixed, with Benchaar et al. (2021) noting a linear increase for 4% FCM yield as CM inclusion in the diet increased from 0 to 24% at the expense of CM and Paula et al. (2018) finding no difference for 3.5% FCM yield between cows fed diets with CM or SBM.

Feed Efficiency

A less comprehensive, albeit still valuable, measure of feed conversion compared to RFI, feed efficiency for lactating dairy cows is frequently expressed as ECM/DMI. In the present experiment, a diet \times week interaction was observed. While ECM/DMI feed efficiency tended to decrease for cows fed the CM diet compared to the SBM diet in week two, it was not

significantly different between diets for the rest of the experiment (Figure 5.4). When comparing ECM/DMI feed efficiency between cows fed diets with CM compared to all other protein sources, Martineau et al. (2013) observed a marginal increase of +0.02 kg ECM/kg DMI per 10% inclusion of CM in the diet. However, when comparing CM to only SBM, there was no difference. Subsequent experiments (Broderick et al., 2015; Paula et al., 2018; Lage et al., 2021) further reinforced no difference for ECM/DMI feed efficiency between cows fed diets with CM compared to SBM during mid-lactation, suggesting that dietary nutrients may be utilized to a similar degree of efficiency for ECM production between diets. While energy partitioned towards milk production is only one portion of RFI, albeit the largest in lactating dairy cows, a tendency for increased ECM/DMI feed efficiency for the low RFI compared to the high RFI cows in the present experiment is not unexpected. Of the two measures, this tendency was due to decreased DMI, as ECM was not affected by RFI status, in agreement with results reported by Xie et al. (2019).

Milk Composition

As was the case for milk yields, while the interaction between RFI status and diet was not significant for any milk composition measure in the present experiment, a diet \times week interaction was observed for milk fat, protein, and total solids percent. For milk fat and total solids percent, a similar response was observed whereby milk fat percent decreased and milk total solids percent either decreased or tended to decrease for cows fed the CM diet compared to the SBM diet from week five through week 10. Additionally, milk fat and total solids percent decreased and tended to decrease for cows fed the CM diet compared to cows fed the SBM diet during week two (Figure 5.4). With regards to milk fat percent, this is potentially due to a “dilution effect” as reported in previous experiments (Couvreur et al., 2007; Bondan et al., 2019). This explanation

is seemingly more likely as the difference for milk fat percent between cows fed the CM diet and SBM diet was the lowest during week one and the highest during week 10, similar to the results for milk yield (Figure 5.4). The temporal results for milk total solids percent followed essentially the same trajectory as milk fat percent, due to fat being an inherently significant portion of total solids (Figure 5.4). While a diet \times week interaction was also observed for milk protein percent, there was no difference between diets for any week of the experiment (Figure 5.4). In contrast to the present experiment, Huhtanen et al. (2011) and Martineau et al. (2013) found no difference for milk fat or protein percent when substituting CM for SBM in diets fed to dairy cows during middle and late lactation. Given the magnitude of the milk production response corresponding to the weeks where milk fat percent was consistently decreased in the present experiment, the lack of response for milk fat percent in Huhtanen et al. (2011) and Martineau et al. (2013) may be due to the lack of a significant “dilution effect.” However, similar to the present experiment, Martineau et al. (2013) observed no difference for milk lactose percent between cows fed diets with CM compared to SBM. Furthermore, recent experiments have observed mixed results when substituting CM for SBM in diets fed to mid-lactation dairy cows. No difference for milk fat, protein, or lactose percent is typically observed when comparing diets with CM or SBM during mid-lactation (Broderick et al., 2015; Paula et al., 2020; Lage et al., 2021). However, a linear decrease was reported for milk fat and lactose percent as CM inclusion in the diet increased from 0 to 24% at the expense of SBM in Benchaar et al. (2021), with the milk fat percent response likely due to a “dilution effect” from a linear increase for milk yield. In contrast to Benchaar et al. (2021), milk lactose percent increased for cows fed diets with CM compared to SBM in Paula et al. (2018).

Milk Component Yields

Similar to milk composition, the interaction between RFI status and diet was not significant for any measure related to milk component yields. Furthermore, diet did not affect milk fat yield in the present experiment. This is likely due to the opposite trends for the diet \times week interactions for milk yield and milk fat percent, whereby as the difference for milk yield between cows fed the CM or SBM diets increased as the experiment progressed, so did the difference for milk fat percent in the opposite direction. Additionally, no effect of diet for 4% FCM can likely be attributed to the trends for these two interactions as well. The diet \times week interaction observed for milk protein and lactose yields largely mimics the diet \times week interaction for milk yield. As the experiment progressed, the milk protein and lactose yields persisted better for cows fed the CM diet compared to the SBM diet. While there was no diet effect for milk fat yield and a diet \times week interaction for milk protein and lactose yields, there was a diet effect for milk total solids yield, with cows fed the CM diet increasing milk total solids yield compared to cows fed the SBM diet. As milk total solids accounts for milk fat, protein, and lactose, this is likely due to a culmination of all the effects, or lack thereof, of the individual milk component yields “smoothing out” to instead achieve a main effect. In agreement with the present experiment, Martineau et al. (2013) observed no difference for milk fat yield between cows fed diets with CM compared to SBM. Furthermore, Martineau et al. (2013) reported increased milk protein yield for cows fed diets with CM compared to SBM and no effect for milk lactose yield. The results of more recent experiments are generally mixed for milk component yields. For milk fat yield, cows fed diets with CM sometimes increase milk fat yield compared to cows fed diets with SBM (Broderick et al., 2015). However, this response is typically not observed (Gidlund et al., 2015; Benchaar et al., 2021; Lage et al., 2021). Contrary

to milk fat yield, more recent experiments typically report increased milk protein yield for cows fed diets with CM compared to SBM (Broderick et al., 2015; Gidlund et al., 2015; Benchaar et al., 2021) but not always (Paula et al., 2018; Lage et al., 2021). Similar to milk fat yield, a positive response for milk lactose yield for cows fed diets with CM compared to SBM is sometimes reported (Paula et al., 2020; Benchaar et al., 2021). However, no difference is typically reported (Broderick et al., 2015; Gidlund et al., 2015; Lage et al., 2021).

Milk Urea Nitrogen and Log₁₀Somatic Cell Count

A toxic byproduct of proteolysis in the rumen, ammonia must be converted to urea in the liver before it gets recycled or excreted from the cow (Getahun et al., 2019). After passing from the rumen to the bloodstream, urea is rapidly equilibrated amongst other pools of liquid in the body such as milk (Broderick and Clayton, 1997). Consequently, MUN is a useful tool to evaluate nitrogen balance due to the high correlation that exists between MUN and dietary nitrogen (Nousianien et al., 2004). Lower concentrations of MUN suggest more efficient utilization of dietary nitrogen, which may be achieved via incorporation of protein sources that better match the AA requirements of the lactating dairy cow, such as CM. A tendency for a diet × week interaction was observed for MUN, with decreased MUN for cows fed the CM diet compared to the SBM diet occurring only in weeks one and two of the experiment, suggesting that cows fed the SBM diet adapted to the AA profile of the SBM for milk protein production relatively quickly and more efficiently than is typically observed. In recent experiments, MUN is commonly decreased for cows fed diets with CM compared to SBM during mid-lactation (Paula et al., 2018; Benchaar et al., 2021; Lage et al., 2021).

