Oversupplying metabolizable protein in late gestation for beef cattle: effects on postpartum ruminal fermentation, blood metabolites, skeletal muscle catabolism, colostrum composition, milk yield and composition, and calf growth performance[1]

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ABSTRACT: The objective of the study was to determine whether oversupplying MP prepartum affects postpartum cow BW, colostrum composition, milk production and composition, protein catabolism in the dam, and calf growth. Crossbred Hereford heifers were individually fed a control treatment designed to meet MP requirements (**CON**; n = 10) or 133% of the MP requirement (**HMP**; n = 11) from day −55 ± 4 until parturition. All cows were provided a common postpartum diet. Cow BW was measured on days 7 ± 1, 14 ± 2, 28 ± 3, 57 ± 4, 82 ± 5, and 111 ± 3 relative to parturition. DMI and ruminal pH were measured daily and summarized by week until day 33. Milk yield was estimated based on a 12-h two-quarter milk yield on days 7 ± 1, 12 ± 1, 28 ± 3, 33 ± 3, 70 ± 3, and 112 ± 3. Urine samples were collected from cows over a 6-d period starting on days 7 ± 1 and 28 ± 3 and the composited samples were analyzed for 3-methylhistidine (**3-MH**) and creatinine. Muscle samples were collected from cows on day 13 ± 1 while calf muscle samples were collected on days 2 and 111 ± 3 of age. Muscle samples from cows were analyzed for markers of protein catabolism, and calf muscle samples were analyzed for genes regulating cell growth and differentiation. Data were analyzed as a randomized complete block design using the MIXED procedure of SAS accounting for repeated measures when necessary. Postpartum BW did not differ ($P \geq 0.30$) by treatment, day, or the interaction of treatment and day (T × D), but rump fat decreased ($P = 0.011$) as lactation progressed. DMI decreased during weeks 2 and 3 compared to 1 and 4, whereas ruminal pH was less during weeks 2, 3, and 4 relative to week 1. Colostrum fat concentration was less ($P = 0.003$) for HMP than CON; but, milk production was not affected by treatment. Milk yield was greatest from days 7 to 33 and decreased thereafter ($P < 0.01$). Urinary 3-MH and the 3-MH:creatinine ratio did not differ by treatment, day, or the T × D ($P \geq 0.22$) interaction, nor was there a difference ($P \geq 0.13$) in the abundance of catabolic proteins. Calf growth was not affected by treatment, but HMP calves had greater expression (T × D, $P = 0.05$) of PPARG while PKM expression increased for CON calves (T × D, $P = 0.04$) at day 111 compared to their expression at day 2. Overfeeding MP during late gestation does not improve postpartum indicators of N balance or maternal muscle turnover but may alter colostrum composition and calf gene expression at weaning.

Key words: beef cow, gene expression, metabolizable protein, milk production, postpartum, ruminal fermentation

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INTRODUCTION

Beef cow nutrient requirements are greatest during early lactation (NASEM, 2016), mainly due to utilization of energetic and nitrogenous substrates for milk production. Milk yield for beef cattle is estimated to range between 4 and 15 kg/d and typically contains up to 4.0% fat, 3.8% protein, and 8.3% total solids-not-fat (Freetly et al., 2006; NASEM, 2016). In early lactation, beef cattle may be unable to consume adequate quantities of feed to meet nutrient demand, particularly with low forage quality. Moreover, when coupled with high nutrient demand, low nutrient intake immediately following parturition may impose a negative nitrogen balance during early postpartum and this response may be affected by the prepartum diet (Larson et al., 2009). However, the extent to which adequate MP supply may be compromised during early lactation and affected by late gestation MP supply in beef cattle is not known.

Prepartum CP supplementation when the basal forage imposed a mild CP deficiency has been reported to improve BW and BCS at calving (Larson et al., 2009; Bohnert et al., 2013), shorten the postpartum interval, and improve conception rates (Richards et al., 1986; Bohnert et al., 2002). The improvements in performance outcomes with added CP suggest that there may be benefits from increasing late MP supply that positively influence early lactation nitrogen balance and cow–calf performance. However, it is not clear if a purposeful oversupply of MP prepartum may have carry over effects affecting postpartum cow and calf performance.

