Does providing metabolizable protein in excess of requirement prior to calving improve nitrogen balance and cow-calf performance?

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ABSTRACT

The objective of this experiment was to evaluate the effect of over-feeding metabolizable protein (**MP**) during late gestation on prepartum N balance, and postpartum cow BW and skeletal muscle catabolism, colostrum composition, and milk yield (**MY**) and composition. Twenty-four (14 cannulated, 10 non-cannulated) crossbred Hereford cows were assigned to a control treatment designed to meet (**CON** = 100% MP, n =12) or exceed (**HMP** = 133% MP, n = 12) MP requirements. Cows consumed a common lactation (103% MP) diet postpartum. One HMP and 1 CON cow-calf pair were removed due to dystocia, while 1 CON cow was removed due to aggression at parturition. Heifers were housed and fed individually from d -55 to d 33 relative to parturition and then group-housed until 112 d post-partum. Dry matter intake was measured throughout the study and summarized by week. Cow BW was recorded on d -55 ± 3.7, -41 ± 3.7, -28 ± 3.6, -8 ± 3.9, 7 ± 1.0, 13 ± 2.0, 28 ± 2.9, 57 ± 3.6, 82 ± 5.1, and 112 ± 2.9 relative to parturition. Total fecal and urinary collections were conducted over a period of 6 d starting on d -33 and -15 to measure N balance, and total tract digestibility. Urine samples from the prepartum collections and postpartum spot samples (starting d 7 and 28) were composited and analyzed for 3-methylhistidine (**3-MH**) and creatinine as indicators of muscle turnover. Digesta samples were collected and ruminal ammonia-N concentration was measured on d -33 ± 5.3, -15 ± 4.4, 7 ± 0.9, and 28 ± 2.9. Blood samples were collected from cows via jugular venipuncture on d -34 ± 5.3, -17 ± 4.4, 7 ± 0.9, 28 ± 2.9, 70 ± 3.2 and 112 ± 2.9 and analyzed for plasma glucose, urea-N, and insulin, and serum NEFA and BHBA. Calf BW and frame measurements were conducted at birth and on d 7 ± 0.9, 28 ± 2.9, 57 ± 3.6, 82 ± 5.1, and 112 ± 2.9. At parturition, colostrum samples were collected and analyzed for nutrient composition and IgG concentration. Milk yield was measured on d 7 ± 0.9, 12 ± 0.9, 28 ± 2.9, 33 ± 2.9, 70 ± 3.2, and 112 ± 2.9 relative to parturition. Milk samples were analyzed for the concentration of fat, crude protein, lactose, urea-N (**MUN**) and somatic cell count (**SCC**). Data for the prepartum and postpartum periods were analyzed separately. All data were analyzed as a randomized complete block design using the MIXED procedure of SAS. Fixed effects included treatment, time, and the two-way interaction of treatment and time, while cow nested in block was considered as the random effect. Time (day or week) was analyzed as a repeated measure when appropriate. Prepartum BW did not differ among days for CON. Whereas, HMP cows increased BW as parturition approached (treatment × day, $P < 0.01$). While not differing by treatment, dry matter intake (% BW) increased ($P < 0.01$) by 18% on week

-2 compared to -8, but then decreased (P <0.01) by 8.0% during week -1. Nitrogen intake, apparent digestion, excretion, and retention (g/d) were all greater (P < 0.01) for HMP heifers than CON. Heifers fed HMP had improved (P < 0.01) DM, OM, and NDF digestibility relative to CON heifers. Concentration of urinary 3-MH and the 3-MH:creatinine ratio did not differ ($P \geq 0.23$) between treatments. Maximum ruminal pH and the total concentration of short-chain fatty acids were not affected by treatment. Prepartum ruminal ammonia-N decreased (treatment × day, P < 0.01) as parturition approached for HMP (10.1 to 8.7 mg/dL) whereas, ammonia-N was not affected for CON (1.0 to 1.3 mg/dL). Plasma urea-N was greater (P < 0.01) for HMP heifers (15.0 vs. 7.5 mg/dL). Postpartum BW did not differ ($P \geq 0.30$) by treatment, day, or the interaction of treatment and day, but rump fat decreased (P = 0.011) as lactation progressed. Dry matter intake decreased during wk 2 and 3 compared to 1 and 4, whereas ruminal pH was less during wk 2, 3, and 4 relative to wk 1. Colostrum fat concentration was less (P = 0.003) for HMP than CON, but milk production and milk component concentrations were not affected by treatment. Milk yield was greatest from d 7 to 33 and decreased thereafter (P < 0.001). Plasma and serum metabolites were not affected by treatment, but NEFA was greater (P < 0.001) on d 7 and 28 relative to d 70 and 112. Urinary 3-MH and the 3-MH:creatinine ratio did not differ by treatment, day, or the interaction of treatment and day ($P \geq 0.22$). Calf growth was not affected by treatment. Over-feeding MP prepartum may improve prepartum heifer BW, NDF and OM digestibility and N balance, but might decrease colostrum fat concentration without affecting lactation or postpartum metabolic indicators of energy balance or protein turnover.

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AA = Amino acid

aNDFom = NDF assay with amylase, sodium sulfite and ash correction

ADICP = Acid detergent-insoluble crude protein

AFBW = Expected SRW

ATP = Adenosine triphosphate

BCS = Body condition score

BHBA = Beta-hydroxybutyrate

BW = Body weight

CNCPS = Cornell Net Carbohydrate and Protein System

CON = Control ration, 100% of predicted MP requirements

CHO = Carbohydrate

CP = Crude protein

DIM = Days in milk

DM = Dry matter

DMI = Dry matter intake

EBW = Empty body weight

EqSBW = Equivalent shrunk body weight

FC = Fibrous carbohydrates

GIT = Gastrointestinal tract

HMP = High metabolizable protein ration, 133% of predicted MP requirements

Ig = Immunoglobulin

LAC = Lactation ration, 103% of predicted MP requirements

NDF = Neutral detergent fiber

NEFA = Non-esterified fatty acids

NFC = Non-fibrous carbohydrates

ME = Metabolizable energy

MCP = Microbial crude protein

MP = Metabolizable protein

MP_m = Metabolizable protein for maintenance

MP_p = Metabolizable protein for pregnancy

MY = Milk yield

N balance = Nitrogen balance

NDICP = Neutral detergent insoluble crude protein

NE = Net energy

NEFA = Non-esterified fatty acids

NLAC = Non-lactating

NP = Net protein

NPr = Not pregnant

NPN = Non-protein nitrogen

OM = Organic matter

peNDF = Physically effective neutral detergent fiber

PPI = Postpartum interval

PR = Pregnant

RDP = Rumen degradable protein

RUP = Rumen undegradable protein

SBW = Shrunk body weight

SCFA = Short-chain fatty acids

SRW = SBW of a standard reference animal

TDN = Total digestible nutrient

TDNI = Total digestible nutrient intake

WSC = Water-soluble carbohydrate

3-MH = 3-methylhistidine

1.0 GENERAL INTRODUCTION

Typically, beef cattle are fed high-fiber low-quality forages (CP < 10%; NASEM, 2016) throughout mid- to late-gestation to reduce feed costs (Adams et al., 1996; Anderson et al., 2005) while often being provided a supplement to improve NDF digestibility (Paterson et al., 1994) or metabolizable protein (**MP**) requirements (Sletmoen-Olsen et al., 2000a,b). Gestational rations are often based on crude protein (**CP**) content and research has focused on CP supplementation relative to marginally CP deficient diets (Stalker et al., 2006; 2007; Martin et al., 2007) to evaluate its impact on cow BW and BCS (Shoup et al., 2015a,b), reproductive efficiency (Lents et al., 2008), and effects on progeny development (Greenwood et al., 2004; Martin et al., 2007; Larson et al., 2009). However, dietary CP content does not equate to the MP supply available. Cattle have a requirement for amino acids (**AA**) rather than CP. Metabolizable protein supply is the predicted quantity of metabolizable AA and peptides resulting from intestinal digestion of rumen undegradable protein, bypass protein, microbial crude protein, and endogenous losses. However, predicting the quantity of available MP encompasses estimation of degradation rates of various protein fractions relative to predicted passage rate (Fox et al., 2004). Therefore, alterations in passage rate and gastrointestinal fill, as is seen in late gestation beef cattle (Gunter et al., 1990; Hanks et al., 1993; Linden et al., 2015), are likely to affect MP supply during the weeks preceding parturition.

Concurrent to decreases in total tract digestibility with advancing gestation (Linden et al., 2015), the AA demand increases as a result advancing pregnancy (Prior et al., 1979) and preparation for lactation (Rattray et al., 1974). During late gestation, fetal mass increases exponentially (Rattray et al., 1974; Bauman and Currie, 1980) causing a correspondingly large increase in AA demand from maternal supply. In fact, fetal muscle protein content increases by 83% from d 160 to 280 (Prior et al., 1979) and there is a corresponding increase in fetal oxidative metabolism, with 30 to 50% of oxidative substrates derived from oxidation of AA. Simultaneously, the dam prepares for parturition and the shift into lactation through mammogenesis (Ferrell et al., 1976) and colostrogenesis (Swanson et al., 2008; Neville et al., 2013), as well as changing hormonal patterns that can affect appetite and satiety (Bell, 1995; Drackley, 1999). The combination of increased nutrient demand due to the aforementioned physiological processes and a pattern of decreasing DMI as parturition approaches (Linden et al., 2015) may challenge the capability of the dam to maintain a positive MP balance relative to her requirements during late

gestation. Furthermore, as cattle transition into early lactation they experience an increase in energy and protein demand to support lactation. For beef cows, daily milk yield is assumed to be equivalent to calf demand and estimated to average between 4 and 15 kg/d (NASEM, 2016), but milk composition and peak yield are not well-defined in cow-calf production systems. These characteristics are assumed to be similar to dairy cattle when modeling predictions for nutrient requirements.

On average, dairy cattle reduce DMI by 30% (Hayirli et al., 2003), while reductions in DMI as great as 50% have been reported (Marquardt et al., 1977) during the 3 wk preceding parturition. The reduction in DMI predisposes cattle to negative energy and protein balances during early lactation (Drackley, 1999). Metabolic adaptations ensue (Grummer, 1995) and allow the cow to prioritize available nutrients towards gestation and lactation requirements (Bauman and Currie, 1980) ahead of maintenance requirements. Consequently, the dam catabolizes adipose tissue as demonstrated by elevated serum NEFA concentrations (Penner et al., 2007), and skeletal muscle tissue as shown by increased ubiquitin expression and abundance in skeletal muscle, and increasing concentrations of urinary 3-MH (Chibisa et al., 2008) during early lactation. Cow BW decreases as a result of increased catabolism and mobilization of adipose and skeletal muscle reserves. These metabolic adaptations provide oxidative substrates and can contribute to the available AA pool in order to support the maternal physiological state (Bauman and Currie, 1980; Drackley, 1999). It is possible that beef cattle employ similar homeorhetic mechanisms during the prepartum transition phase; however, the magnitude of the negative energy and protein balance has not been assessed. Support for a negative protein balance has been provided by Wood et al. (2013) and Du et al. (2005) where increased abundance of ubiquitin in skeletal tissue has been reported when beef cows are mildly nutrient restricted in mid- to late-gestation. More importantly, these changes are not expressed in fetal tissues (Du et al. 2005), indicating that the dam is preferentially catabolizing skeletal tissue to support the nutrient demands of gestation. However, evidence of skeletal muscle catabolism in early lactation has not yet been evaluated in beef cattle. The extent to which prepartum nutritional status incurs carry-over effects on postpartum protein and N balance is not well understood in beef cattle.

It is possible that late gestation beef cattle may experience MP deficiency, leading to increased skeletal muscle catabolism to support fetal AA demand. A late gestation AA deficiency could have carry-over effects on the performance during postpartum transition phase. Furthermore,

maternal nutrition is known to impact progeny growth and development (Larson et al., 2009), and reproductive performance (Martin et al., 2007). Thus, any deficits in AA supply during late gestation or early lactation may impact progeny performance. Though literature is available to evaluate the effect of CP supplementation relative to CP restriction and the effect on cow-calf performance, researchers have yet to examine the effect of over-supplementation of MP on maternal nitrogen balance and its influence on cow-calf performance and the beef cow lactation curve and milk composition.

2.0 LITERATURE REVIEW

2.1 Cornell Net Carbohydrate and Protein System

Animal nutritionist and researchers use a variety of models to predict ruminant nutrient requirements, including the Dairy NRC (2001), the Cornell Net Carbohydrate and Protein System (**CNCPS**), NASEM (2016), and Molly cow model (Hanigan et al., 2013), that estimate nutrient requirements using either an empirical or a mechanistic model. The CNCPS 6.5 was developed and is regularly revised (Lanzas et al., 2007a,b; Tylukti et al., 2007; Higgs et al., 2015; Van Amburg et al., 2015) to provide a mechanistic model for quantifying ruminant carbohydrate and protein requirements and nutrient supply based on ruminal fermentation and postruminal digestion (Sniffen et al. 1992). Previously, requirements were predicted using an empirical model that was limited in its ability to estimate microbial production from fermentation of carbohydrates, peptides, and amino acids as the model was developed under strictly controlled research conditions that were not representative of industry scenarios (Fox et al., 2004). However, the model applications have been expanded and, to date, it is widely used.

The CNCPS model classifies carbohydrates and proteins by their physical characteristics, chemical composition (shown in Table 2.1; Higgs et al. 2015), availability and rate of degradation (shown in Figure 2.1 and 2.2; Lanzas et al., 2007a,b) within the rumen to predict their contribution microbial growth, and metabolizable end products supplied to the ruminant. The CNCPS 6.5 model was originally developed for use with beef and dairy cattle (Fox et al., 1992; Russell et al., 1992; Sniffen et al., 1992; O'Connor et al., 1993), though the model has been adjusted to predict nutrient requirements of sheep as well (Cannas et al., 2004). Many physiological sub-models have been developed within CNCPS for maintenance, growth, pregnancy, lactation, body reserves, feed intake and composition, rumen fermentation, intestinal digestion, metabolism, and nutrient excretion (Fox et al., 2004). Most of these sub-models rely on mechanistic equations, though empirical equations are used for some sub-models that have not yet been well-characterized. The CNCPS current focus is modelling rumen fermentation in order to increase accuracy of the rumen sub-model predictions and address limitations of the current model. Future goals may focus on improving the sensitivity of the production sub-models and incorporate a mechanistic metabolism

Table 2.1 Equations used by the Cornell Net Carbohydrate and Protein System (version 6.5) to calculate carbohydrate and protein fractions[3]

Fraction	Description	Equation[1,2]
CHO	Carbohydrates	100 - CP - EE - Ash
CC	Indigestible fiber	aNDFom × uNDFom
CB3	Digestible fiber	aNDFom - CC
NFC	Nonfiber CHO	CHO - aNDFom
CB2	Soluble fiber	NFC - CA1 - CA2 - CA3 - CA4 - CB1
CA1[4]	Short-chain fatty acids	Acetic + Propionic + (Butyric + Isobutyric)
CA2[4]	Lactic acids	Lactic
CA3[4]	Other organic acids	Organic acids
CA4[4]	Water-soluble CHO	WSC
CB1[4]	Starch	Starch
PA1	Ammonia	Ammonia × (SP/100) × (CP/100)
PA2	Soluble true protein	SP × CP/100 - PA1
PB1	Insoluble true protein	CP - (PA1 - PA2 - PB2 - PC)
PB2	Fiber-bound protein	(NDICP - ADICP) × CP/100
PC	Indigestible protein	ADICP × CP/100
SUM	Sum of composition	100 = CP + EE + ash + NDF + acetic + propionic + isobutyric + lactic acid + other organic acids + WSC + starch + soluble fiber

[1]EE = ether extract; WSC = water-soluble CHO; SP = soluble protein; ADICP = acid detergent-insoluble CP; aNDFom = NDF assay with amylase, sodium sulfite and ash correction; uNDFom = undigested NDFom after a 240-h in vitro fermentation and ash correction.

[2]Chemical components are expressed as percent DM except: SP = % CP; ADICP = % CP; NDICP = % CP; ammonia = % SP; lignin = % NDF; uNDFom = unavailable aNDFom, % NDF.

[3]Adapted from Higgs et al. (2015).

[4]Analyzed directly.

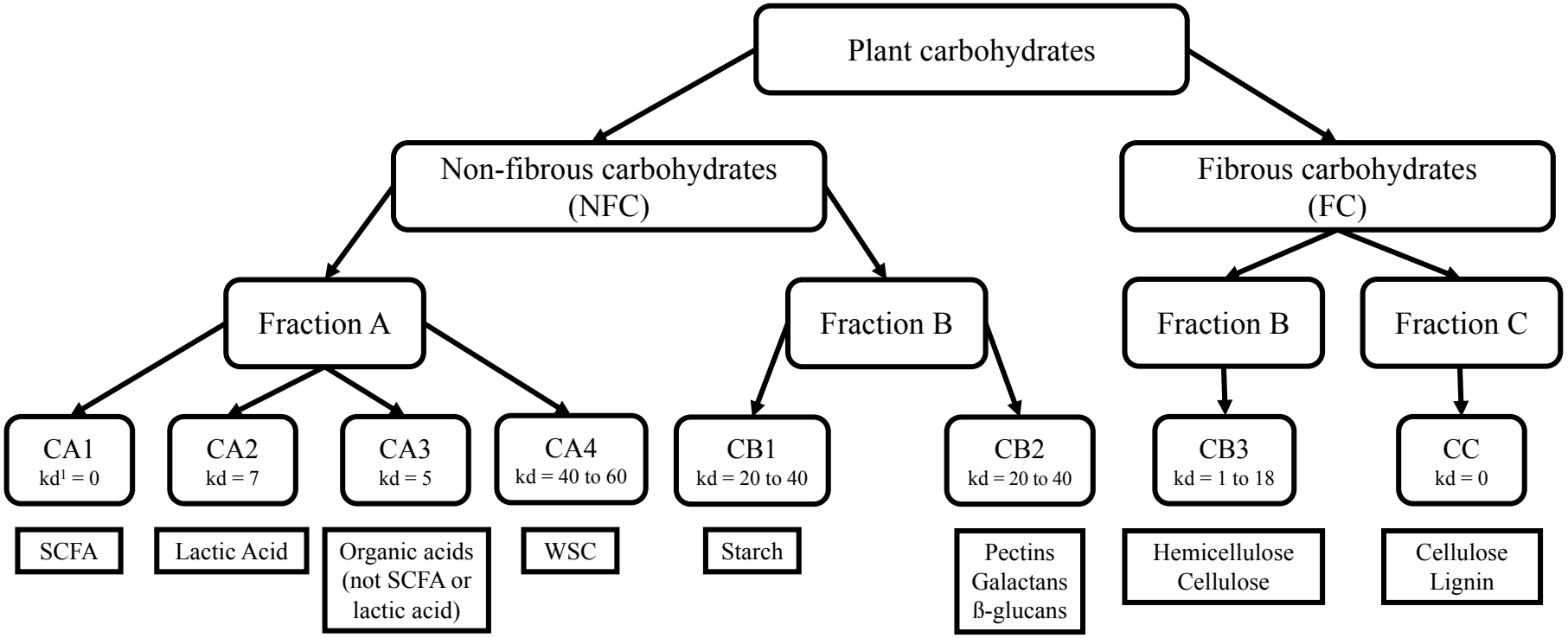

Figure 2.1 The Cornell Net Carbohydrate and Protein System (CNCPS) version 6.5 carbohydrate fractionation scheme detailing sub-fractions of NFC (fractions CA1 to 4 and CB1 and 2) and NDF (fractions CB3 and CC) with estimated degradation rates (kd = %/hr). Adapted from Lanzas et al. (2007a).

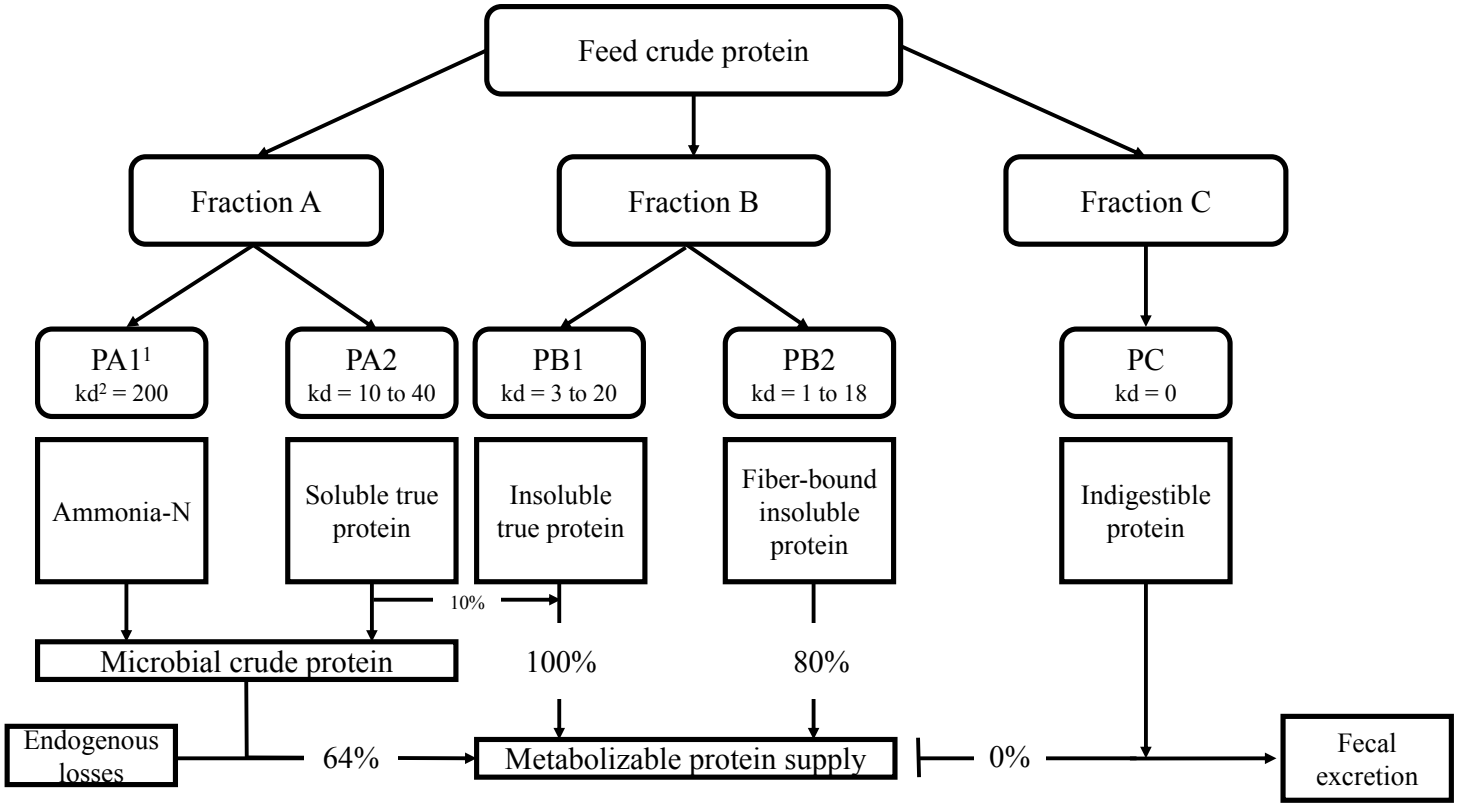

Figure 2.2. The Cornell Net Carbohydrate and Protein System (CNCPS) version 6.5 protein fractionation scheme detailing fractions of soluble protein (PA1 and PA2), insoluble protein (PB1 and PB2), and indigestible protein (PC), estimated degradation rates of protein fractions (2kd = %/hr), and assumed intestinal digestion that compose the metabolizable protein supply. Adapted from Lanzas et al. (2007b). 1The NPN fraction within previous version of the model was redesignated as PA1 (representing ammonia-N) while the AA and peptide components of the NPN fracture are redesignated to fraction PA2 (Van Amburg et al., 2015).

model once CNCPS is capable of accurately predicting the quantities and subsequent absorption of fermentative end-products (Fox et al., 2004).

2.1.1 Classification of Carbohydrates

Carbohydrates can be partitioned as being either fibrous (**FC**) or non-fibrous carbohydrates (**NFC**). Fibrous carbohydrates are the structural components comprising the plant cell wall including celluloses, hemicelluloses, and lignin (Figure 2.1). The plant cell wall components are relatively slowly fermented compared to NFC. The NFC consist of organic acids, simple sugars, water-soluble carbohydrates, starch, and neutral detergent soluble carbohydrates (Lanzas et al., 2007a; NASEM, 2016). While classification of carbohydrates by their chemical composition provides valuable information to describe the feed value, further classification is needed to partition carbohydrates by their relative rates of fermentation. By predicting relative rates of fermentation, the CNCPS model is capable of predicting the extent of degradation and digestion of the feed. Figure 2.1 shows the revised CNCPS classifications of carbohydrate feed fractions. Soluble, insoluble and indigestible carbohydrate fractions are prefaced with the letters A, B, and C, respectively (Van Amburg et al., 2015). Fractions CA1-CA4 consist of short-chain fatty acids (**SCFA**), lactic acid, organic acids other than SCFA and lactic acid, and water-soluble carbohydrates (**WSC**). Fractions CB1 and CB2 are starch and the neutral detergent soluble fibers (cellulose and hemicellulose), respectively. Fibrous carbohydrates are fractioned into CB3, the digestible fibers, and CC, the indigestible fibers (celluloses and lignin).

Acetic, propionic, and butyric acid are the predominant SCFA (fraction CA1) that originate in silages during the ensiling process (Cherney and Cherney, 2003) and high moisture cereals (Hall and Eastride, 2014). As SCFA represent fermentation end products, they do not contribute substantially as a substrate for microbial growth and their ruminal degradation rates are defined as 0 (Lanzas et al., 2007a). That said, SCFA are readily absorbed across the ruminal epithelium and contribute directly to the energy supply of the ruminant. Fraction CA2 represents lactic acid: the most abundant organic acid within fermented feed (McDonald et al., 1991) representing 3 to 8% and 4 to 6% of DM in small grain cereals and corn silages, respectively (McAllister et al., 1995). During the ensiling process, *Lactobacillus* and other bacterial cultures lead to the accumulation of lactate within corn and alfalfa silage (Cherney et al., 2004; Kung, 2008) and alfalfa haylage

(Gordon et al., 1961; Lynch et al., 2014). Innoculants can be used with high moisture corn (Schaeffer et al., 1989; Taylor and Kung, 2002; Kung et al., 2004) to improve preservation, digestibility, and rumen fermentation patterns. Lactate is readily fermented to propionate to acetate (to a lesser extent), contributing to microbial growth. Fraction CA3 represents organic acids other than SCFA and lactic acid. While other organic acids are not common within fermented feeds, they are often detected in fresh forages (Jones and Barnes, 1967; Russell and Van Soest, 1984). Examples of organic acids include, but are not limited to, citric, malic, quinic, succinic, and fumaric acids found in citrus by-products (Bampidis and Robinson, 2006), ryegrass, cocksfoot (Jones and Barnes, 1967), alfalfa, and bermudagrass varieties (Callaway et al., 1997) with concentrations varying depending on stage of maturity of the plant. Organic acids are fermented to acetate within the rumen (Russell and Van Soest, 1984). Fraction CA4 represents the WSC encompassing monosaccharides (fructose and glucose), disaccharides (lactose, maltose, and sucrose), oligosaccharides (raffinose, stachyose; Rooke and Hatfield, 2003), and fructans (levan, inulin; Van Soest, 1994). Water-soluble carbohydrates are rapidly fermented within the rumen, producing propionate and butyrate as common end-products, though lactate can be a fermentation end-product at low ruminal pH (Strobel and Russell, 1986). Although the CNCPS model predictions classify individual feeds based on their rate of degradation, the CNCPS designates all WSC within fraction CA4 to one rate of degradation. The assumption of a common rate of ruminal degradation (40 to 60%/hr) requires further attention as it has been demonstrated that rate of fermentation differs between sugar types (Weisbjerg et al. 1998). The assumption that all sugar degradation rates are equal may over- or under-predict the contribution of fraction CA4 towards ME supply.

Starch has been allocated to fraction CB1 of the CNCPS carbohydrate class (Lanzas et al., 2007a) and can be further detailed as either amylose or amylopectin, dependent on the type of linkages between glucose molecules and degree of branching. Amylose is a linear glucose polymer with α-1,4 glycosidic linkages, whereas amylopectin is differentiated by having α-1,6, as well as α-1,4 glycosidic linkages, causing it to be highly branched and less degradable with the degree of branching and degradability dependent on the number of α-1,6 glycosidic bonds (Svihus et al., 2005). Starch is deposited as granules within grains (Gallant et al., 1992), varying in relative quantities of amylose, amylopectin, and prolamins that influence the rate and site of starch digestion (Huntington, 1997). Granules are classified as either A, B, or C, dependent on the

crystalline arrangement of amylose and amylopectin macromolecules within the granule (Gallant et al., 1992). The interaction between macromolecule arrangement within the granule and type and quantity of prolamins determines the extent of ruminal starch fermentation and intestinal digestion (McAllister et al., 1993). Prolamins are a form of grain-specific protein that comprise 50 to 60% of the total feed protein and affect the degradability of protein and starch within the rumen dependent on the type and quantity of prolamin (van Barneveld, 1999).

Carbohydrate fraction CB2 consists of neutral detergent soluble fibers that include ß-glucans and pectins. The CB2 fraction is not digested by mammalian enzymes. Pectic substances are commonly found in legumes and by-product feeds and are readily fermented at similar rates as starch (20 to 40%/hr; Figure 2.1). ß-glucans are also readily fermented within the rumen and are found in small concentrations within grasses and grains. Fraction CB3 is comprised of the potentially degradable NDF, hemicellulose and cellulose accounting for a large portion (30 to 75%) of the DM in forages and some by-product feeds (Lanzas et al., 2007a). Neutral detergent fiber is stratified within the plant cell wall and can interact with lignin, thereby reducing digestibility (Weiss et al., 1992; Raffrenato, 2011). It has been postulated that the relative concentrations of hemicellulose and cellulose, the orientation of the cellulose microfibrils within the layer (Akin, 1993; Wilson, 1993), and the quantities of other compound such as lignin, pectin, proteins, and phenolic compounds within the cell wall matrix all affect the rate and extent of NDF digestibility. The remaining carbohydrate fraction CC is composed of the indigestible or unavailable NDF within the plant cell wall. The CC fraction was originally estimated as 2.4 × lignin/NDF (Chandler et al., 1980). Lignification increases as forages mature and has a negative relationship with NDF degradability within the rumen (Himmelsbach, 1993), though the lignin content and composition will vary widely between different feeds. Higgs et al. (2015) and Van Amburgh et al. (2015) describe the changes made to calculating the indigestible carbohydrate fraction. The FC fraction is treated with amylase and corrected for ash content to calculate aNDFom, which addresses issues with overestimation of NDF due to ash contamination (Mertens, 2002) and alters predictions of NDF k_d. The indigestible carbohydrate fraction CC is then calculated as the aNDFom by the undegradable NDF content that is determined from a 240-h (**uNDF240**) in vitro incubation (Raffrenato, 2011). Using 240 h uNDF reflects variation in availability of NDF due to growing conditions and plant genetics to better represent ruminal fermentation (Huhtanen et al., 2006; Cotanch et al., 2014). Carbohydrate fraction CB3 is the

difference between aNDFom content and fraction CC. The CNCPS model predicts k_d rates of 20 to 40%/h for fraction CB2 and 1 to 18%/h for fraction CB3 (shown in Figure 2.1) and accounts for hindgut fermentation of CB2 and CB3 fractions that does not contribute to the ME supply of the host.

Aside from classifying carbohydrate fractions, the CNCPS uses sub-models to determine physically effective neutral detergent fiber (**peNDF**) and the contribution of feed peNDF in combination with carbohydrate composition to predict ruminal pH (Fox et al., 2004). A Penn State Particle Separator can be used to determine the peNDF content of the feed, calculated as the percentage of NDF retained on either the first two sieves (19 and 8 mm; $peNDF_{8.0}$) or the first three sieves (19, 8, and 4 mm; $peNDF_{4.0}$) (Jiang et al., 2016) The peNDF content of a feed is relevant as it stimulates saliva flow, and ruminal fluid dilution rate through stimulation of chewing, rumination, and rumen motility (Fox et al., 2004). While the CNCPS models a requirement for peNDF and its influence on ruminal pH (Boston et al., 2000), it neglects to account for the influence of peNDF on the ruminal fluid dilution rate (Fox et al., 2004; Tylutki et al., 2007). The SCFA production from fermentation of carbohydrate fractions is not integrated with the rate of NFC degradation, nor does the model account for the influence of fluid dilution on SCFA removal from the rumen and subsequent ruminal pH fluctuations. Fluid dilution rate is high with high forage diets, but decreased when grain-based rations are fed (Russell, 1999). Failure to account for rate of NFC digestion and fluid dilution rate and their combined effect on ruminal pH are limitations of the model. Acidic pH can drastically affect microbial growth and fiber digestion, ultimately altering the contribution of microbial crude protein (**MCP**) yield to MP supply and ME energy supplied by carbohydrate digestion.

2.1.2 Classification of Protein

Proteins are nitrogenous compounds within the feed that can be classified as rumen degradable, leading to the production of MCP, or undegradable protein (**RDP**, **RUP**) thereby, directly contributing to the MP supply. Intestinal digestion and absorption of microbial crude protein, endogenous protein losses, and RUP constitute the MP supplied to the ruminant. The CNCPS model offers two levels of solution to calculate the MP supplied to the ruminant. Solution level 1 uses empirical calculations to estimate MP supplied, based off of TDN provided relative to

maintenance energy requirements (Fox et al., 2004). The model calculates MCP as 13% of TDN (assuming that MCP is 64% true protein) and can be discounted for level of dry matter intake (**DMI**; Tedeschi, 2001). In solution level 2 of the CNCPS model, protein is classified into five fractions: PA1, PA2, PB1, PB2, and PC (shown in Figure 2.2); that are prefaced with letters A, B, and C to represent the soluble, insoluble, and indigestible protein fractions. Similar to the carbohydrate fractionation scheme, the CNCPS model classifies protein by their chemical composition, rates of degradation (shown in Figure 2.2) and passage in the rumen environment, and postruminal digestibility (Higgs et al. 2015).