Somatic cell count measured in milk can be used as a proxy for mammary gland health, with decreased concentrations suggesting better mammary gland health. Due to its right-skewed

distribution, SCC is sometimes log-transformed before statistical analysis so that the data follows a normal distribution. In the present experiment, diet did not affect \log_{10} SCC values. This observation agrees with the results of previous experiments that also found no difference for SCC values between cows fed diets with CM compared to SBM (Christen et al., 2010; Broderick et al., 2015; Benchaar et al., 2021). However, it is important to note that SCC values analyzed in previous experiments were raw values and not \log_{10} -transformed prior to analysis.

Previous Experimental Designs and Diet \times Week Interaction

Compared to most previous experiments that analyzed the effect of including CM versus SBM in diets fed to dairy cows during mid-lactation, the present experiment has a couple of distinct advantages. Perhaps the most obvious distinction is the overarching experimental design itself. To our knowledge, this is the second experiment comparing the effects of including CM versus SBM in diets fed to mid-lactation dairy cows that did not utilize some form of a changeover. The majority of previous experiments utilized a Latin square or rectangle design with an adaptation period either 14 or 18 days long and a data collection period between three and 14 days long that occurred sometime during week three through five after starting a new diet. This distinct difference allowed the present experiment to evaluate the temporal effect of including CM versus SBM in diets fed to dairy cows for a period of 10 weeks from around peak lactation until approximately halfway through mid-lactation. Furthermore, this made it possible for the present experiment to test for the presence of a diet \times week interaction for all measures that were collected over multiple time points, unlike the majority of previous experiments. To our knowledge, the first experiment that did not utilize a changeover design when evaluating the effect of including SBM versus CM in diets fed to mid-lactation dairy cows was Paula et al. (2018). After feeding diets with CM or SBM for 12 weeks, Paula et al. (2018) reported that the

diet \times week interaction was not significant for any production measure. Despite utilizing a Latin square design with 10 days of data collection per period, Lage et al. (2021) incorporated the interaction of treatment \times day in the statistical model for BW, DMI, milk yield, and feed efficiency (MY/DMI). However, the interaction was not significant for any production measure, potentially due to the shorter period for data collection.

Another advantage of the present experiment is that while cows enrolled at a similar average DIM (86.5) compared to the other experiments mentioned previously, the SD (± 6.7) for average DIM at the start of the experiment was considerably lower than is typically observed, strengthening the argument that the diet \times week interactions of the present experiment may correspond to specific weeks (i.e. \sim week 13 to 22) of lactation. Of the experiments encompassed by the Martineau et al. (2013) meta-analysis and thereafter that evaluated CM versus SBM included in diets fed to dairy cows during mid-lactation, SD for DIM at the beginning of the experiment ranged from 19 (Brito and Broderick, 2007) to 40 (Oba et al., 2010). In Paula et al. (2018), the higher SD for DIM (± 23) for cows at the beginning of the experiment may have made it more difficult to observe a diet \times week interaction for production measures if the interactions did correspond to specific weeks of lactation.

Plasma Hormones and Metabolites

Plasma samples collected during week eight of the experiment were analyzed for hormones and metabolites related to production in hopes of explaining production differences. Similar to most production measures, there was no interaction between RFI status and diet for any of the analyzed plasma hormones and metabolites.

The thyroid hormones T3 and T4 are critical for overall metabolism and numerous pathways related to energy expenditure in dairy cows, including those linked to carbohydrate, lipid, and protein metabolism (Kaneko, 2008). Rapeseed meal (RSM), the genetic precursor to CM, was utilized to a lesser extent in diets fed to dairy cows compared to other protein sources due to its high concentrations of glucosinolates (Bell, 1984; Canola Council of Canada, 2019), anti-nutritional factors that sometimes exhibited an adverse effect on DMI and milk yield due to its goitrogenic nature (Waldern, 1973). Goitrogenic compounds reduce the availability of iodine in dairy cows, subsequently diminishing the synthesis of T4 (Gross and Pitt-Rivers, 1952; Premachandra et al., 1961), a hormone critical for milk production (Grenet and Journey, 1971). In the present experiment, plasma T3 concentrations decreased for cows fed the diet with CM compared to SBM, which corresponded to increased milk yield. This observation is consistent with previous data that demonstrated a negative correlation between plasma T3 and milk production (Blum et al., 1983; Tiirats, 1997; Steinhoff et al., 2019) and may be due to overall energy metabolism and decreased utilization of nutrients in tissues peripheral to the mammary gland (Shetty 1990; Vernon and Pond, 1997; Vernon et al., 2002). A similar phenomenon was observed in Kuehnl and Kalscheur (2021), whereby temporal plasma T3 concentrations were consistently decreased, as milk yield was concurrently increased, during early lactation for cows fed diets with CM compared to SBM. However, Kuehnl et al. (2021) found no effect of diet on temporal plasma T3 concentrations during early lactation between cows fed low or high protein diets with SBM or CM. Furthermore, similar to the present experiment, Kuehnl and Kalscheur (2021) reported no effect of postpartum diet on plasma T4 concentrations. However, Kuehnl et al. (2021) observed an interaction between protein source and week of lactation for plasma T4 concentrations.

Comprising one glucose and one galactose molecule, lactose is a major osmoregulator of milk production (Bleck et al., 2009). While the lactating mammary gland does not depend on insulin for glucose uptake, tissues that are peripheral to the mammary gland, such as adipose and skeletal muscle, are dependent (Zhao, 2014). In the present experiment, plasma glucose and insulin concentrations were not affected by RFI status, which is in agreement with previous experiments (Xi et al., 2016; Xie et al., 2019; Martin et al., 2021). Although plasma glucose concentrations were not affected by diet, similar to previous results (Maxin et al., 2013a; Kuehnl and Kalscheur, 2021; Kuehnl et al., 2021), plasma insulin concentrations increased for cows fed the CM diet compared to the SBM diet in the present experiment. The increased plasma insulin concentrations corresponded to increased milk protein yield for cows fed the CM diet compared to the SBM diet during week eight of the experiment. While insulin has long been recognized to influence milk protein synthesis in dairy cows (Griinari et al., 1997; Mackle et al., 1999), its full array of metabolic influence is likely not yet elucidated. Menzies et al. (2008) demonstrated the vastly multifaceted influence of insulin on protein synthesis, finding that insulin stimulated the expression of 28 genes directly involved with protein synthesis.