The hypothesis for this study was that oversupplying MP (133% of the predicted requirement) prepartum would improve N-balance prepartum and postpartum indicators of N balance, milk production, and calf growth. The objective was to evaluate whether increasing MP supply prepartum would affect milk production and composition, indicators of skeletal muscle catabolism, calf growth performance, and gene expression. A secondary objective was to characterize postpartum phase of the transition period for beef cattle.

MATERIALS AND METHODS

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

Experimental Design, Cow Husbandry, and Dietary Treatments

This paper is one of two companion papers addressing the effects arising from increased prepartum MP supply. The companion paper (Hare et al., submitted) addressed prepartum responses and the current paper addresses postpartum responses. Detailed materials and methods have been presented in the companion paper (Hare et al., submitted). Briefly, a total of 24 primiparous pregnant Hereford-cross heifers, including 14 that were fit 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 Angus sire (Cole Creek Cedar Ridge 1V, Reg. No. 1659099 (CAN), Genex Co-operative Inc., Shawano, WI) to minimize genetic and gender variation. The experiment was arranged as randomized complete block design with the expected date of parturition as the blocking factor. Within block and between blocks, heifers were assigned to treatments while balancing for the number of cannulated heifers and initial BW. Starting on day −55 ± 3.7 relative to parturition, heifers were housed in individual pens (9 m^2) with rubber mats. Pens were cleaned and washed daily. Eight 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, the cow–calf pair was moved into individual bedded outdoor pens (36 m^2) until day 33. Cows were then group-fed by prepartum treatment starting on day 34 relative to calving, with three to four cow–calf pairs per group until day 112 relative to parturition.

Prepartum treatments consisted of the control treatment (**CON**; n = 12) or a treatment where 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 isocaloric based on net energy. The predicted supply was based on a mature cow BW of 550 kg BW (BCS 5) at 260 d of gestation and a calf birth weight of 36 kg. Exposure to the dietary treatments was initiated on day −55.3 ± 3.7 relative to

parturition. The diets consisted of the same whole-crop barley hay and wheat straw with cows being offered one of two prepartum supplemental pellets based on their respective treatment (Table 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. 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 heifers 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 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 d in milk (**DIM**), and 6 kg/d milk yield with expected composition of 3.6% milk fat, 3.3% milk protein, and 4.9% milk lactose. Total mixed rations were fed twice daily at 0900 and 1630 hours 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, when calves were separated from their dams for measurement of 12-h milk yield (described below), calves had constant access to their dams over the pre-weaning period. Water was available free choice and dry feed (i.e., forage and concentrate) was accessible for calves after day 33 as feed bunk design (height and depth) prevented calves from consuming the diet fed to their dams.

Data and Sample Collection

DMI. DMI was determined daily from the day of calving to day 33 by measuring the weight of the feed offered and the weight of the feed that was refused. The weights were then corrected for their respective DM content. Throughout the study, samples of forages were collected once weekly, and a concentrate sample was collected every 2 wk. Samples of refusals were composited weekly by day (proportionally by the quantity refused per day on an as-is basis). The feed and composited refusal samples were dried using a forced-air oven at 55 °C until they achieved a constant weight.

Table 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) diets fed during the final 8 wk of gestation, and the lactation (LAC = formulated to 103% of MP requirements) diet fed from parturition to day 112.

	Prepartum diet		Postpartum diet
Item	CON	HMP	LAC
Ingredient composition, % DM			
Whole-crop barley hay	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 24.03% canola meal solvent, 14.79% soybean meal 47.5 solvent, 1.85% oat hulls, 14.79% molasses beet, 36.98% ground barley grain, and 7.56% 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 24.03% oat hulls, 27.73% molasses beet, 40.67% ground barley grain, and 7.56% 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, 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 5H20.

[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.

Body weight and rib and rump fat thickness. Heifers were weighed at calving and on days 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 days 14 ± 2.0, 28 ± 3.0, 57 ± 3.6, 84 ± 3.0, and 112 ± 2.9.