Fraction PA1 represents non-protein nitrogen (**NPN**), or ammonia, peptides, and amino acids, that are assumed to be completely degraded within the rumen at a k_d of 200%/h (Van Amburgh et al., 2015) for all feeds. Fraction PA2 represents the soluble true protein that is hydrolyzed in the rumen environment. Protein classified within PA2 is assumed to have a k_d ranging from 5 to 50%/h (Van Amburg et al., 2015), reflecting that the degradability of larger soluble proteins is slower than originally predicted (Sniffen et al., 1992) and that microbial uptake of peptides is rate-limiting (Chen et al., 1987; Broderick and Wallace, 1988). Microbial growth is proportional to the quantities of fractions of PA1 and PA2 that are degraded within the rumen and the energy supplied by carbohydrate fermentation. Microbial crude protein is then digested postruminally and can provide 50 to 100% of the MP requirement of the ruminant (Spicer et al., 1986).

The insoluble true protein content of the feed represents fractions PB1 and PB2, distinguished by their insusceptibility to degradation within rumen environment. It is assumed that fractions PB1 and PB2 are bound to other feed compounds that render them unavailable for ruminal degradation. Fraction PB1 represents the insoluble true protein that contributes directly to the MP supply of the host (in combination with the MCP supply), as it is hydrolyzed post-ruminally and absorbed as peptides and amino acids within the small intestine. Fraction PB2 represents the fiber-bound protein that is insoluble in neutral detergent (**NDICP**) minus the protein fraction that is insoluble in acid detergent (**ADICP**). The ADICP content of the feed represents fraction PC that is the indigestible protein portion of the feed as it cannot be degraded within the rumen and nor does it contribute to the amino acid pool of the host (Krishnamoorthy et al., 1982). Collectively, intestinal digestion and absorption MCP, endogenous losses and secretions, and fractions PB1 and PB2 form the MP supplied to the host. The CNCPS assumes that intestinal

digestibility and absorption is consistent within the protein fractions. Fraction PA2 and PB1 are assumed to be 100% digested post-ruminally, whereas fraction PB2 is assumed to have 80% digestibility post-ruminally (O'Connor et al., 1993). These assumptions limit the accuracy of predicting MP supplied to the host as they fail to recognize the impact of differences in protein AA composition within a fraction on digestibility, the influence of passage rate on digestion and absorption of AA. In contrast to the CNCPS model, NASEM (2016) uses an empirical approach to estimate the MP supplied by a feed. Within the model, digestible organic matter and RDP are used to calculate the MCP yield using a constant efficiency of 13% of TDN intake for diets that contain greater than 40% forage (Watson et al., 2017). For diets containing less than 40% forage, the NASEM (2016) model predicts a 2.2% decrease in MCP yield per 1% decrease in dietary peNDF < 20% (Russell et al., 1992). Rumen degradable protein requirement is then equal to MCP yield (NASEM 2016). The model then predicts the RUP requirement as the difference between the MP requirement and MCP yield and the predicted rumen bypass protein. Often, diets fed to beef cattle are sufficient in RDP to meet microbial requirements (Fu et al., 2001); however, cow-calf production systems often use low-quality forages that may not contain adequate RDP for maximum MCP yield (NASEM, 2016).

The CNCPS aims to further improve its model for MCP yield and protein digestibility through its AA sub-model. In this sub-model, ruminant AA requirements are calculated using a factorial method based on expected AA composition of tissue (William and Hewitt, 1979; Diaz et al., 2001; Tylutki et al., 2007) and milk (Fox et al., 2004), as well as the recommended methionine:lysine ratio for optimal milk production and ruminal microbial growth. The capability of feed to meet these requirements are based on efficiencies of utilization for individual AA. Data from O'Connor et al. (1993) was originally used to estimate the efficiency of AA use for pregnancy and lactation; however, coefficients of efficiency were revised using the ratio of uptake to output of individual AA by the gravid uterus based on Chung et al. (1998) and uptake/output of individual AA by the mammary gland summarized from experiments using dairy cattle as discussed by Fox et al. (2004). The estimated AA supplied by MCP yield, and protein fractions PA1, PB1, and PB2 are compared to the predicted AA requirements for maintenance, growth, lactation, and pregnancy to identify deficiency or excess. The CNCPS uses individual coefficients of AA efficiencies under the assumption that 100% MP is being supplied. Researchers (Doepel et al., 2004; Lapierre et al., 2007) have highlighted the biological correctness of using different efficiencies of AA utilization

for different physiological processes. Lapierre et al. (2007) has further extended this work to examine the importance of calculating coefficients of efficiency for individual AA at different levels of MP supply. Currently, the CNCPS model is continuing to use estimations of AA requirements based on 100% MP supplied under the assumption that the user will formulate rations to meet predicted requirements (Van Amburgh et al., 2015).

2.1.3 Contribution of Feeds Towards ME and MP Supply

The CNCPS model allows users to formulate rations using either solution level 1 or 2, dependent on the extent of knowledge of the chemical composition of feeds. Chemical composition for specific feeds can be determined though lab analysis, allowing users to utilize solution level 2 to formulate rations. Lab analysis reduces the variation in feed inputs that may arise from the sampling process and handling, preparation, and the assay (Hall and Mertens, 2012), meaning that users can expect their ration formulation to be closer to meeting animal requirements and predicting performance. However, it is not always possible to have chemical composition of all feeds and CNCPS is equipped with an extensive feed database that includes expected chemical composition of the feed (Higgs et al. 2015). The feed database was developed from the work of Van Soest (1994, 2015) and Sniffen et al. (1992), but many updates have been added since, including the addition of an AA database originating from the work of O'Connor et al. (1993). Currently, the CNCPS feed library includes approximately 800 feed ingredients, consisting of forages, concentrates, vitamins, minerals, and commercial products (Higgs et al. 2015). The developers of CNCPS regularly aim to adjust the feed library database so that database values are closer aligned with values generated from laboratories, allowing the model to better predict animal performance.

Calculations for carbohydrate and protein fractions are shown in Table 2.1 with some descriptions of analytical techniques. Recent changes to the characterizations of carbohydrate and protein pools have altered the CNCPS model predictions for ruminal degradation and post-ruminal digestion of each fraction (Van Amburgh et al. 2015). Within the original CNCPS model it was assumed that soluble carbohydrate and protein fractions were rapidly degraded within the rumen, so as no portion would reach the intestine for digestion (Russell et al., 1992). Carbohydrate fraction CA1 was predicted to have no contribution to microbial growth and MCP yield, but to be simply

absorbed across the rumen epithelium to contribute directly to the ME supply of the host (Lanzas et al., 2007a). Revisions to the CNCPS model assigned fraction CA1 to the solid phase of the rumen without accounting for the soluble properties of SCFA that suggest they are more appropriately assigned to the liquid phase. Van Amburgh et al. (2015) discusses the reassignment of soluble feed components to the liquid phase and consequential outflow to the intestine of components that were previously predicted to be 100% within the rumen environment. The remaining carbohydrate fractions CA2 to 4 and CB1 do contribute to microbial energy supply and their catabolism by microbes with the rumen environment produces SCFA that are available to the ruminant as a ME source after absorption across the rumen epithelium. Furthermore, microbial utilization of the NFC fraction is assumed to contribute to rapid microbial growth that utilizes primarily amino acids and, to a lesser extent, ammonia N. As the microbial community flows out the rumen, the MCP yield the contributes to the MP supply of the host with an assumed 80% true protein content and 80% digestibility.

The FC fractions CB2 and CB3 are expected to flow with the solid phase of the rumen environment and are fermented to varying degrees dependent on passage rate and form of structural carbohydrate. Cellulolytic bacteria ferment fractions CB2 and CB3 and utilize ammonia N for slow microbial growth that will eventually contribute to the host MP supply as MCP. Characterization of the degradability of the CB2 and CB3 fractions by aNDFom and uNDF240 was previously discussed.

Passage rate is essential in determining the extent of ruminal and intestinal digestion of feedstuffs and their contribution to ME and MP supply of the host. Owens and Goetsch (1986) discuss factors that affect passage rate, such as feed additives, particle size and processing of the feed, diet composition and feeding method, as well as animal variation and level of feed intake. Increased DMI can increase passage rate (Balch and Campling, 1965), allowing for an increase in energy and nutrient intake, though total tract digestion may be reduced (Firkins et al., 1998). Digestion is predicted to follow first-order kinetics and assumed to equal the rate of digestion as a proportion of combination of the rate of digestion and rate of passage [digestion = kd/(kd+kp)] (Russell et al, 1992; Sniffen et al., 1992; Van Amburgh et al. 2015). Thus, passage rate can significantly impact digestion of carbohydrate and protein fractions and affect the quantity of MCP flowing to the duodenum. Passage rate can be estimated in vivo by the recovery of internal or external markers (NASEM, 2016). However, CNCPS uses the passage rate predictions of Seo et

al. (2006) and reassigns the soluble fractions (CA1 to 4 and PA1 and 2) to the liquid phase passage rates within to better reflect the utilization of these fractions for fermentation and digestion (Van Amburg et al., 2015). Whereas protein fraction PA1 was originally thought to be composed of solely NPN and to be completely degraded in the rumen (Lanzas et al., 2007b), the CNCPS model now recognizes the contribution of peptides and free AA to PA1 (Higgs et al., 2015). The reassignment of fraction PA1 to the liquid passage rate reflects that the peptides and free AA may contribute to the MP supply (Givens and Rulquin, 2004) by as much as 10% of the dietary AA flowing to the small intestine (Choi et al., 2002; Reynal et al., 2007). The inclusion of this change decreases predictions of ammonia production and microbial yield and alters predictions of ruminal N requirements.

The insoluble protein and carbohydrate fractions, as well as the soluble fractions that bypass ruminal degradation by rate of passage, flow to the small intestine of the ruminant where they are subjected to intestinal digestion. Once again, extent of digestion is assumed to follow the previously described first-order kinetics. Bypass CB1 is digested by pancreatic amylases and brush-border disacharidases to contribute to the ME supply of the host as absorbed glucose. It is assumed that little digestion of CB2 and CB3 occurs within the small intestine due to lack of pancreatic cellulolytic enzyme production; however, fractions CB2 and CB3 may be fermented in the large intestine and cecum with minimal contribution to the ME supply of the host. Microbial crude protein, bypass PA1, the insoluble protein fractions PB1 and PB2 are hydrolyzed by proteolytic enzymes and absorbed as peptides and amino acids across the intestinal epithelium. After absorption, they undergo first-pass hepatic metabolism prior to contributing to peripheral MP supply. Within the CNCPS model, it is assumed that MCP is 80% CP to be 80% digested within the small intestine, that PA1 and PB1 are 100% digested within the intestine, and that PB2 is 80% digested (O'Connor et al., 1993). Assumption of these digestion constants limits the accuracy of the CNPCS model in predicting MP supply, as discussed in the previous section.

2.1.4 Predicting Nutrient Requirements

Accurate prediction of ME and MP requirements for ruminants is essential to formulating rations in order to optimize use of dietary ingredients in meeting cow nutrient requirements. Animal requirements are calculated in terms of net energy (**NE**) and protein (**NP**), then converted

to ME and MP to reflect the efficiency of conversion of feed energy and protein supply in meeting net requirements. Numerous models (National Research Council (**NRC**), Agricultural Research Council, Institut National de la Recherche Agronomique, Commonwealth Scientific and Industrial Research Organization) are available to predict requirements based from either empirical or mechanistic estimations of requirements (Tedeschi et al., 2015). NASEM (2016) uses an empirical level of solution that relies on tabular values for total digestible nutrient intake (**TDNI**) to predict MCP yield and RDP requirements relative to MCP yield, as well as a mechanistic level of solution that uses ruminal degradation kinetics for carbohydrate and protein fractions to calculate TDN, MCP and RUP requirements relative to feed intake and composition. However, CNCPS uses a mechanistic model to provide a quantitative estimation of cow requirements for both ME and MP that explicitly includes the effect of DMI (discussed in the following sections) on MP requirements and the supply of nutrients available within provided rations for meeting animal requirements.

2.1.4.1 Prediction of Metabolizable Protein Requirement

Metabolizable protein requirement is predicted using a factorial approach calculated from estimation of NP needed and efficiency of conversion of MP for maintenance (**MP_m**), growth, lactation, and pregnancy. Cow body weight (**BW**) is essential for prediction of maintenance, growth, and pregnancy requirements, and BW is standardized as equivalent shrunk body weight (**EqSBW**) for calculation and cross-reference of requirements between different breeds and growing animals. The maintenance MP requirement is calculated from excretion of urinary protein and scurf protein by shrunk BW, and metabolic fecal protein that is assumed to be 9% of indigestible DM (Tedeschi et al., 2015). The efficiency of conversion of MP_m to NP is assumed to be 67% and MP requirements account for this conversion when predicting the contribution of formulated rations to MP supply. Growth MP requirements are calculated using the same model as the beef NRC (2001) and are dependent on efficiency of use of EqSBW and NP required to be retained for growth after MP_m requirement is satisfied. Prediction of MP requirements for lactation are the same as those used by the NRC (2000; 2001). The prediction uses milk yield and true protein but varies by the coefficient used for efficiency of conversion from MP to NP. In contrast to beef cattle, dairy cattle are assumed to have an efficiency of conversion of MP to NP for lactation of 67%, whereas the efficiency of conversion is assumed to be 65% for beef cattle. For predicting MP required for pregnancy (**MP_p**), the CNCPS uses an identical equation for predicted MP_p to NP

between beef and dairy cattle. Dairy cattle are assumed to have an efficiency of conversion of 33%, whereas beef cattle are assumed to have an efficiency of 50% for conversion of MP to NP within CNCPS 6.5. Calculations for MP_p are based on calf birth weight and day of gestation to reflect the exponential growth of the fetus through gestation and the corresponding exponential increases in MP_p required.

2.1.4.2 Prediction of Metabolizable Energy Requirement

Similar to the predictions for MP requirements, CNCPS uses a factorial approach to estimate ME requirements from NE requirements and the efficiency of energy conversions. These predictions are computed from calculations of ME requirement for maintenance (**ME_m**), growth, pregnancy, and lactation, and can be adjusted for numerous factors within the CNCPS model.

Maintenance ME requirement varies drastically by BW, can be adjusted for breed, sex, level of activity, the energetic cost associated with heat or cold stress (adjusted based on surface area), physiological state, and the influence of environmental acclimatization (Fox and Tylutki, 1998). Body weight significantly affects the ME required for maintenance, as its reflects differences in organ mass and energy utilization as well as impacts the prediction of body reserves between growing and mature animals. The model also accounts for the impact of body condition score (**BCS**) as a way to estimate the impact of body reserves on the ME requirements by increasing or decreasing net energy of maintenance by 5% for each BCS above or below 5 when reported on a 1 to 9 scale. Body condition score is included within the model to represent the influence of previous plane of nutrition on organ size and the associated energetic costs (Fox et al., 2004). The energetic cost of urea synthesis is also included when diets provide protein in excess of predicted requirements to account for the increase in urea synthesis.

The ME required for growth is calculated within the model from BW, rate of BW gain, chemical composition of gain, and expected mature BW. To calculate chemical composition of gain, the CNCPS model standardizes shrunk BW (BW × 0.96; **SBW**) to the EqSBW of a standard reference animal at the same stage of growth (Tylutki et al., 2007). Equivalent shrunk body weight is calculated as SBW × (SRW/AFBW), where SRW is the SBW of the standard reference animal and AFBW is the expected SRW (Tylukti et al., 2007). Using an empirical model, the NEg is calculated using EqSBW × 0.891 and empty body gain as shrunk weight gain × 0.956. Using BW and net energy of maintenance, the net energy of growth can be used to predict average daily gain

allowing for estimation of NE required for the gain. Mammary gland requirements for ME and MP are estimated based on EqSBW to represent the effect of varying BW on mammary development. The model may not truly represent requirements for mammary development as the gland follows an exponential growth curve during the final trimester of gestation (Rattray et al., 1974; Swanson et al., 2008; Neville et al., 2013). Metabolizable energy requirements for pregnancy are estimated from conceptus growth, developed from the work of Bell et al. (1995) and the NRC (2000; 2001) as the day of gestation and expected calf birth weight. Weight of fetus and uteroplacental tissue is included to calculate energy requirements for conceptus growth and these requirements are added to ME requirements. Net energy of lactation requirements can be calculated from milk production and composition. The efficiency of conversion of ME to NE is estimated using a coefficient of 64.4% (Moe, 1981). Milk production and composition are not traditionally measured in beef cattle, so ME estimates are based on age of cow, expected time of peak lactation and peak milk yield (calculated from calf weaning weights, day of lactation, breed), milk fat content, milk solids not fat, and protein content (NRC, 2000).

2.1.5 Strengths and Limitations of the CNCPS Model

Previously, modelling nutrient requirements for ruminants relied on empirical predictions and factorial calculations to predict ruminal fermentation and intestinal digestion of feeds (Fox et al., 1992; Russell et al., 1992; Sniffen et al., 1992; O'Connor et al., 1993). Empirical predictions lack the accuracy and sensitivity of mechanistic models. The CNCPS uses a combination of empirical and mechanistic models to predict ruminal fermentation and intestinal digestion of carbohydrate and protein fractions and their contribution to ME and MP supply of the host. The dynamic rumen sub-model allows for prediction of carbohydrate digestion and SCFA supply through the use of digestion and passage rates for all carbohydrate fractions. The model also considers protein fraction degradation and passages rates relative to microbial growth and carbohydrate supply to prediction MCP yield and the RUP requirement to meet MP requirements. Ruminal pH is recognized to affect microbial growth and fiber digestion (Tylutki et al., 2007); however, the CNCPS model does not account for the rate of NFC degradation on ruminal pH, nor the influence of fluid dilution rate on SCFA wash-out from the rumen (Fox et al., 2004; Tylutki et al., 2007). Empirical calculations of the intestinal digestion of MCP are used in combination with

constant rates for intestinal digestion of protein fraction PA2, PB1, and PB2. These assumptions limit the sensitivity of the model as they do not consider protein composition or the AA supply for the host. Essential AA requirements are modelled in CNCPS and use different efficiencies of AA use for different physiological process. The use of AA-specific efficiencies allows users to formulate for specific levels of AA, but uses constant rates of efficiency of AA use under the assumption that 100% of MP is supplied. Under these conditions, users cannot predict AA requirements when MP is limiting or in excess supply. The CNCPS model has a high degree of accuracy in modelling nutrient requirements in a variety of production systems. The program allows users to use either empirical or mechanistic calculations depending on the level of knowledge of the chemical composition of the feeds. Furthermore, the CNCPS developers recognize the limitations of the model (Fox et al., 2004) and consistently revise and update the model (Lanzas et al., 2007a,b; Higgs et al., 2015; Van Amburgh et al., 2015) to improve predictions of animal requirements, ruminal fermentation dynamics, carbohydrate and protein degradation and absorption, ME and MP supply, nutrient excretion, and ultimately animal performance given a specific physiological state. The regular and ongoing updates are a particular strength of the CNCPS model.

2.2 Transitioning from Gestation to Lactation

In cow-calf production systems, the profitability and efficiency of the operation depends on the proper transition of beef heifers and cows from gestating to lactating. While the transition from gestation to lactation is not a common focal point of extension or research publications, a successful transition impacts progeny survival (Wittum et al., 1994a; Sanderson and Dargatz, 2000) and development (Wittum et al., 1994b; Wittum and Perino, 1995), as well as the subsequent concept rate (Richards et al., 1986; Bohnert et al., 2002). In contrast, for dairy cows, the transition period has been a focal point of research and is defined as the time extending from 3 weeks prior to and 3 weeks following parturition (Drackley, 1999). The transition period is a critical period with substantial physiological, hormonal, and behavioural changes that ultimately determines the success of the subsequent lactation (Grant and Albright, 1995). Although beef cattle likely experience similar but less dramatic changes, the term 'transition' is not commonly used or well defined with cow-calf production systems. Nevertheless, during the prepartum phase of the transition period, fetal mass increases exponentially (Du et al., 2010), mammary and colostrum development occur (Rattray et al., 1974; Ferrell et al., 1976; Neville et al., 2013), passage rate increases (Gunter et al., 1990; Hanks et al., 1993; Scheaffer et al., 2001), and overall organic nutrient metabolism changes (Bell, 1995) as the cow transitions from gestating to lactating. Prepartum maternal nutrition during late gestation has been reported to impact fetal development (Du et al., 2010), mammogenesis and colostrogenesis (Swanson et al., 2008; Meyers et al., 2011), and cow pre- and post-partum performance. Additionally, following parturition there is an increased nutrient requirement due to the lactogenesis (CNCPS 6.5; NASEM, 2016); however, relative to dairy cattle, less is known about how beef cows respond in terms of DMI, ruminal fermentation, and digestive characteristics during the early postpartum period. The lack of scientific literature highlights the uncertainty that beef cows are capable of consuming adequate nutrients to meet the nutrient demands of lactation.

2.2.1 Gestation and Fetal Development

Gestation begins with the fertilization of oocytes, implantation of the oocyte into the uterine wall, and embryonic development. To prevent early embryonic loss and ensure embryonic

development, uterine function must be properly established and maintained (Bridges et al., 2013). The connection between poor maternal nutrition during late gestation and early lactation and failure to conceive or maintain pregnancy has been well-characterized (Murphy et al., 1991; Mackey et al., 1999; Bridges et al., 2013). Thus, research has focused on gestational and early lactation nutritional strategies to improve postpartum reproductive performance and conception rates.

The first trimester of gestation is characterized by placental growth and differentiation, as well as fetal organogenesis, indicating a sensitive time-period of gestation to nutritional insults dependent on their severity (Funston et al., 2010). Ruminants have a unique cotyledonary placenta where cotyledons and caruncles form a unit termed the placentome that facilitates physiological exchanges of nutrients and gases between the dam and the fetus (Reynolds and Redmer, 1995; 2001). Uteroplacental development occurs early during gestation (Patten, 1964; Ramsey, 1982) with the growth rate slowing during mid- to late-gestation (Ferrell et al., 1976; Reynolds and Redmer; 1995). Fetal organogenesis occurs during the first trimester in coordination with placental development, but follows a sigmoidal growth pattern as opposed to a linear growth curve (Richardson et al., 1991). Prioritization of organ system development leads to altered post-conception initiation of differentiation and development, as well as rate of development (Hubbert et al., 1972; Richardson et al., 1991). Severe nutrient restrictions can affect fetal and uteroplacental development and function. But, overall accumulation of nitrogen and energy by the gravid uterus causes during early- to mid-gestation minimal disruption to maternal nutrient supply, especially relative to late-gestation when maternal nutrient requirements are estimated to be 75% greater than those of a non-pregnant cow (Bauman and Currie, 1980).

Although uteroplacental growth slows, fetal nutrient requirements increase as the fetus grows exponentially during the third trimester (Redmer et al., 2004). A review by Du et al. (2010) discussed the nutritional controls of skeletal muscle development in ruminants with an emphasis on the concept of developmental programming (Barker et al., 1993; Godfrey and Barker, 2000). Briefly, the initiation of skeletal muscle development occurs during early gestation (Cossu and Borello, 1999) when mesenchymal stems cells are signalled by neighboring tissues to the myogenic lineage (Kollias and McDermott, 2008). Following the initiation of skeletal muscle development, fetal body composition changes in response to four distinct development phases: 1) primary myogenesis during early gestation; 2) secondary myogenesis during mid gestation; 3)

adipogenesis beginning around 4 months of gestation; and 4) muscle fiber hypertrophy beginning around 6 months of gestation (Du et al., 2010). In contrast to adipose tissue, muscle fiber hyperplasia does not occur postnatal (Stickland, 1978; Karunaratne et al., 2005), highlighting the importance of the maternal nutrient supply during early- to mid-gestation (Zhu et al., 2004). Adipogenesis and muscle fiber hypertrophy, collectively, account for the exponential increase in fetal mass during late gestation that has been estimated to represent 60 (Bauman and Currie, 1980) to 75% (Robinson et al., 1977) of fetal mass. Relative to muscle accumulation, the contribution of adipogenesis during mid- to late-gestation to fetal mass is minimal (Prior and Laster, 1979), suggesting that the maternal AA contribution to the developing fetus is substantial and potentially limiting. Additionally, it is estimated that 30 to 50% of fetal oxidative substrates are derived from the catabolism of AA (Bauman and Currie, 1980). Thus, maternal supplies must provide AA for the concurrent increases in fetal nitrogen accretion and oxidative capacity. During the late gestation transition phase, catabolism of skeletal muscle reserves has been demonstrated in dairy cows (Chibisa et al., 2008) in response to increased fetal AA demand and inadequate dietary supply of MP. In beef cattle, skeletal muscle catabolism has been demonstrated in periods of mild nutrient restriction (Du et al., 2005; Wood et al., 2013), but the magnitude of late gestation catabolic state has not been characterized in beef cows.

Maternal metabolizable nutrient supply for fetal demand is dependent on DMI, ration nutrient density, ruminal fermentation dynamics, and the digestibility and absorption of major fermentative and digestive end-products. Late gestation DMI and digestive characteristics in beef cows has been partially characterized. Early studies reported that particulate and fluid passage rates were greater in late-gestation ewes compared to nonpregnant (**NPr**) ewes, with no impact on ruminal fermentation or total tract digestibility (Gunter et al., 1990). Hanks et al. (1993) reported similar findings, demonstrating that particulate and fluid passage rate was greater and ruminal retention times and gastrointestinal (**GIT**) fill were less for pregnant (**PR**) cows in comparison to NPr cows, with no differences in DMI and total tract digestibility. However, both studies (Gunter et al., 1990; Hanks et al., 1993) had used 4 ruminants for both PR and NPr groups, indicating that statistical power was limited in detecting differences in total tract digestibility and DMI. Conversely, many authors have found that DMI decreases during late gestation (Stanley et al., 1993; Allen, 1996; Scheaffer et al., 2001), as the ratio of fetal:GIT mass within the visceral cavity increases. Prepartum decreased DMI can cause BW loss pre-calving and poor performance during

the postpartum period (Godfrey et al., 1988). More recent work evaluated the effect of gestation on forage intake, digestion, and passage rates, including the effect of parity within the model (Linden et al., 2015). These authors found DMI increased for PR heifers until two weeks prepartum, at which point it declined. In contrast, multiparous cows did not alter their DMI as parturition approached (Linden et al., 2015). Additionally, DM digestibility was observed to decrease with advancing gestation, but was still greater than for NPr cows and heifers. Coinciding with the results of Gunter et al. (1990) and Hanks et al. (1993), Linden et al. (2015) found that passage rate was increased in PR animals. Collectively the data suggests that passage rate increases, consequently altering ruminal degradation and total tract digestion during the late gestation transition period; although, parity, differences in diet composition and predicted DMI must be accounted for in modelling changes in transition cow feeding behaviour and organic nutrient metabolism.

As discussed in the previous section, CNCPS 6.5 predicts nutrient requirements for late gestation using estimates of fetal and mammary MP and ME requirements. The MP and ME required for fetal growth includes associated uterine tissues and bases predictions from calf birth weight and day of gestation to reflect the exponential growth curve, whereas the MP and ME required for mammary development are based on EqSBW and do not account for the exponential growth and demand of the mammary gland (discussed in the following section). In the case of heifers, the model adds requirements for growth to reach an estimated mature BW to the total ME and MP requirements. Yet, the model fails to account for increasing fetal oxidative metabolism and transitional changes. The CNCPS model uses the pre-determined values for prediction of passage rate and degradation of CHO and protein based on feed nutrient composition to estimate the contribution of feeds towards ME and MP supplied. This approach neglects the observed changes in passage rate, ruminal degradation, and nutrient digestion that are observed during late gestation (Allen, 1996; Scheaffer et al., 2001; Linden et al., 2015) and the ensued alterations to available ME and MP supply from feed. Therefore, it is possible that the CNCPS model may underestimate nutrient requirements during late gestation and overestimate the dam's capacity for efficient ME and MP capture from supplied rations. Yet, no literature is available as to whether or not beef cattle experience a nitrogen or energy deficiency during late gestation, indicating a knowledge gap in our understanding of transition beef cattle.

2.2.2 Mammary Development, Colostrogenesis, and the onset of Lactation

In heifer fetuses, development of the mammary gland begins during early gestation, but neonatal mammary development is minimal and post-natal development of the mammary gland does not progress until attainment of puberty (Anderson, 1975). At this time, nutritional plane can have large impacts on future milk production. Past studies have reported that feeding Holstein heifers diets with a high energy to protein ratio can increase adiposity in the mammary gland and decrease lifetime milk production (Reid et al., 1957; Swanson et al., 1957).

In addition to development in response to puberty, numerous authors have demonstrated in ewe and beef models that mammary development of the dam increases dramatically with increase in fetal mass (Rattray et al., 1974; Ferrell et al., 1976; Neville et al., 2013) However, the demand that mammogenesis instills on maternal nutrient requirements during late gestation is often considered to be negligible (Bauman and Currie, 1980), despite the increase in cell proliferation and differentiation and associated protein and energy requirements during this time-period. Mammary gland energy accretion is reported to increase by 205 and 293% from d 70 to 140 for single- and twin-bearing ewes respectively (Rattray et al., 1974), while total nitrogen content increased by from 1.98 to 2.76% between d 70 and 140. In beef heifers, total nitrogen content of the mammary gland has been observed to increase by 152% from d 237 to 264 of gestation representing 67 and 102 g of nitrogen, respectively in primiparous beef heifers (Ferrell et al., 1976). This is much greater than the estimated 37 g of nitrogen for open heifers (Ferrell et al., 1976). Other research has demonstrated that global nutrient restriction during late gestation decreases mammary gland weight in ewes (Swanson et al., 2008; Neville et al., 2013). However, results on mammary development when ewes are fed in excess of requirements are conflicting. Neville et al. (2013) found that ewes fed in excess (overfed by 40%) had greater mammary gland weight in comparison to control and restricted (underfed by 40%) groups; whereas, Swanson et al. (2008) demonstrated that mammary gland weight did not differ between control (100% of total requirements) and excess (40% greater than requirements) ewes. When mammary weight was standardized by BW, restricted and excess ewes had decreased mammary gland weight per unit of empty BW (**EBW**) relative to the control ewes (Swanson et al., 2008). Yet, Neville et al. (2013) saw no differences in relative mammary gland weight between restricted, control, and excess ewes. By 20 d post-parturition, the differences in total mammary gland weight and mammary gland

weight as a proportion of EBW due to prepartum treatment were not detected (Neville et al., 2013). The lack of differences occurring in early lactation (Neville et al., 2013) suggests that lamb milk demand may compensate for the limited or delayed mammary gland weight occurring due to inadequate or excessive prepartum maternal nutrition. However, postpartum correction of mammary gland development will increase maternal nutrient requirements simultaneously with the increase in requirement due to lactogenesis.

Colostrogenesis must occur during late gestation simultaneously with mammogenesis, yet the rate and initiation of colostrogenesis relative to mammogenesis has not been quantified. Colostrum yield and quality are critical as colostrum functions as an energy and nutrient bolus for the neonate. Relative to milk secretions, colostrum composition is generally high in fat, protein, vitamin A, and urea-N, and decreased in lactose content. Total solids in colostrum range between 21 to 27% in contrast to 12 to 13% in regular milk for dairy cows (Jaster, 2005). The high nutrient content of colostrum represents a significant demand of metabolizable substrates from maternal supplies during late gestation. Furthermore, unlike other mammalian species, ruminant neonatal immunity is derived postnatally from immunoglobulins (**Ig**) within the colostrum (Khalaf et al., 1979), as opposed to in utero placental transfer of immunity. Colostrum Ig content accounts for more than 90% of the total protein content (Butler, 1974; Larson, 1992). Dairy cattle colostrum contains 6.0% Ig in the first milking, thereafter decreasing as the cow transitions from colostrum to milk production (Godden, 2008). Transfer of immunity is complicated by the fact that neonatal ruminants experience gut-closure that begins as early as 6 to 12 h and ceases passive transfer by 24 h of life (McCoy et al., 1970; Stott et al., 1979a; Jaster, 2005), limiting the time-frame in which immunoglobulins, among other bioactive compounds, can cross the intestinal epithelium. It is recommended that calves consume 2 to 3 L of colostrum within the first 6 h postnatal and passive transfer failure is defined when calf serum IgG concentrations are less than 10 mg/mL (Godden, 2008). Therefore, sufficient colostrum yield and Ig concentrations delivered in a timely manner are critical for establishment of adequate passive immunity.

Early studies examining the impact of maternal nutrient restriction, using either sheep or beef models, on colostrum quantity (McCance, 1959; McCance and Alexander, 1959; Meyers et al., 2011) and quality (Mellor and Murray, 1985; Mellor et al., 1987; Meyers et al., 2011) have found that nutrient restricted groups have decreased colostrum production and quality. Most models have focused on the effect of undernutrition; however, Meyers et al. 2011 found that ewes

fed at 140% of requirements had decreased colostrum yield relative to ewes fed at 100% of requirements. Similar results were observed by Swanson et al. (2008). In terms of quality, ewes fed in excess had greater non-fat solids than restricted ewes, but restricted ewes had increased fat content relative to excess and control treatments. Ewes fed control had greater fat, lactose, and protein yield than restricted (60% of requirements) and excess (Meyers et al., 2011). Moreover, Swanson et al. (2008) found that total IgG yield tended to be lower for restricted and excess compared to the control due to decreased colostrum yield while Ig composition remained the same. Decreased IgG yield due to maternal over-supplementation has been previously demonstrated (Wallace et al., 2006). However, the previously discussed experimental models focused solely on global dietary nutrient profiles. To the author's knowledge, no studies have specifically evaluated the impact of CP or MP supplementation on mammary development and colostrogenesis. The increased nutrient demand of the mammary gland during late gestation due to colostrogenesis may indicate that increased AA supply could improve quality and yield; however, these results indicate that contrary to expectations, over- vs. adequate-global supply during the close-up prepartum period may decrease colostrum yield and quality. Though these have been modeled within ewes and in dairy cattle in terms of Ig content and yield, research covering beef cattle is limited.