While GH is widely accepted as the galactopoietic hormone in ruminants (Tucker, 2000), its lack of binding with GH receptors on the bovine mammary gland (Keys and Djiane, 1988) suggests that its galactopoietic effect is fulfilled outside of the mammary gland. Increased partitioning of nutrients toward the mammary gland for milk synthesis is one avenue that helps to achieve this (Peel and Bauman, 1987; Bauman et al., 1989). Galactopoiesis may also be achieved, in part, via binding of GH to receptors on hepatocytes, stimulating secretion of IGF-1, a potent mitogen. While mammary epithelial cells are known to have IGF-1 receptors (Gluckman et al., 1987), the mediation of GH's galactopoietic effects via IGF-1 is inconclusive (Shamay et

al., 1988; Prosser et al., 1990). Given the substantial milk production increase for cows fed the CM diet compared to the SBM diet during week eight of the experiment, increased GH and/or IGF-1 concentration for the cows fed the CM diet may be expected. Contrary to results from Osorio et al. (2013), whereby milk yield and plasma GH concentrations were concurrently increased during early lactation for cows fed diets supplemented with rumen-protected methionine (RPM) compared to cows fed the control diet, plasma GH concentration was not affected by diet in the present experiment. However, similar to the present experiment, plasma IGF-1 concentrations were not affected by diet in Osorio et al. (2013). Furthermore, Kuehnl and Kalscheur (2021) reported no difference for plasma IGF-1 concentrations between cows fed diets with SBM or CM during early lactation. While a diet \times week interaction was present for feed efficiency, there was no difference between diets during week eight of the experiment. This suggests a similar degree of nutrient partitioning to the mammary gland for milk production and supports no effect of diet on plasma GH concentration. Interestingly, however, Osorio et al. (2013) reported no difference for feed efficiency (ECM/DMI) despite increased plasma GH concentrations for cows fed diets supplemented with RPM. In contrast to the present experiment, RFI status did not affect plasma GH concentration in Xi et al. (2016). However, plasma IGF-1 concentration was also not affected by RFI status in Xi et al. (2016), similar to the present experiment.

Urinary Excretion

Intestinal microbial N flow, which is calculated using the daily urinary excretion rate of PD (allantoin and uric acid), is commonly used as a proxy for quantifying microbial protein synthesis (MPS) in the rumen. Given its similarities with the AA profile of milk (Sok et al., 2017; NASEM, 2021), maximizing MPS is considered to be desirable. Historically, CM has been

considered a poorer source of RUP compared to SBM, with values of 35.7% and 42.6% reported for each, respectively (NRC, 2001). However, subsequent experiments (Maxin et al., 2013b; Broderick et al., 2016) suggested that the RUP value of CM increased and even surpassed that of SBM. Despite these results, NASEM (2021) reported RUP values of 32 and 33% for CM and SBM, respectively. The RUP value of CM and SBM is a potentially important factor for MPS as it dictates how much protein is available as substrate. In agreement with the present experiment, no difference for intestinal microbial N or CP flow is typically observed when cows are fed diets with CM or SBM during mid-lactation (Maxin et al., 2013a; Paula et al., 2018; Lage et al., 2021). However, a tendency for decreased microbial N flow for cows fed the diet with CM compared to SBM during mid-lactation was reported in Brito and Broderick (2007). While urea and UUN excretion were not affected by diet, there was an interaction between RFI and diet for TUN due to decreased TUN excretion for low RFI cows fed the SBM diet. Furthermore, low RFI cows decreased intestinal microbial N flow and excretion of urea and UUN compared to high RFI cows, which may be due to decreased DMI for low RFI cows. This result is in contrast to Xie et al. (2019), which reported no difference for MPS between high and low RFI cows. Similar to the present experiment, no difference for UUN between cows fed diets with CM or SBM during mid-lactation has been observed (Brito and Broderick, 2007; Maxin et al., 2013a, Paula et al., 2018). However, decreased UUN is also frequently noted for cows fed diets with CM compared to SBM during mid-lactation (Broderick et al., 2015; Paula et al., 2020; Lage et al., 2021). Interestingly, a concurrent decrease for urine volume is also commonly (Broderick et al., 2015; Lage et al., 2021) but not always (Paula et al., 2020) observed when UUN is decreased. Lage et al. (2021) hypothesized that this is due to lower osmotic pressure related to decreased UUN excretion, given that urine osmolality remains constant (Bannink et al., 1999).

Interestingly, despite no effect of diet on UUN, cows fed the CM diet tended to decrease urine volume compared to cows fed the SBM diet.

Conclusions

In conclusion, replacement of SBM with CM in diets fed to dairy cows during mid-lactation improves production measures and alters various aspects related to physiology, with most differences independent of RFI status. Cows fed the CM diet increased BW and DMI compared to cows fed the SBM diet and a diet \times week interaction was present whereby cows fed the CM diet persisted better than cows fed the SBM diet as the experiment progressed. A diet \times week interaction was also observed for milk fat, protein, and total solids percent, and milk protein and lactose yields. The interactions for component percentages acted in opposition to the interaction for milk yield, whereas the interactions for component yields behaved similarly as the interaction for milk yield. Plasma T3 and insulin concentrations decreased and increased, respectively, for cows fed the CM diet compared to the SBM diet, corresponding to increased milk yield or milk protein yield. Diet did not affect intestinal microbial N flow, suggesting that it did not affect MPS in the rumen. Collectively, these results suggest that substitution of SBM with CM increases production measures during mid-lactation, with milk yield responding more positively to CM as the experiment progressed. Furthermore, the results for MPS suggest that the production effects are mediated postruminally, potentially due to the increased methionine content of CM. More long-term experiments are needed to support the results of the present experiment.

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Tables and Figures

Table 5.1. Ingredient composition of the herd, SBM, and CM diets.

Ingredient (% DM)	Herd ¹	SBM ¹	CM ¹
BMR Corn Silage	30.7	45.0	45.0
Alfalfa Silage ²	27.4	15.0	15.0
Soybean Meal	-	11.7	-
Soybean Hulls	-	3.80	-
Canola Meal ²	11.0	-	15.5
Corn Gluten Meal	-	2.00	2.00
Roasted Soybeans	7.64	-	-
High-Moisture Corn ²	20.8	19.0	19.0
Rumen inert fat ³	-	1.00	1.00
Mineral/Vitamin Mix ⁴	2.49	2.50	2.50

¹Herd diet fed during the 4-week screening period in early lactation; SBM = soybean meal; CM = canola meal.

²Different sources of alfalfa silage, canola meal, and high-moisture corn used for herd diet versus SBM and CM diets.

³Energy Booster 100 (Milk Specialties, Dundee, IL).

⁴Lactating cow mineral contained (on a DM basis): 16.7% Ca, 10.5% salt, 14.4% Na, 5.6% Mg, 0.3% K, 0.6 ppm Se, 274,500 IU vitamin A/kg, 54,900 IU vitamin D3/kg, 1,250 IU vitamin E/kg, and 0.46 g Monensin/kg (Vita Plus Corporation, Madison, WI).

Table 5.2. Chemical composition of diets.