Ruminal fermentation, urine and blood metabolites. For cannulated heifers, 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 hours on days 7 ± 0.9 and 28 ± 2.9 relative to parturition. Digesta was strained through two 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 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% sulfuric acid and was stored frozen (−20 °C) until used for determination of ammonia-N concentration (Fawcett and Scott, 1960).

Ruminal pH was measured every 5 min in cannulated cows from parturition until day 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 millivolt 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 days 7 ± 1.0 and d 28 ± 2.9 relative to parturition. Eight samples were collected with 18-h intervals between samples (Chizzotti et al., 2007), and were stored frozen (−20 °C) until being composited on an equal volume basis (to represent one full day of sample collection every 3 h). Samples were analyzed for urea-N concentration, total N concentration, 3-methylhistidine, and creatinine. Urine urea-N was analyzed by the method described by Fawcett and Scott (1960). Nitrogen concentration was analyzed according to the AOAC (1994) method for Kjeldahl determination of CP. Urine 3-methylhistidine (**3-MH**; Rathmacher et al., 1992) and creatinine (Slot, 1965) concentrations were analyzed by Heartland Assays (Ames, IA).

Blood samples from the jugular vein were collected the day of ruminal samples at 1900 hours 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 collected for serum was allowed to sit at room temperature for 15 min to facilitate clotting prior to centrifugation. 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). 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%.

Abundance of proteins relating to energy balance and protein catabolism. Western blot was used to determine the abundance of proteins related to muscle synthesis and degradation (similar to methods described by Wood et al., 2013; Nichols et al., 2017). Approximately 400 mg of muscle tissue from the longissimus lumborum (caudal to 13th rib, ~10 cm ventral from spine) were collected using a Bergstrom biopsy needle or a disposable biopsy punch (Acu-Punch, Acuderm, Ft. Lauderdale, FL) from cows on day 13 ± 0.9 relative to parturition. Tissue was snap frozen in liquid N_2 and stored at −80 °C until analysis when samples were homogenized in ice cold radio-immunoprecipitation assay buffer (1% Triton X-100, 0.1% SDS, 50 mM Tris–HCl, pH 8.0, 150 m*M*, NaCl, and 0.5% sodium deoxycholate) containing protease and phosphatase inhibitors (Thermo Scientific, Nepean, ON). Concentrations of total protein in the homogenate were determined using a commercially available bicinchoninic acid kit (Pierce BCA Protein Assay Kit, Pierce Biotechnology) using bovine serum albumin as a standard. Protein concentrations were standardized across samples to 1 mg/μL with Laemmli sample buffer [Bio-Rad Laboratories Ltd, Mississauga, ON (65.8 mM Tris–HCl at pH 6.8, 2.1% SDS, 26.3%,

(w/v) glycerol, 0.01% bromophenol blue] such that 15 mg of protein for each sample were loaded onto precast Mini-PROTEAN TGX Stain-Free gels (4% to 15%, Bio-Rad Laboratories Ltd). Gels were vertically electrophoresed using a Mini-PROTEAN Tetra Cell (Bio-Rad Laboratories Ltd) for 15 min at 220 V. Gels were then transferred using a Trans-Blot Turbo Transfer System (Bio-Rad Laboratories Ltd) to 0.2 µm polyvinylidene difluoride membrane (Trans-Blot Turbo, Mini Transfer Membranes, Bio-Rad Laboratories Ltd). Membranes were then blocked in a blocking solution (10 mM Tris–HCl, 200 mM NaCl, 1 mL/L Tween-20), and 20 g/L Blotting-Grade Blocker (Bio-Rad Laboratories Ltd)] at room temperature for 1 h. Primary antibodies for mTOR (1:1,000; mouse monoclonal; Cell Signaling Technologies #4517, Danvers, MA) Phospho-mTor Ser 2448 (1:1,000; rabbit polyclonal; Cell Signaling Technologies #2971), AKT (1:1,000, rabbit monoclonal; Cell Signaling #4691), Phospho-AKT SER 473 (1:2,000, rabbit monoclonal, Cell Signaling Technologies #4060), ubiquitin (1:1,000; rabbit polyclonal, Cell Signaling Technologies #3933), calpain 2 (m-type; 1:1,000; rabbit polyclonal, Cell Signaling Technologies #2539), and calpastatin (1:1,000; rabbit polyclonal, Cell Signaling Technologies #4146) were diluted in the blocking solution, and membranes were incubated overnight at 4 °C. The next morning, the membranes were washed three times in tris-buffered saline (10 mM Tris–Cl, 200 mM NaCl, 1 mL/L Tween-20) and then incubated with secondary antibody (goat anti-rabbit [1:2,000; Abcam ab205718, Cambridge, MA] or goat anti-mouse [1:2,000, Abcam, ab205719]) for 1 h at room temperature and were then washed six times for 5 min/wash in tris-buffered saline. Following the final wash, membranes were developed using Clarity Western ECL Substrate Solutions (Bio-Rad Laboratories Ltd) luminol/enhancer solution and peroxide solution according to the manufacturer's instructions.