2.2.3 Lactation

Milk production and composition following parturition is essential to determining extent of nutrient demand and for lactation. In dairy cattle, the lactation curve and expected milk composition is well characterized (NRC, 2001), but similar measurements and characterization is limited for beef cattle due to the nature of the cow-calf production system. Beef cattle are generally maintained on pasture with milk being consumed by the calf. The technical constraints associated with extensive management of beef cattle have hindered characterization of the beef cow lactation. Milk production and component yield are of particular importance during the transition period as cows have concurrent nutritional demands for other physiological processes. The nutrient demands include, but are not limited to, uterine involution, resumption of cyclic estrous activity, and subsequent conception. Despite the extensive production system, researchers (detailed in NASEM, 2016) have attempted to measure milk yield in beef cows to evaluate breed effects and for the estimation of milk yield as a factor determining pre-weaning growth of the calf. Researchers have

used numerous approaches to determine milk production in beef cows (Anthony et al., 1959; Lamond et al., 1969; Totusek et al., 1973), but the most common approach is the weigh-suckle-weigh technique (Knapp and Black, 1941). For the weigh-suckle-weigh approach, the cow and calf are separated for a period of time that varyies in duration from 4 (Williams et al., 1979) to 19 h (Beal and Akers, 1990). To estimate milk production, the calf is weighed prior to and following being allowed access to the dam once the separation interval is complete. The difference in calf BW between the two measurements is assumed to equal milk yield. While the weigh-suckle-weigh approach allows for estimation of milk production, it makes the assumptions that the calf consumes all the milk produced and that calf BW is only affected by milk consumption. Milk composition cannot be analyzed using this technique. Therefore, the weigh-suckle-weigh approach does not provide adequate information about the nutrient and energy expenditure arising from lactation.

Lactation curves are commonly estimated using an equation developed by Wood (1980) for dairy cattle. The Wood equation requires information like breed, parity, and the stage of lactation. However, few data points for beef cattle milk production limits the use of the Wood's equation to estimate beef cow milk production throughout lactation. The NASEM (2016) recognizes that beef cow milk production is dissimilar to dairy cattle (distinguished by lesser milk production and differing milk composition) and uses an equation developed by Jenkins and Ferrell (1984) that can be fit using fewer data points. The equation calculates a generalized lactation curve and predicts daily milk yield using week of lactation, dam age, parity, and adjusts for estimated peak milk yield. Using the Jenkins and Ferrell's (1984) equation and data from previous research, NASEM (2016) predicts peak milk yield (6 to 12 kg/d, dependent on breed) to occur at 8.5 weeks post-parturition and assumes that peak and yield are relative to calf nutrient demand. The NASEM (2016) model assumes that calves consume their complete requirements from milk, but researchers recognize that forage intake will comprise a significant portion (forage intake equal to 1.5% of BW) of the calf's diet as young as 60 d of age (Lusby et al., 1976; Wyatt et al., 1977; Boggs et al., 1980). In contrast to NASEM (2016), CNCPS 6.5 predicts beef cow MP and ME requirements, based on a dairy cow model using Wood's equation (1980), adjusting for age of cow, breed, and expected milk composition. When predicting nutrient requirements, the CNCPS 6.5 model allows the user to adjust expected milk composition for fat, protein, and lactose content. This option is also available with NASEM (2016) model. Beef cow milk composition will vary with average fat (3.5 to 4.0% milk fat), protein (3.3 to 3.8% milk protein), and lactose percentage dependent on

breed (assumed to be straightbred cattle) and expected milk yield. Total non-fat solids are expected to be 8.3% and consistent between breeds (NASEM, 2016). The CNCPS model calculates NE expenditure for milk production as the heat of combustion for fat, protein, and lactose by the nutrient composition (NE_L, Mcal/kg = (0.0929 × fat %) + (0.0547 × protein %) + (0.0395 × lactose %), whereas NASEM (2016) accounts for the heat of combustion of milk fat and milk solids-not-fat (NE_L, Mcal/kg = (0.092 × fat %) + (0.049 × MkSNF – 0.0569) in predicting NE_L. Net energy is then converted to ME to reflect the efficiency of energy utilization. Both the CNCPS and NASEM models use an efficiency of 65% for conversion of net protein to MP required for lactation (MP_L, kg/week = Total yield/0.65). Therefore, maternal nutrient requirements for lactation, with particular focus on the postpartum transition phase, are determined by the level of milk yield and milk component yield.

As previously discussed, nutritional plane during the prepartum transition period can alter mammogenesis and colostrogenesis. Few researchers have evaluated the impact of transition period nutrition on subsequent beef cow milking performance, but some authors have found, using an ewe model, that prepartum nutrition can impact lactation. Meyer et al. (2011) found that restricted ewes had lower milk yield and total solids-not-fat, lactose, protein, and milk urea-N in comparison to control and over-supplied ewes, with no differences observed between control and over-supplied ewes. However, in contrast, the percentage of proliferating alveolar cells was greater for over-supplied ewes in comparison to restricted and control ewes (Swanson et al., 2008), suggesting that over-supplied ewes potentially have a capacity to increase milk production in response to lamb demand. Substrate supply for milk synthesis is regulated by mammary gland blood flow and restriction of substrate supply has been shown to alter mammary metabolic activity (Davis and Collier, 1985); thus, milk and milk component yield are bound to be regulated by maternal nutrient supply in conjunction with blood flow. Collectively, the data indicates that nutrition during the prepartum transition phase in combination with nutrient and substrate availability during the postpartum transition phase can at least partially program postpartum milking performance with production being strongly correlated with progeny demand.

Knowledge of nutrient intake and digestion characteristics in early lactation beef cattle is similarly limited as the knowledge of the prepartum characteristics. To the author's knowledge, one study has evaluated ruminal fermentation characteristics and digesta kinetics, using the ewe as a model in comparison to non-lactating ewes. Gunter et al. (1990) found that digesta kinetics

did not vary between lactating and non-lactating ewes; however, isobutyrate, isovalerate and serum urea-N concentrations were lower in lactating ewes, indicating minor changes in ruminal fermentation dynamics and potentially AA demand during lactation. Gastrointestinal fill was less, yet DM digestion was greater for lactating ewes. In dairy cows, it is well-established that DMI and digestion decrease sharply as parturition approaches and remains depressed for start of the postpartum phase but recovers quickly (Lean et al., 2013). The depression in DMI during the transition phase causes the dairy cow to be in a state of negative energy and protein balance relative to nutrient demands for lactation. Homeorhetic mobilization of maternal fat and protein reserves occurs to offset the negative energy and protein balance in order to meet requirements. Typical dairy cattle transition phase adaptations include: increased skeletal muscle catabolism, increased lipolysis, decreased lipogenesis, increased gluconeogenesis, increased glycogenolysis, increased oxidation of NEFA and decreased oxidation of glucose as an energy source (Bauman and Currie, 1980). The dramatic disruptions in organic nutrient metabolism result in the dairy cow being susceptible to interrelated metabolic disorders (Lean et al., 2013), such as ketosis, fatty liver, pregnancy toxemia, retained fetal membranes and metritus, and poor fertility and reproduction.

Though the dairy cow is an extreme example, it is plausible that beef cattle would experience similar changes. Over- and under-nutrition in beef cows during late gestation can cause changes in BW, increase risk for dystocia, increase risk for retained placenta and metritis, and impact the postpartum interval. During early lactation, decreased DMI can affect the ME and MP supply and nutrient availability for lactation causing cows to lose BW and BCS, lengthen the postpartum interval and decrease conception rates. Global nutrient and CP supplementation in excess of requirements and the effect of MP supplementation in excess of requirements (140%; Van Emon et al., 2014; 2015) on performance in ewes during the postpartum transition phase have been studied. Supplementation of CP prepartum can improve cow BW and BCS at calving (Bohnert et al., 2013) and may be a more effective strategy for maintaining appropriate BW and BCS postpartum to decrease the length of time until first estrous. However, little is known regarding digestive kinetics during late gestation and early lactation and their subsequent effects on organic nutrient metabolism.

2.3 Metabolizable Protein Status during the Transition Period

2.3.1 Ruminant Nitrogen Metabolism

Feed CP consists of RDP and RUP fractions that differ in their availability for microbial proteolytic activity. Rumen undegradable protein is either unavailable for microbial proteolytic degradation or passes through the rumen prior to complete degradation thereby allowing for digestion and absorption in the abomasum and small intestine. In contrast, rumen degradable protein is available for proteolysis with peptides, AA, SCFA, and ammonia being produced as fermentative end-products. The rumen microbial population will attach to undigested feed particles (Craig et al., 1987) and begin protein degradation by the activity of cell-bound microbial proteases (Brock et al., 1982). Microbes absorb the resulting AA and peptides where intracellular peptidase activity degrade peptides to AA that can be used for synthesis of microbial protein or deaminated to SCFA, CO_2, and ammonia (Tamminga, 1979; Figure 2.3). Energy availability from carbohydrates influence whether AA will be used for microbial growth or transaminated. A greater proportion of the absorbed AA are deaminated when energy is limiting. The carbon skeleton is fermented to yield adenosine triphosphate (**ATP**) and SCFA (Bach et al., 2005). The resulting NH_3-N produced from deamination can be utilized for microbial protein synthesis with cellulolytic bacteria predominantly utilizing NH_3-N as opposed to AA (Bach et al., 2005). Degradation of NPN, such as urea and nucleic acids, also contributes to the rumen ammonia pool as NPN is degraded rapidly and extensively within the rumen environment to NH_3 (Leng and Nolan, 1984). When NH_3-N concentration is in excess it will either pass out of the rumen with the fluid phase or be absorbed across the ruminal epithelium and converted to urea in the liver (Tan and Murphy, 2004). Urea recycling is central to ruminant nitrogen metabolism. The NH_3 is absorbed across the epithelium of the rumen, small intestine, and large intestine to collectively enter the liver through the portal vein, where 70 to 95% of the ammonia will be extracted (Tan and Murphy, 2004) and detoxified by conversion to urea or glutamine through the ornithine cycle (Wright, 1995). It has been reported that 93.5 and 6% of the portal NH_3-N will be converted to urea-N and glutamine, respectively (Lobley et al., 1995), stressing the importance of converting NH_3 to urea as the major pathway for NH_3 detoxification. The liver returns urea to arterial circulation where it can be recycled back into the lumen of the gastrointestinal tract (**GIT**) and utilized for production of

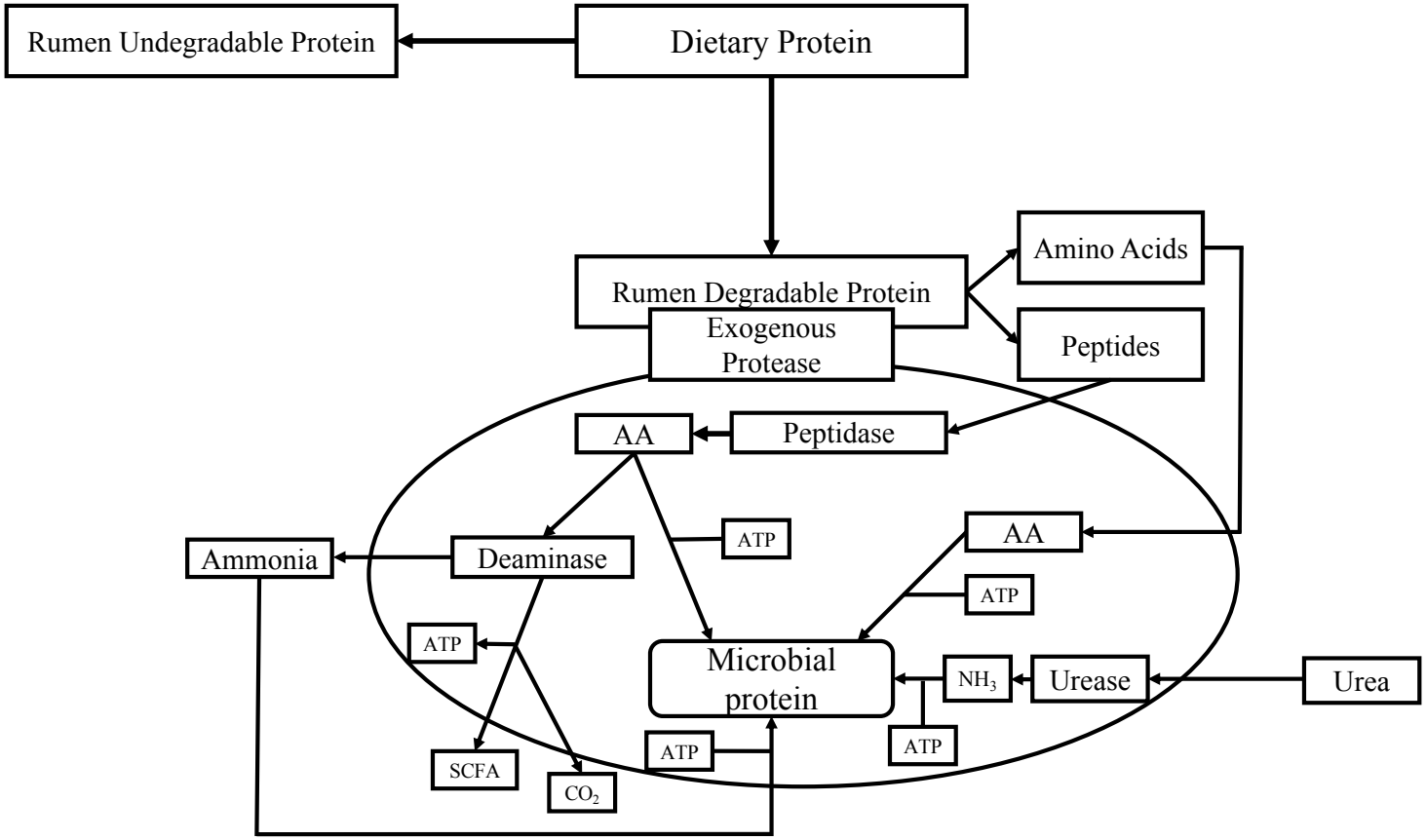

Figure 2.3 Schematic representation of process of degradation of ruminally degradable protein and pathways to synthesize microbial crude protein. Adapted from Bach et al. (2005).

microbial protein. Approximately 40 to 80% of the urea produced will be returned to the GIT, with an estimated 27 to 60% of the urea entering the rumen (Lapierre and Lobley, 2001) and the remainder being recycled to the hindgut. Additionally, urea is recycled to saliva, where urea-N represents 80 to 85% of the total salivary nitrogen (Phillipson and Mangan, 1959), and returned to the GIT via saliva. One mole of recycled urea is degraded to 1 mol of CO_2 and 2 moles of NH_3-N, the latter of which being salvaged for microbial protein production (Figure 2.3). Microbes are washed-out of the rumen within the fluid phase or attached to outflowing feed particles to be digested downstream in the small intestine by the activity of pancreatic proteolytic enzymes. Endogenous secretions and RUP fractions are additionally digested. Collectively, the digestion and absorption of microbial CP, endogenous secretions, rumen bypass protein and undegradable protein are absorbed as AA or peptides to contribute to the MP pool available for the ruminant.

2.3.2 Nitrogen Partitioning during the Transition Period

The nitrogenous substrates that enter the small intestine for digestion and absorption are derived from MCP, endogenous losses, soluble protein fractions (PA2) that wash-out of the rumen prior to degradation, and insoluble protein fractions (PB1 and PB2) that are not degraded in the rumen (RUP). As discussed in a the CNCPS sub-chapter, MCP and endogenous protein losses are assumed to be 80% CP and 80% intestinally digested and absorbed. Bypass soluble protein and fraction PB1 of the insoluble fractions are assumed to be 100% digested and absorbed, and the insoluble fraction PB2 is assumed to be 80% digested and absorbed. Collectively, these AA sources contribute to the MP supply of the ruminant. Metabolizable protein is defined as the true protein that is digested and absorbed as peptides and amino acids (NASEM, 2016). The MP supply can then be partitioned to satisfy requirements for maintenance, growth, pregnancy, lactation, and return to estrous and conception (listed in approximate order of priority, dependent on the ruminant's physiological state; NASEM, 2016). However, AA partitioning is described to be regulated by two antagonistic processes, homeostasis and homeorhesis during late gestation or lactation (Bauman and Currie, 1980). Homeostasis is the maintenance of physiological equilibrium, or 'steady-state', whereas homeorhesis is the opposing process the prioritization of AA partitioning to support other physiological states such as gestation or lactation.

Amino acids are required during late gestation for maintenance of tissues and organ systems, as well as fetal development, uteroplacental function, mammary development, and colostrogenesis. Beef heifers have additional requirements for growth, as they are typically bred at 55 to 65% of their mature BW and expected to gain between 30 to 35% of their mature BW by parturition (Larson, 2007). The CNCPS model predicts requirements for maintenance using a factorial method based on the excretion of urinary, scurf, and fecal protein and when standardized for metabolic BW to reflect protein-turnover by various tissues and organ systems. For growing ruminants, protein requirements for growth are estimated by modelling the relationship between stage of growth (percentage of the mature BW), rate of gain, and the composition of gain as either fat or protein accretion. As heifers mature, the chemical composition of body tissues changes, reflecting the increased rate of deposition of adipose tissue relative to skeletal tissue (Owens et al., 1995). The AA required for partitioning to gestation is calculated from the estimated fetal AA requirement for myogenesis, late gestation muscle fiber hypertrophy, the increase in fetal oxidative metabolism AA requirement, and the AA requirement for uteroplacental function and associated amniotic fluids. The estimation of MP required for gestation is calculated (using an exponential model) from birth BW and day of gestation (Tedeschi et al., 2015). In contrast, mammogenesis AA requirements and partitioning are estimated using a linear model, though past research demonstrates that mammary gland development follows an exponential growth pattern similar to the fetus (Rattray et al., 1974; Ferrell et al., 1976; Neville et al., 2013). Following parturition, AA is partitioned to and required for lactogenesis for maintenance of mammary tissues as well as formation of milk proteins. Requirements of AA for lactation is based from predicted milk yield, expected milk protein composition, and thus, milk protein yield. During the early postpartum period, AA will be partitioned to allow for uterine involution, the resumption of estrous, and subsequent conception.

The process of homeostasis suggests that beef cattle should partition AA to maintenance prior to partitioning for maintenance of pregnancy, mammogenesis, lactation, return to cyclic estrous activity and conception. However, the concept of and evidence for homeorhesis suggests that during late gestation and early lactation there is indeed a reprioritization of AA partitioning to meet requirements for those physiological processes. In dairy cows, skeletal muscle mobilization (using plasma 3-methylhistidine (**3-MH**) as a marker of degradation) has been observed during the 4 weeks prior to parturition (van der Drift et al., 2012) and shown to slow when methionine is

supplemented (Philips et al., 2003). In beef cattle, Du et al. (2005) and Wood et al. (2013) both demonstrated that mild nutrient restriction during mid- to late-gestation will increase abundance of ubiquitin (a marker for protein catabolism) in maternal skeletal muscle tissue. More importantly, the increase in ubiquinylation was not observed within fetal tissues (Du et al., 2005), suggesting that the dam repartitioned AA towards fetal growth and catabolized her own skeletal protein reserves to meet fetal AA requirements. The increase in skeletal muscle mobilization during the transition period has also been shown by Chibisa et al. (2008) who evaluated gene expression profiles for major protein degradation pathways in skeletal muscle using transition dairy cattle. Abundance of mRNA for m- and μ-calpain and ubiquitin was increased at d 15 compared to -14 and 38 relative to parturition. Moreover, it was reported that the dairy cattle mobilized 14 kg of body protein between d -14 and 38 (Chibisa et al., 2008), demonstrating the degree of homeorhetic muscle catabolism to meet N requirements for other physiological processes.

Muscle degradation is a continuous protein process that occurs through three main pathways: the lysosomal pathway; the cytosolic protease pathway via calpains and caspases; and the ubiquitin-proteasome pathway (Lecker et al., 1999). The lysosomal pathway primarily degrades extracellular proteins, such as plasma proteins, hormones, and cell-surface receptors through endocytosis of the proteins across the cell membrane and proteolysis within the lysosome. The lysosomal pathway does not have a major role in turn-over of cytosolic proteins (Furuno and Goldberg, 1986; Lowell et al., 1986). In contrast, calpains and caspases are both cytosolic cysteine proteases that respectively respond to either cell injury (Goll et al., 1992) or toxic stimuli that signal apoptosis (Salvesen and Dixit, 1997). Lastly, the ubiquitin-proteasome pathway is responsible for the majority of protein turnover within the cell. The protein is first marked for degradation by covalent attachment of the ubiquitin molecule that catalyzes the attachment of the proteasome sub-unit (Kisselev et al., 1998). The proteasome sub-unit is responsible for proteolysis of the protein to peptides and AA and their release into the cytosol. Upregulation of the ubiquitin-proteasome pathway can occur when in a state of negative energy or protein balance.

Excessive mobilization of adipose and skeletal tissue is correlated with incidence of postpartum metabolic disease in dairy cattle (Drackley, 1999). Increased delivery of protein in prepartum diets has been shown decrease incidence of ketosis (Curtis et al., 1985; Van Saun, 1993) during the postpartum transition period. Using blood albumin as a proxy for transition cow protein status has indicated that decreased blood albumin concentrations (≤ 35 g/L) pre- and postpartum

are associated with greater incidence of postpartum metabolic disease whereas albumin concentration ≥ 35 g/L are indicative of healthy fresh cows (Van Saun and Sniffen, 2016). Blood albumin concentration can reflect protein status, but can be misleading as an indicator since it also decreases during periods of liver dysfunction and active inflammatory responses (Bertoni et al., 2008; Overton and Burhans, 2013). Thus, caution is needed when interpreting blood albumin as an indicator of overall protein status. The early postpartum period is marked by inflammatory responses as the cow transitions between gestating to lactating and furthermore, metabolic diseases such as fatty-liver syndrome can cause liver dysfunction. Excessive mobilization of non-esterified fatty acids (**NEFA**) during the transition period to meet energy requirements for gestation and lactation can cause build-up of triglyceride deposition in hepatocytes to impair liver function. Furthermore, dairy cows will catabolize skeletal muscle tissue postpartum to mitigate deficiencies in AA supply and support lactation requirements (Bell et al., 1995). Myocyte diameter has been observed to decrease by 25% immediately following parturition (Reid et al., 1980) and estimates suggest that 25 to 27% of skeletal protein reserves (10 to 17 kg total body protein; Parquay et al., 1972; Belyea et al., 1978; Botts et al., 1979) can be mobilized during the postpartum transition phase. Early lactation skeletal catabolism directs AA to the mammary gland for maintenance and milk protein synthesis (Lean et al., 2013), as well as the liver as gluconeogenic precursors to support energy and mammary glucose demands (Aschenbach et al., 2010).

The dairy cow is well-recognized as experiencing severe metabolic strain due to the extensive organic nutrient metabolism and repartitioning during late gestation and early lactation and is an extreme model for homeorhetic AA partitioning during the transition period. However, these homeorhetic responses have been indirectly modeled in prepartum beef cattle during global nutrient restriction in either mid- or late-gestation. Researchers have focused on the response of beef cows to nutrient and protein supplementation or restriction usually in terms of BW and BCS, and their subsequent impact on calving success, progeny development, and reproductive performance by length of the postpartum interval (**PPI**) and conception rates. Although the previously described performance responses are not sensitive measures, less attention has been given to metabolic indicators of nutrition stress or protein status and impact on early lactation milk production and component yield. These transition phase characteristics are yet to be thoroughly described in the beef cattle, though extensive knowledge of beef cattle performance responses to CP supplementation exists.

2.3.3 Effect of Protein Supplementation on Transition Beef Cow Performance

Cow-calf production systems maintain cattle in extensive grazing systems, usually on pasture, corn-residues, or swath-grazing through the final trimester prior to calving. Concerns with maintaining cattle on pasture are mainly related to pasture quality that can vary dramatically due to the forage source and growing season conditions. Producers may supplement in order to meet nutrient requirements during late gestation and improve cow-calf performance. The effects of protein supplementation relative to restriction or requirements on cow BW, BCS, PPI and conception rates have been studied extensively (Stalker et al., 2007; Martin et al., 2007; Lents et al., 2008) and will be discussed in the following paragraph. Regularly, supplementation strategies focus on either CP (Martson et al., 1995; Lents et al., 2008; Larson et al., 2009) or high-protein by-product feeds (Bohnert et al., 2013; Summer et al., 2015a; Shoup et al., 2015a). The focus on use of CP or high-protein by-product feeds makes it difficult to draw conclusions on cow responses to protein supplementation as they provide little detail on the quantity of MP available for the ruminant.

Cow BW and BCS at calving can impact the postpartum anestrous interval and conception rates at rebreeding. The influence of weight loss prior to calving on the PPI is dependent on the severity of weight loss and resulting BCS pre-calving. Depending on the severity of the weight loss, cattle that are maintained at a BCS ≥ 5 (on a scale of 1 to 9) pre-calving can tolerate weight loss during the pre- or post-partum periods with minimal to no effects on the PPI (Richards et al., 1986; Bohnert et al., 2002). However, cows that are under-conditioned (BCS ≤ 4) at calving can experience dystocia, lengthened PPI, and decreased conception rates (Richards et al., 1986; Bohnert et al., 2002). Crude protein and by-product supplementation compared to nitrogen restriction during late gestation has been repeatedly shown to positively impact BW (Rusche et al., 1993; Shoup et al., 2015a; Summers et al., 2015b) and BCS gains prepartum (Bohnert et al., 2013; Stalker et al., 2007; Larson et al., 2009). However, supplementation may be more effective for cows that are in low BCS as opposed to a high BCS prior to calving (Bohnert et al., 2013). Bohnert et al. (2013) showed the protein supplementation prepartum did not influence cow BW at weaning, but rather cow BW at weaning was greater for cows that had calved in a higher BCS. In contrast, Shoup et al. (2015a) found that prepartum supplementation increased BW and BCS post-calving

and post-breeding relative to cows that were not supplemented or received low quantities of supplement. The results of Larson et al. (2009) agree with those of Shoup et al. (2015a) in that prepartum supplementation of cows improved pre-breeding BW and BCS. However, these differences in BW and BCS pre-breeding due to protein supplementation did not persist at weaning (Larson et al., 2009). The variation in postpartum cow response due to prepartum protein supplementation may result from maintaining cows on pasture that is likely to differ in quality. Thus, pasture quality is likely to impact nutrient supply during the postpartum period and affect cow BW and BCS responses.

Another strategy to improve cow condition during the postpartum phase to increase protein supplementation during early lactation (Houghton et al., 1990; Lalman et al., 1997). However, previously discussed increased energy and protein demands during early lactation suggest that supplementing during this period to compensate for poor BCS at calving will be challenging and increase feed costs (NASEM, 2016). Ultimately, the aim is to maintain adequate BCS to shorten the PPI and improve conception rates (Randel, 1990). However, the impact of prepartum protein supplementation on the PPI and conception rates has been inconsistent. The PPI can be shortened by prepartum protein supplementation (Sasser et al., 1988; Martson et al., 1995) suggesting that increased MP availability prepartum should improve time-frame of rebreeding and conception rates. Yet, conception rates do not appear to be affected by prepartum protein supplementation (Stalker et al., 2007; Larson et al., 2009; Summers et al., 2015b). During of the anestrous period and conception rates appear to be more regulated by BCS than by prepartum protein supplementation (Lents et al., 2008; Bohnert et al., 2013). Managing cattle to maintain or achieve adequate BCS at calving can be accomplished through CP and by-product supplementation during the prepartum period. However, supplementing MP should provide a more targeted approach to manage cow BCS pre-calving while more accurately matching cow requirements while reducing feed input costs and environmental impacts.

2.3.4 Progeny Response to Prepartum Protein Supplementation of the Dam

The concept of development programming states that progeny are susceptible to nutritional insults incurred during gestation that can have long-term impacts on growth and development (Barker et al., 1993; Godfrey and Barker, 2000). As previously highlighted, fetal organogenesis

(Richards et al., 1991), adipogenesis, myogenesis (Cossu and Borello, 1999), and muscle fiber hypertrophy occur at different rates and stages of gestation (Du et al., 2010), indicating that the timing of maternal malnutrition might matter. Developmental prioritization typically partitions nutrient supply to organogenesis (Bauman et al., 1982; Close and Pettigrew, 1990) and nervous system development (Richards et al., 1991) prior to myogenesis and muscle fiber hypertrophy. Therefore, variation in nutrient supply during gestation, particularly mid- to late-gestation, can determine progeny skeletal muscle development and growth performance.

Fetal skeletal muscle tissue is sensitive to maternal nutrition and of importance as muscle fiber hyperplasia does not occur postnatally (Glore and Layman, 1983; Greenwood et al., 2000; Nissen et al., 2003). Over- and under-supplying energy and protein have been utilized to study the impact of maternal nutrition and timing of nutritional insults throughout gestation on progeny birth weight and growth performance (Greenwood et al., 2004; Larson et al., 2009). Nutrient deficient rations are associated with decreased birth BW and reduced progeny performance that is likely mediated through reduction of muscle fibers size and abundance (Dwyer and Stickland, 1994) especially when occurring in mid- to late-gestation. In addition, a reduction in satellite cell nuclei has been observed in offspring from nutritionally restricted dams (Bedi et al., 1982; Wilson et al., 1988; Ward and Stickland, 1991). As protein restriction can decrease birth weight and hinder post-natal progeny performance, it is reasonable to postulate that supplementation relative to requirements may increase birth BW to progeny growth and development.

Traditional protein supplementation strategies have focused on high-protein by-product feeds (Shoup et al., 2015a; Summers et al., 2015a) to supply CP during early (Martin et al., 2007) and late gestation (Martin et al., 2007; Larson et al., 2009). Yet, results are mixed regarding the effect of protein supplementation during late gestation with some authors reporting that birth BW tended to increase when dams were supplemented (Larson et al., 2009), while others have reported that birth BW does not change in response to protein supplementation (Martin et al., 2007). This difference in progeny birth weight response exists despite similarities in protein supplementation strategies (supplement = 32 and 42 CP %DM, respectively for Larson et al. (2009) and Martin et al. (2007)) and cows being maintained on the similar native range (CP = 6.4 to 6.8 %DM; Adams et al., 1998) during the last trimester of gestation. However, Larson et al. (2009) provided supplements that contained 80 mg of monensin per cow/d in contrast to Martin et al. (2007), where no ionophores were supplemented. Ionophores modify the rumen environment to improve

fermentation and SCFA production, ultimately increasing the maternal ME supply and availability of energetic substrates for fetal growth. Additionally, all cows in the study of Martin et al. (2007) were moved to a drylot and fed hay (CP = 8.6% of DM) with no protein supplementation approximately one month prior to parturition: a time when fetal growth increases exponentially (Du et al., 2010). The difference in birth weight response to protein supplementation between the two studies could be attributed to monensin supplementation (Larson et al., 2009) or the ceasing of protein supplementation prior to parturition (Martin et al., 2007).

Although birth BW did not differ due to supplementation in the work of Martin et al. (2007), they observed increased weaning weights in offspring from protein supplemented dams and this response is in agreeance with the results of others (Stalker et al., 2006; 2007; Larson et al., 2009). Furthermore, maternal protein supplementation can increase progeny value by increasing hot carcass weight (Stalker et al., 2007; Larson et al., 2009), marbling score, and the proportion of steers grading choice (Greenwood et al., 2004; Larson et al., 2009). The improvements in carcass yield and quality may be mediated through substrates available during late gestation for fetal growth resulting in an increased quantity of myocytes or adipocytes. Alternatively, gluconeogenic AA precursors from protein supplementation may also be used as an energetic substrate for intramuscular adipocytes (Smith and Crouse, 1984) to increase the degree of adipogenesis in utero and have a downstream influence on progeny carcass quality and yield grade. In addition, calves that were born from protein supplemented dams required less treatment for bovine respiratory disease at the feedlot (Larson et al., 2009) and research has shown that calves receiving treatment have lower quality grades than their counterparts (Gardner et al., 1999; Busby et al., 2004). Research indicates that nutritional restriction during gestation can impair progeny immune function (Corah et al., 1975; Wittum et al., 1994a; Hammer et al., 2007) and directly impact the susceptibility of calves to bovine respiratory disease. Therefore, the improvements in quality grade may be partly mediated through improved calf health and immune function resulting from maternal protein supplementation.

There are clear benefits for progeny performance arising from maternal protein supplementation, but it is imperative to recognize the constraints of the previously mentioned studies when evaluating the influence of maternal MP supply on fetal development and programming of progeny performance. Firstly, the use of high-protein by-product feeds to provide supplemental CP (Shoup et al., 2015a; Summers et al., 2015a) may contribute more than CP,

confounding the model and progeny responses. Secondly, the use of CP as a supplementation strategy (Stalker et al., 2006; Martin et al., 2007; Larson et al., 2009) limits the usefulness of the research model as CP supplementation does not represent the MP supply, making it difficult to draw conclusions in cross-study comparisons. Lastly, the recent research in beef cattle has focused on supplementation relative to restriction or rations that are marginally deficient in meeting protein or energy requirements, whereas over-supplementation relative to predicted requirements has only been evaluated using ewes and lambs as a model (Van Emon et al., 2014; 2015). Therefore, the current understanding of maternal MP requirement and supply and its subsequent effect on beef progeny is limited and represents a knowledge gap in the available literature.