Item (% DM, unless noted)	Diet		
	Herd	SBM ¹	CM ¹
DM, %AF	45.1	44.1	44.2
CP ²	17.8	16.4	16.7
NDICP, ² %CP	8.36	7.74	7.91
ADICP, ² %CP	4.53	4.40	4.47
aNDF ²	28.5	28.0	28.4
aNDFom ²	27.0	26.9	26.9
ADF ²	20.5	19.2	19.5
NFC ^{2,3}	42.8	45.6	45.2
Starch ²	25.3	29.4	29.4
EE ²	5.46	4.77	4.98
Ash ²	5.55	4.67	4.69
Ca	1.00	1.05	1.03
P	0.42	0.34	0.41
Mg	0.42	0.38	0.46
K	1.51	1.37	1.14
S	0.31	0.24	0.32
RDP ⁴	11.7	9.67	10.3
RUP ⁴	6.14	6.73	6.43
Dig Lys, ^{4,5} g/d	169	175	172
Dig Met, ^{4,5} g/d	50	53	56
RDP ⁶	12.6	11.0	11.2
RUP ⁶	5.20	5.44	5.46
Metab Lys, ^{6,7} g/d	179	175	178
Metab Met, ^{6,7} g/d	54	55	60
NE _L , ^{2,8} Mcal/kg DM	1.68	1.67	1.65
NE _L , ⁹ Mcal/kg DM	1.62	1.60	1.58
NE _L , ¹⁰ Mcal/kg DM	1.79	1.84	1.80

¹SBM = soybean meal, CM = canola meal.

²Calculated using nutrient analysis of individual feed ingredients.

³NFC = 100 - ((%aNDFom - %NDICP) + %CP + %EE + %Ash); NDICP expressed as % DM.

⁴Estimated based on NRC (2001) using average DMI and BW of cows during the experiment.

⁵Dig Lys = digestible lysine; Dig Met = digestible methionine.

⁶Estimated based on NASEM (2021) using average DMI and BW of cows during the experiment.

⁷Metab Lys = metabolizable lysine; Metab Met = metabolizable methionine.

⁸NE_L = net energy for lactation, calculated using nutrient analysis of individual feed ingredients.

⁹Estimated based on NRC (2001) using average DMI and BW of cows during the experiment.

¹⁰Estimated based on NASEM (2021) using average DMI and BW of cows during the experiment.

Table 5.3. Chemical composition of diet ingredients.

Item (% DM, unless noted)	BMR, CS ¹	Alfalfa Silage		CM ¹		HMC ¹				
		Herd ²	Expt ²	SBM ¹	SH ¹	Herd	Expt	RSB ¹	Herd	Expt
DM, % AF	34.9	34.8	35.1	87.3	93.2	87.9	87.9	91.7	72.8	69.4
CP	7.64	21.9	22.5	52.0	14.0	45.8	45.8	71.3	8.85	8.70
NDICP, %CP	10.7	8.62	6.46	1.04	23.6	8.29	7.68	3.78	6.93	4.65
ADICP, %CP	5.64	5.92	5.62	3.16	7.67	3.59	4.70	2.94	2.88	1.59
aNDF	38.1	41.8	41.3	10.1	63.4	24.7	26.2	3.60	7.22	5.21
aNDFom	36.5	40.0	39.6	9.77	61.9	21.6	22.6	3.47	6.94	5.06
ADF	23.8	37.1	37.2	6.64	46.6	17.8	18.4	1.34	2.21	1.78
NFC ³	49.0	24.9	23.6	28.7	19.4	25.6	24.2	21.1	79.5	81.3
Starch	32.9	0.39	0.39	0.81	0.96	0.30	0.64	13.5	72.3	74.4
EE	4.17	4.66	4.90	2.27	3.08	3.78	3.83	1.80	3.76	3.89
Ash	3.52	10.6	10.9	7.79	5.06	7.27	7.25	3.65	1.59	1.47

¹BMR, CS = BMR, corn silage; SBM = soybean meal; SH = soybean hulls; CM = canola meal; CGM = corn gluten meal; RSB = roasted soybeans; HMC = high-moisture corn.

²Herd = used for the screening diet; Expt = used for the experimental diets.

³NFC = $100 - ((\%aNDFom - \%NDICP) + \%CP + \%EE + \%Ash)$; NDICP expressed as % DM.

Table 5.4. Penn State Particle Separator descriptive analysis.

Item	>19 mm	8 to 19 mm	4 to 8 mm	1.18 to 4 mm	Pan
BMR Corn Silage (AF ¹)	4.21 ²	68.8	15.2	11.1	0.66
Alfalfa Silage (AF)	25.4	55.6	11.2	6.44	1.44
TMR – SBM (AF)	3.69	50.6	13.0	21.8	11.0
TMR – CM (AF)	3.64	52.4	13.0	17.8	13.2
BMR Corn Silage (DM ¹)	3.72	66.0	17.0	12.5	0.86
Alfalfa Silage (DM)	25.3	55.0	11.1	6.73	1.82
TMR – SBM (DM)	3.17	45.1	13.5	24.5	13.8
TMR – CM (DM)	3.09	46.6	13.4	20.4	16.5

¹AF = as-fed basis, DM = dry matter basis.

²Proportion remaining on each sieve and pan for each ingredient and diet.

Table 5.5. Comparison of residual feed intake status during the screening period and during the 10-week experimental period.

RFI ¹ Status	High → High or Low → Low	High → Low or Low → High	High or Low → Medium ²	High or Low → Medium but not past SD = 0 ³	High → High, Low → High or Low → Medium but not past SD = 0
n=cows	35	13	24	15	50
Proportion	35/72 = 0.49	13/72 = 0.18	24/72 = 0.33	15/72 = 0.21	50/72 = 0.69

¹RFI = residual feed intake.

²Denotes cows that switched from either low (≤ -0.5 SD) or high (≥ 0.5 SD) to medium RFI (> -0.5 and < 0.5 SD from expected intake).

³Denotes cows that switched from either low (≤ -0.5 SD) or high (≥ 0.5 SD) to medium RFI up to SD = 0 (> -0.5 and < 0 for low or > 0 and < 0.5 SD for high, respectively, from expected intake).

Table 5.6. Body weight, BCS, DMI, milk yield, and feed efficiency.

Item	Hi ¹		Lo ¹		P-value ^{2,3}							
	SBM ⁴	CM ¹	SBM	CM	SEM	RFI	Diet	RFI×Diet	Week	RFI×Week	Diet×Week	RFI×Diet×Week
Body Weight, kg	636	649	632	639	4.76	0.17	0.03	0.53	<0.01	0.63	0.48	0.48
Body Weight Change, kg Δ/wk	4.42	4.63	2.58	4.01	1.07	0.26	0.45	0.58	-	-	-	-
BCS, units	3.10	3.17	3.15	3.11	0.02	0.77	0.47	0.02	<0.01	0.92	0.59	0.09
BCS Change, units Δ/wk	0.010	0.026	0.023	0.009	0.005	0.69	0.78	<0.01	-	-	-	-
DMI, kg/d	26.3	28.0	24.7	26.0	0.50	<0.01	<0.01	0.70	0.61	0.66	0.11	0.60
Milk Yield, kg/d	42.3	44.2	40.1	44.7	1.38	0.55	0.02	0.90	<0.01	0.90	<0.01	0.68
ECM Yield ⁴ , kg/d	47.1	48.1	44.3	47.7	1.17	0.17	0.06	0.31	<0.01	0.82	0.40	0.57
4% FCM Yield ⁵ , kg/d	44.0	44.7	41.6	44.4	1.13	0.22	0.11	0.34	<0.01	0.70	0.38	0.60
Feed Efficiency ⁶	1.79	1.72	1.80	1.83	0.03	0.08	0.55	0.11	<0.01	0.27	0.03	0.14

¹Hi = high RFI; Lo = low RFI; SBM = soybean meal; CM = canola meal.