Band intensities were measured using Chemidoc Imaging System and analyzed with Image Lab imaging software version 6.0 (Bio-Rad Laboratories Ltd) and band specificity was confirmed using standard molecular weight markers (Precision Plus Standards, All Blue, 10 to 250 kDa; Bio-Rad Laboratories Ltd). Samples were normalized for unequal loading by quantifying total protein loaded using stain-free protein imaging (Bio-Rad Laboratories Ltd) and subtracting from band intensity measurement. Band intensities are expressed as corrected arbitrary units (**AUs**) and expressed relative to control treatment.

Colostrum composition, milk production, and milk 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 using a portable milking machine (Deluxe Portable Pump, E-Zee Milking Equipment, Gordonville, PA). 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 days 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, two-quarter milk yield was determined as described by Wall and McFadden (2007) with minor modifications for beef cattle. 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 hours 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. After 12 h, the cow was moved back to the chute, injected with 4 mL oxytocin, and two 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 four-quarter 24-h yield.

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. Linear regression was used to calculate ADG based on the day of measurement and calf BW.

Calf gene expression. Approximately 100 mg of muscle tissue from the longissimus lumborum (caudal to 13th rib, ~10 cm ventral from spine) were collected using a disposable biopsy punch (Acuderm,

Ft. Lauderdale, FL) from calves on days 2 and 111 ± 3 relative to parturition. Tissue was snap frozen in liquid N_2 and then stored at −80 °C until analysis. Muscle samples were weighed and 40 to 90 mg of muscle was transferred into a 2-mL homogenization tube (Precellys Hard tissue grinding MK28, Bertin Technologies, Rockville, MD) containing 1 mL TRIzol reagent (Ambion, Life Technologies Inc., Carlsbad, CA). Tissue was homogenized using the Precellys 24 Tissue Homogenizer with the Cryolys accessory (Bertin Technologies) at 4 °C, two times for 30 s at 5,500 rpm with a 10 s pause between the homogenization steps. Tissue homogenate was incubated at room temperature for 5 min before centrifugation at 12,000 × *g*, at 4 °C, for 10 min. Supernatant was transferred to a new tube and the rest of the RNA extraction procedure followed the TRIzol manufacturer's protocol, commencing from the addition of 200 µL chloroform to the supernatant. Resulting RNA pellets were resuspended in 40 µL nuclease-free H2O (Ambion, Life Technologies Inc.).

Total RNA concentration in the samples was quantified using a spectrophotometer ND-1000 (NanoDrop, Wilmington, DE). RNA was also evaluated for RNA integrity (**RIN**) using an Agilent 2200 TapeStation RNA screen tape device (Agilent, Santa Clara, CA) and stored at −80 °C until cDNA synthesis. The RIN value of RNA isolated from all samples was >7. Total RNA (2 µg) from each individual tissue was reverse transcribed using a High Capacity cDNA Reverse Transcription Kit (Life Technologies Inc.) according to the manufacturer's instructions. RNaseOUT (Life Technologies Inc.) was also added to the reaction at a concentration of 2 U/µL. After reverse transcription, the cDNA was diluted to 1.25 ng/µL with nuclease-free H_2O (Ambion, Life Technologies Inc.).