2.4 Conclusion

The transition period in beef cattle is a dynamic period in the cow-calf production cycle that is characterized prepartum by increasing maternal AA demand due to exponential fetal growth, mammogenesis, and colostrogenesis, while during the postpartum transition phase the cow must meet the nutrient requirement of lactation and resume estrous activity. It is known that particulate and fluid passage rates will increase during late gestation while rumen fill is reduced and total tract digestibility decreases. Due to the inverse relationship of degradation to passage rate, increased passage rate will decrease ruminal degradation of carbohydrate and AA sources and reduce the production of SCFA and MCP. Decreasing total tract digestibility imposes additional challenges in meeting MP requirements as the availability of AA for absorption is then reduced during late gestation. Though current nutritional models account for increasing fetal mass during late gestation on MP requirement, they fail to account for how the transient changes in rumen fermentation dynamics and total tract digestibility during late gestation affect capacity for MP capture. It is probable that nutritional models may underestimate MP requirements during late gestation. Despite the transient changes that occur near parturition and their impact on AA absorption and availability, there is limited knowledge to provide insight as to how over-supplementation of MP relative to predicted requirements may influence the homeorhetic state of the dam during either late gestation or the subsequent lactation. Mild global nutrient restriction during mid- to late-gestation has been shown to increase maternal skeletal muscle catabolism while the fetus remains in an anabolic state. Yet, research has not evaluated the impact of over-supplementation of MP on

maternal skeletal muscle degradation in spite of the potential underestimation of MP requirements. Focus has been on CP supplementation relative to protein deficient diets when evaluating cow-calf responses to increased prepartum protein supply. From these studies, it is clear that prepartum nutrition has the capacity to influence performance during the postpartum transition phase. However, CP supply does not represent the available MP for the previously described reasons and confounds inference about maternal MP supply. Additionally, the lack of uniformity in CP source in rations and supplementation strategies between studies makes it difficult to form conclusions regarding the dam's MP requirement immediately prior to parturition. The common indirect measurement of cow-calf performance via cow BW and BCS, and calf birth BW, and growth performance are useful, but do not allow for direct interpretation of the dam's MP balance during late gestation and the resulting consequences on colostrogenesis and lactation. Evaluation of the resulting N balance during late gestation from protein supplementation would be a more reliable strategy to determine the MP requirement of late gestation beef cattle. However, no studies have evaluated pre- or post-partum nitrogen balance in relation to over-supplementation of MP during late gestation, or the implications on the transition beef cow and progeny performance, which represents a knowledge gap in the available literature.

2.5 Hypothesis

The hypothesis was that feeding predicted MP in excess of requirements (133%; based on CNCPS 6.5) during late-gestation would improve nitrogen balance for the dam, causing her to decrease catabolism of maternal skeletal muscle protein reserves, and improve cow (postpartum) and calf performance.

2.6 Objectives

The objective of this study was to evaluate the effect of over-feeding predicted MP during late-gestation on pre- and post-partum cow-calf performance, markers of protein turnover, and maternal nitrogen balance, and to evaluate the carry-over effects on calf growth performance. Secondary objectives of this project were to characterize the beef cow transition phase (pre- and post-partum transition periods) as well as characterize beef cow lactation characteristics in terms of milk yield, peak yield, persistency, and milk composition over the lactation.

3.0 OVER-SUPPLYING METABOLIZABLE PROTEIN IN LATE GESTATION FOR BEEF CATTLE: EFFECTS ON PREPARTUM NITROGEN BALANCE, RUMINAL FERMENTATION, AND HEIFER BODY WEIGHT

3.1 Abstract

The objective of the study was to determine the effect of oversupplying metabolizable protein (**MP**) during late gestation on prepartum BW, nutrient intake, ruminal fermentation, and N balance. Twenty-four primiparous crossbred Hereford cows (blocked by date of expected parturition) were assigned to a control treatment designed to meet MP requirements (**CON**) or a treatment providing 133% of the MP requirement (**HMP**). Cows were individually fed their treatment diet from d -55 ± 3.7 relative to parturition. Cow BW was measured on d -55 ± 3.7, -41 ± 3.7, -27 ± 3.6, and -8 ± 3.9 and DMI was summarized by week. Ruminal pH was measured daily and ruminal digesta samples were collected on d -33 ± 5.3 and -15 ± 4.4 for short-chain fatty acid (**SCFA**) and ammonia concentration. Nitrogen balance was measured over a 6-d period starting on d -33 ± 5.3 and -15 ± 4.4. Plasma and serum samples were collected the day prior to ruminal digesta samples and analyzed for NEFA, BHBA, glucose, insulin, and plasma urea-N. Data were analyzed as a randomized complete block design using the MIXED procedure of SAS. Prepartum BW did not differ among days for CON; whereas, HMP cows increased BW as parturition approached (treatment × day, $P < 0.01$). Dry matter intake increased ($P < 0.01$) by 18% in week -2 compared to -8, but then decreased ($P < 0.01$) by 8.0% during week -1, but did not differ by treatment. Nitrogen intake, apparent total tract digestion, excretion, and retention (g/d) were all greater ($P < 0.01$) for HMP heifers than CON. When reported as a proportion of N intake, apparent total tract N digestion, N excretion, and retention were not different ($P > 0.05$) between treatments. Heifers fed HMP had improved ($P < 0.01$) DM, OM, and NDF digestibility relative to CON heifers. Concentration of urinary 3-methylhistidine (**3-MH**) and the 3-MH:creatinine ratio did not differ ($P \geq 0.23$) between treatments. Maximum ruminal pH and total SCFA concentration were not affected by treatment. However, prepartum concentrations (% of total SCFA) of isobutyric (0.6 vs. 0.9, $P < 0.01$), isovaleric (0.6 vs. 0.9, $P < 0.01$), and valeric acid (0.96 vs. 1.24, $P < 0.01$) were less for CON than HMP. Prepartum ruminal ammonia-N decreased (treatment × day, $P < 0.01$) as parturition approached for HMP (10.1 to 8.6 mg/dL) whereas, ammonia-N was not

affected for CON (1.0 to 1.3 mg/dL). Plasma urea-N was greater ($P < 0.01$) for HMP heifers (15.0 vs. 7.5 mg/dL). Based on ammonia-N concentration, increased nitrogen retention, and improved BW at parturition for HMP heifers, feeding higher levels of MP during late gestation may improve prepartum ruminal fermentation, N balance, and BW.

3.2 Introduction

Late gestation and early lactation represent physiological stages for beef cattle where nutrient requirements are greatest due to fetal growth (Du et al., 2010), mammary tissue development (Rattray et al., 1974), colostrogenesis, and initiation of lactation (NASEM, 2016). The increase in nutrient requirements during late gestation is primarily due to an exponential increase in fetal mass (Robinson et al., 1977; Bauman and Currie, 1980). Fetal muscle protein concentration increases from approximately 60 to 110 mg/g of muscle tissue between d 160 and 280 of gestation (Prior et al., 1979). Much of the growth of the fetus during the last trimester is due to fetal muscle fiber hypertrophy and hyperplasia (Prior et al., 1979; Du et al., 2010). In addition to being used for protein synthesis, amino acids (**AA**) are estimated to provide 30 to 50% of the oxidized substrates used by the fetus (Bauman and Currie, 1980). The combined effect of AA demand for protein synthesis and energy substrates causes a substantial increase in AA demand that must be met by maternal supply. Instances of maternal AA deficit during late gestation, dependent on their severity, may result in the dam experiencing homeorhetic skeletal muscle catabolism in order to meet fetal AA demand.

As metabolizable energy (**ME**) and protein (**MP**) requirements increase, fetal growth causes a reduction in gastrointestinal (**GIT**) capacity, increased digesta passage rate, and decreased DMI (Scheaffer et al., 2001). Du et al. (2005) and Wood et al. (2013) demonstrated increased abundance of ubiquitin in skeletal muscle for gestating beef cows exposed to a mild nutrient restriction during gestation, supporting the proposition that beef cows may enter a negative MP balance during late gestation due to increasing AA demand and transient GIT changes that hinder the dam's capacity to efficiently capture consumed MP. Loss of skeletal muscle has been reported for periparturient dairy cattle (Chibisa et al., 2008), but the occurrence and extent of the negative ME and MP balance for beef cattle, without an imposed feed restriction, is not known.

The hypothesis was that oversupplying MP would mitigate a maternal N deficiency thereby improving N balance. The primary objective was to evaluate whether increasing MP supply would improve N balance and decrease metabolic indicators of skeletal muscle catabolism. A secondary objective was to characterize DMI, ruminal fermentation, and plasma insulin and metabolites during the prepartum phase of the beef cow transition period.

3.3 Materials and Methods

All procedures used in this study were pre-approved by the University of Saskatchewan Animal Research Ethic Board (protocol 20100021).

3.3.1 Experimental Design, Cow Husbandry, and Dietary Treatments

This chapter is 1 of 2 that address the effects of increasing MP supply for beef cattle prepartum N balance and resultant indications of skeletal muscle catabolism and metabolic energy status. The current chapter addresses the prepartum responses while the following chapter (Chapter 4.0) addresses post-partum indicators of maternal skeletal muscle protein catabolism, postpartum cow responses, and calf performance. A total of 24 pregnant Hereford-cross heifers were used in this experiment. Of the 24 heifers, 14 were previously fit with a ruminal cannula (model 9C, Bar Diamond, Parma, ID). Heifers were bred using fixed-time artificial insemination with sexed semen (90% female) from one sire (Cole Creek Cedar Ridge 1V, Reg. No. 1659099 (CAN), Genex Co-operative Inc., Shawano, WI), to minimize bias in calf growth performance due to genetic variation. The experiment was arranged as a randomized complete block design with the expected date of parturition (278 d post-breeding date) as the blocking factor. Within and between blocks, heifers were assigned to treatments while balancing for the number of cannulated and non-cannulated heifers and initial, non-conceptus corrected BW. Non-conceptus corrected heifer BW was measured immediately prior to the start of each block's experimental period. Heifers were managed similarly during gestation until being transported to the Livestock Research Building at the University of Saskatchewan on d -55 ± 3.7 d relative to parturition. At this time, heifers were housed in individual pens (9 m^2) with rubber mats. Pens were cleaned and washed daily. Ten days

prior to parturition, heifers were moved to pens (18 m^2) bedded with straw and maintained there until 7 d following parturition.

Treatments consisted of the control treatment (CON; n = 12; non-cannulated = 6, cannulated = 6) or a treatment where MP was purposely over-fed (HMP; n =12; non-cannulated = 6, cannulated = 6). The CON was designed to provide 100% of the predicted MP requirement based on CNCPS 6.5 using the Nutritional Dynamic System software (RUM&N Sas, Via Sant'Ambrogio, Italy), while the HMP was formulated to provide 133% of the predicted MP requirements. Providing MP in excess of requirement by 33% was chosen as it would enable the over-supply of MP conforming with previous studies and was expected to elicit changes in performance (Van Emon et al., 2014; 2015) without adverse effects. Diets were formulated to be isoenergetic providing 101.6 and 94.6% of predicted ME requirements for HMP and CON, respectively. Exposure to the dietary treatments was initiated on d -55.3 ± 3.7 relative to parturition to reflect the final 2 mo of gestation where maternal MP requirement is expected to increase exponentially. Date of experiment initiation was calculated for each block as 8 wk prior to the expected date of parturition (278 d post-breeding). The diets consisted of the same barley green feed forage and wheat straw with cows being offered 1 of 2 prepartum supplemental pellets based on their respective treatment (Table 3.1). Diets contained the same forage-to-concentrate ratio (60:40). Nutrient requirements were predicted using an average heifer BW of 550-kg (BCS of 5 on a scale of 1 to 9) from BW measurements taken during breeding as it was impractical to match MP requirements to each heifer as she began the experiment. A 13-month calving interval was assumed and requirements were predicted based on d 260 (median d of gestation during period of treatment provision) in gestation with a calf birth weight of 36 kg (based on the sire's estimated progeny data). Diets were fed twice daily at 0900 and 1630 h targeting ad libitum intake (5 to 10% refusals on an as fed basis).

3.3.2 Data and Sample Collection

3.3.2.1 Dry matter intake

Dry matter intake was determined daily by measuring the weight of the feed offered and the weight of the feed that was refused. The as-fed feed consumption was then corrected for DM

Table 3.1 Ingredient and chemical composition of high metabolizable protein (HMP = formulated to 133% of requirements) and control (CON = formulated to be 100% of requirements) rations fed during the final 8 wk of gestation.

	Prepartum diets	
Item	CON	HMP
Ingredient composition, % DM		
Barley green feed	37.4 ± 0.5	37.4 ± 0.5
Wheat straw	23.3 ± 0.7	23.2 ± 0.6
Supplemental pellet[1,2]	39.3 ± 0.9	39.4 ± 0.9
Chemical composition, % DM		
DM, %	90.3 ± 2.4	89.4 ± 2.7
OM	91.4 ± 0.3	91.0 ± 0.3
CP	9.3 ± 0.5	14.4 ± 0.3
Predicted MP[4]	7.4 ± 0.0	9.4 ± 0.0
ADF	32.7 ± 1.4	31.2 ± 1.4
aNDFom[5]	50.5 ± 1.6	46.4 ± 1.5
Starch	14.8 ± 1.3	13.8 ± 1.0
Ether extract	1.4 ± 0.1	1.4 ± 0.2
Calcium	0.6 ± 0.05	0.7 ± 0.03
Phosphorous	0.2 ± 0.02	0.3 ± 0.01

[1]High protein supplement contained 24.0% canola meal solvent, 14.8% soybean meal 47.5 solvent, 1.9% oat hulls, 14.8% molasses beet, 37.0% ground barley grain, and 7.6% custom mineral and vitamin supplement[3]; chemical composition: 9.244 kIU/kg of Vitamin A, 0.843 kIU/kg of Vitamin D_3, 0.185 kIU/kg of Vitamin E, 708.26 ppm of Cu, 192.02 ppm of Fe, and 986.74 ppm of Zn.

[2]Control and lactation supplements contained 24.0% oat hulls, 27.7% molasses beet, 40.7% ground barley grain, and 7.6% custom mineral and vitamin supplement[3]; chemical composition: 9.245 kIU/kg of Vitamin A, 0.843 kIU/kg of Vitamin D_3, 0.185 kIU/kg of Vitamin E, 709.40 ppm of Cu, 153.92 ppm of Fe, and 971.86 ppm of Zn.

[3]Mineral and vitamin supplement contained 1.7% zinc oxide, 4.9% vitamin E premix, 0.98% vitamin A premix, 0.49% vitamin D, 0.13% Sel-Plex 2000 (Alltech), 7.62% white salt, 9.77% magnesium oxide, 24.42% ground limestone, 21.88% ground barley grain, 24.42% manganese oxide, and 3.66% copper sulfate $5H_20$.

[4]Metabolizable protein was predicted using CNCPS 6.5 using tabular values.

[5]Amylase- and sodium sulfite-treated NDF corrected for ash content.

content of the diet and refusals, and the calculated DM consumption was assumed to be equal to DMI. Throughout the study, samples of forages were collected once weekly and samples of concentrate (CON and HMP pellets) were collected every two weeks. Samples of refusals were taken daily and composited by week (proportionally by quantity refused per day on an as is basis). The feed samples were placed in a forced-air oven at 55ºC until achieving a constant weight to determine DM concentration.

3.3.2.2 Body weight, and rib and rump fat thickness

Heifers were weighed on 2 consecutive days at the start of the study (d -56 and -55 relative to expected parturition) and every 2 weeks thereafter until and including the day of parturition. Heifer BW was corrected for conceptus weight using the NASEM (2016) equation. Ultrasounography (Aloka SSD-500; 17 cm 3.5 MHz linear transducer: Aloka UST-5044-3.5) was used to determine rib (between the 12th and 13th rib) and rump fat thickness (Broring et al., 2003) using the scale described by Lowman et al. (1976) on d -55 ± 3.7 and -13 ± 4.6 relative to parturition.

3.3.2.3 Ruminal fermentation, apparent total tract digestibility, nitrogen balance, and blood metabolites

For cannulatd heifers, ruminal digesta was collected from three different locations (250 mL/region): cranial, ventral, and caudal regions of the rumen. Samples were collected at one time-point (at 1300 h as to not conflict with other concurrent measurements) on d -33 ± 5.3 and -15 ± 4.4 relative to parturition. Digesta was strained through 2 layers of cheesecloth and two 10-mL aliquots of ruminal fluid were collected. One 10-mL aliquot was added to 2 mL of 25% metaphosphoric acid (w/v) and was stored frozen (-20°C) until being used for determination of short chain fatty acid (SCFA) concentration by gas chromatography (Khorasani et al., 1996). The second 10-mL aliquot was added to 2 mL of 1% sulphuric acid and was stored frozen (-20°C) until being used for analysis of ammonia-N concentration (Fawcett and Scott, 1960).

Ruminal pH was measured every 5 min in CAN heifers from d -35 until parturition using

the Lethbridge Research Center ruminal pH measurement system (LRCpH; Penner et al., 2006). The LRCpH was standardized, inserted through the ruminal cannula into the ventral sac of the rumen. The LRCpH was removed, data were downloaded, and the system was standardized and re-inserted weekly. The standardization process, used prior to and following in vivo measurement, included the use of standard buffer solutions (pH 4 and 7; Ricca Chemical Company, Arlington, TX) and was conducted at 39°C. Data were transformed from mV recordings to pH using beginning and ending linear regressions with the assumption of linear drift over time (Penner et al., 2006).

Nitrogen balance was determined in 2 separate periods (6-d measurement period) with the first starting on d -33 ± 5.3 and the second starting on -15 ± 4.4 relative to parturition. Samples of each feed ingredient and feed refusals were collected daily and the daily samples were composited (proportionally to the quantity refused per day on an as is basis), and analyzed for DM and nutrient concentration (described below). Dietary N intake was determined daily based on the amount of feed offered and refused. Over the 6-d measurement period, total feces and urine excreted were collected, weighed, and recorded. The total fecal collection was conducted by weighing the total feces (scraped off the pen floor) excreted per 6-h interval. At each time-point, the feces were mixed thoroughly and 5% of the total fecal weight was collected and used to prepare a proportional composite sample (6-d composite for each heifer for each period). Total urine collection was conducted using a Foley catheter (Bard Medical, Covington, GA, USA) that was inserted the day prior to the initiation of total urine collection with urine diverted into 25-L carboys. Although total urine collection was imposed, in some instances heifers expelled the catheter. When a catheter was eliminated, re-catheterization occurred once. A total of 11 and 8 HMP heifers, and 11 and 8 CON heifers successfully completed the N balance sampling periods occurring from d -33 to 27 ± 5.3 and d -15 to 9 ± 4.4, respectively. Urine collected via total urine collection was acidified with 200 mL 12 M hydrochloric acid to prevent N loss. The total urine weight was recorded daily and a 50-mL sample was collected and stored at -20ºC until analysis (described below).

Dry matter content of offered feed, feed refused, and fecal samples for were determined by drying the composite sample in a forced-air convection oven until a constant weight was achieved. The feed, refusal, and fecal samples were then ground to pass through a 1-mm sieve using a Christy Norris hammer mill (Christy and Norris, Christy Turner Ltd., Chelmsford, U.K.) and sent to Cumberland Valley Analytical Services (Abbotsford, BC, Canada) for analysis of DM, OM, CP,

EE, aNDFom (amylase treated NDF corrected for ash content), ADF, and starch (Rosser et al., 2013). Apparent total tract digestibility of DM, OM, CP, and EE and total tract digestibility of aNDFom, ADF, and starch were calculated as the difference between nutrient intake and excretion as a proportion of the nutrient intake on a DM basis. Urine was analyzed for urea-N using the protocol described by Fawcett and Scott (1960) (mean inter- and intra-run CV ± SD was 1.1 ± 0.1 and 0.9 ± 1.1%) and CP content (N content × 6.25) was analyzed according to the AOAC (1994) method for Kjeldahl determination of CP (mean inter- and intra-run CV ± SD was 1.8 ± 0.6 and 1.1 ± 1.2%). Urinary 3-MH (Rathmacher et al., 1992) and creatinine (Slot, 1965) concentrations were analyzed by Heartland Assays (Ames, Iowa, USA) and values were accepted when the CV was < 7.5%.

Blood samples from the jugular vein were collected the day prior to ruminal samples at 1000 h to coincide with urinary catheterization (d -33.9 ± 5.6 and d -16.2 ± 4.0) relative to parturition). Sample vials contained either 158 IU heparin for plasma or a clot activator for serum (BD Vacutainer, BD and Company, Franklin Lakes, NJ, USA). Blood was immediately centrifuged (2,000 × g at 4°C for 15 min) to separate plasma while blood samples for serum collection were allowed to sit at room temperature for 10 min to facilitate clotting prior to centrifugation (2,000 × g at 4°C for 15 min). Harvested plasma and serum were stored at -20°C until analysis. Serum NEFA (mean inter- and intra-plate CV ± SD was 3.5 ± 6.9 and 2.3 ± 1.5%) was analyzed using the NEFA-HR (2) kit from Wako (Richmond, VA, United States of America). Plasma glucose (mean inter- and intra-plate CV was 0.5 ± 0.3 and 1.7 ± 0.8%; product numbers P7119 and number F5803, Sigma Aldrich, Oakville, ON, Canada) and serum urea-N (mean inter- and intra-run CV was 0.8 ± 0.0 and 1.8 ± 1.5%; Fawcett and Scott, 1960), BHBA (mean inter- intra-plate CV was 3.2 ± 0.2 and 2.7 ± 1.7%; Williamson et al., 1962) concentrations were determined using colorimetric methods. Plasma insulin was analyzed using an ELISA (Mercodia Bovine Insulin ELISA, Mercodia, Uppsala, Sweden). The inter- and intra-plate assay variation were (mean ± SD) 4.5 ± 3.4 and 2.9 ± 0.4% for plasma insulin.

3.3.3 Statistical Analysis

All data were analyzed as a randomized complete block design using the PROC MIXED of SAS 9.4 (SAS Institute, Cary, NC, USA). Treatment, time, and the interaction of treatment and

time were included as fixed effects within the model, and cow nested in block was included as a random effect. In all cases, time was considered as a repeated measure. When data were equally spaced, autoregressive, heterogeneous autoregressive, compound symmetry, heterogeneous compound symmetry, toeplitz, heterogeneous toeplitz, unstructured, and ante-dependence covariance structures were used to find best fit for the data (lowest AIC and BIC). When data were unequally spaced, unstructured, ante-dependence, simple, compound symmetry, and heterogeneous compound symmetry covariance structures were used to find best fit for the data. Significance was declared when $P < 0.05$ and tendencies were considered when $0.10 \geq P \geq 0.05$. When the F-test was significant, means were separated (Tukey's) to determine means that differed.

3.4 Results

3.4.1 Cow BW and Rib and RumpFat Depth

A treatment by day interaction was observed for BW ($P = 0.007$; Fig. 3.1). Body weight did not differ between treatments ($P > 0.05$). Yet, HMP heifers increased BW by 16.5 and 33.8 kg on d -8 relative to d -41 and -56, respectively; whereas, CON heifers only increased BW by 13.5 kg from d -56 to -8. Prepartum rib and rump fat depth did not differ ($P \geq 0.31$; Table 3.2) by treatment, day, or the interaction of day and treatment.

3.4.2 Intake and Apparent Digestibility

When measured on a weekly basis, DMI as a percentage of BW or kg/d summarized from week -8 to parturition was not affected ($P \geq 0.36$; Table 3.3) by treatment or the interaction of treatment and week (data not shown). Dry matter intake (% BW) was greater ($P < 0.001$) during wk -3 and -2 compared to wk -8 by 16.2 and 17.7%, respectively. From wk -2 to wk -1, DMI (% BW) decreased by 8.0%. During the N balance periods, DMI (kg/d) was not affected ($P = 0.21$; Table 3.4) by treatment or the interaction of treatment and day but increased by 1 kg/d ($P < 0.001$) for measurements initiated on d -15 relative to d -33. Corresponding to the increase in DMI on d -15, the intake of OM, ADF, NDF, and starch increased ($P < 0.001$).An interaction for treatment and day was detected for CP intake ($P = 0.005$; Fig. 3.2), with HMP having greater overall CP intake

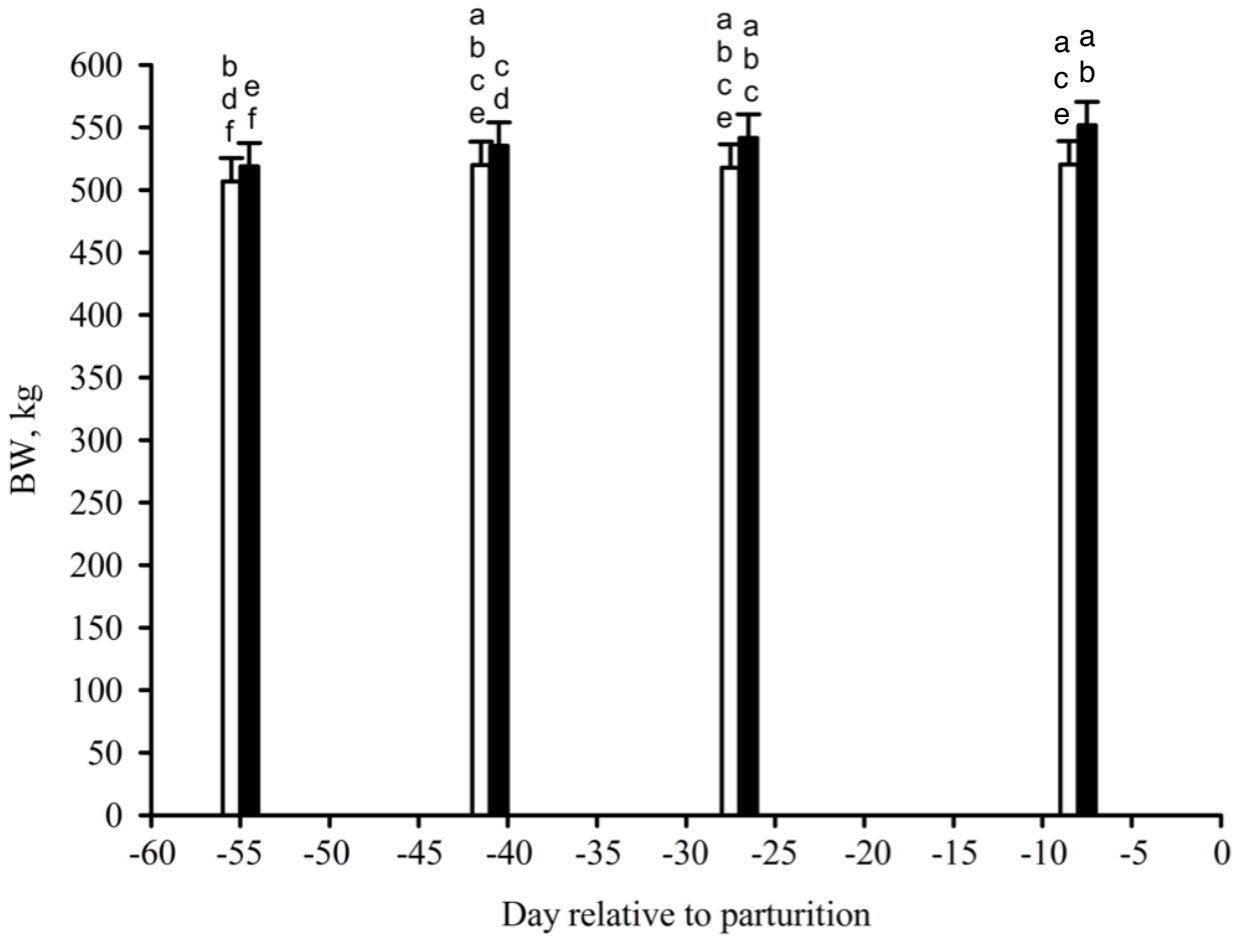

Figure 3.1 The interaction of treatment and day (P = 0.007) for conceptus-corrected BW. Heifers on the CON treatment (white columns) were fed a prepartum designed to meet 100% of the MP requirement and HMP (black columns) were fed a prepartum diet designed to supply 133% of MP requirement. The P-values for the main effects of treatment and day were 0.44 and < 0.001, respectively. High MP heifers gained 16.5 and 33.8 kg on d -8 ± 3.9 relative to d -41 ± 3.7 and -55 ± 3.7; whereas CON heifers only gained 13.5 kg from d -55 ± 3.7 to -8 ± 3.9. Means within a dependent variable that have uncommon letters differ (P < 0.05).

Table 3.2 Prepartum cow BW and rib and rump fat depth for heifers fed diets formulated to supply 100 (CON) or 133% (HMP) of predicted metabolizable protein requirements during the final 8 wk of gestation.

	Treatment			Day[1]					*P*-values		
Item	CON	HMP	SEM[2]	d -55	d -41	d -28	d -8	SEM[2]	Treatment	Day	T × D[3]
n	12	12		24	24	24	24				
Cow BW[4], kg	516	537	18.5	513[c]	528[b]	530[ab]	536[a]	13.2	0.44	< 0.001	0.007
Rib fat depth[5], mm	5.4	6.6	0.8	5.8	—	—	6.2	0.6	0.31	0.41	0.41
Rump fat depth[5], mm	7.9	8.0	1.3	7.9	—	—	8.0	0.9	0.96	0.86	0.37

[a,b,c]Means within a dependent variable that have uncommon letters differ ($P < 0.05$).

[1]Day relative to parturition: d -55 ± 3.7; d -41 ± 3.7; d -27 ± 3.6; d -8 ± 3.9.

[2]Largest SEM shown.

[3]T × D = Treatment and day interaction.

[4]Interaction data described elsewhere.

[5]Rib and rump fat depth measurements conducted on d -13 ± 4.6 as opposed to -8 ± 3.9.

Table 3.3 Prepartum dry matter intake by week of heifers fed diets formulated to supply 100 (CON) or 133% (HMP) of predicted metabolizable protein requirements during the final 8 wk of gestation.

	Treatment			Week Relative to Parturition									*P*-value		
	CON	HMP	SEM	-8	-7	-6	-5	-4	-3	-2	-1	SEM[2]	Treatment	Week	T × W[1]
n	12	12		17	22	24	24	24	22	22	22				
DMI % BW	2.10	2.23	0.10	1.96[c]	2.12[abc]	2.13[abc]	2.19[abc]	2.20[abc]	2.28[ab]	2.31[a]	2.12[bc]	0.11	0.36	< 0.001	0.94
DMI, kg	10.8	11.4	0.5	10.1[d]	10.8[cd]	11.2[bcd]	11.5[abc]	11.6[abc]	12.0[ab]	12.2[a]	11.3[bcd]	0.5	0.42	< 0.001	0.67

[1]T × W = Treatment by week interaction.

[2]Largest SEM shown.

Table 3.4 Prepartum nutrient intake and apparent total tract digestibility for heifers fed rations formulated to supply 100 (CON) or 133% (HMP) of predicted metabolizable protein requirements during the final 8 wk of gestation.

	Treatment			Day[1]			*P*-values		
Item	CON	HMP	SEM[2]	d -33	d -15	SEM[2]	Treatment	Day	T × D[3]
Intake, kg/d									
n	12	12		24	24				
DM	11.4	12.3	0.5	11.3	12.5	0.4	0.21	< 0.001	0.21
OM	10.4	11.2	0.5	10.3	11.3	0.4	0.23	< 0.001	0.18
CP[4]	1.1	1.9	0.1	1.4	1.5	0.1	<0.001	< 0.001	0.005
NDF	5.5	5.5	0.3	5.3	5.8	0.2	0.84	< 0.001	0.20
ADF	3.6	3.7	0.2	3.5	3.8	0.2	0.87	< 0.001	0.11
Starch	1.8	1.8	0.1	1.7	1.9	0.1	0.55	< 0.001	0.67
Apparent total tract digestibility, % DM									
n[2]	9	9		18	14				
DM	58.3	63.5	1.0	60.3	61.4	1.1	0.002	0.45	0.78
OM	60.6	66.3	1.0	62.9	64.0	1.1	< 0.001	0.44	0.93
CP	57.2	71.9	1.2	64.8	64.3	1.3	< 0.001	0.75	0.57
Total tract digestibility, % DM									
NDF	45.6	52.5	1.6	47.7	50.3	1.6	0.004	0.25	0.45
ADF	39.4	43.7	1.8	40.6	42.6	1.9	0.11	0.44	0.47
Starch	87.1	86.3	1.9	88.4	82.9	1.9	0.78	0.024	0.97

[a,b,c]Means within a dependent variable that have uncommon letters differ ($P < 0.05$)

[1]Collections occurred from d -33.2 to -27.2 ± 5.3 and from d -15.0 to -9.0 ± 4.4 relative to parturition.

[2]Largest SEM is reported.

[3]T × D = Treatment and day interaction.

[4]Interaction data described elsewhere.