²RFI = effect of RFI status; Diet = effect of SBM or CM diet; Week = week of experiment.

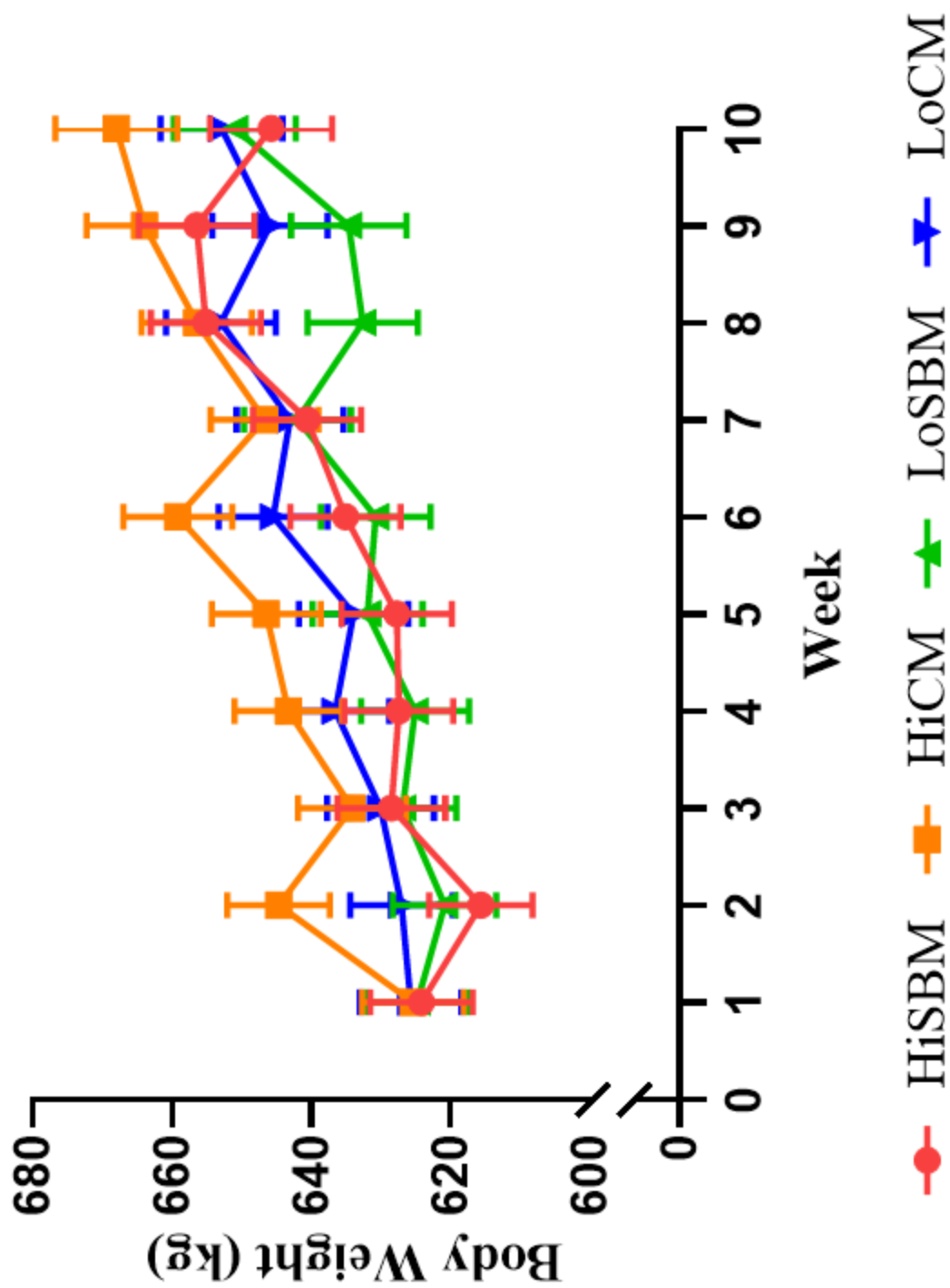
³Parity: BCS and DMI ($P < 0.01$), body weight change ($P = 0.02$); Week × Parity: milk yield, ECM, 4% FCM, and feed efficiency ($P < 0.01$). All other effects containing parity were not significant for any item ($P > 0.10$).

⁴ECM = $0.327 \times \text{Milk Yield (kg/d)} + 12.95 \times \text{Milk Fat Yield (kg/d)} + 7.20 \times \text{Milk Protein Yield (kg/d)}$.

⁵FCM = $0.4 \times \text{Milk Yield (kg/d)} + 15 \times \text{Milk Fat Yield (kg/d)}$.