Real-time PCR analysis was performed in duplicate using 5 ng of cDNA in 96-well plates using KAPA SYBR FAST Master Mix (2X) (Kapa Biosystems Inc., Woburn, MA) and the Step-One Plus Real-time PCR system (Life Technologies Inc.). A blank sample and an RNA sample were added to control for nonspecific amplification. Relative standard curves, made from a serial dilution of pooled cDNA from the samples of interest and ranging from 20 to 0.15 ng, were used to determine the relative quantity of each sample. The primers were designed using Primer3 software using species-specific sequences found in GenBank and were designed to cover exon–exon junctions when possible and ran with an annealing/extension temperature in the real-time PCR reaction of 60 °C (Table 2). The amplification efficiency for each gene was determined using serial dilution of tissue-specific cDNA and was found to be 100 ± 10% for all genes (Table 2). The resulting quantitative PCR amplicons were also sequenced to confirm their identity (data not shown). Five endogenous controls were tested and the best individual or combination of endogenous control was chosen using NormFinder (Andersen et al., 2004). As a result, ribosomal protein L19 (*RPL19*) and peptidylprolyl isomerase A (*PPIA*), were used as the endogenous controls to correct for RNA extraction and reverse transcription efficiency in days 2 and 111 LD muscle, respectively. The endogenous controls were also tested for any treatment effect and were found to be stable among samples within each tissue type confirming their usefulness as suitable endogenous controls. For all genes, only the expression of transcripts that could be reliably detected in each sample is reported.

Statistical Analysis

Of the calves born, three were bull calves (two from HMP dams and one from CON). Gender of the calf did not affect birth weight or growth and therefore gender was not included in the statistical model. All data were analyzed as a randomized complete block design using the PROC MIXED of SAS 9.4 (SAS Institute, Cary, NC). 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. Apart from analysis of colostrum composition and maternal protein abundance data, all data were analyzed using repeated measures. 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. Colostrum and protein abundance data were analyzed as a randomized complete block design with the fixed effect of treatment and random effect of cow nested in block. 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.

Table 2. Primer sequences of the target and endogenous genes used for real-time PCR analysis of longissimus muscle biopsy from calves born to dams fed metabolizable protein at their predicted requirements (100%, **CON**) or greater than their predicted requirements (133%, **HMP**) during the final 8 wk of gestation