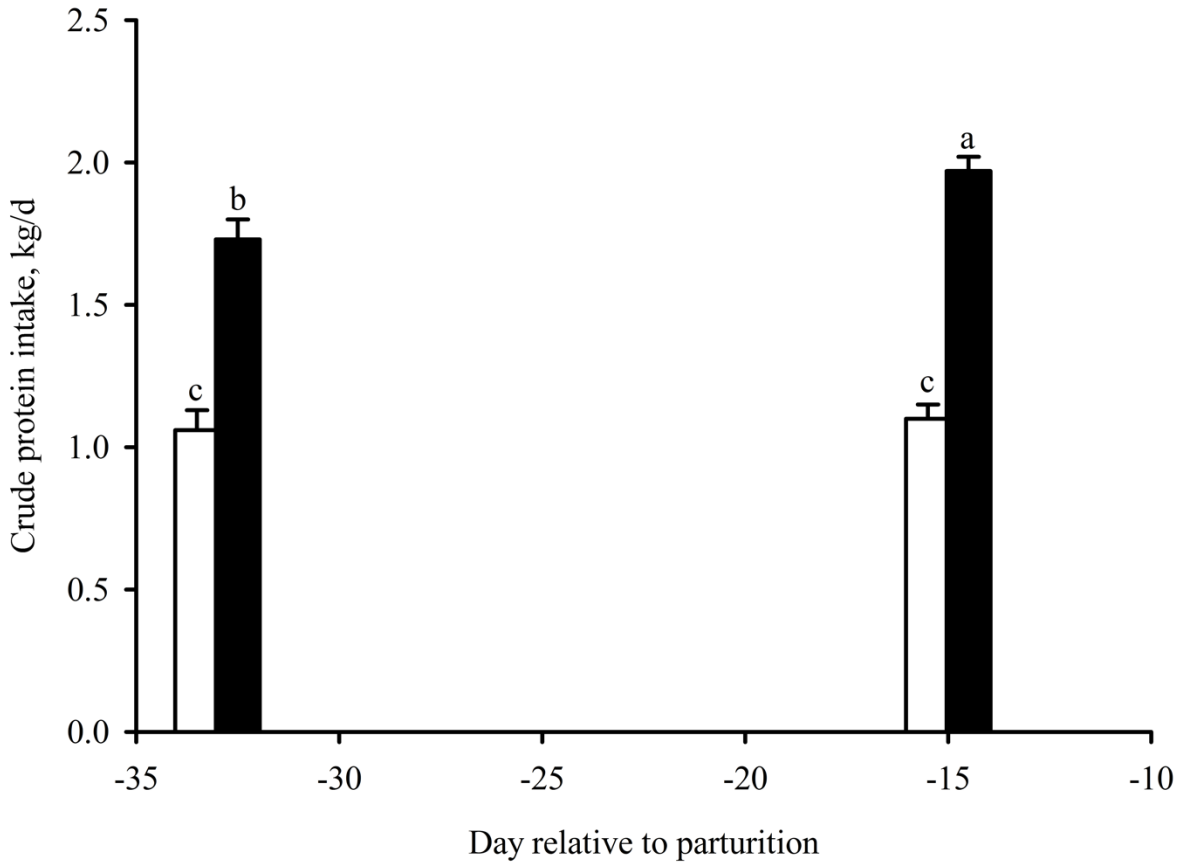

Figure 3.2 The interaction of treatment and day (P = 0.005) for crude protein intake. Heifers on the CON treatment (white columns) were fed a prepartum designed to meet 100% of the MP requirement and HMP (black columns) were fed a prepartum diet designed to supply 133% of MP requirement. The P-values for the main effects of treatment and day were < 0.001 and 0.005, respectively. High MP heifers had greater CP intake relative to CON heifers and HMP CP intake was greater at d -15.0 ± 4.4 relative to -33.2 ± 5.3. Means within a dependent variable that have uncommon letters differ (P < 0.05).

and an increase in CP intake from d -33 to d -15; whereas, CP intake for CON heifers did not differ between the two collection periods. Apparent total tract digestibility of DM, OM, CP, and NDF was greater ($P \leq 0.002$) for HMP heifers than CON heifers. Starch digestibility decreased ($P = 0.024$) by 5.5 percentage units from d -33 to d -15 relative to parturition.

3.4.3 Ruminal Fermentation

Treatment and the interaction of treatment and week did not affect ($P \geq 0.14$; Table 3.5) minimum, mean, and maximum ruminal pH. However, minimum pH tended to be greater during wk -1 relative to wk -4 ($P = 0.066$) and maximum ruminal pH was greater ($P = 0.001$) during week -1 compared to week -4 relative to parturition. Total SCFA concentration did not differ ($P \geq 0.46$; Table 3.6) by treatment, day, or the interaction of treatment and day. The molar proportions of acetic, propionic, and butyric acids were not affected by treatment ($P \geq 0.27$); however, isobutyric, isovaleric, and valeric acid were greater ($P < 0.001$) for HMP than CON. Other than an increase in the molar proportion of acetate ($P = 0.013$) from d -33 to d -15, no differences were detected for the molar proportion of SCFA based on day of measurement prepartum. The ruminal ammonia-N concentrations for HMP were greater (treatment × day; $P = 0.002$) than CON at d -33 and -15, despite a decrease in concentration from d -33 to d -15 for HMP heifers (Fig. 3.3).

3.3.4 Urine Output and Metabolite Excretion

Heifers consuming HMP had 2.6 kg/d greater urine output than CON heifers ($P = 0.025$; Table 3.7). Regardless of treatment, urine output tended to increase ($P = 0.080$) from d -33 to d -15 relative to parturition. However, urinary creatinine and 3-MH concentrations did not differ ($P \geq 0.19$) by treatment, day, or the interaction of treatment and day. Consequently, greater urine output and urinary urea concentration translated to greater ($P < 0.001$) urea output for HMP heifers than CON. The HMP heifers also had greater creatinine output ($P = 0.014$) and 3-MH output ($P = 0.012$) compared to CON heifers. Creatinine output also increased by 1.7 g/d, regardless of treatment, from d -33 to d -15 ($P = 0.022$).

Table 3.5 Prepartum minimum, mean, and maximum ruminal pH for heifers fed diets formulated to provide 100 (CON) or 133% (HMP) of predicted metabolizable protein requirements during the final 8 wk of gestation.

	Treatment			Week relative to parturition						P-values		
Item	CON	HMP	SEM[1]	-5	-4	-3	-2	-1	SEM[1]	Treatment	Week	T × W[2]
n	6	7		9	12	13	13	12				
Ruminal pH												
Minimum	6.25	6.27	0.03	6.25	6.23	6.26	6.29	6.27	0.05	0.81	0.066	0.14
Mean	6.69	6.70	0.04	6.69	6.65	6.69	6.69	6.75	0.04	0.81	0.91	0.90
Maximum	7.10	7.14	0.04	7.09[ab]	7.04[b]	7.15[ab]	7.11[ab]	7.19[a]	0.05	0.46	0.001	0.31

[a,b,c]Means within a dependent variable that have uncommon letters differ ($P < 0.05$).

[1]Largest SEM shown.

[2] T × W = Treatment by week interaction.

Table 3.6 Prepartum ruminal short-chain fatty acids and ammonia-N concentrations for heifers fed diets formulated to supply 100 (CON) or 133% (HMP) of predicted metabolizable protein requirements.

	Treatment			Day[1]			*P*-values		
Item	CON	HMP	SEM[2]	d -33	d -15	SEM[2]	Treatment	Day	T × D[3]
n	6	7		13	13				
Total SCFA, m*M*	114.9	109.9	6.8	114.4	110.4	5.3	0.60	0.46	0.46
SCFA, mol/100 mol									
Acetic acid	68.4	68.0	0.4	67.9	68.5	0.3	0.52	0.013	0.54
Propionic acid	17.2	16.6	0.4	17.0	16.8	0.3	0.27	0.32	0.94
Isobutyric acid	0.6	0.9	0.03	0.7	0.7	0.02	< 0.001	0.55	0.89
Butyric acid	12.0	12.0	0.2	12.1	11.9	0.2	0.80	0.43	0.51
Isovaleric acid	0.6	0.9	0.04	0.8	0.7	0.04	< 0.001	0.79	0.79
Valeric acid	1.0	1.2	0.03	1.1	1.1	0.03	< 0.001	0.29	0.41
Caproic acid	0.4	0.3	0.03	0.4	0.4	0.03	0.15	0.42	0.32
Rumen ammonia-N[4], mg/dL	1.1	9.3	0.5	5.5	4.9	0.3	< 0.001	0.027	0.002

[1]Collections occurred on d -33.2 ± 5.3 and d -15.0 ± 4.4 relative to parturition.
[2]Largest SEM is reported.
[3]T × D = Treatment and day interaction.
[4]Interaction data are described elsewhere.

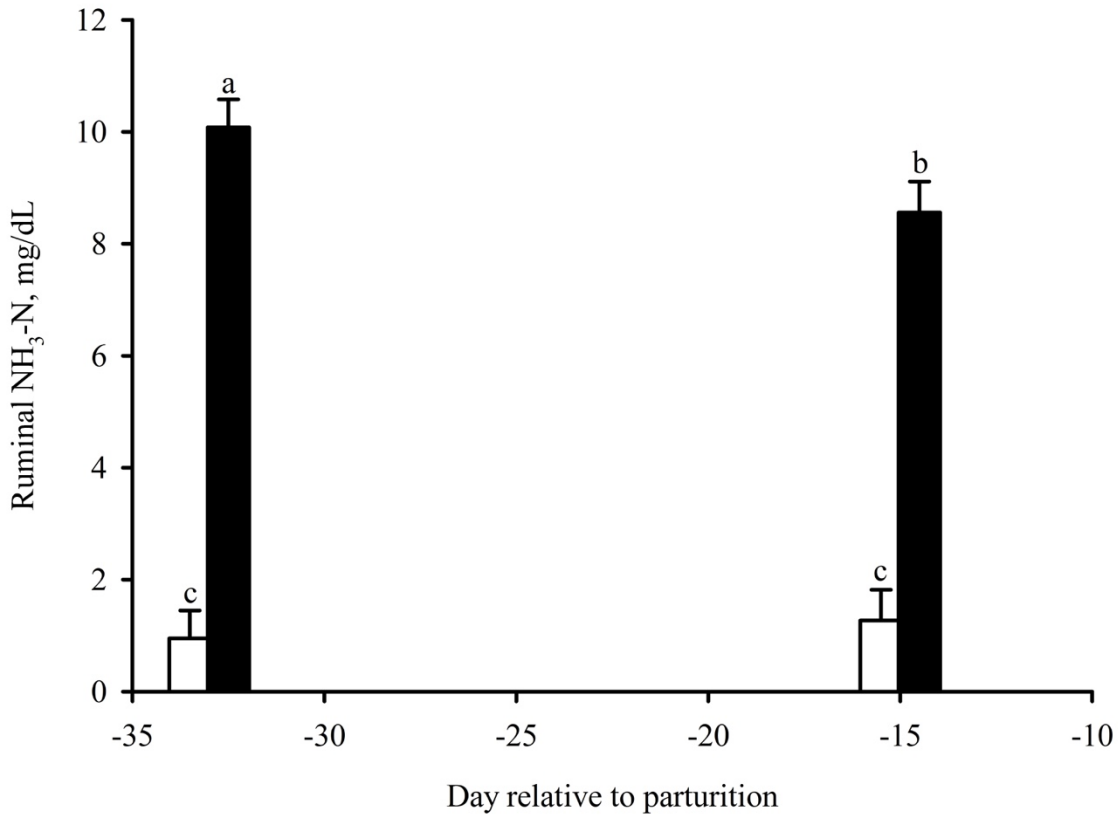

Figure 3.3 The interaction of treatment and day (P = 0.002) for ruminal NH3-N. Heifers on the CON treatment (white columns) were fed a prepartum designed to meet 100% of the MP requirement and HMP (black columns) were fed a prepartum diet designed to supply 133% of MP requirement. The P-values for the main effects of treatment and day were < 0.001 and 0.27, respectively. Ruminal ammonia-N concentrations were greater for HMP heifers compared to CON, but concentration decreased at d -15.0 ± 4.4 relative to -33.2 ± 5.3 for the HMP heifers. Means within a dependent variable that have uncommon letters differ (P < 0.05).

Table 3.7 Prepartum urine output and metabolite concentrations for heifers fed diets formulated to supply 100 (CON) or 133% (HMP) of predicted metabolizable protein requirements during the final 8 wk of gestation.

	Treatment			Day[1]			*P*-values		
Item	CON	HMP	SEM[2]	d -35	d -17	SEM[2]	Treatment	Day	T × D[3]
n	11	11		22	16				
Urine output, kg/d	7.9	10.5	0.8	8.9	9.5	0.6	0.025	0.080	0.90
Urine metabolite concentrations									
Urea-N concentration, mg/dL	317.5	567.0	39.6	433.8	450.7	42.6	< 0.001	0.76	0.42
Creatinine concentration, mg/dL	188.1	170.6	9.3	175.0	183.7	10.0	0.19	0.52	0.63
3-Methylhistidine concentration, nmol/mL	122.4	124.7	6.7	122.1	125.0	7.2	0.81	0.77	0.75
3-Methylhistidine:creatinine ratio	0.67	0.75	0.05	0.71	0.71	0.05	0.23	0.97	0.72
Urine metabolite output									
Urea-N, g/d	24.5	59.3	5.2	40.4	43.4	5.6	< 0.001	0.69	0.76
Creatinine, g/d	14.5	17.1	0.7	14.9	16.6	0.7	0.014	0.021	0.93
3-Methylhistidine, mmol/d	1.0	1.3	0.1	1.1	1.2	0.1	0.012	0.30	0.78

[1]Collections occurred from d -33.2 to -27.2 ± 5.3 and from d -15.0 to -9.0 ± 4.4 relative to parturition.

[2]Largest SEM is reported.

[3]T × D = Treatment and day interaction.

[4]Interaction data are described elsewhere.

3.3.5 Blood Metabolites and Insulin

Plasma glucose, serum NEFA, and serum BHBA were not affected ($P \geq 0.19$; Table 3.8) by treatment, day, or the interaction of treatment and day. Plasma insulin tended ($P = 0.067$) to be affected by the interaction of treatment and day with HMP heifers tending to have increased insulin concentrations (0.65 vs. 0.41 µg/L) relative to the CON at d -17 while CON and HMP did not differ on d -33. Plasma urea-N was greater for HMP heifers compared to CON heifers (15.0 vs. 7.5 mg/dL; $P < 0.001$).

3.3.6 Nitrogen Balance

The interaction of treatment and day affected nitrogen intake ($P = 0.007$; interaction data not shown as CP intake has been reported in Fig. 3.2). The interaction was a result of HMP heifers consuming 65% more N at d -33 and 78% more N at d -15 than CON heifers. Fecal N output tended to be greater for HMP than CON ($P = 0.054$; Table 3.9) and was greater on d -16 than d -35 relative to parturition. The quantity of N excreted in urine was affected by a treatment × day interaction ($P = 0.016$; Fig. 3.4). When fecal- and urinary-N were combined, heifers consuming HMP rations excreted 70 and 96% more nitrogen on measurements initiated on d -33 and -15 than heifers consuming CON ($P = 0.011$; Fig. 3.5). When reported as a percentage of N intake, HMP heifers had less ($P < 0.001$) fecal N output than CON, but more ($P < 0.001$) urinary-N output, resulting in no difference for total output as a proportion of N intake. Apparent total tract nitrogen digestion was greater for HMP heifers than CON and the magnitude of difference between treatments increased from 106% on d -33 to 134% on d -15 ($P = 0.004$; Fig. 3.6). However, when reported as a percentage of nitrogen intake, the interaction was not detected ($P = 0.53$) with HMP heifers having greater nitrogen digestibility as a percentage of nitrogen intake than CON heifers (71.9 vs. 57.1%; $P < 0.001$). The quantity of nitrogen retained was greater ($P < 0.001$) for HMP heifers, but not when reported as a percentage of N intake ($P = 0.45$).

Table 3.8 Prepartum circulating blood metabolite concentrations for heifers fed diets formulated to supply 100 (CON) or 133% (HMP) of predicted metabolizable protein requirements during the last 8 wk of gestation.

	Treatment			Day[1]			*P*-values		
Item	CON	HMP	SEM[2]	d -34	d -16	SEM[2]	Treatment	Day	T × D[3]
n	12	12		24	24				
Plasma glucose, mg/dL	58.8	56.4	1.7	54.3	56.0	1.8	0.30	0.35	0.54
Plasma insulin, µg/L	0.5	0.6	0.1	0.6	0.5	0.1	0.62	0.43	0.067
Serum non-esterified fatty acids, µEq/L	270.4	205.6	40.3	234.6	241.3	38.2	0.27	0.83	0.19
Serum BHBA, mg/dL	8.1	8.2	0.5	7.9	8.4	0.5	0.81	0.54	0.37
Plasma urea-N, mg/dL	7.5	15.0	0.4	11.1	11.4	0.7	< 0.001	0.65	0.14

[1]Collections occurred on d -34.2 ± 5.3 and from d -16.0 ± 4.4 relative to parturition.

[2]Largest SEM is reported.

[3]T × D = Treatment and day interaction.

Table 3.9 Prepartum nitrogen balance of heifers fed diets formulated to supply 100 (CON) or 133% (HMP) of predicted metabolizable protein requirements during the final 8 wk of gestation.

	Treatment			Day[1]			*P*-values		
Item	CON	HMP	SEM[2]	d -35	d -16	SEM[2]	Treatment	Day	T × D[3]
n	7	9		18	13				
Nitrogen intake[4], g/d	172.7	296.9	8.6	223.4	246.2	7.3	< 0.001	< 0.001	0.007
Nitrogen excretion, g/d									
Feces	72.0	82.3	3.6	71.7	82.6	3.9	0.054	0.043	0.70
Urine[5]	35.4	115.1	4.9	71.6	78.8	4.9	< 0.001	0.095	0.016
Total[4]	106.6	195.1	7.7	140.3	161.4	6.8	< 0.001	0.002	0.011
Nitrogen excretion, % N intake									
Feces	42.9	28.1	1.2	35.2	35.9	1.3	< 0.001	0.70	0.53
Urine	21.0	38.4	1.2	29.1	30.4	1.3	< 0.001	0.39	0.23
Total	64.5	66.5	1.8	65.5	65.5	1.9	0.45	1.00	0.66
Apparent N digestion									
g/d[4]	95.0	208.9	7.0	142.0	161.9	5.6	< 0.001	< 0.001	0.004
% of N intake	57.1	71.9	1.2	64.8	64.1	1.3	< 0.001	0.70	0.53
N Retained									
g/d	59.4	97.1	4.8	73.4	83.1	5.1	< 0.001	0.15	0.60
% of N intake	33.5	35.5	1.8	34.5	34.5	1.9	0.45	0.99	0.65

[1]Collections occurred from d -33.2 to -27.2 ± 5.3 and from d -15.0 to -9.0 ± 4.4 relative to parturition.

[2]Largest SEM is reported.

[3]T × D = Treatment and day interaction.

[4]Interaction data are described elsewhere.

[5]The Tukey's post-hoc test did not produce means that differed for the interaction of treatment and day.

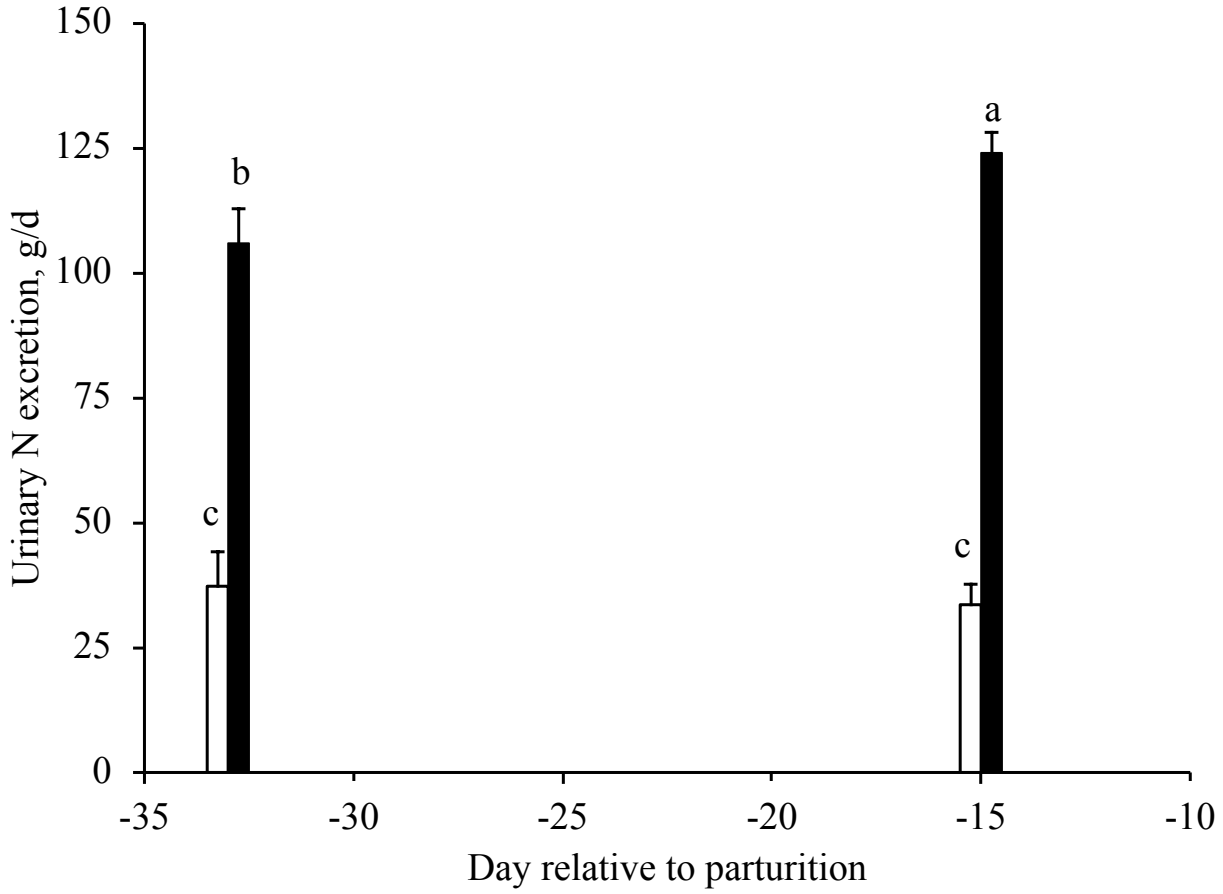

Figure 3.4 The interaction of treatment and day (P = 0.016) for urinary N excretion (g/d). Heifers on the CON treatment (white columns) were fed a prepartum designed to meet 100% of the MP requirement and HMP (black columns) were fed a prepartum diet designed to supply 133% of MP requirement. The P-values for the main effects of treatment and day were < 0.001 and 0.095, respectively. Urinary N excretion was greater for HMP heifers compared to CON, but excretion increased at d -15.0 ± 4.4 relative to -33.2 ± 5.3 for the HMP heifers. Means within a dependent variable that have uncommon letters differ (P < 0.05).

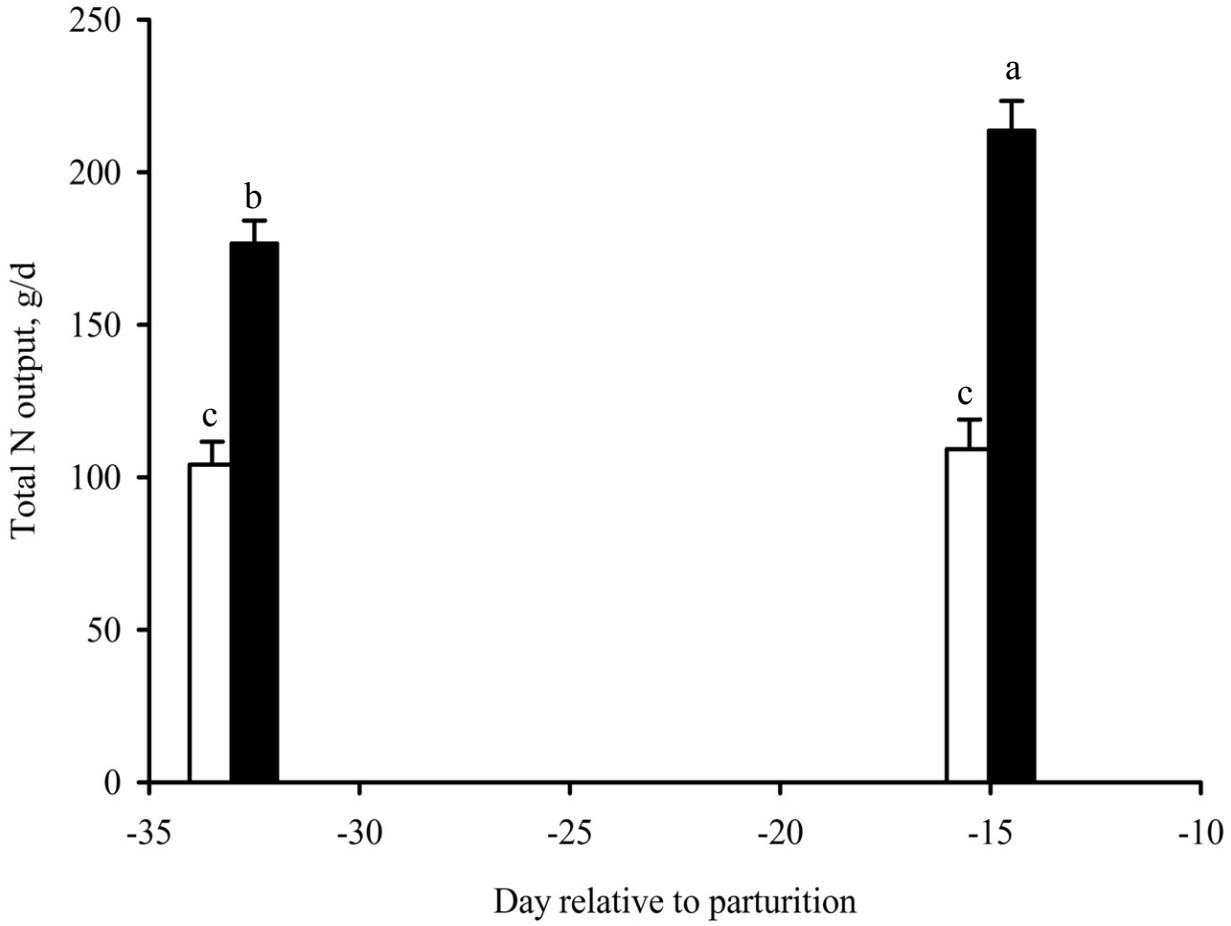

Figure 3.5 The effect of the interaction of treatment and day (P = 0.011) on total nitrogen excretion. Heifers on the CON treatment (white columns) were fed a prepartum designed to meet 100% of the MP requirement and HMP (black columns) were fed a prepartum diet designed to supply 133% of MP requirement. The P-values for the main effects of treatment and day were < 0.001 and 0.002, respectively. Means were separated using the Tukey's test and means with uncommon letters differ (P < 0.05).

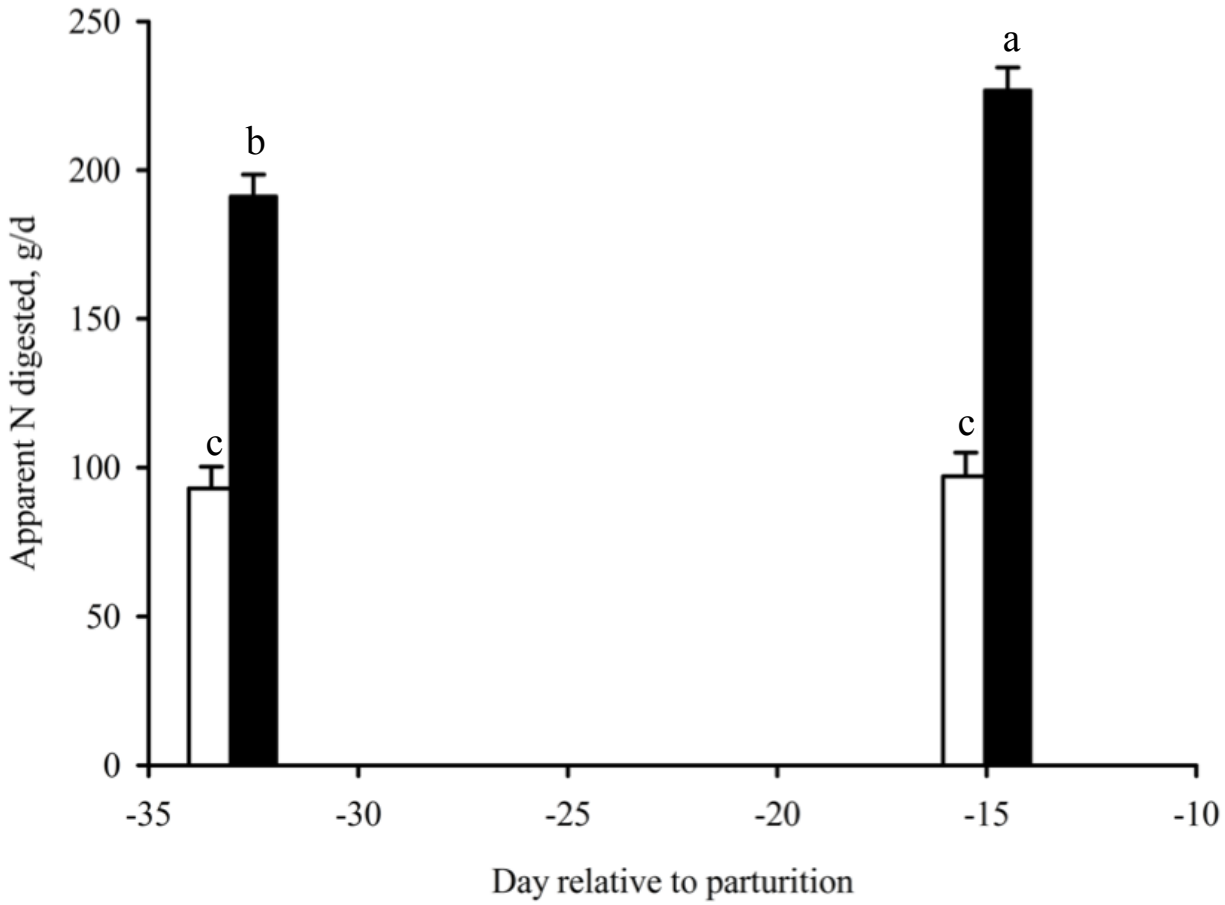

Figure 3.6 The effect of the interaction of treatment and day (P = 0.004) on apparent N digestion for CON (white columns; 100% of MP requirement) and HMP (black columns; 133% of MP requirement) heifers, whereby HMP had greater apparent N digestion than CON heifers on d -15.0 ± 4.4 and d -33.2 ± 5.3. Means within a dependent variable that have uncommon letters differ (P < 0.05). The P-values for the main effects of treatment and day were < 0.001 and < 0.001, respectively. High MP had greater apparent N digestion than CON heifers on d -15.0 ± 4.4 and d -33.2 ± 5.3.

3.5 Discussion

3.5.1 Effect of Increasing Prepartum MP Supply

The current study was designed to evaluate the effect of oversupplying MP to beef heifers during late gestation on nutrient intake, ruminal fermentation, and nitrogen balance. Nutrient supply was predicted using CNCPS 6.5 to provide 831 and 1137 g MP/d for CON and HMP heifers at a predicted consumption of 11.4 and 12.3 kg DM/d. Diets were predicted to differ in MP content offered by 33%, while CP concentration of the diet (% DM) for HMP heifers was 154.8% of that offered to CON. Thus, the study model was effective in over-supplying MP during late gestation to beef heifers, relative to a CON treatment that was near predicted requirements.

Supplementation of CP during late gestation can increase BW prior to parturition for beef cows when compared to diets that are marginally adequate for CP (Martin et al., 2007; Larson et al., 2009), and increases in MP supply during the last trimester of gestation linearly increases ewe BW for ewes fed MP at 60, 100, and 140% of predicted requirements (Van Emon et al., 2014). It should be noted that the studies of Martin et al. (2007) and Larson et al. (2009) applied supplementation strategies that may have also increased the net energy supply in the diet and, thus, improvements in BW cannot solely be attributed to greater quantities of CP supplied. The improvement in BW observed in the current study when feeding HMP is therefore supported by the previously mentioned studies (Martin et al., 2007; Larson et al., 2009; Van Emon et al., 2014). It is not clear what body components account for the added conceptus corrected BW with increased MP supply.

In the current study, it was hypothesized that increasing the supply of MP would improve N balance. We observed that nitrogen intake, apparent total tract nitrogen digestion, and retained nitrogen were greater for HMP heifers than CON heifers. Thus, our hypothesis was confirmed. However, both the CON and HMP heifers had a positive N balance. Though N balance experiments commonly underestimate nitrogen losses (Owen, 1967; MacRae et al., 1993; Spanghero and Kowalski, 1997), the observed N retention values in the present study were 59.4 and 97.1 g N/d for CON and HMP and were relatively similar to calculated values used to

estimate N accretion. To validate the observed N retention values, heifer BW gain was corrected for conceptus weight (NASEM, 2016). All BW gain was assumed to be skeletal muscle accretion as the authors cannot distinguish composition of BW gain. Efficiency of conversion of MP to net protein (**NP**) for conceptus-corrected BW gain was predicted to be 0.50 (NASEM, 2016) and tissue nitrogen composition was assumed static at 26 g N/kg of BW gain (Kohn et al., 2005). The nitrogen required for the observed heifer BW gain between d -55 and -8 was then calculated as 15.6 and 36.4 g N/d, respectively. Conceptus weight was partitioned as predicted for uterine (Koong et al., 1975), fluid, membrane, and fetal weights (based on d of gestation; Ferrell et al., 1976). The nitrogen content of the uterine, fluid, membrane, and fetal components were then individually estimated. Uterine N composition at d -55 and -8 was predicted to be 2.05 and 2.40% (Ferrell et al., 1976). Fluid N composition at d -55 and -8 was predicted to be 0.39 and 0.46% (Ferrell et al., 1976) while membrane N composition at d -55 and -8 was predicted to be 1.40 and 1.65%, respectively (Ferrell et al., 1976). An efficiency of conversion of MP to NP for uterine, fluid, and membrane N was assumed as 0.65 (NRC, 2000), resulting in N accretion estimations of 2.1, 0.93, and 0.95 g N/d for the respective tissues. The nitrogen accretion of the mammary gland was estimated from the data of Ferrell et al. (1976) that reflects the exponential growth of the mammary gland, and, using an efficiency of conversion of MP to NP of 0.65 (NRC, 2000; NASEM, 2016), was calculated to be 1.3 g N/d. Lastly, fetal N accretion between d -55 to -8 was predicted based on estimated N contents of 1.84 and 2.52% (Ferrell et al., 1976) and an assumed efficiency of conversion of MP to NP of 0.50 (Tedeschi et al., 2015) equated to a value of 59.1 g N/d. The estimated fetal nitrogen accretion in the current study is similar to the value estimated by Bell (1995) for a 35 kg Holstein fetus at d 250 of gestation (38 g N/d). Admittedly, there may be differences in the estimation of fetal nitrogen accretion between Bell (1995) and the current study as result of calves in this study having an average birth BW of 36 kg at d 278 of gestation and differing efficiencies of conversion for MP to NP for pregnancy between dairy and beef cattle (Tedeschi et al., 2015). Collectively, N accretion due to conceptus-corrected BW gain, uterine, fluid, placental component gain, mammary gland development, and fetal gain while accounting for the efficiency of nitrogen conversion was estimated to be 71.9 and 100.7 g N/d for CON and HMP heifers, respectively. These values are approximately similar to the measured N retention values of 59.4 and 97.1 g N/d for CON and HMP heifers, thereby validating the N balance measurements and confirming the positive response due to increased

supply of MP during the last 55 d of gestation. The discrepancy in the predicted N accretion for CON heifers (59.4 vs. 71.9 g N/d) may reflect insufficient N retention to match requirements, but more likely reflects the inherent variability in measurement due to unaccounted N losses during sampling or processing of samples (Spanghero and Kowalski, 1997) or the inherent lack of accuracy in estimating N accretion as a result of using constant efficiencies of conversion for MP to NP. For example, using 0.32 as the efficiency of conversion of MP to NP for fetal growth of dairy calves (Bell, 1995) results in an estimated N accretion of 50.4 and 71.2 g N/d for CON and HMP heifers, respectively. For lactation, efficiency of conversion of MP to NP is shown to respond to excess or deficient MP provision (Lapierre et al., 2007), yet the responsiveness of various tissues (i.e. BW gain, fetal growth, uterine development) to over- or under-provision is currently not well-understood in beef cattle.