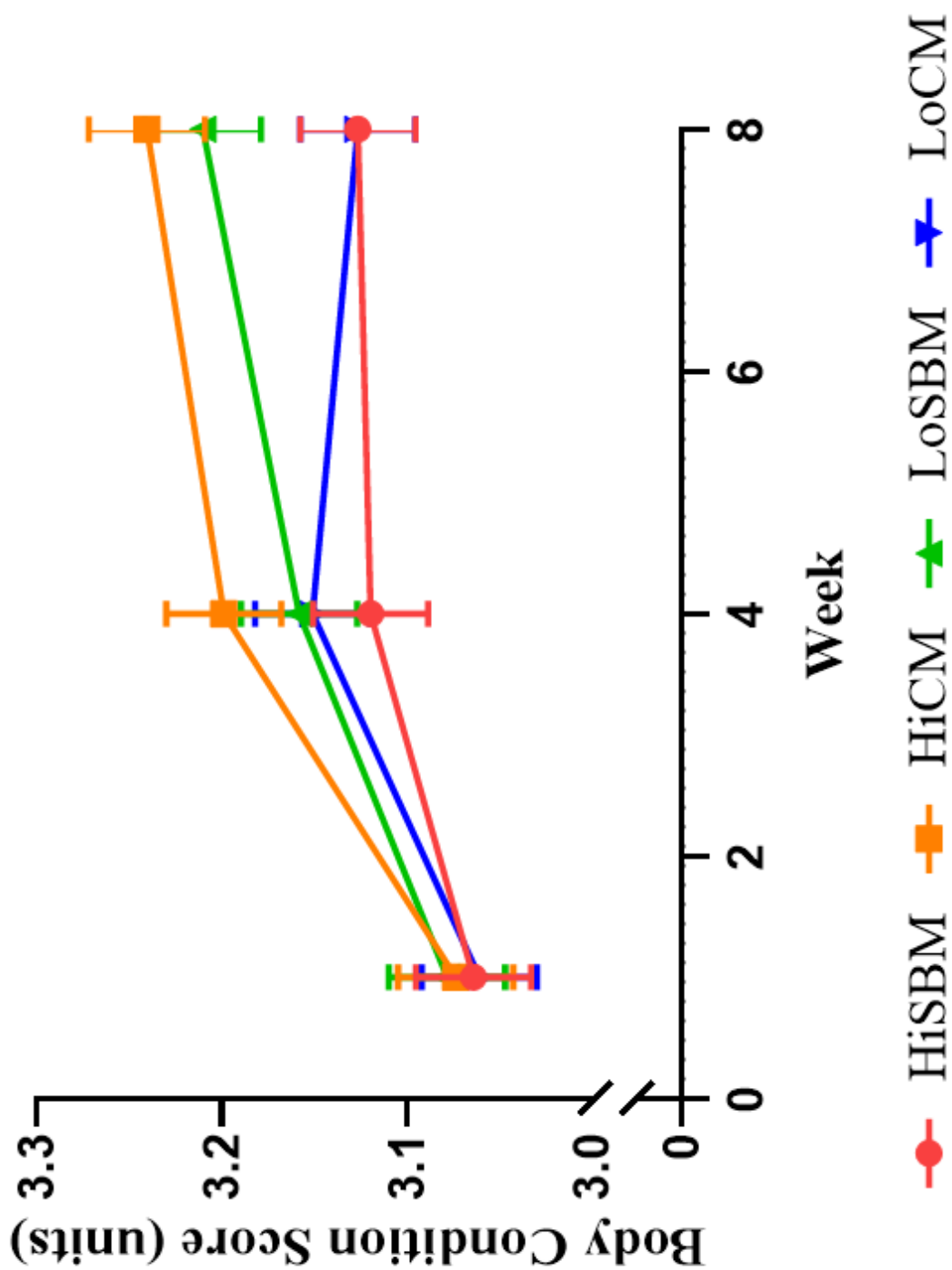
⁶Feed Efficiency = ECM/DMI.

Figure 5.1. Body weight LSM for each treatment group by week of experiment.



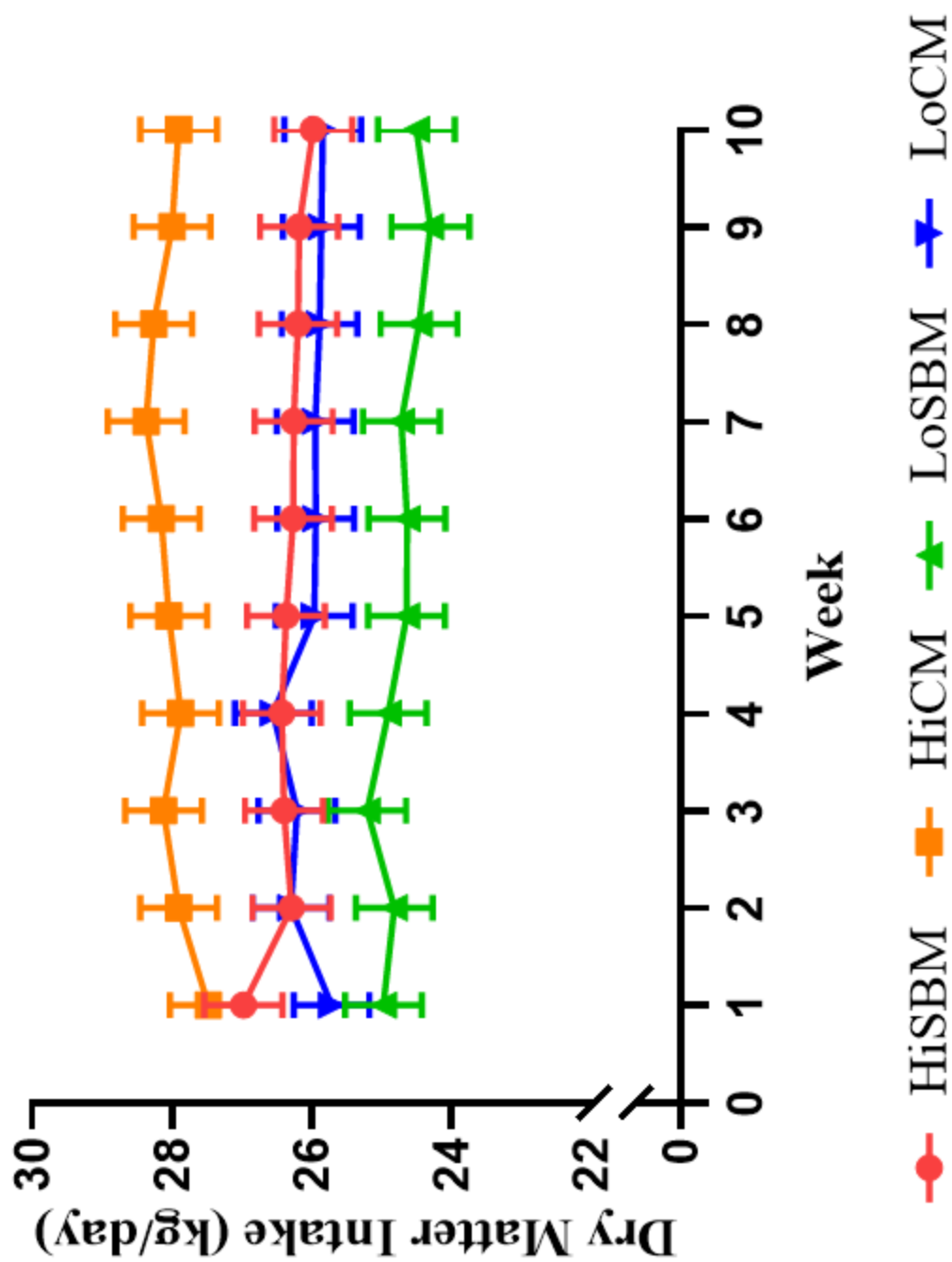
Diet: $P = 0.03$.

Figure 5.2. Body condition score LSM for each treatment group by week of experiment.



RFI×Diet×Week: $P = 0.09$.

Figure 5.3. Dry matter intake LSM for each treatment group by week of experiment.



Diet: $P < 0.01$.

Table 5.7. Milk composition.