Gene	Gene name	GenBank accession #	Primer[1]	Sequence 5′–3′	Amplicon size, bp	Efficiency, %	R^2
IGF-I	Insulin-like growth factor-I	NM_001077828	Fwd	GATGCTCTCCAGTTCGTGTG	141	97.7	0.993
			Rev	CTCCAGCCTCCTCAGATCAC			
IGF-IR	Insulin-like growth factor-I receptor	NM_001244612	Fwd	CAAAGGCAATCTGCTCATCA	139	93.8	0.998
			Rev	CAGGAAGGACAAGGAGACCA			
IGF-II (ex9-10)	Insulin-like growth factor-II	NM_174087.3	Fwd	GGGATGTGTCTGCCTCTACG	95	98.1	0.992
			Rev	GGTGGACTGCTTCCAGATGT			
IGF-IIR	Insulin-like growth factor-II receptor	NM_174352	Fwd	TCCTCAATCCCATAGCCAAC	188	95.1	0.994
			Rev	CACACACACTGGTCCTCAGC			
ACO2	Aconitase 2	NM_001102015.2	Fwd	GTTACGAAGCCGCCATTAAG	119	90.3	0.994
			Rev	GCCCAAATCTCCAGAAACAC			
DLK1	Delta-like homolog	NM_174037.2	Fwd	ATTCTGCGACGATGACAGTG	165	97.8	0.990
			Rev	CGGATGTCTAGGTCACAGAGG			
EGR3	Early growth response 3	XM_002689773	Fwd	ACTCGGAGCCTGTGTCTTTC	105	102.5	0.990
			Rev	GGAAGAGGTTGCTGTCCAAG			
GPD1	Glycerol-3-phosphate dehydrogenase 2	NM_001035354.1	Fwd	TCTGCCACCTTCTTGGAGAG	193	90.9	0.998
			Rev	TGTGCTGGAGGATACTGTGC			
HACL1	2-hydroxyacyl-CoA lyase 1	NM_001098949.1	Fwd	TCAAGAACAACTACCCAGAGAGTG	147	106.7	0.957
			Rev	GCAAATCCCAAACCGACTCC			
INSR	Insulin receptor	XM_002688832	Fwd	CCTATGCCCTGGTGTCACTT	114	93.2	0.989
			Rev	GCTGCCTTAGGTTCTGGTTG			
MBNL1	Musclebind-like splicing regulator 1	NM_001099725.1	Fwd	CACAGTCACCGTGTGTATGG	122	92.0	0.999
			Rev	CCTGGTTGACCTGGTATTGG			
MEF2A	Myocyte enhancer factor 2A	NM_001083638	Fwd	CAATGCCAACTGCCTACAAC	130	104.8	0.996
			Rev	TGTCCTAAATGGTGCTGCTG			
MYF5	Myogenic factor 5	NM_174116	Fwd	TGGTCCAGAAAGAGCAGCAG	123	101.2	0.994
			Rev	GGTGATCCGATCCACTATGC			
MYF6	Myogenic factor 6	NM_181811	Fwd	CCCTTCAGCTACAGACCCAAG	144	94.0	0.992
			Rev	CGATGAATCAATGCTTGTCC			
MYOD1	Myogenic differentiation 1	NM_001040478	Fwd	GAACACTACAGCGGCGACTC	121	96.6	0.988
			Rev	AGTAAGTGCGGTCGTAGCAG			
MYOG	Myogenin	NM_001111325	Fwd	CAGTGAATGCAGCTCCCATA	164	98.9	0.993
			Rev	CGACATCCTCCACTGTGATG			
PFKM	Phosphofructokinase, muscle associated	NM_001075268.1	Fwd	AGCGTCTGGGATATGACACC	116	90.8	0.999
			Rev	TGCCATTACTGCTTCCACAC			
PKM	Pyruvate kinase, muscle associated	NM_001205727.1	Fwd	ATCGTCCTCACCAAGTCTGG	146	92.7	0.994
			Rev	GGGTCCTTACACACCACAGG			

Table 2. *Continued*

Gene	Gene name	GenBank accession #	Primer[1]	Sequence 5′–3′	Amplicon size, bp	Efficiency, %	R^2
PLCB4	Phospholipase C β4	NM_001166510.1	Fwd	GAGAGCGGATCAAATGAGAGC	106	90.2	0.998
			Rev	TTTGGCTGTGTTGGCTTTCC			
PYGM	Glycogen phosphorylase, muscle associated	NM_175786.2	Fwd	TCGGACTGTGATGATTGGAG	199	98.7	0.990
			Rev	GATCTGCTCGGAGAGGTCAG			
PPARG	Peroxisome proliferator-activated receptor gamma	NM_181024,2	Fwd	ACCACCGTTGACTTCTCCAG	137	100.9	0.988
			Rev	ACAGGCTCCACTTTGATTGC			
SDHA	Succinate dehydrogenase complex flavoprotein subunit A	NM_174178.2	Fwd	CGTGAAAGGCTCCGACTGG	165	103.9	0.976
			Rev	CCGAACTTGAGGCTCTGTCC			
SFRP1	Secreted frizzled-related protein 1	NM_174460.2	Fwd	AGATGCTCAAGTGCGACAAG	146	103.9	0.976
			Rev	ATGCTCAATGATGGCTTCAG			
SRF	Serum response factor	NM_001206016	Fwd	CGGCTTTGAAGAGACAGACC	101	101.2	0.992
			Rev	GCAGGTTGGTGACGGTAAAC			
TTN	Titin	XM_002685260.1	Fwd	CGAGTATGTGTTCCGAGTGG	165	98.7	0.995
			Rev	GTAAGTGTCATGCCCTCACG			
PPIA	Peptidylprolyl isomerase A	NM_178320.2	Fwd	GTCAACCCCACCGTGTTCT	132	96.0	0.998
			Rev	TCCTTTCTCTCCAGTGCTCAG			
RPL19[2]	Ribosomal protein L19	NM_001040516.1	Fwd	ACCCCAATGAGACCAATGAA	101	93.9	0.998
			Rev	ATGGACAGTCACAGGCTTCC			