Corresponding to improved N balance, it was theorized that HMP heifers would have reduced reliance on skeletal muscle as a source of AA for maternal oxidative metabolism or mammary development and fetal skeletal protein synthesis or oxidative metabolism relative to CON heifers, given the hypothesis that CON heifers may have be N deficient despite their rations being formulated to provide adequate MP to match their predicted requirement. As an approach to estimate skeletal muscle catabolism, urinary excretion of 3-MH and creatinine were used as indicators. McCarthy et al. (1984) suggested that 3-MH is a suitable marker of protein turnover in cattle as: 1) 90% of bound 3-MH is in skeletal muscle; 2) 3-MH is not reused for protein synthesis; and 3) 3-MH is excreted rapidly in urine (Harris and Milne, 1981). In contrast to the improved N balance observed in the present study, the excretion of 3-MH by HMP heifers was greater that CON suggesting increased muscle catabolism. The contradictory findings between N balance and 3-MH excretion highlight a challenge when evaluating biomarkers to indicate a biological process as 3-MH is only indicative of protein turnover in muscle and does not provide information regarding the rate of protein synthesis. Moreover, given HMP heifers had a greater BW, it could be postulated that body protein content was also greater. 3-MH excretion can be standardized with creatinine (Simmons et al., 1994) as a proxy for total muscle mass relative to protein mobilization (Pires et al., 2013). In the current study, there was no difference in the urinary 3-MH:creatinine ratio for HMP and CON heifers, further confirming that there was no difference in protein catabolism per unit muscle mass between treatments. Thus, the data suggest that neither the CON nor HMP were in a negative N balance.

Heifers in this study were fed a low-quality forage basal diet as a strategy to manage MP supply. While the previously mentioned approach was used from an experimental perspective, cattle are often fed low-quality forages as a strategy to reduce feed costs and enable extended winter grazing settings (Adams et al., 1996; Anderson et al., 2005). Increasing the supply of MP generally improved apparent total tract digestibility for HMP relative to CON. Often forage-based rations are deficient in rumen degradable protein and supplementation is provided to improve NDF degradability (Paterson et al., 1994), by meeting microbial N demands. Heifers fed HMP had greater ruminal ammonia-N concentrations compared to the CON. Although CON ruminal ammonia-N concentrations were relatively low (1.1 mg/dL), it is important to note that the mean ammonia-N concentration represents one sample collected 4.0 h after feeding (due to constraints on the frequency of sample collection), suggesting that the sample may have been collected near peak ammonia-N demand and concentration. For low-quality forages, ammonia-N from degradation of ruminally degradable protein is typically first-limiting nutrient (Horn and McCollum, 1987) and CP supplementation frequently increases diet digestibility (Wallace, 1988; DelCurto et al., 1990; Beaty et al., 1994). Supporting the notion that rumen degradable protein may have been limiting for CON, the concentration of branched-SCFA was greater for HMP than CON (Andries et al., 1987) suggesting some of the amino acids were deaminated as a source of N for the ruminal microbial population. Thus, the improvements for OM and NDF digestibility for HMP over CON are likely mediated through increased proliferation or activity of cellulolytic microbial species (and thus, capacity for NDF degradation) as greater quantities of substrates, such as ammonia-N (Russell et al., 1992) and branched SCFA (Russell and Sniffen, 1984) were available for microbial growth (Russell and Hespell, 1981).

However, caution must be applied when using the concentration of ruminal ammonia-N collected at one time-point to determine adequacy of RDP provision for CON heifers. Recently, concentration of SCFA have been contested in their validity as an estimate of treatment effects on rumen fermentation in vivo (Hall et al., 2013; Hall et al., 2015) due to unaccounted variation in other factors such as ruminal absorption, fluid passage rate, fluid dilution, and ruminal liquid volume (Hall et al., 2015). It follows logically that the same factors may influence ruminal ammonia-N concentration. Increased water inflow and the arising dilution may help explain the observed effect of treatment and time on HMP ruminal ammonia-N concentration where despite increased DMI and N intake, ruminal ammonia-N concentration was less on d -15 relative to d -

33. Additionally, the time of ruminal fluid collection (4 h post-feeding) in the present study occurred when ruminal fluid fill is expected to be greatest and therefore the low ammonia-N for CON heifers may be the result of increased fluid dilution rather than insufficient RDP.

3.5.2 The Prepartum Transition Period for Beef Cattle

For dairy cattle, the transition period has been highlighted as the 3 wk prior to and 3 wk following parturition (Drackley, 1999) and it is accompanied by significant alterations in organic nutrient metabolism and metabolic and hormonal function (Bell, 1995). Major changes occurring during the prepartum portion include a marked reduction in DMI (Hayirli et al., 2003), where DMI (%BW) can decrease by 25 to 50% from wk -2 until calving (Marquardt et al., 1977) with much of the reduction occurring in the last week of gestation. The combined increase in nutrient requirements and reduction in DMI predispose dairy cattle to a negative energy and protein balance (Drackley, 1999). However, there are limited data describing whether beef cattle experience similar challenges as dairy cattle during the transition period. In the present study, we observed that DMI (%BW) decreased during the week prior to parturition equating to an 8.0% decline. Linden et al. (2015) observed a 9.1% reduction in DMI (%BW) for pregnant beef heifers during the last week of gestation. While the pattern for a reduction in DMI relative to parturition appears to be similar in beef cattle relative to dairy cows, the depression in DMI in transition dairy cattle is more exaggerated (Hayrili et al., 2003). Reasons for differing magnitude for the reduction in DMI may include the inherent composition of the diets commonly fed to beef or dairy cattle. Often energy dense rations are fed to close-up dairy cattle to reduce the extent of negative energy balance. Yet, more rapidly fermentable starch diets can reduce meal size by 17% and DMI by 8% (Oba and Allen, 2003) and more energy dense rations can increase the magnitude of reduction in DMI (Rabelo et al., 2003; Penner et al., 2007). In contrast, increasing the proportion of forage, and particularly low-quality forage (as is typical for beef cow diets) in periparturient rations can mitigate the severity of DMI depression prepartum (Dann et al., 2007). Thus, part of the discrepancy for the severity of DMI depression between beef and dairy cattle may be due to differences in the proportion of forage in the diet and overall diet fermentability fed during the prepartum transition phase.

In addition to changes in DMI, we observed that maximum ruminal pH increased and there was a tendency for an increase in mean ruminal pH as parturition approached. The decreased DMI coupled with decreased apparent total tract starch digestion likely contributed to the increases observed for ruminal pH. Other studies have reported increased passage rate (Gunter et al., 1990; Hanks et al., 1993; Linden et al., 2015) and increased passage rate may also contribute to greater ruminal pH through reduced SCFA production and potentially greater proton outflow (Allen, 1997).

3.6 Conclusion

Results of the current study indicate that provision of MP in excess of predicted requirements improved N balance and retention, nutrient digestion, and prepartum BW gain for HMP heifers relative to CON. In addition, feeding HMP improved ruminal fermentation indicated by greater concentrations of ruminal ammonia-N and NDF digestibility. Despite decreased DM (%BW) during the week prior to parturition, neither HMP nor CON heifers appeared to be in a state of negative nitrogen balance as indicated a positive N balance and the lack of difference in urinary 3-MH and 3-MH:creatinine between treatments. In conclusion, supplying MP in excess of predicted requirements improved nitrogen balance in late gestation beef heifers that were consuming adequate amounts of metabolizable energy. Yet, over-provision of MP may not be a practical applied strategy for late gestation beef heifers, as CON heifers consumed adequate quantities of MP and were in a positive N balance.

4.0 OVER-SUPPLYING METABOLIZABLE PROTEIN TO LATE GESTATION BEEF CATTLE: EFFECTS ON POSTPARTUM RUMINAL FERMENTATION, BLOOD METABOLITES, COLOSTRUM COMPOSITION, AND MILK YIELD AND COMPOSITION

4.1 Abstract

The objective of the study was to determine the effect of oversupplying metabolizable protein (**MP**) during late gestation on postpartum BW and BCS, colostrum composition, milk production and composition, and blood and urinary metabolites. Twenty-four primiparous crossbred Hereford heifers were assigned to either a control diet designed to meet MP requirements (**CON**; n = 12) or a treatment providing 133% of the MP requirement (**HMP**; n =12) with 10 CON and 11 HMP cow-calf pairs used postpartum. Cows were individually fed their treatment diets from d -55 ± 3.7 until parturition and were then provided a common diet during the postpartum period. Cow BW was measured on d 7 ± 1.0, 14 ± 2.0, 28 ± 2.9, 57 ± 3.6, 82 ± 5.1, and 112 ± 2.9 d relative to parturition. Ruminal pH and DMI were measured daily and summarized by wk until d 28. Daily milk yield was estimated using a 12-h 2-quarter milk yield protocol on d 7 ± 0.9, 12 ± 0.9, 28 ± 2.9, 33 ± 2.9, 70 ± 3.2, and 112 ± 2.9. Plasma and serum samples were collected on d 7 ± 0.9, 28 ± 2.9, 70 ± 3.2 and 112 ± 2.9 and analyzed for NEFA, BHBA, glucose, insulin, and plasma urea-N. Spot urine samples were collected over a 6-d period beginning on d 7 ± 0.9 and 28 ± 2.9 and the composited samples were analyzed for concentrations of urea, CP, 3-MH (**3-MH**), and creatinine. Data were analyzed as a randomized complete block design using the MIXED procedure of SAS accounting for repeated measures. Postpartum BW did not differ ($P \geq 0.30$) by treatment, day, or the interaction of treatment and day, but rump fat thickness decreased ($P = 0.011$) as lactation progressed for both CON and HMP heifers. Dry matter intake decreased during wk 2 and 3 compared to 1 and 4, whereas ruminal pH was less during wk 2, 3, and 4 relative to wk 1. Colostrum fat concentration was less ($P = 0.003$) for HMP than CON. Milk production and milk component concentrations were not affected by treatment. Milk yield was greatest from d 7 to 33 and decreased thereafter ($P < 0.001$). Plasma and serum metabolites were not affected by treatment, but NEFA was greater ($P < 0.001$) on d 7 and 28 relative to 70 and 112. Urinary 3-MH

and the 3-MH:creatinine ratio did not differ by treatment, day, or the interaction of treatment and day ($P \geq 0.22$). Calf growth was not affected by treatment. Overfeeding MP during late gestation does not improve postpartum indicators of N balance, but may alter colostrum composition, without affecting milk production and composition or calf growth.

4.2 Introduction

Late gestation and early lactation represent physiological stages for beef cattle where nutrient requirements are greatest due to fetal growth (Du et al., 2010), mammary tissue development (Rattray et al., 1974), colostrogenesis, and the initiation and onset of lactation (NASEM, 2016). Colostrogenesis occurs during the last month prior to parturition and colostrum is higher in fat, protein, and total solids content than milk (Jaster, 2005). Following parturition, lactating heifers are expected to have maintenance and growth requirements that are 20% greater than those of non-lactating heifers (Larson, 2007) in addition to their nutrient requirements for lactation. Milk yield is reported to range between 4 and 15 kg/d, depending on breed and parity (NASEM, 2016). Milk composition varies among breeds but typically contains 3.5 to 4.0% fat, 3.3 to 3.8% protein, and 8.3% total solids-not-fat (NASEM, 2016). The resulting milk energy output may be sufficient to induce a negative energy and protein balance.

Gastrointestinal fill has been observed to be lower in early lactation ewes compared to non-lactating ewes (Gunter et al., 1990) and a pattern of depressed, but rapidly increasing DMI at the start of lactation in dairy cows is well-established (Lean et al., 2013). The low feed intake immediately following parturition followed by a subsequent increase may suggest that a negative nitrogen balance may occur early postpartum. However, the extent to which adequate MP supply may be compromised during early lactation and the influence of late gestation nitrogen balance on the postpartum transition phase is not known. Prepartum CP and high-protein by-product supplementation relative to a mild nitrogen restriction has consistently improved BW and BCS at calving (Bohnert et al., 2013; Stalker et al., 2007; Larson et al., 2009) and can shorten the postpartum interval and improve conception rates (Bohnert et al., 2002; Richards et al., 1986), suggesting that there may be benefit from increasing MP supply in late gestation to positively influence early lactation nitrogen balance and cow-calf performance.

The hypothesis for this study was that oversupplying MP (133% of the predicted requirement) prepartum would mitigate a maternal nitrogen deficiency prepartum (data presented in previous chapter) and improve postpartum indicators of nitrogen balance and milk production. The primary objective was to evaluate whether increasing MP supply prepartum would affect milk production and composition, indicators of skeletal muscle catabolism, and calf growth performance. A secondary objective was to characterize postpartum phase in terms of DMI, ruminal fermentation patterns, and lactation characteristics of the transition period for beef cattle.

4.3 Materials and Methods

All procedures used in this study were pre-approved by the University of Saskatchewan Animal Research Ethic Board (protocol 20100021).

4.3.1 Experimental Design, Cow Husbandry, and Dietary Treatments

This chapter is 1 of 2 addressing the effects of increasing MP supply prepartum. Chapter 3.0 addressed prepartum responses and the current chapter addresses postpartum responses. Detailed materials and methods have been presented in chapter 3.0. Briefly, a total of 24 primiparous pregnant Hereford-cross heifers, including 14 that were fitted with a ruminal cannula (model 9C, Bar Diamond, Parma, ID), were used in this experiment. Heifers were bred using fixed-time artificial insemination with sexed semen to a single sire (Cole Creek Cedar Ridge 1V, Reg. No. 1659099 (CAN), Genex Co-operative Inc., Shawano, WI, USA) to minimize genetic variation. The experiment was arranged as general randomized complete block design with the expected date of parturition as the blocking factor. Within block, heifers were assigned to treatments while balancing for the number of cannulated heifers and initial BW. Starting on d -55 ± 3.7 d relative to parturition, heifers were housed in individual pens (9 m^2) with rubber mats. Pens were cleaned and washed daily. Ten days prior to parturition, heifers were moved to pens (18 m^2) bedded with straw and maintained there until 7 d following parturition. On day 8 of lactation, cow-calf pairs were moved into individual bedded outdoor pens (36 m^2) until d 33. Cows were group-fed by prepartum treatment starting on d 34 relative to calving, with 3 to 4 cow-calf pairs/group until d 112 relative to parturition.

Treatments consisted of the control treatment (**CON**; n = 12) or a treatment in which MP was purposely over-fed (**HMP**; n =12). The CON was designed to provide 100% of the predicted MP requirement based on CNCPS 6.5 using the Nutritional Dynamic System software (RUM&N Sas, Via Sant'Ambrogio, Italy), while the HMP was formulated to provide 133% of the predicted MP requirements. Prepartum diets were formulated to be isoenergetic providing 101.6 and 94.6 % of predicted ME requirements for HMP and CON, respectively. The predicted supply was based on a heifer BW of 550 kg (BCS 5) at 260 d of gestation and a calf birth weight of 36 kg. Exposure to the dietary treatments was initiated on d -55.3 ± 3.7 relative to parturition. The diets consisted of the same barley green feed forage and wheat straw with cows being offered 1 of 2 prepartum supplemental pellets based on their respective treatment (Table 4.1). At parturition, two cow-calf pairs were removed due to dystocia. Additionally, one cow became too aggressive after calving to safely continue the experiment following parturition. As a result, there were 21 cow-calf pairs available for data collection during the postpartum period (CON = 10; HMP = 11). During the postpartum period, all cows were fed a common lactation diet (60 to 40 forage-to-concentrate ratio) formulated to meet the requirements for lactation based on CNCPS 6.5 (Table 4.1). Nutrient requirements for lactation were predicted using a mature BW of 550 kg, BCS of 5 on a scale of 1 to 9, average of 30 DIM, and 6 kg/d milk yield with expected composition of 3.60% milk fat, 3.60% milk protein, and 4.90% milk lactose. Cows were individually fed lactation rations from parturition to d 33 and were subsequently group-housed (3 to 4 cow-calf pairs based on maternal prepartum treatment) until d 112. Total mixed rations were fed twice daily at 0900 and 1630 h, targeting ad libitum intake (5 to 10% the weight of the feed offered refused daily on an as fed basis). Apart from six 12-h intervals where calves were separated from their dams for measurement of 24 h theoretical milk yield (described below), calves had constant access to their dams over the pre-weaning period. Water was constantly available and dry feed (i.e. forage and concentrate) was accessible for calves after d 33.

4.3.2 Data and Sample Collection

4.3.2.1 Dry matter intake

Dry matter intake was determined daily from the day of calving to d 33 by measuring the weight of the feed offered and the weight of the feed that was refused. Feed bunk design (height

Table 4.1 Ingredient and chemical composition of high metabolizable protein (HMP = formulated to 133% of MP requirements) and control (CON = formulated to 100% of MP requirements) rations fed during the final 8 wk of gestation, and the lactation (LAC = formulated to 103% of MP requirements) ration fed from parturition to d 112.

	Prepartum diets		Postpartum diet
Item	CON	HMP	LAC
Ingredient composition, % DM			
Barley greenfeed	37.4 ± 0.5	37.4 ± 0.5	37.2 ± 0.7
Wheat straw	23.3 ± 0.7	23.2 ± 0.6	23.4 ± 0.9
Supplemental pellet[1,2]	39.3 ± 0.9	39.4 ± 0.9	39.4 ± 0.8
Chemical composition, % DM			
DM, %	90.3 ± 2.4	89.4 ± 2.7	90.0 ± 2.0
OM	91.4 ± 0.3	91.0 ± 0.3	91.4 ± 0.3
CP	9.3 ± 0.5	14.4 ± 0.3	8.6 ± 0.4
Predicted MP[4]	7.4 ± 0.0	9.4 ± 0.0	7.7 ± 0.0
ADF	32.7 ± 1.4	31.2 ± 1.4	33.7 ± 1.0
aNDFom[5]	50.5 ± 1.6	46.4 ± 1.5	50.7 ± 1.5
Starch	14.8 ± 1.3	13.8 ± 1.0	14.6 ± 0.7
Ether extract	1.4 ± 0.1	1.4 ± 0.2	1.3 ± 0.1
Calcium	0.6 ± 0.05	0.7 ± 0.03	0.6 ± 0.03
Phosphorous	0.2 ± 0.02	0.3 ± 0.01	0.2 ± 0.01

[1]High protein supplement contained 24.0% canola meal solvent, 14.8% soybean meal 47.5 solvent, 1.9% oat hulls, 14.8% molasses beet, 37.0% ground barley grain, and 7.6% custom mineral and vitamin supplement[3]; chemical composition: 9.244 kIU/kg of Vitamin A, 0.843 kIU/kg of Vitamin D_3, 0.185 kIU/kg of Vitamin E, 708.26 ppm of Cu, 192.02 ppm of Fe, and 986.74 ppm of Zn.

[2]Control and lactation supplements contained 24.0% oat hulls, 27.7% molasses beet, 40.7% ground barley grain, and 7.6% custom mineral and vitamin supplement[3]; chemical composition: 9.245 kIU/kg of Vitamin A, 0.843 kIU/kg of Vitamin D_3, 0.185 kIU/kg of Vitamin E, 709.40 ppm of Cu, 153.92 ppm of Fe, and 971.86 ppm of Zn.

[3]Mineral and vitamin supplement contained 1.74% zinc oxide, 4.88% vitamin E premix, 0.98% vitamin A premix, 0.49% vitamin D, 0.13% Sel-Plex 2000 (Alltech), 7.62% white salt, 9.77% magnesium oxide, 24.42% ground limestone, 21.88% ground barley grain, 24.42% manganese oxide, and 3.66% copper sulfate $5H_20$.

[4]Metabolizable protein was predicted using CNCPS 6.5 using average values for RUP and RDP.

[5]Amylase- and sodium sulfite-treated NDF corrected for ash content.

and depth) prevented calves from consuming the ration. Throughout the study, samples of forages were collected once weekly and samples of the concentrate were collected every two weeks. Samples of refusals were composited weekly by day (proportionally by the quantity refused/d on an as is basis). The feed and composited refusal samples were placed in a forced-air oven at 55ºC until dried to a constant weight.

4.3.2.2 Body weight and rib and rump fat thickness

Cows were weighed at calving and on d 7 ± 1.0, 14 ± 2.0, 28 ± 3.0, 57 ± 3.6, 84 ± 3.0, and 112 ± 2.9. Ultrasonography (Aloka SSD-500; 17 cm 3.5 mhz linear transducer: Aloka UST-5044-3.5) was used to determine rib (between the 12th and 13th rib) and rump fat thickness (Broring et al., 2003) on d 14 ± 2.0, 28 ± 3.0, 57 ± 3.6, 84 ± 3.0, and 112 ± 2.9.

4.3.2.3 Ruminal fermentation, urine and blood metabolites

For cannulated cows, ruminal digesta was collected from three different locations (250 mL/region): cranial, ventral, and caudal regions of the rumen at the ruminal fluid rumen mat interface. Samples were collected at 1300 h on d 7 ± 0.9 and 28 ± 2.9 relative to parturition. Digesta was strained through 2 layers of cheesecloth and two 10-mL aliquots of ruminal fluid were collected. One 10-mL aliquot was added to 2 mL of 25% metaphosphoric acid (w/v) and was stored frozen (-20°C) until being used for determination of short chain fatty acid (**SCFA**) concentration by gas chromatography (Khorsani et al., 1996). The second 10-mL aliquot was added to 2 mL of 1% sulphuric acid and was stored frozen (-20°C) until being used for determination of ammonia-N concentration (Fawcett and Scott, 1960).

Ruminal pH was measured every 5 min in cannulated cows from parturition until d 28 using the Lethbridge Research Center ruminal pH measurement system (**LRCpH**; Penner et al., 2006). The LRCpH was standardized and inserted through the ruminal cannula into the ventral sac of the rumen. On a weekly basis, the LRCpH was removed, data were downloaded, and the system was standardized and re-inserted. The standardization process, used prior to and following in vivo measurements, included the use of standard buffer solutions (pH 4 and 7; Ricca Chemical Company, Arlington, TX) and was conducted at 39°C. Data were transformed from mV recordings to pH using beginning and ending regressions with the assumption of linear drift over time (Penner et al., 2006).

Spot urine samples were collected over a 6-d period beginning on d 7 ± 1.0 and d 28 ± 2.9 relative to parturition. Eight samples were collected at 18-h intervals between samples (Chizzotti et al., 2008), and were stored frozen (-20°C) until being composited on an equal volume basis (to represent 1 full day of sample collection every 3 h). Samples were analyzed for urea-N concentration, 3-MH, creatinine, and CP content. Urine urea-N was analyzed by the method described by Fawcett and Scott (1965). Crude protein content was analyzed according to the AOAC (1994) method for Kjeldahl determination of CP. Urine 3-MH (Rathmacher et al., 1992) and creatinine (Slot, 1965) concentrations were analyzed by Heartland Assays (Ames, Iowa).

Blood samples from the jugular vein were collected the day of ruminal samples at 1900 h to coincide with 12-h milk yield measurements. Sample vials contained either 158 IU of heparin for plasma or a clot activator for serum (BD Vacutainer, BD and Company, Frankin Lakes, NJ). Blood was immediately centrifuged (2,000 × *g* at 4°C for 15 min) to separate plasma while blood samples for serum collection were allowed to sit at room temperature for 10 min to facilitate clotting prior to centrifugation (2,000 × g at 4°C for 15 min). Harvested plasma and serum were stored at -20°C until analysis. Serum NEFA was analyzed using the NEFA-HR (2) kit (Wako USA, Richmond, VA, United States of America). Plasma glucose (product numbers P7119 and number F5803, Sigma Aldrich, Oakville, ON, Canada), serum urea-N (Fawcett and Scott, 1960), and BHBA (Williamson et al., 1962) concentrations were determined using colorimetric methods. Plasma insulin was analyzed using an ELISA (Mercodia Bovine Insulin ELISA, Mercodia, Uppsala, Sweden). The inter- and intra-plate assay variation was 4.5 ± 3.4 and 2.9 ± 0.4%.

4.3.2.4 Colostrum composition, milk production and composition

Colostrum was collected immediately following parturition (within 1 h) and prior to the calf nursing. Colostrum yield was not determined due to difficulty in obtaining a complete collection as the colostrum was too viscous to extract sufficiently from the mammary gland using a portable milking machine (Deluxe Portable Pump, E-Zee Milking Equipment, Gordonville, PA, USA). The colostrum sample was diluted at a ratio of 1:3 colostrum:water (purified through distillation and reverse osmosis) and analyzed at Central Milk Testing Lab (Dairy Herd Improvement, Edmonton, AB, Canada) for composition (lactose, fat, CP, urea-N, SCC and total solids). The remaining colostrum was available for the calf to nurse. The concentration of IgG was

determined at the Saskatoon Colostrum Company using a radial immunodiffusion assay (Fleenor and Stott, 1981).

Milk yield and composition were determined on d 7 ± 0.9, 12 ± 0.9, 28 ± 2.9, 33 ± 0.9, 70 ± 3.2, and 111 ± 2.9 relative to parturition. At each time point, the 12-h milk yield 2-quarter milk yield was determined. Cattle were moved into a chute and administered with 4 mL of oxytocin (OXY-20 NW, Rafter 8, Calgary, AB, Canada). All four quarters were milked at 0630 h and the cow and calf were separated using fence-line contact to prevent nursing for 12 h while still allowing for visual and some physical contact. Calves had access to water and were offered forage and concentrate during the 12 h separation. After 12 h, the cow was moved back to the chute, injected with 4 mL oxytocin, and 2 diagonal quarters (back right and front left) were milked. Two-quarter milk production (12-h yield) was measured and representative samples were collected for analysis of lactose, MUN, fat, protein, and SCC at Dairy Herd Improvement (Edmonton, AB). Milk net energy output was calculated from milk composition (NRC, 2001) based on a predicted 4-quarter 24-h yield (12-h 2-quarter measured yield × 4).

4.3.2.5 Calf growth performance

Calves were weighed immediately after birth and frame size was measured (crown to rump length, heart-girth, and hip and wither height). Calf BW and frame measurements were collected at 2 and 4 wk of age. After 4 wk of age, calves were weighed and measured every 28 d until weaning at 112 d. Average daily gain was assumed to be equal to the slope arising from the linear regression between calf BW and day of age.

4.3.3 Statistical Analysis

All data were analyzed as a randomized complete block design using the PROC MIXED of SAS 9.4 (SAS Institute, Cary, NC, USA). Treatment, time (day relative to parturition), and the interaction of treatment and time were included as fixed effects within the model. Cow nested in block was included as a random effect. In all cases, time was considered as a repeated measure. When data were equally spaced, autoregressive, heterogeneous autoregressive, compound symmetry, heterogeneous compound symmetry, toeplitz, heterogeneous toeplitz, simple, unstructured, and ante-dependence covariance structures were used to find best fit for the data

(lowest AIC and BIC). When data were unequally spaced, unstructured, ante-dependence, simple, compound symmetry, and heterogeneous compound symmetry covariance structures were used to find the best fit for the data. Significance was declared when $P < 0.05$ and tendencies were considered when $0.10 > P > 0.05$. When the F-test was significant, means were separated (Tukey's) to determine means that differed.

4.4 Results

4.4.1 Cow BW and Rib and Rump Fat Depth

Cow BW was not affected ($P \geq 0.30$; Table 4.2) by treatment, day, or the interaction of treatment and day. An interaction between treatment and day was detected for rib fat depth ($P = 0.022$); however, the Tukey's post-hoc mean separation test did not identify means that differed (data not shown). Rump fat depth did not differ ($P \geq 0.15$) by treatment or the interaction of treatment and day, but decreased ($P = 0.011$) in thickness from d 14 and 28 to d 112 resulting in respective reductions of 1.8 and 1.9 mm.

4.4.2 Dry Matter Intake and Ruminal Fermentation

Cows fed the CON and HMP treatments prepartum did not differ ($P = 0.80$; Table 4.3) for DMI when reported as kg/d or as a proportion of BW, nor was DMI affected ($P = 0.51$) by the interaction of treatment and day. Dry matter intake (kg/d and % BW) was less ($P \leq 0.009$) during wk 2 and 3 relative to wk 1 and 4 post-parturition.

Treatment and the interaction of treatment and week did not affect ($P \geq 0.20$) minimum, mean, or maximum pH. However, minimum, mean and maximum pH were all greatest ($P \leq 0.013$) during the first wk following parturition relative to the following 3 wk. Total SCFA concentration did not differ ($P \geq 0.53$; Table 4.4) by treatment, day, or the interaction of treatment and day. When expressed as a molar proportion, acetic acid tended to decrease ($P = 0.076$) and isovaleric acid was greater ($P = 0.002$) on d 7 compared to d 28. Isovaleric acid also tended to be greater ($P = 0.070$) for CON compared to HMP cows. Although cows were fed the same diet postpartum, ruminal ammonia-N concentration was greater ($P = 0.013$) for HMP relative to CON cows.

Table 4.2 Postpartum cow BW, and rib and rump fat depth for cows fed diets with either 100 (CON) or 133% (HMP) of predicted metabolizable protein requirements during the final 8 wk of gestation.

	Treatment			Day[1]							*P*-values		
Item	CON	HMP	SEM[2]	d 7	d 14	d 28	d 57	d 82	d 112	SEM[2]	Treatment	Day	T × D[3]
Cow BW, kg	530	557	17.4	540	553	534	545	541	545	13.6	0.30	0.30	0.54
Rib fat depth[4], mm	5.3	6.3	0.8	—	5.6	5.8	5.8	5.7	5.9	0.6	0.36	0.96	0.022
Rump fat depth, mm	6.4	8.7	1.1	—	8.2[a]	8.3[a]	7.3[ab]	7.4[ab]	6.4[b]	1.1	0.15	0.011	0.39

[a,b]Means within row differ significantly ($P < 0.05$).

[1]Day relative to parturition: d 6.6 ± 1.0; d 14.0 ± 2.0; d 27.9 ± 2.9; d 57.4 ± 3.6; d 82.3 ± 5.1; and d 111.9 ± 2.9.

[2]Largest SEM shown.

[3]T × D = Treatment and day interaction.

[4]Means for treatments within a point in time were not different after having been separated using Tukey's mean separation test.

Table 4.3 Postpartum DMI (% BW) and minimum, mean, and maximum ruminal pH of cows fed with either 100 (CON) or 133% (HMP) of predicted metabolizable protein requirements during the final 8 wk of gestation.

	Treatment			Week relative to parturition					*P*-values		
Item	CON	HMP	SEM[1]	1	2	3	4	SEM[1]	Treatment	Week	T × W[2]
DMI, n	10	11		21	21	21	21				
DMI, kg/d	10.8	11.4	0.5	12.1	10.6	10.4	11.4	0.5	0.42	< 0.001	0.67
DMI, % BW	2.1	2.1	0.1	2.3[a]	1.9[b]	1.9[b]	2.2[a]	0.1	0.80	0.009	0.51
Ruminal pH, n	5	7		12	11	11	11				
Minimum	5.87	5.96	0.05	6.20[a]	5.87[b]	5.3[b]	5.76[b]	0.08	0.20	0.013	0.91
Mean	6.36	6.42	0.07	6.68[a]	6.39[b]	6.29[b]	6.21[b]	0.07	0.54	<0.001	0.79
Maximum	6.83	6.88	0.10	7.10[a]	6.94[ab]	6.73[c]	6.66[bc]	0.08	0.68	0.004	0.93

[a,b,c]Means within row are different ($P < 0.05$).

[1]Largest SEM shown.

[2]T × W = Treatment and week interactions.

Table 4.4 Postpartum ruminal SCFA and ammonia-N concentrations for cows fed rations with either 100 (CON) or 133% (HMP) of predicted metabolizable protein requirements during the final 8 wk of gestation.

	Treatment			Day[1]			*P*-values		
Item	CON	HP	SEM[2]	d 7	d 28	SEM[2]	Treatment	Day	T × D[3]
n	5	7		12	12				
Total SCFA, m*M*	119.2	121.4	5.2	120.5	120.0	4.8	0.75	0.94	0.53
SCFA, mol/100 mol									
Acetic acid	66.6	67.7	0.9	68.4	65.9	1.1	0.33	0.076	0.75
Propionic acid	18.5	16.9	1.1	16.8	18.6	1.4	0.28	0.24	0.52
Isobutyric acid	0.6	0.6	0.03	0.6	0.6	0.03	0.46	0.81	0.51
Butyric acid	12.0	12.9	0.4	12.2	12.7	0.4	0.11	0.38	0.25
Isovaleric acid	0.8	0.7	0.1	0.6	0.9	0.1	0.070	0.002	0.25
Valeric acid	1.0	1.0	0.04	1.0	1.0	0.03	0.18	0.12	0.87
Caproic acid	0.5	0.4	0.1	0.5	0.4	0.1	0.21	0.37	0.42
Rumen ammonia-N, mg/dL	0.6	1.0	0.1	0.9	0.8	0.1	0.013	0.45	0.48

[1]Collections occurred from d 6.7 to 11.7 ± 0.9 and from d 27.8 to 32.8 ± 2.9.