Item	Hi ¹		Lo ¹		P-value ³							
	SBM ¹	CM ¹	SBM	CM	SEM	RFI	Diet	RFI×Diet	Week	RFI×Week	Diet×Week	RFI×Diet×Week
Fat, %	4.30	4.20	4.43	4.00	0.12	0.76	0.03	0.16	<0.01	0.06	<0.01	0.63
Fat, kg/d	1.77	1.80	1.69	1.76	0.05	0.22	0.32	0.73	<0.01	0.49	0.56	0.65
Protein, %	3.24	3.25	3.23	3.17	0.06	0.40	0.66	0.52	<0.01	0.71	<0.01	0.42
Protein, kg/d	1.37	1.42	1.27	1.41	0.03	0.08	<0.01	0.23	0.10	0.73	0.03	0.32
Lactose, %	4.82	4.76	4.79	4.79	0.02	0.96	0.13	0.15	<0.01	0.98	0.97	0.79
Lactose, kg/d	2.02	2.10	1.90	2.13	0.07	0.50	0.02	0.25	<0.01	0.84	0.03	0.63
Total Solids ⁴ , %	13.4	13.3	13.5	13.0	0.18	0.59	0.07	0.41	<0.01	0.13	<0.01	0.15
Total Solids, kg/d	5.60	5.81	5.31	5.77	0.15	0.24	0.02	0.41	<0.01	0.70	0.13	0.11
MUN, mg/dL	12.6	12.7	12.7	11.8	0.28	0.18	0.16	0.11	0.77	0.99	0.10	0.99
Log ₁₀ SCC	4.97	4.97	4.95	4.77	0.12	0.35	0.46	0.44	0.10	0.88	0.69	0.90

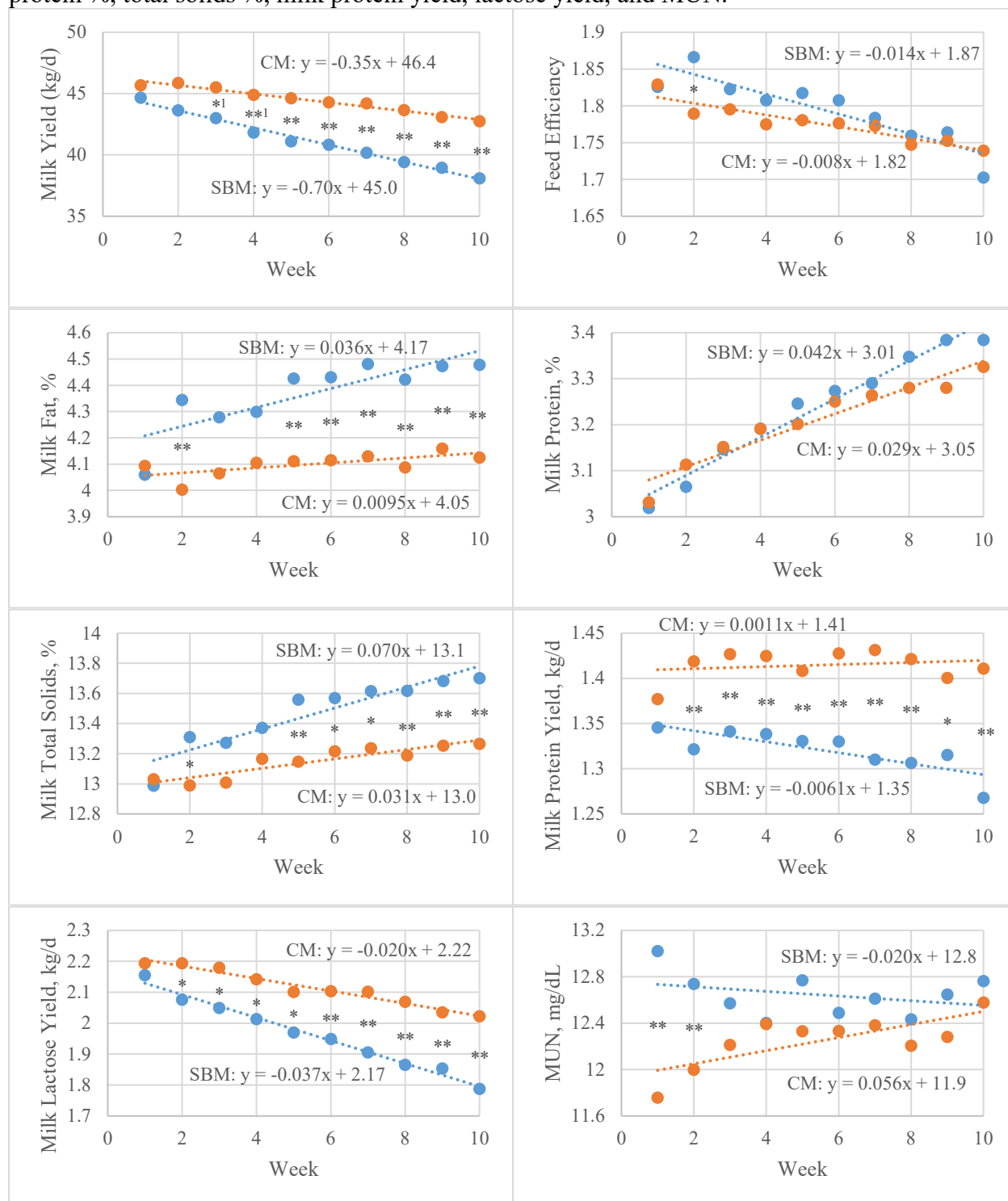
¹Hi = high RFI; Lo = low RFI; SBM = soybean meal; CM = canola meal.

²RFI = effect of RFI status; Diet = effect of SBM or CM diet; Week = week of experiment.

³Parity: fat and lactose % ($P < 0.01$), total solids % ($P = 0.05$); Week × Parity: fat, protein, lactose, and total solids yield ($P < 0.01$), MUN ($P = 0.06$); RFI × Parity: MUN ($P = 0.10$); RFI × Week × Parity: protein % ($P = 0.07$). All other effects containing parity were not significant for any item ($P > 0.10$).

⁴Total Solids = Fat + Protein + Lactose + Ash.

Figure 5.4. Diet \times week interactions for milk yield, feed efficiency (ECM/DMI), milk fat %, protein %, total solids %, milk protein yield, lactose yield, and MUN.



● SBM ● CM Linear (SBM) Linear (CM)

¹ $P \leq 0.05$ **; $0.05 < P \leq 0.10$ *.

Table 5.8. Plasma hormone and metabolite concentrations.

Item	Hi ¹		Lo ¹		SEM	P-value ^{2,3}		
	SBM ¹	CM ¹	SBM	CM		RFI	Diet	RFI×Diet
T3 ⁴ , ng/mL	1.08	0.95	1.08	0.87	0.06	0.50	<0.01	0.50
T4 ⁴ , µg/dL	4.32	4.42	4.38	4.28	0.17	0.81	0.98	0.55
Glucose, µg/mL	583	574	575	575	14.1	0.81	0.72	0.75
Insulin, µg/L	0.92	1.31	0.92	1.19	0.13	0.65	0.02	0.66
GH ⁴ , ng/mL	4.03	2.97	3.27	3.05	0.45	0.43	0.15	0.34
IGF-1 ⁴ , ng/mL	44.5	46.0	45.8	50.5	2.39	0.23	0.20	0.51

¹Hi = high RFI; Lo = low RFI; SBM = soybean meal; CM = canola meal.