[2]Largest SEM is reported.

[3]T × D = Treatment and day interaction.

4.4.3 Colostrum Composition, Milk Production and Milk Composition

The majority of colostrum components (concentrations of protein, lactose, urea-N, total solids, and IgG) did not differ ($P \geq 0.17$; Table 4.5) by maternal prepartum treatment. However, colostrum fat concentration was less ($P = 0.003$) for HMP cows compared to CON (3.4 vs. 7.0 ± 0.8%). The net energy concentration of colostrum tended ($P = 0.052$) to be greater for CON cows compared to HMP cows.

Treatment and the interaction of treatment and day did not affect ($P \geq 0.20$; Table 4.6) milk yield, milk composition, and energy output in milk. Milk yield did not differ between d 7, 12, 28, and 33, but was less ($P < 0.001$) on d 70 relative to d 7 and 12. The lowest milk yield occurred at d 112 ($P < 0.001$) compared to all previous d apart from d 70. Milk protein and lactose concentration were less ($P < 0.001$) on d 28 and 33 relative to d 7, 12, 70, and 112. Milk urea-N concentration was lower on d 33 than d 12 and d 112 ($P = 0.007$). The concentration of total solids was less ($P = 0.022$) on d 28, 33, and 70 compared to d 112. Milk protein and lactose yields gradually declined ($P < 0.001$) with advancing days in milk. Day affected milk fat yield ($P = 0.043$); however, the Tukey's post-hoc means separation test did not detect means that differed by d of measurement for milk fat yield. Milk urea-N excretion decreased ($P = 0.013$) by 19.2% from d 7 relative to 33.

4.4.4 Urine and Blood Metabolite Concentrations

Treatment and the interaction of treatment and day did not affect urine metabolite concentrations ($P \geq 0.18$; Table 4.7). Standardization of 3-MH concentration by creatinine concentration showed no effect ($P \geq 0.64$) of treatment, day, or the interaction of treatment × day. Urinary CP concentration decreased ($P < 0.001$) and urea tended to decrease ($P = 0.099$) from d 7 to 28 relative to parturition.

Non-esterified fatty acid concentration was greater ($P < 0.001$; Table 4.8) at d 7 and 28 relative to 70 and 112. On average, NEFA concentration was 70.6% greater at d 7 and 28 as compared to 70 and 112. Although a treatment × day interaction ($P = 0.070$) was detected for plasma glucose concentration, the Tukey's post-hoc test did not detect means that differed. Insulin

Table 4.5 Colostrum composition for cows fed rations with either 100 (CON) or 133% (HMP) of predicted metabolizable protein requirements during the final 8 wk of gestation.

	Treatment			
Item	CON	HMP	SEM[1]	*P*-value
n	11	10		
Colostrum composition				
Fat, %	7.0	3.4	0.8	0.003
Protein, %	18.1	17.3	1.3	0.66
Lactose, %	2.1	2.4	0.2	0.17
Urea-N, mg/dL	58.0	55.8	16.0	0.99
Total solids, %	31.7	27.5	1.9	0.26
Net energy, Mcal/kg	1.7	1.4	0.1	0.052
IgG, g/L	153.7	146.5	16.7	0.78

[1]Largest SEM is reported.

Table 4.6 Milk production, composition, and component yield for cows fed rations with either 100 (CON) or 133% (HMP) of predicted metabolizable protein requirements during the final 8 wk of gestation.

	Treatment			Day[1]							*P*-values		
Item	CON	HMP	SEM[2]	d 7	d 12	d 28	d 33	d 70	D 112	SEM[2]	Treatment	Day	T × D[3]
n	10	11		21	21	21	21	18	21				
Milk yield, kg/d	6.1	6.5	0.5	6.9[a]	6.8[a]	6.6[ab]	6.7[ab]	5.7[bc]	5.1[c]	0.4	0.59	< 0.001	0.46
Milk composition													
Fat, %	3.50	3.72	0.13	3.59	3.62	3.43	3.46	3.44	4.12	0.20	0.25	0.15	0.75
Protein, %	3.64	3.69	0.06	3.89[a]	3.69[bc]	3.38[d]	3.45[d]	3.62[c]	3.96[ab]	0.08	0.47	< 0.001	0.81
Lactose, %	4.60	4.65	0.06	4.55[c]	4.56[bc]	4.80[a]	4.77[ab]	4.56[bc]	4.51[c]	0.08	0.55	< 0.001	0.51
Somatic cell count, 1,000 cells/mL	157.2	196.8	79.3	191.8	413.5	51.9	92.0	188.2	124.6	272.3	0.72	0.058	0.41
Urea-N, mg/dL	10.1	10.4	0.5	10.2[ab]	10.4[a]	10.1[ab]	8.9[b]	10.1[ab]	11.8[a]	1.1	0.63	0.007	0.20
Total solids	12.8	13.0	0.2	13.1[ab]	12.9[ab]	12.6[b]	12.6[b]	12.6[b]	13.6[a]	0.2	0.35	0.022	0.44
Net energy, Mcal/L	0.7	0.7	0.0	0.7[ab]	0.7[ab]	0.7[b]	0.7[ab]	0.7[ab]	0.8[a]	0.0	0.31	0.049	0.63
Milk component yield, g/d													
Fat[4]	212.1	242.8	20.9	246.8	242.5	229.4	235.5	200.0	210.4	18.6	0.3	0.043	0.64
Protein	220.2	235.6	14.5	266.8[a]	248.2[ab]	221.7[bc]	228.2[bc]	203.9[c]	198.5[c]	13.0	0.45	< 0.001	0.65
Lactose	284.9	197.4	21.3	313.0[a]	309.9[ab]	316.6[a]	318.1[a]	261.5[bc]	227.5[c]	18.9	0.68	< 0.001	0.48
Milk urea-N excretion, mg/d	598.6	651.9	46.4	710.0[a]	706.0[ab]	669.4[ab]	595.4[b]	476.9[ab]	594.7[ab]	106.0	0.43	0.013	0.060
NE_L expenditure, Mcal/d	4.3	4.7	0.4	5.0[a]	4.8[a]	4.6[ab]	4.7[ab]	4.0[b]	3.9[b]	0.3	0.40	< 0.001	0.59

[a,b,c]Means within row are different ($P < 0.05$).

[1]Day relative to parturition: d 6.7 ± 0.9; d 11.7 ± 0.9; d 27.8 ± 2.9; d 32.8 ± 2.9; d 70.2 ± 3.2; d 111.9 ± 2.9.

[2]Largest SEM is reported.

[3]T × D = Treatment and day interaction.

[4]Means did not differ once separated using Tukey's.

Table 4.7 Postpartum urine metabolite concentrations for cows fed rations with either 100 (CON) or 133% (HMP) of predicted metabolizable protein requirements during the final 8 wk of gestation.

	Treatment			Day[1]			*P*-values		
Item	CON	HMP	SEM[2]	d 7	d 28	SEM[2]	Treatment	Day	T × D[3]
n	10	11		20	21				
Urea-N, mg/dL	324.1	332.4	13.2	343.5	313.0	12.5	0.64	0.099	0.56
3-methylhistidine, nmol/mL	154.4	133.4	12.0	152.3	135.5	12.0	0.22	0.32	0.77
Creatinine, mg/dL	172.4	161.7	14.9	175.8	158.2	12.1	0.61	0.16	0.68
3-methylhistidine:creatinine	0.94	0.89	0.1	0.89	0.93	0.1	0.64	0.76	0.75
Crude protein, %	2.7	3.1	0.2	3.1	2.6	0.1	0.11	< 0.001	0.25

[1]Collections occurred from d 6.7 to 11.7 ± 0.9 and from d 27.8 to 32.8 ± 2.9 relative to parturition.

[2]Largest SEM shown.

[3]T × D = Treatment and day interaction.

Table 4.8 Postpartum circulating blood metabolites for cows fed rations with either 100 (CON) or 133% (HMP) of predicted metabolizable protein requirements during final 8 wk of gestation.

	Treatment			Day[1]					*P*-values		
Item	CON	HMP	SEM[2]	d 7	d 28	d 70	d 112	SEM[2]	Treatment	Day	T × D[3]
n	10	11		21	21	18	21				
Non-esterified fatty acids, µEq/L	253.6	267.6	21.8	321.2[a]	336.0[a]	188.9[b]	196.2[b]	23.5	0.49	< 0.001	0.22
Glucose[4], mg/dL	59.2	58.2	1.5	61.8	59.5	56.0	57.5	1.9	0.64	0.048	0.070
Insulin, µg/L	0.6	0.6	0.1	0.7	0.6	0.6	0.6	0.1	0.78	0.55	0.30
BHBA, mg/dL	7.5	7.7	0.3	7.5	7.4	7.3	8.1	0.4	0.64	0.37	0.29
Plasma urea-N, mg/dL	6.6	6.6	0.4	6.0[b]	6.3[ab]	6.3[ab]	7.5[a]	0.5	0.84	0.067	0.76

[a,b,c]Means within row and column for each item differ significantly ($P < 0.05$).

[1]Collections occurred on d 6.7 ± 0.9, 27.8 ± 2.9, 70.2 ± 3.2, and 111.9 ± 2.9.

[2]Largest SEM is reported.

[3]T × D = Treatment and day interaction.

[4]Means did not differ once separated using Tukey's.

and BHBA concentrations were not affected by treatment, day, or the interaction of treatment and day. Plasma urea-N concentration tended to increase ($P = 0.067$) from 6.0 to 7.5 mg/dL from d 7 to 112.

4.4.5 Calf Growth Performance

At birth and d 112, calf BW, chest circumference, wither height, hip height, and body length did not differ ($P \geq 0.21$; Table 4.9) due to maternal prepartum treatment. Calf ADG (kg/d), calculated by linear regression, did not differ ($P = 0.82$) by treatment.

4.5 Discussion

The current study was designed to evaluate the effects of over-supplying MP to beef heifers during late gestation on postpartum DMI and ruminal fermentation, indicators of skeletal muscle catabolism and metabolic energy status, lactation and cow-calf performance. Nutrient supply was predicted using the CNCPS 6.5 model and retrospective analysis predicted that CON and HMP heifers were provided with 831 and 1137 g MP/day when consuming 11.4 and 12.3 kg DM/d, respectively. By design, diets were predicted to differ in MP content relative to requirements by 33% (100 vs. 133% for CON and HMP) while the CP content (% of DM) for the HMP diet was 154.8% of that in the CON diet. The prepartum responses have been previously reported (Chapter 3.0). During the postpartum period, heifers were fed a common ration that was in excess of ME and adequate in MP supply (126 and 103% of ME and MP, respectively; CNCPS 6.5). As such, the treatments imposed were appropriate to evaluate carry-over effects of prepartum MP supply and nitrogen balance on postpartum responses.

Carry-over effects from oversupplying MP prepartum were not detected despite improved nitrogen balance during late gestation (Chapter 3.0). The experimental design did not allow for direct measurement of N balance during the postpartum period as measurement of total urinary and fecal output were not possible while the dam was rearing a calf. As such, indicators of N balance and skeletal muscle catabolism were used to interpret the carry-over effects arising from the oversupply of prepartum MP. Major routes of nitrogen excretion (i.e. milk, urine, and feces)

Table 4.9 Body weight and frame measurements at birth and d 112, and ADG of calves born to heifers fed diets formulated to supply 100 (CON) or 133% (HMP) of predicted metabolizable protein requirements during the final 8 wk of gestation.

	Treatment			
Item	CON	HMP	SEM[2]	*P*-value
n[1]	10	11		
Birth				
Birth BW, kg	36.4	35.6	1.15	0.65
Chest circumference	76.8	75.5	0.70	0.21
Wither height	71.5	70.2	0.83	0.27
Hip height	75.0	73.7	0.88	0.29
Body length	58.2	55.4	1.65	0.24
112-d of age				
BW, kg	122.0	120.8	4.9	0.86
Chest circumference	117.5	117.4	1.8	0.98
Wither height	92.8	93.3	1.1	0.77
Hip height	95.6	96.4	1.2	0.76
Body length	82.0	83.1	1.0	0.45
ADG[3], kg/d	0.74	0.76	0.04	0.82

[1]Smallest 'n' shown.

[2]Largest SEM shown.

[3]ADG calculated by the linear regression of the difference between weaning and birth BW by d post-parturition.

showed no difference in concentration of nitrogenous compounds between treatments and the urinary 3-MH:creatinine ratios did not differ among CON and HMP cows. Doepel et al. (2002) also reported no carry-over effects of prepartum protein supply on postpartum N balance; however, that study utilized dairy cattle and the prepartum feeding period only persisted for the last 25 d of gestation. Moreover, milk and milk component yield and calf growth were not affected suggesting that oversupplying MP prepartum will likely have little effect on postpartum N balance.

Oversupplying MP prepartum reduced colostrum milk fat concentration and tended to reduce the colostrum net energy content. It is not clear why such a response was observed as global nutrient over-supply (140% of nutrient requirements) has not previously affected colostrum fat concentration, although colostrum and milk fat yield have been reported to be lower when pregnant ewes are over-fed (Swanson et al., 2008; Meyer et al., 2011). As colostrum yield was not measured in the present study, we cannot confirm whether the reduction in colostrum fat percentage was a result of reduced fat output or if it was due to a dilution response arising from increased colostrum yield. However, given the results of previous studies using global nutrient over supply (Swanson et al., 2008; Meyer et al., 2011; McGovern et al., 2015), it is unlikely that oversupplying MP during late gestation would improve colostrum yield. Moreover, the concentrations of other components were not affected suggesting the reduction in colostrum fat concentration was likely due to reduced colostrum fat production. Mammary lipid output results from de novo synthesis of short- and medium-chain fatty acids (4 to 8 and 10 to 16 carbons, respectively) and incorporation of long-chain fatty acids (>16 carbons) from peripheral supply (Bauman and Griinari, 2003). Without knowing the relative composition of short-, medium-, and long-chain fatty acids within the colostrum fat content, nor their metabolic regulators, we cannot determine which metabolic pathway was up- or down-regulated (and by what mechanism) by over-supplying MP during late gestation and cannot speculate the causation of the reduction in colostrum fat content for HMP heifers.

Daily estimated milk yield did not differ between HMP and CON cows over the 112-d lactation, but day relative to parturition affected the milk yield response. Others have also reported no effect of prepartum protein (Doepel et al., 2002) or general oversupply of prepartum nutrients on milk yield (Meyer et al. 2011). In the present study, milk yield was greatest at d 7, 13, 28 and 33, relative to more advanced days in milk. The reduction in milk yield corresponded to a time that cows were group-housed and feeder design no longer prohibited calves from consuming the

diet fed to the dam. While this pattern of milk yield differs from that in NASEM (2016) with an expected peak milk yield occurring around 8.5 wk post-parturition (NASEM, 2016), the decline in milk production was likely regulated by calf demand. In the present study, milk production theoretically represented 16.6, 11.7, 6.6, and 4.2 of fluid intake as a percentage of BW for calves at 7, 28, 70, and 112 d of age. The observed consumption of milk in the present study, at times when calves relied solely on milk (until d 33) and when reported as a percentage of BW are similar to that reported for dairy calves fed ad libitum (Drackley et al. 2008). Moreover, Boggs et al. (1980) reported that heifer calves should be expected to consume forage at 1.5 % of BW by 60 d of age. The substitution in forage intake for milk consumption at d 60 coincides with the observed decrease in milk yield at d 70 and 112. Supporting the notion that calves reduced reliance on milk following d 33, calf ADG was 0.75 kg/d between d 7 and 28, was 0.68 kg/d between d 28 and 70, and was 1.12 kg/d between d 70 and 112. The growth response for the calves suggests that nutrient density decreased between d 28 and 70 supporting reduced reliance on milk as part of their diet.

Characterization of the postpartum portion of the transition period for beef cattle is limited. Data from the current study indicate that DMI decreased from wk 1 to that observed during wk 2 and 3 and then increased for wk 4 relative to parturition. It is unclear as to why DMI decreased in wk 2 and 3 following parturition, although the cause is likely multifactorial. Ruminal fill and hepatic oxidation are primary theories explaining regulation of DMI (Allen et al., 2009). Ruminal DM fill and fluid fill are greater in early lactation beef cattle than in late gestation along with slower ruminal passage rate during the first month following parturition relative to that prepartum (Stanley et al., 1993). Though rumen distention is a regulator of DMI (Allen, 2000), Stanley et al. (1993) observed increased DMI at d 8 and 22 after calving (n = 4) and concluded that distention is likely not the sole factor regulating feed intake in the periparturient period. Diets in the present study were purposely diluted with the inclusion of straw (23% of DM offered) to avoid overfeeding of nutrients postpartum. The low digestibility of straw coupled with potential decreases in passage rate after parturition may have partially limited DMI postpartum. Additionally, the hepatic oxidation theory (HOT) suggests that feed intake is metabolically controlled by hepatic oxidation (Allen et al., 2009). In dairy cattle, elevated NEFA concentrations prepartum may cause a reduction in DMI during the periparturient period as a result of increased hepatic uptake and oxidation of fatty acids (Reynolds et al., 2003; Drackley and Anderson, 2006). As serum NEFA concentrations were observed to be greatest on d 7 and 28 in our study, the reduction in DMI

during wk 2 and 3 relative to parturition may have also been partially regulated by greater availability of fatty acids as a hepatic energetic substrate.

Net energy output in milk was greatest during the first month of lactation, coinciding with the greatest recorded milk yield. Correspondingly, NEFA concentrations were greater at d 7 and 28 than at d 70 and 112 and rump fat, but not rib fat, thickness was less at d 112 compared to 14 and 28. Catabolism of adipose tissues increases serum NEFA concentrations (McNamara, 1994) and increases the available oxidative substrates for hepatic oxidation (Drackley, 1999). While serum NEFA concentrations increased, the observed NEFA and BHBA concentrations are relatively low and far below that used to indicate risk for clinical and sub-clinical ketosis (Leblanc, 2010; Roberts et al., 2013).

Although rump fat decreased and NEFA increased during early lactation, cow BW was not affected. Body weight is not a reliable indicator of energy or protein balance (Roche et al., 2009) as the relationship between BW and body reserves is highly variable and affected by DMI and gastrointestinal fill, parity, frame size, and breed (Enevoldsen and Kristensen, 1997; Stockdale, 2001; Berry et al., 2006). Nevertheless, cows in the present study were calculated to have a positive energy balance as predicted net energy intake was in excess (15.5 Mcal/d) of the predicted requirements for maintenance and lactation (13.2 Mcal/d; data not shown). The energy requirement for uterine involution and reparation of the reproductive tract was not considered in calculated of net energy expenditure. Additionally, there is the potential that milk production and, consequently, net energy of lactation expenditure may have been under estimated as a result of measurement techniques and the application of the 2-quarter 12-h milk yield model to approximate the theoretical 24-h milk yield. Though energy expenditure during early lactation cannot be fully described, it can be postulated that calculated energy balance may have over predicted energy supply as cows mobilized rump fat and NEFA concentration increased.

Ruminal pH decreased from wk 1 to wk 2 and 3, and remained low during wk 4. The magnitude of change varied from maximum ruminal pH of 7.1 at wk 1 to 6.7 at wk 4 and minimum ruminal pH decreased from 6.2 at wk 1 to 5.8 in wk 4. Despite the reduction for ruminal pH, values observed do not indicate a risk for ruminal acidosis. It is counter-intuitive that ruminal pH decreased during wk 2, 3, and 4 while fermentative substrates were less available as a result of reduced DMI. However, during the early post-parturient period, DMI was highly variable among days (data not shown) and we speculate that the variability in feed intake may increase risk for low

ruminal pH. Future research is needed to confirm this theory. In applied situations, producers should carefully monitor concentrate supplementation postpartum to ensure lactating cattle are not predisposed to ruminal or sub-acute ruminal acidosis.

4.6 Conclusion

In conclusion, supplying MP greater than requirements by 33% during late gestation had marginal carry-over effects on the postpartum transition phase. However, the considerable changes in DMI and ruminal fermentation patterns during the early lactation transition phase likely impact nutrient balance and potentially confound determination of any carry-over effects. Future research is warranted to understand the mechanisms of these changes in DMI and ruminal pH, as well as their subsequent impacts on cow-calf performance.

5.0 GENERAL DISCUSSION

5.1 Benefits of MP Supplementation and the Arising Economic and Environmental Consequences

The general objective of this project was to determine whether provision of predicted MP greater than requirements (33% above requirements) would improve maternal N balance prepartum to decrease catabolism of maternal skeletal muscle tissue and improve cow-calf performance during the postpartum period. Though neither CON nor HMP heifers were in a negative N balance during the prepartum transition phase, HMP heifers had increased nitrogen intake, apparent digestibility, and retention than the CON heifers. Due to the purposeful over supply of prepartum MP, HMP heifers had improved OM and NDF total tract digestibility than the CON heifers and consequently, had greater BW gains during the prepartum transition phase. The prepartum results suggest that over-feeding MP during late gestation has beneficial impacts on prepartum heifer performance without affecting BCS or rib and rump fat depth. Research has shown that over-conditioned heifers are at an increased risk of dystocia, lengthened postpartum interval, and decreased conception rates (Richards et al., 1986; Bohnert et al., 2002). Yet, apart from HMP heifers tending to have decreased 3-MH:EBW$^{0.75}$ (data not shown) relative to the CON heifers during the postpartum period, carry-over effects of prepartum maternal treatment were minimal. Postpartum cow BW and BCS were not different between maternal prepartum treatment and neither was calf growth and development. Unfortunately, reproductive measures such as postpartum interval or conception rates were not monitored in the current study and as such, I cannot confirm whether improved BW prepartum for the HMP heifers would have impacted subsequent reproductive efficiency. It can be postulated that, as CON and HMP heifers did not vary in BCS at calving and both treatments were in a positive N balance, reproductive efficiency would not have varied between treatments.

Managing pregnant beef cows over-winter represents up to two thirds of the production costs throughout the year (Larson, 2013; Damiran et al., 2016) and supplemental protein can be the most expensive dietary ingredient. However, protein supplementation for the dam during late gestation can reduce the age at which heifer progeny reach puberty (Martin et al., 2007), increase

weight gain during lactation, and increase subsequent conception rates relative to heifers that are fed CP to meet requirements (Patterson et al., 2003). Supplementation of protein during lactation to achieve the improve BW and BCS is often more expensive than prepartum supplementation as there are additional nutrient requirements arising from lactation and uterine involution (NASEM, 2016). Additionally, late gestation protein supplementation may improve birth BW (Larson et al., 2009), weaning weights (Stalker et al., 2006; Martin et al., 2007), hot carcass weight (Stalker et al., 2007), marbling score, and the proportion of steers grading choice (Greenwood et al., 2004; Larson et al., 2009). Therefore, even though additional MP provision during late gestation may increase feed cost, producers could receive economic benefit from the improved reproductive efficiency and increased progeny value at weaning and slaughter.

Public attention and criticism of animal agriculture has escalated in recent years, largely due to the perceived environmental impact from nitrogen excretion (Ndegwa et al., 2008; Lee et al., 2012; Reed et al., 2015). Overfeeding MP prepartum increased urinary nitrogen excretion for HMP heifers relative to CON heifers thereby, increasing the risk for N-volatilization. Increased nitrogen excretion occurs primarily through the urine once RDP and MP requirements are satisfied (Vansconcelos et al., 2009; Koenig and Beauchemin, 2013a,b). Given that HMP and CON heifers had urinary nitrogen excretion rates that were 1.40 and 0.49 times greater than fecal nitrogen excretion, respectively, it would seem that HMP heifers were being provided either RDP or MP in excess of their physiological requirements relative to the CON heifers. In the current study, we did not evaluate the quantity RDP or RUP provision that would result in positive heifer performance responses while optimizing the rate of urinary nitrogen excretion to fecal nitrogen excretion. Future research may focus on evaluating the optimum provision of RDP and supplementation of RUP to match MP requirements while decreasing urinary nitrogen excretion.

5.2 Implications of Late Gestation for RDP Requirement and Supply

The data from the study within this thesis may be interpreted to suggest that the CON heifers were deficient in RDP, while still consuming adequate MP. The previous interpretation is based on ruminal ammonia-N concentrations from CON heifers that were less than concentrations deemed to be adequate for efficient microbial synthesis (1.1 mg/dL vs. 3 to 16 mg/dL; NASEM, 2016). Research shows that particulate and fluid passage rates increase during late gestation

(Gunter et al., 1990; Hanks et al., 1993) and increased passage rate may increase the flow of NH_3-N out of the rumen. In addition, as the rate of ruminal degradation of soluble protein fractions is inversely related to passage rate. Any increases in fluid and particulate outflow from the rumen may decrease degradation of dietary sources, thereby decreasing degradation of the RDP supplied. However, the current mechanistic and empirical equations for modelling the capability of a ration to meet predicted nutrient requirements do not account for the increase in passage rate during late gestation. As the CON treatment was designed to provide an adequate quantity of RDP, it is possible that inaccurate estimation of passage rate during late gestation may have affected the supply. Furthermore, ruminal degradation of soluble protein fractions contributes NPN, peptides, and AA for microbial protein synthesis that are preferentially used by cellulolytic bacterial species (Russell et al., 1992). In particular, the production of ammonia-N from protein degradation is necessary for growth of cellulolytic microbial species. When NPN-sources are not available, the rate of microbial proliferation decreases and a reduction in NDF digestibility occurs (Russel et al., 1992). In addition to a lesser ammonia-N concentration, CON heifers had reduced NDF and OM digestibility. As NDF provides the majority of available dietary energy in forage-based beef cattle rations (NASEM, 2016), the observed decrease in NDF and OM digestibility for CON heifers constrains their ability to capture the available ME. A reduction in NDF digestibility can also limit DMI. Although not statistically different, DMI was numerically less for CON than HMP heifers during the prepartum phase. Nutritional models should account for the impact of increasing passage rate on RDP degradability and the resulting ME supplied. Rations may be supplemented with RUP to meet MP requirements (Sletmoen-Olson et al., 2000a,b), but despite RUP supplementation, it may be worthwhile to consider increasing the predicted RDP requirement, or discount the potentially available RDP, to reflect prepartum transition phase changes in passage and NDF digestibility.

5.3 Crude protein vs. Metabolizable protein

Crude protein content is determined by the total nitrogen content multiplied by a factor of 6.25 to reflect the average proportion of nitrogen in AA. Traditionally, CP has been measured using the Kjeldahl method (AOAC, 1993) that involves digestion of the sample in concentrated H_2SO_4 at 400°C and the conversion of the feed nitrogen to ammonia-N. The ammonia is then

converted to ammonium by acidification and the sample is titrated to determine the concentration of ammonia-N in the sample. As stated above, the measured nitrogen is then multiplied by 6.25 to determine the CP concentration. The Kjeldahl method is useful as a preliminary analysis of the nitrogen content of the feed, but CP content fails to represent a unit related to nutrient requirements of cattle. Metabolizable protein represents the AA absorbed across the small intestine, reflecting a unit that more similar to requirements. Calculation of MP involves estimated rates of ruminal degradation, ruminal passage rate, and the intestinal digestion absorption of AA and peptides supplied by the feed and microbial CP (discussed in Chapter 2.1). It is assumed that 10% of fraction PA2 passes out of the rumen and is available for intestinal digestion, whereas fractions PA1 and PA2 are degraded in the rumen to contribute to MCP. Microbial CP and endogenous losses are assumed to contribute 64% of their respective CP content to MP supply. The remaining MP supply is derived from the escape soluble true protein (fraction PA2) and insoluble protein fractions. Fraction PB1 (insoluble true protein) is assumed to be 100% digested and absorbed while fraction PB2 (fiber-bound insoluble true protein) is assumed to be 80% digested and absorbed (Fig. 2.2).

Typically, producers and researchers in cow-calf production systems focus on the CP content of forages and concentrates, rather than the quantity of MP available. Predicted CP requirements are advised relative to the total digestible nutrient content of the ration (NASEM, 2016) and often producers follow a 'golden rule' of 7, 9, and 11% total CP content as a proportion of the DM content to meet mid- and late-gestation, and early lactation protein requirements (Alberta Agriculture and Forestry, 2004). However, calculating CP by the total nitrogen content of the feed accounts for sources of NPN within the ration, such as ammonia-N, urea, or nucleic acids. Ammonia-N and urea are utilized within the rumen as nitrogen sources for microbial AA synthesis and therefore are of biological value to the ruminant. Yet, some sources of nitrogen are not used as nitrogenous substrates for microbial protein production. Additionally, defining beef cattle protein requirements by CP assumes a constant digestibility of all CP sources. Crude protein digestibility can vary significantly due to protein-type (e.g. animal vs. plant protein) or degree of maillard reactions that render the proteins indigestible. Therefore, CP does not truly represent the quantity of AA available to the ruminant as it does not discriminate between nitrogenous sources. Furthermore, expressing protein content simply as CP content does not account for the influence of passage rate on ruminal degradation and intestinal digestion of feed protein. As such, CP content is an inaccurate representation of the MP supply available to the ruminant. Industry and research

should alternatively focus on the MP supplied and eventually progress to predicting the capability of a ration to meet AA requirements (termed 'ideal protein' when discussing poultry and swine nutrition) of beef cattle during gestation and lactation. Currently, beef cattle AA requirements have not been specifically defined for either physiological state.

5.4 Predictions for urine and fecal output

Quantifying N balance during the postpartum phase was one of the objectives originally included in my research conducted within this thesis. Given that postpartum cows were housed with their nursing their calves, there was no ability to insert and maintain urinary catheters for total urine and fecal collection in order to quantify postpartum N balance. Our intention then was to predict postpartum urine and fecal output by using regression equations generated from prepartum urine and fecal data in combination with endogenous markers. From these predictions, we planned to estimate N balance during the postpartum period.

5.4.1 Postpartum urine output

Urine spot samples were collected during the postpartum phase and were analyzed CP, urea, 3-methylhistine, and creatinine concentrations. Eight spot urine samples (50 mL) were collected from d 6 to 12 and 28 to 33 at 18 h intervals (d 1 1000 h; d 2 0400 and 2200 h; d 3 1600 h; d 4 0700 h; d 5 0100 and 1900; d 6 1300) and were composited on an equal-volume basis to account for the diurnal variation in creatinine excretion. We intended to predict urine output by day during the postpartum period using an equation generated from prepartum total urine collection data. Previous studies have used urinary creatinine concentration to predict urine output (Valaderes et al., 1999; Chizzotti et al., 2008; Pina et al., 2009). Given that this approach was supported by past research, it was deemed a reasonable strategy to estimate urine output.

To generate predictions, I intended to use total collection during the prepartum phase. Total collection of urine was conducted from d -33 to -28 and -16 to -10 with urine samples taken daily to be measured for CP, urea, 3-MH, and creatinine concentrations. Using the prepartum urine samples from the total collection periods, we had planned to create a linear regression equation to relate creatinine concentration to daily urine output (shown in Eq. 5.1). The use of creatinine as an

endogenous marker relies on the assumption that creatinine is excreted via urine at a constant rate relative to body mass (Cetinkaya et al., 2006) and use of creatinine as an endogenous marker has been successful in dairy cattle to predict urine output (Chizzotti et al., 2008).

Equation 5.1 Linear regression equation derived from prepartum total urine production and creatinine concentration in urine data.

$$\text{Predicted urine output} = (-0.0021 \times \text{Creatinine concentration}) + 12.79$$
$$P < 0.001;\ r^2 = 0.23$$

The correlation between creatinine concentration and urine output during the prepartum sampling periods was too weak and only accounted for 23% of the variation in daily urine output. We could not confidently apply the linear regression equation to postpartum data set to predict postpartum urine output.

Alternatively, I also attempted to predict urine output from the prepartum data sets using a multiple regression model. Urea and creatinine concentration, conceptus-corrected BW, metabolic BW, DMI, CP intake, creatinine concentration/conceptus-corrected BW, and creatinine concentration/metabolic BW were all tested as variables in the multiple regression model. A *P*-value of 0.05 was used to determine if the variable was significant within the model and variables were tested to determine if they were auto-correlated. Step-wise elimination was used to remove variables that were not significant. The variables that were found to be significant within the model were creatinine concentration ($P < 0.001$), conceptus-corrected cow BW ($P = 0.033$), and CP intake ($P < 0.001$). Thus, the multiple regression model (shown in Eq. 5.2) was fitted to the data to predict daily urine output.

Equation 5.2 Multiple regression equation derived from prepartum total urine production, creatinine concentration in urine, conceptus-corrected heifer BW, and CP intake.

$$\text{Predicted urine output} = (-0.00249 \times \text{creatinine concentration}) + (-0.00786 \times \text{conceptus-corrected heifer BW}) + (2.95309 \times \text{CP intake}) + 13.19751$$
$$P < 0.001;\ r^2 = 0.66$$

Though the multiple regression model had a greater correlation relative to the linear regression model, there was still too much unaccounted variation to predict urine output. Therefore, it was concluded that we could not confidently predict daily urine output during the postpartum period and would be unable to predict postpartum urinary N excretion.

The failed use of creatinine excretion to predict urine output is likely caused by many factors. Differences in intake and diet, particularly nitrogen intake, can cause variation in creatinine excretion. As well, creatinine excretion in late gestation beef heifers relative to urine output has not, to my knowledge, been evaluated. It is possible that gestating cattle may have variation in creatinine excretion as the fetus may contribute to maternal creatinine concentration in serum or plasma.

Creatinine may also be used to standardize other urinary substrates, such as catecholamines (Spierto et al., 1997) or purine derivatives (Broderick and Merchen, 1992), under the assumption that daily creatinine excretion is nearly constant. Standardization of urinary purine derivatives by creatinine concentration is common method to estimate microbial protein synthesis (Broderick and Merchen, 1992; Webster et al., 2003) However, we found that creatinine excretion prepartum was too variable by day (CV = 24.96%), suggesting that normalizing the concentration of other urinary metabolites by creatinine concentration is an inaccurate approach to evaluate their daily excretion. Some studies estimate glomerular filtration rate from creatinine concentration in plasma or serum and urine to determine urine output. Estimated glomerular filtration rate is then standardized by metabolic BW and is a more reliable measure to predict urine production (Chizotti et al., 2008) than creatinine concentration in urine. Yet, this approach requires taking blood samples that coincide with urine samples and would have been unfeasible under the current experimental conditions.

5.4.2 Postpartum Fecal Output

In addition to predicting postpartum urinary output, I had planned to predict postpartum fecal output using uNDF240 intake as an indigestible marker to predict DM total tract digestibility (Combs et al., 2013). Indigestible NDF240 is the indigestible NDF portion that remains after a 240-h incubation (Raffrenato, 2011). Fecal output of uNDF240 is then assumed to be equal to the

uNDF240 consumed. As it is the indigestible fiber fraction of the feed, it is reasonable to assume that intake of uNDF240 will be equal to output.

Daily feed intake (from d 6 to 12 and 28 to 33) was calculated as the difference between the amount of feed offered and refused when corrected for DM. Samples were taken daily for forages and concentrates and used for analysis of DM by drying in a forced-air convection oven until a constant weight was achieved. Samples of feed refusals were taken daily (10% of refusals) and composited on an as-is basis relative to the quantity refused per day. Composited feed refusal samples were dried using a forced-air convection oven until a constant weight was achieved. Following DM determination, samples were ground through a 1-mm sieve using a Christy Norris hammer mill (Christy and Norris, Christy Turner Ltd., Chelmsford, U.K). Feed and refusal samples were then analyzed by for nutrient content by Cumberland Valley Analytical Services (described by Rosser et al., 2013). Indigestible NDF240 was determined by a 240-h in vitro incubation (Raffrenato, 2011). Intake of uNDF240 was measured directly as the difference in uNDF240 content in feed offered and refused. Prepartum total collection data (intake and fecal excretion of uNDF240) was intended to validate the use of uNDF240 as an indigestible to marker to predict postpartum fecal excretion.

When plotting the correlation (r^2 = 0.59) between measured uNDF240 intake and output, fecal excretion of uNDF240 was overestimated by an offset of 0.83 kg/d (DM basis; shown in Fig 5.1). The 0.83 kg/d intercept suggests that intake of uNDF240 was underestimated by 830 g. Given that intake was measured directly from feed offered and refused, it is unlikely that uNDF240 intake was underestimated and more likely that fecal output may have been overestimated. Prepartum intake of uNDF240 by fecal concentration of uNDF240 was used to predict prepartum fecal DM output. Relative to measured prepartum fecal DM output, use of uNDF240 concentration in feces overestimated fecal DM output relative to measured fecal output (shown in Fig. 5.2). The correlation (r^2 = 0.66) between measured fecal uNDF240 output to predicted fecal uNDF240 output was weak. Due to non-consistent relationship between uNDF240 intake and output (Fig. 5.1) and over-estimation of predicted output relative to measured fecal DM output (Fig. 5.2), we were unable to confidently apply postpartum uNDF240 intake and fecal uNDF240 concentration to determine postpartum fecal DM output.

Past research has used uNDF240 or iNDF as endogenous markers to determine total tract digestibility (Combs, 2013; Lopes et al., 2015) and fecal DM output. Lopes et al. (2015) compared

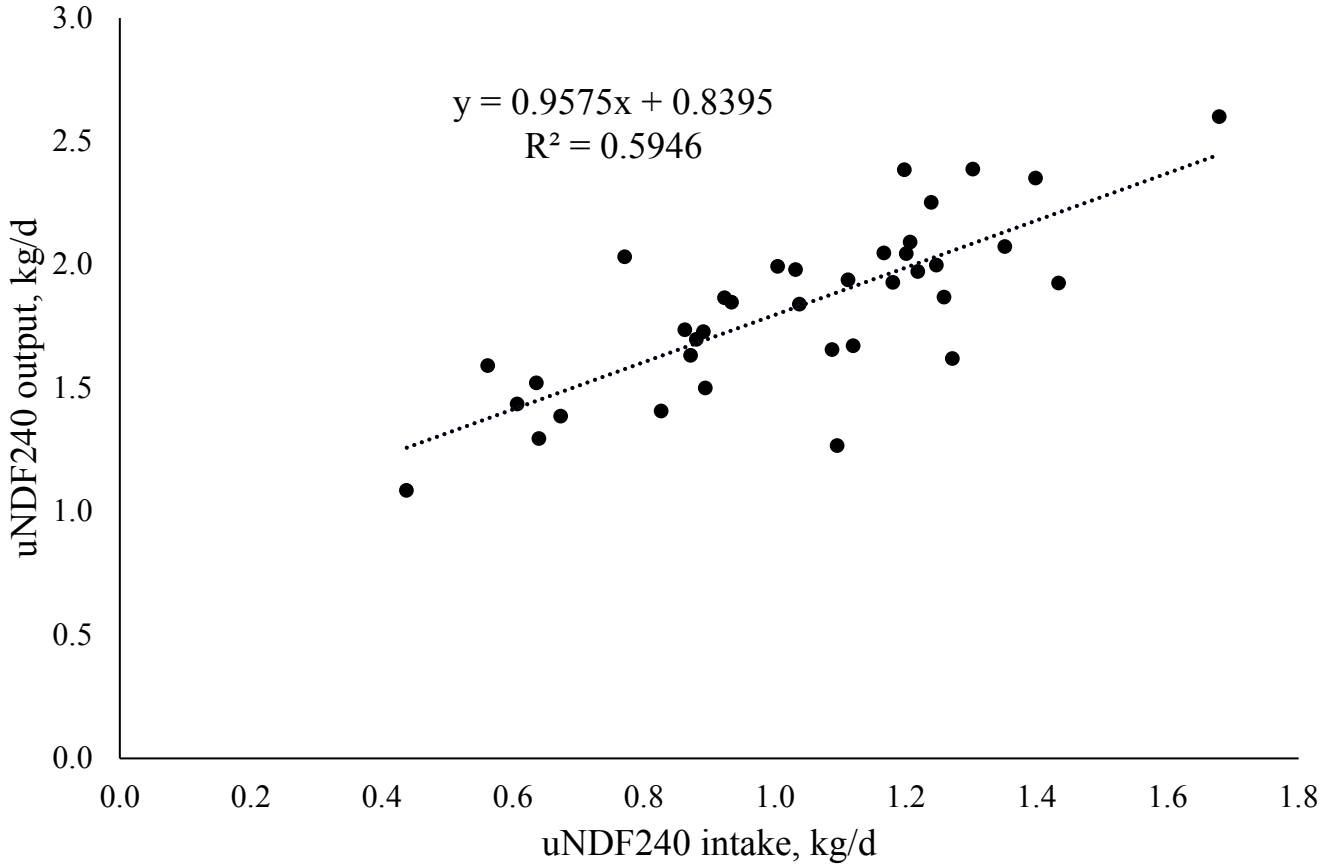

Figure 5.1 The relationship between uNDF240 intake and uNDF240 output for CON and HMP heifers during the prepartum total collection periods (d -33 to -28 and -16 to -10).

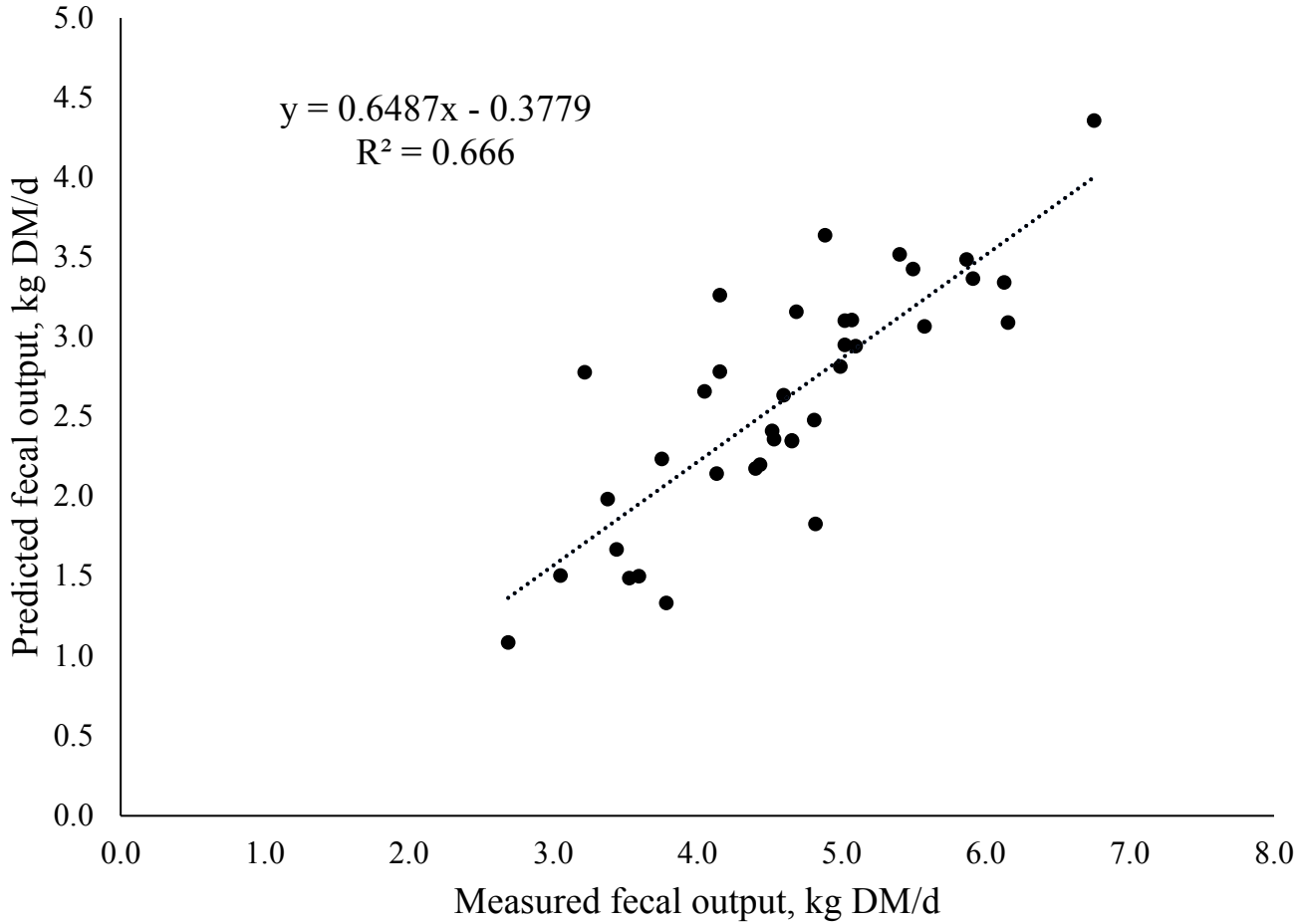

Figure 5.2 The relationship between measured and predicted fecal output (using uNDF240 intake and fecal uNDF240 concentration) for CON and HMP heifers during the two prepartum total collection periods (d -33 to -28 and -15 to -9).

in vitro and in vivo measurements of total tract digestibility using the digestible NDF fraction. These authors found that when diets that had high fiber digestibility were fed, in vitro and in vivo determination of total tract digestibility were not different. However, when diets that had low fiber digestibility were fed, in vitro measurements underestimated the total tract NDF digestibility (Lopes et al., 2015). Rations fed in the current study were highly fibrous due to being composed largely of barley greenfeed and wheat straw to represent typical on-farm feeding schemes. Our results were similar to those of Lopes et al. (2015) in that total DM digestibility was underestimated when predicting fecal DM excretion, resulting in an over-estimation of fecal output in the prepartum periods. Thus, it is likely that the high fiber content of the diets confounded our predictions of fecal DM output.

Unfortunately, as validation of both models (urine and fecal output) generated from the prepartum data were unsuccessful, I was incapable of applying these models to the postpartum data to predict fecal and urine output. Without prediction of postpartum fecal and urine output, I was unable to estimate nitrogen loss through feces and urine and consequently unable to determine N balance during the postpartum transition phase.

5.5 Transition beef cow characterization

In dairy cattle, the transition period is defined as the 3 wk preceding and following parturition (Drackley, 1999). The transition period is a time when cows experience significant changes in DMI and ruminal fermentation patterns (Marquart et al., 1977; Penner et al., 2007). Homeorhetic metabolic adaptations occur to support gestation and lactation (Bell, 1995; Drackley, 1999) and the cow experiences alterations in organic nutrient metabolism (Bell, 1995; Grummer, 1995). Conversely, the concept of transitioning between two vastly different physiological states (i.e. late gestation to early lactation) is not commonly focused on within beef cattle production systems. Comparatively little is known about how beef cattle adapt to the transition between gestating and lactating or the intake and digestive changes that occur during this time, but there is a growing body of research available to make preliminary characterizations of the pre- and prepartum transition phases.

5.5.1 DMI and Total Tract Digestibility

Dry matter intake has been reported to decrease in beef cattle as parturition approaches (Hanks et al., 1993; Stanley et al., 1993; Linden et al., 2015) which is similar to the response observed for dairy cattle (Marquardt et al., 1977), though the reduction in DMI is not nearly as severe. Whereas dairy cattle experience a 25 to 50% decrease in DMI in the three wk prior to parturition (Marqhardt et al., 1977), primiparous beef cattle appear to only experience a decrease in the range of 8 to 9.2% (DMI, %BW) the wk before calving (Chapter 3.0; Linden et al., 2015); though some researchers have reported that DMI (kg/d) decreased by as much as 16.3% in wk -1 compared to wk -5 relative to parturition or that it will sharply decrease in the 3 days preceding parturition (Stanley et al., 1993). Comparatively, DMI does not change in multiparous cows as parturition approaches (Linden et al., 2015). These differences in DMI between studies may exist due to numerous reasons, such as type of forage and supplement provided, or differences in visceral cavity capacity and ruminal fill (Hanks et al., 1993; Scheaffer et al., 2001; Wood et al., 2013) between late gestation beef heifers and cows. As DMI changes during the prepartum transition phase, passage rate also appears to increase (Gunter et al., 1990; Vanzant et al., 1991; Hanks et al., 1993) while total tract digestibility decreases (Linden et al., 2015). In the current study, we found no difference in total tract digestibility of most nutrient constituents by d relative to parturition, apart from starch digestibility decreasing by 5.5% from d -33 to -15. Evidence also suggests that DMI may increase during late gestation until 3 to 7 d prior to parturition in beef heifers (Linden et al., 2015; Chapter 3.0). Simultaneous with changes in DMI, digestibility decreases during the prepartum transition phase. The reduction in DMI and digestibility may challenge nutrient supply and nutritional balance.

Less is known about how DMI and total tract digestibility change in early lactation beef cattle. Heifers appear to have greater DMI (%BW) than multiparous cows during early lactation (Linden et al., 2015), but results are mixed on the patterns in DMI by wk relative to parturition. Stanley et al. (1993) found that DMI increased by 4 to 6 kg/d during the postpartum transition phase. However, our results contradict the work of Stanley et al. (1993) in that they demonstrate an 11% decrease in DMI (%BW) during wk 2 and 3 relative to wk 1 prepartum. The magnitude of change in DMI as a percentage of BW during the postpartum phase is greater than that observed the wk prior to parturition (Chapter 3.0), suggesting that beef cattle may be more critically

challenged in meeting nutrient requirements postpartum than prepartum. In dairy cattle, DMI increases shortly after parturition (Lean et al., 2013), relating more closely to the patterns in DMI observed by Stanley et al. (1993). However, concurrent decreases in DM digestibility are reported in dairy cattle as DMI increases (Colucci et al., 1982; Okine and Matheson, 1991). Specifically, for beef cattle DM digestibility does not appear to decrease during early lactation (Ovenell et al., 1991; Vanzant et al., 1991; Martson and Lusby, 1995), but may increase for heifers with as d post-parturition increases (Linden et al., 2015). Unfortunately, in the current study we were unable to measure postpartum DM digestibility.

5.5.2 Ruminal Fermentation

Few studies have evaluated ruminal fermentation patterns in transition beef cattle. Some studies have found that concentration of butyric acid decreases as parturition approaches (Hanks et al., 1993; Stanley et al., 1993), whereas others have reported no change (Scheaffer et al., 2001; Chapter 3.0). Acetic acid has been observed to increase (Chapter 3.0), decrease (Scheaffer et al., 2001), but most often is not reported to change as parturition approaches (Hanks et al., 1993; Stanley et al., 1993). Only one study has indicated that the concentration of propionic acid will increase as parturition is near (Scheaffer et al., 2001) with the remaining literature showing no influence of pregnancy or day relative to calving on concentration of propionic acid (Hanks et al., 1993; Stanley et al., 1993; Chapter 3.0). It is plausible that the noted differences in concentration of acetic, propionic, and butyric acids between studies is more related to difference in diet composition than pregnancy itself. Comparatively, early literature consistently shows decreases in molar proportions of branched-SCFA and isoacids (isobutyric, and isovaleric acid) in pregnant beef cows relative to non-pregnant cows. As well, concentration of branched-SCFA will decrease as parturition approaches (Coffey et al., 1989; Hanks et al., 1993; Stanley et al., 1993). The decrease in concentration may reflect increased ruminal wash-out. Gunter et al. (1990) used ewes as model and found that the interaction of treatment and time effected the concentration of isovaleric acid. Non-pregnant and pregnant ewes had similar concentrations of isovaleric acid immediately after feeding, but pregnant ewes had decreased concentrations at 4, 8 and 12 h after feeding compared to 0 h after feeding and when compared the non-pregnant ewes at the same time-points. In the current study, there was no observed effect of day on the molar proportions of the

branched-SCFA or isoacids; however, heifers that were fed the HMP rations had greater concentrations of isobutyric, isovaleric, and valeric acid relative to heifers consuming CON rations. It is likely that HMP heifers consumed greater quantities of ruminally degradable branched-chain AA. Though evidence is limited, it has been suggested that there may be indirect effects on mammogenesis associated with branched-SCFA (Andries et al., 1987), whereby increased branched-SCFA in the mammary gland may result in decreased catabolism of their precursor AA to improve milk production (Chang et al., 1985).

Similarly, concentration of ammonia-N is regularly demonstrated to be lower in pregnant relative to non-pregnant ruminants and decrease as parturition approaches (Gunter et al., 1990; Hanks et al., 1993; Scheaffer et al., 2001; Chapter 3.0). Increased passage rate during late gestation likely caused increased ammonia-N wash-out (Vanzant et al., 1991; Stanley et al., 1993). Alternatively, the decreased ammonia-N concentration may be related to increased efficiency of nitrogen use in maternal tissues that could decrease urea recycling to the rumen (Hanks et al., 1993).

Ruminal pH is another parameter used to evaluate rumen fermentation dynamics. To the authors' knowledge, five studies (Gunter et al., 1990; Vanzant et al., 1991; Hanks et al., 1993; Stanley et al., 1993; Scheaffer et al., 2001) have evaluated ruminal pH in pregnant ruminants relative to non-pregnant ruminants and d relative to parturition. All five studies found no difference in ruminal pH due to pregnancy and no change in ruminal pH as parturition approached. Yet, in the current study, ruminal pH was observed to increase when DMI dropped in the wk prior to parturition. The rise in pH is expected when there are less fermentative substrates available for SCFA-H and H^+ production (Penner et al., 2007) due to decreased DMI. The difference between my results and the previous studies is that they took 1 ruminal fluid sample at each time point to determine pH, whereas my work utilized indwelling ruminal pH probes that allowed for continuous pH measurement during the 5 wk preceding parturition. That said, it is unlikely that the change in pH would have consequences for the dam during the transition period and that the observed change is more related to DMI and alterations in passage rate rather than pregnancy.

Very few studies have evaluated rumen fermentation parameters such as SCFA and ammonia-N concentrations or ruminal pH during the early postpartum period. Results of the current study agree with the work of Stanley et al. (1993), whereby molar proportion of isovaleric acid increased with d post-parturition and molar proportion of acetic acid decreased. Stanley et al.

(1993) saw increases in isobutyric and valeric acid that were not observed in the current study. Using single time-point ruminal fluid samples, it was determined that ruminal pH did not change during the postpartum transition phase relative to the prepartum transition (Stanley et al., 1993). However, our results using continuous pH measurements summarized by wk relative to parturition showed a trend in decreasing ruminal pH in wk 2, 3, and 4 relative to wk 1. The degree of decrease in not necessarily worrisome, more so the relative trend of decrease is concerning; minimum ruminal pH in wk 2 and 3 reached 5.8. Mean ruminal pH levels of 5.8 are generally accepted as the level at which fiber digestibility begins to decrease; therefore, if ruminal pH in the current study continued to decrease with increasing wk post-parturition, there may be negative repercussions on fiber digestibility for lactating beef cattle.

5.5.3 Energy and Nitrogen Balance

Plasma and serum indicators of energy status are useful to distinguish the cow's metabolic state during pregnancy. Yet, very few studies (Stanley et al., 1993; Radunz et al., 2010; Wood et al., 2013; Linden et al., 2015) have evaluated indicators of energy status, such as glucose, NEFA, and BHBA, and how they differ between pregnant and non-pregnant cows. Generally, late gestation beef cattle elevated concentrations of NEFA (Radunz et al., 2010; Wood et al., 2013) and BHBA (Wood et al., 2013b; Linden et al., 2015) relative to non-pregnant cows. Plasma glucose is reported to range between 60 (Linden et al., 2015) to 85 mg/dL (Prior et al., 1979), dependent on the level of concentrate supplied in the ration. The results of this study show similar levels of serum NEFA, BHBA, and plasma glucose as the previous research. Together, the data indicates that late gestation beef cattle will have increased metabolic indicators of energy status compared to non-pregnant cattle due to the increased energy demand for fetal growth and mammogenesis. The concentration of serum NEFA and BHBA in beef cattle do not indicate a potential for development of metabolic disorders as they do in dairy cattle (Drackley, 1999; Penner et al., 2007). But, it may be more practical to evaluate at what concentration these metabolites could indicate clinical, but more likely, sub-clinical metabolic disorders in beef cattle.

There is a similar lack of characterization of plasma and serum metabolites in early lactation beef cattle. Doornenbal et al. (1988) characterized plasma glucose concentrations in lactating beef cattle, as did Linden et al. (2015). Both studies found that plasma glucose

concentration was lower in lactating than non-lactating beef cattle, reflecting the utilization of glucose by the mammary gland. Beta-hyrdroxybutyrate was increased for lactating cattle (Linden et al., 2015) relative to non-lactating cattle, but there was no difference in BHBA concentration over the entire lactation period, in agreeance with our BHBA results (Chapter 4.0). However, we did find that serum NEFA concentration changed over the course of the lactation. Our data was the first to demonstrate that NEFA concentrations are increased in early than late lactation, though this is not an unexpected result as the postpartum transition period is marked by energetically demanding processes, such as milk production and uterine involution.

Our study was novel in investigating nitrogen balance during the transition phase in beef cattle. Though research has evaluated protein supplementation and the impact of mild nutrient restriction on skeletal muscle catabolism during late gestation beef cattle, until the present work no studies had evaluated the dam's nitrogen balance during the transition period. Within our experimental conditions, neither CON nor HMP heifers were in a negative nitrogen balance during the late gestation period. Yet, HMP heifers were in a more positive nitrogen balance relative to the control. High MP heifers also tended to have lower urinary 3-MH concentration per unit of empty metabolic body weight during the early lactation period than CON heifers, indicating that they catabolized less skeletal muscle than their counterparts. My data suggests that over-supplementing MP during the remaining 8 wk of gestation relative to parturition has positive carry-over effects for early lactation beef cattle, whereby positively increasing nitrogen balance during late gestation will decrease the magnitude of protein turnover postpartum.

5.5.4 Characteristics of the beef cow transition phase

Though data are scarce for characterizing the beef cow transition phase, some preliminary properties of the period can be defined. In terms of initiation of the prepartum and completion of the postpartum transition phase, beef cattle differ from dairy in which they appear to decrease DMI most often at 1 to 2 wk prior to parturition, with the greatest decrease in DMI occurring 2 to 3 d before calving. It could be appropriate to term the start of the transition phase in beef cattle as 2 wk as opposed to 3 wk prior to parturition. Dry matter digestibility may either increase or decrease during this time frame, mainly due to changes in passage rate and what rations are fed. The combination of low-quality forage provision (with or without protein and energy

supplementation), increasing passage rate, and potentially decreasing digestibility can cause beef cattle to be in a catabolic state to meet the demands of gestation. Ruminal fermentation is altered at 2 wk prior to parturition, though not in a manner that significantly affects SCFA concentration. In conjunction with decreased DMI and digestibility, and the increase in passage rate, the quantity of SCFA produced will be theoretically reduced. Plasma and serum metabolites, such as NEFA and BHBA, are elevated and reflect the energetic demands during this period.

More research is needed to properly define the postpartum transition phase. Results are conflicting in how DMI responds to the stress of parturition immediately following the event. Digestibility does not appear to be influenced by increasing day post-parturition but ruminal fermentation may be altered even up to 4 wk postpartum. While serum NEFA and BHBA are elevated in lactating beef cattle compared to non-lactating beef cattle, NEFA concentrations are greatest during the early lactation period up to wk 4 postpartum whereas BHBA does not change over the course of the lactation. The threshold for which concentrations of NEFA and BHBA in transition beef cattle indicate energy balance and susceptibility to sub-clinical or clinical metabolic disorders is not known.

Based on the time-frame in which DMI, digestibility, ruminal fermentation, and blood metabolites are altered relative to parturition, the transition period in beef cattle may be preliminarily defined as the 2 wk prior and 4 wk following parturition.

5.6 Milk production

NASEM (2016) utilizes an equation developed by Jenkins and Ferrell (1984) that accounts for DIM, relative milk yield, predicted peak milk yield, and cow age to predict milk yield at various points during the lactation. Milk production in beef cattle is then assumed to follow a similar lactation curve to dairy cattle, though the time of peak milk yield is estimated to be 8.5 wk post-parturition. Dairy cattle differ in that peak milk yield is predicted to occur 10 wk after calving. Milk production will range between 4.5 to 14 kg depending on the breed and parity of the cow (NASEM, 2016). However, most estimations of milk yield have been based off of the weigh-suckle-weigh technique (Knapp and Black, 1941; Williams et al., 1979; Beal et al., 1988). Weigh-suckle-weigh assumes that milk production over a period of separation is equal to the change in calf weight after the calf has been reunited with dam to nurse. It does not allow for analysis of milk

composition or the associated energy expenditure through milk. It is also possible that the calf does not consume the total volume of milk produced to result in under-prediction of milk yield and that there may be weight changes unrelated to milk consumption, such as urination or defecation.

In the current study, milk yield and composition at 6 time-points over the course of a 112-d lactation was evaluated using a portable milking machine to completely milk 2-quarters of the mammary gland after a 12-h period of cow-calf separation. Therefore, our experimental protocol was not limited by the same constraints as the commonly used weigh-suckle-weigh technique. Milk yield and energy expenditure through milk was greatest at the start of lactation and thereafter declined, with no observed peak milk yield at d 70. Although NASEM (2016) predicts that peak milk yield and expenditure will occur at d 60, our results do not confirm increased milk yield at this time. Instead, the result suggest that beef cattle may not experience a peak in milk yield. Radunz et al. (2010) found similar results whereby milk production was static between d 31 and 100, and decreased by d 170. These authors used a modified weigh-suckle-weigh technique whereby calves were separated from the dams for 3 h, then allowed to nurse for 30 minutes, and separated again for 6 h on d 31 and for 12 h at d 100 and 170. Using this technique, the authors assumed that the calves had completely nursed the udder following the 3 h separation and the 6 and 12 h separations. Milk production per day after the 6 or 12 h separation was calculated as the difference in calf weight following nursing and corrected for the interval of separation. Measured milk yield was greater by 4.5 and 5.6 kg at d 33 and 112 (Radunz et al., 2010) than observed in our study, but likely related to the differences in experimental protocol for measuring production, parities, and breeds. The slight decreases in milk production over the course of the lactation period observed by Radunz et al., 2010 and described in Chapter 4.0 is expected as calves will begin consuming DM and rely less on milk to their meet nutrient requirements. Composition of fat and protein was lower for Radunz et al. (2010), but overall yield of fat and protein were similar between the studies (Randunz et al., 2010; Chapter 4.0). However, lactose composition and yield were greater (Radunz et al., 2010) than our reported values. The differences in reported milk composition may be related to sampling techniques. In our study, milk samples were representative of the entire yield, whereas 50 mL milk samples were stripped directly from the udder in the work of Radunz et al. (2010) and as such, are not representative of the entire milk yield. The milk composition changes may otherwise be related to differences in breed, environment, or diet. However, prepartum nutrition does not appear to influence milk composition and yield as

composition in the current study and that of Radunz et al. (2010) did not vary by diet fed to the dam prepartum. Therefore, milk production and composition is more feasibly influenced by postpartum nutrition.

5.7 Strengths and Limitations of the Current Experiment

5.7.1 Strengths

The current study has yielded novel data to contribute to the available literature regarding protein supplementation in late gestation and transition beef cow characteristics. To the author's knowledge, N balance in beef heifers fed MP at 100 or 133% of their requirements during late gestation has not been previously studied. We were capable of detecting direct prepartum effects (i.e. apparent total tract digestibility, BW gain, improved N balance), as well as carry-over effects during the postpartum period (reduction in colostrum fat content for HMP heifers). Our lactation data conflicts with the current predictions of beef cow lactation production and persistency, yet the data was derived quantitatively from 2-quarter 12-h milk yield and representative milk samples. Therefore, it may more accurately match milk production as regulated by calf demand. Additionally, this experiment contributes to our knowledge of the beef cow transition period, as our DMI and ruminal pH data confirm that the transition period is a dynamic period during the production cycle for beef cattle. The use of indwelling pH probes was a novel approach to evaluate ruminal pH relative to parturition.

5.7.2 Limitations

Despite the previously described strengths and benefits of this research, there were challenges associated with specific experimental protocols. We were unable to measure colostrum at calving as it was too viscous to remove without our portable milking machine. Hand-stripping to collect colostrum would have overcome this issue but is a time-consuming practice and time of collection (due to the timing of parturition) was unpredictable. Though our lactation data is novel and potentially more representation of beef lactation characteristics, our milk yields may be underestimated. The high level of stress associated with cow and calf separation and novel stimulation arising from maching milking could have reduced milk production or inhibited

sufficient milk let-down. That said, the weigh-suckle-weigh approach also requires cow-calf separation. Alternatively, cow-calf pairs could have been housed together for the measurement period with a device to prevent the calf from nursing. We initially attempted this method; however, we were unsuccessful in preventing suckling and used fenceline separation as a solution. Development of a device to prevent calves from suckling while in the presence of the dam would be a valuable methodological contribution in the future.

Lastly, we were interested in predicting urine and fecal output during the postpartum period to determine whether prepartum N balance had carry-over effects on postpartum estimated N balance. Our prediction equations developed from the prepartum data sets were not accurate enough to apply them to the postpartum data. We had used creatinine concentration and excretion within the regression model, assuming that creatinine concentration in urine was stable relative to body muscle mass. However, glomerular filtration rate can be estimated from the relationship of plasma and urinary creatinine concentrations to metabolic empty body weight and would provide a more reliable prediction of postpartum urine output.

5.8 Future research recommendations

A. Future research needs to evaluate the ideal MP supplementation level and progress to defining late gestation requirements in terms of AA requirement for specific physiological processes.
B. Future research needs to determine the adequate quantity of RDP supplied during late gestation, relative to passage rate and ruminal degradation of soluble protein fractions, to maintain proper rumen function and fiber digestibility.
C. Future research needs to evaluate the optimum quantity of RDP provision and RUP supplementation meet MP requirements while reducing the rate of urinary nitrogen excretion to fecal nitrogen excretion.
D. Future research is needed to evaluate whether the observed decrease in ruminal pH during the early lactation period in the current study is typical of forage-grazing beef cows in cow-calf production systems and the extent to which the decrease in ruminal pH effects fiber digestibility and susceptibility to sub-acute rumen acidosis.

E. Future research needs to validate the theory that beef cow milk yield matches calf demand. Furthermore, the average age at which calves begin consuming DM should be established to determine when beef cattle may experience a decrease in nutrient demand for lactation relative to calves alternatively consuming forages and concentrates.
F. Future research could evaluate postpartum nutritional strategies to maximize milk production or component yield in order to improve calf performance.

6.0 CONCLUSION

Late gestation represents a period of time where AA demand (alternatively MP demand) increases dramatically for beef cattle, primarily reflecting the exponential increase in fetal mass prior to parturition. It is known that gestational nutrition can have carry-over effects during the early postpartum period. The research performed and data collected within this project indicates that over-supplementation of MP during late gestation improves heifer N balance prepartum with beneficial impacts on rumen fermentation and heifer BW prior to parturition. Carry-over effects of improved prepartum N balance during the postpartum period are minimal. Contradictory to the hypothesis, improved N balance during late gestation did not alter the degree of skeletal muscle catabolism between heifers supplemented at or above requirements. Research needs to be conducted to evaluate the carry-over influence of prepartum N balance on postpartum N balance.

The secondary objective of this project was to evaluate parameters, such as DMI, rumen fermentation, and urinary and blood metabolites, relative to the transition between gestation and lactation. The term transition is often used in dairy production, but, until the current research, it has received little scientific or industrial focus when discussing late gestation or early lactation beef cattle. Data within this project are indicative that the transition period is indeed a dynamic time-frame in the cow-calf production cycle that warrants future investigation. For example, research could evaluate alterations in total tract digestibility or N balance during the early lactation period and their subsequent impacts on cow energy balance or milk production. As well, the alterations in rumen fermentation patterns and total tract digestion should be incorporated into nutritional models to reflect the changes in available nutrient supply during late gestation.

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