²RFI = effect of RFI status; Diet = effect of SBM or CM diet.

³Parity: GH ($P = 0.09$). All other effects containing parity were not significant for any item ($P > 0.10$).

⁴T3 = triiodothyronine; T4 = thyroxine; GH = growth hormone; IGF-1 = insulin-like growth factor 1.

Table 5.9. Urinary excretion of purine derivatives and urea.

Item	Hi ¹		Lo ¹		SEM	P-value ^{2,3}		
	SBM ¹	CM ¹	SBM	CM		RFI	Diet	RFI×Diet
Creatinine, mmol/d	162	168	160	166	3.60	0.58	0.09	0.58
Urine Volume, L/d	33.5	29.2	31.4	30.2	1.57	0.72	0.07	0.30
Allantoin, mmol/d	682	635	575	607	34.4	0.04	0.82	0.23
Uric Acid, mmol/d	75.2	71.1	66.7	58.6	3.76	<0.01	0.09	0.56
PD ⁴ , mmol/d	757	706	642	665	36.3	0.03	0.69	0.28
PD Absorption ⁵ , mmol/d	832	772	697	724	42.5	0.03	0.68	0.28
Intestinal Microbial N Flow ⁵ , g/d	605	561	507	526	30.9	0.03	0.68	0.28
Urea, g/d	352	352	329	339	10.7	0.08	0.62	0.58
UUN ⁴ , g/d	164	164	153	158	5.00	0.08	0.62	0.58
TUN ⁴ , g/d	296	289	262	292	9.60	0.09	0.20	0.04
UUN, % TUN	56.3	56.9	58.3	54.3	1.36	0.85	0.19	0.09

¹Hi = high RFI; Lo = low RFI; SBM = soybean meal; CM = canola meal.

²RFI = effect of RFI status; Diet = effect of SBM or CM diet.

³Parity: urine volume ($P = 0.03$), urea and UUN ($P = 0.04$); RFI × Parity: TUN and UUN, % TUN ($P = 0.09$). All other effects containing parity were not significant for any item ($P > 0.10$).

⁴PD = purine derivatives (allantoin + uric acid); UUN = urinary urea-N; TUN = total urinary N.

⁵Estimated using equations from Chen and Gomes (1992).

General Conclusions

In the first experiment, substituting canola meal (CM) for soybean meal (SBM) in diets fed during the close-up dry period and early lactation increased production measures in Holstein dairy cows. Dry matter intake (DMI) increased for cows fed the diets with CM compared to SBM during the close-up dry period and early lactation. Furthermore, milk yield tended to increase and milk urea nitrogen decreased for cows fed the diets with CM compared to SBM during early lactation. Plasma triiodothyronine concentrations decreased during the close-up dry period and early lactation for cows fed the diets with CM compared to SBM, which corresponded to increased milk yield. This may have been due to decreased nutrient utilization in tissues peripheral to the mammary gland. Diet digestibility decreased during the close-up dry period and early lactation and intestinal microbial N flow decreased during early lactation for cows fed the diets with CM compared to SBM. Despite this, milk yield increased for cows fed the diets with CM postpartum, suggesting that the protein quality of CM, due to increased methionine, overcame the amount of CM protein digested. Furthermore, decreased intestinal microbial N flow suggests that the positive production effects of CM occurred postruminally.

In the second experiment, substituting CM for SBM in diets fed during mid-lactation increased production measures in Holstein dairy cows. Dry matter intake increased for cows fed the diet with CM compared to SBM. A diet \times week interaction occurred for milk yield, with increased persistence for cows fed the diet with CM compared to SBM. Plasma triiodothyronine concentrations decreased and insulin concentrations increased for cows fed the diet with CM compared to SBM, corresponding to increased milk and milk protein yields. Intestinal microbial N flow was not affected by diet, suggesting no difference for microbial protein synthesis.

Overall, the results from both experiments support previous observations of increased production for cows fed diets with CM compared to SBM during early and mid-lactation. Furthermore, various physiological aspects were affected for cows fed the diets with CM compared to SBM, helping to expand the framework for why the production effects occurred. However, further research is needed to determine the scenarios in which dairy cows perform most optimally when fed diets formulated with CM compared to SBM from the dry period through all stages of lactation.

While similar ingredients were utilized to formulate the diets in both experiments, the most obvious dietary difference between experiments was the higher inclusion of corn silage in the second experiment. The higher inclusion of corn silage helped to increase dietary energy density compared to the diets in the first experiment, which may have helped to facilitate the more drastic response observed for milk yield in the second experiment. While the basis of any sound dairy cow diet is high quality forages, future experiments should not only consider how differing inclusion rates of different forages interact with differing inclusion rates of SBM and CM, but to also pay attention to differing inclusion rates of other dietary ingredients such as other protein sources and concentrates.

In the first experiment, cows began consuming the close-up diets approximately four weeks before parturition and remained on a postpartum diet with either the same or other protein source for the first 16 weeks of lactation. The primary focus of this experiment was to determine whether there was an interaction between prepartum and postpartum diets on postpartum production and physiological parameters based on these durations of feeding. However, future experiments should focus on longer term feeding of diets with either SBM or CM during the dry period to determine potential effects on the fetus. Cows fed the close-up diet with CM increased

prepartum DMI and decreased plasma T3 concentrations compared to cows fed the close-up diet with SBM, demonstrating that utilization of the extra consumed nutrients was likely affected for cows fed the CM diet. While diet DM, OM, and CP digestibility also decreased for cows fed the close-up diet with CM compared to SBM, cows fed the close-up diet with CM still received a greater supply of methionine due to the substantially greater methionine content of CM compared to SBM. This begs the question of whether the greater supply of methionine exerted any fetal programming effects in terms of birth weight, average daily gain, and subsequent production performance. As approximately 2/3 of fetal growth occurs during the last 1/3 of gestation, longer term feeding of diets with SBM or CM may be necessary to adequately determine whether consuming diets with CM affects the fetus. Ideally, cross-lactational experiments would help to uncover long-term effects of the methionine supply difference on production in dairy cows and their offspring.

In the second experiment, a diet \times week interaction was observed for milk yield and several milk composition measures. While this experiment was the first to report this interaction, it is only the second experiment, to our knowledge, conducted in a way that makes it possible to assess whether it is present. The vast majority of previous experiments have utilized either a Latin square design or some form of a changeover design, therefore reducing temporal data, which in most cases represents a few days to a few weeks of feeding to begin with, to averages by cow during a period. Given the shorter term duration of testing, these forms of experimental designs inherently remove the effect of week from the statistical model, making it impossible to determine whether a diet \times week interaction would have been present in that given scenario. While longer term experiments are challenging to perform from planning, cost, and labor standpoints, they are undoubtedly necessary to more accurately determine the long term

production effects of feeding diets with SBM or CM. Ultimately, this information will be paramount to both nutritionists and producers alike.