[1]Fwd = forward; Rev = reverse.

[2]Endogenous gene *RPL19* was different ($P < 0.05$; data not shown) for HMP and CON calves at day 111 relative to parturition. As such, gene expression day 111 for all target genes was normalized by endogenous gene PPIA, while gene expression at day 2 for all target genes was normalized by RPL19.

RESULTS

Cow BW and rib and rump fat depth. Cow BW was not affected ($P \geq 0.30$; Table 3) 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 differ (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 days 14 and 28 to 112 resulting in respective reductions of 1.8 and 1.9 mm.

DMI and ruminal fermentation. Cows fed the CON and HMP treatments prepartum did not differ ($P = 0.80$; Table 4) for DMI when reported as kilogram per day or as a proportion of BW, nor was DMI affected ($P = 0.51$) by the interaction of treatment and day. DMI (kg/d and % BW) was less ($P \leq 0.009$) during weeks 2 and 3 relative to weeks 1 and 4 postparturition.

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 ($P = 0.013$, <0.001, 0.004) during the first week following parturition relative to the following 3 wk. Total SCFA concentration did not differ ($P \geq 0.53$; Table 5) 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 day 7 compared to 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.

Colostrum composition, milk production, and milk composition. The majority of colostrum components (concentration of protein and lactose, urea-N, total solids, and IgG concentration) did not differ ($P \geq 0.17$; Table 6) 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

Table 3. 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]	day 7	day 14	day 28	day 57	day 82	day 112	SEM[2]	Treatment	Day	T × D[3]
n	10	11		21	20	20	18	21	21				
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: day 6.6 ± 1.0, 14.0 ± 2.0, 27.9 ± 2.9, 57.4 ± 3.6, 82.3 ± 5.1, and 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. 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 5. Postpartum ruminal SCFA and ammonia-N concentrations 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]					
Item	CON	HP		Day 7	Day 28		*P*-values		
n	5	7	SEM[2]	12	12	SEM[2]	Treatment	Day	T × D[3]
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	0.0	0.6	0.6	0.0	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	0.0	1.0	1.0	0.0	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
Ruminal 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 days 6.7 to 11.7 ± 0.9 (mean ± SD) and from days 27.8 to 32.8 ± 2.9.

[2]Largest SEM is reported.

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

Table 6. 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		
n	11	10	SEM[1]	*P*-value
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.

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

Urine and blood metabolite concentrations. Treatment and the interaction of treatment and day did not affect urine metabolite concentrations ($P \geq 0.18$; Table 8). Standardization of 3-methylhistidine 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 ($P = 0.099$) at day 28 compared to day 7 relative to parturition.

Non-esterified fatty acid concentration was greater ($P < 0.001$; Table 9) at days 7 and 28 relative to days 70 and 112. On average, NEFA concentration was 70.6% greater at days 7 and 28 as compared to days 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 and BHBA 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 days 7 to 112.

Abundance of proteins relating to energy balance and protein catabolism. Prepartum treatment had no effect ($P \geq 0.13$; Table 10) on the abundance of mTOR, p-mTOR, p-mTOR/mTOR, Akt, p-Akt, p-Akt/Akt, calpain, calpastatin, and ubiquitin in maternal skeletal muscle postpartum.

Table 7. Milk production, composition, and component yield 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]									
Item	CON	HMP		day 7	day 12	day 28	day 33	day 70	D 112		*P*-values		
n	10	11	SEM[2]	21	21	21	21	18	21	SEM[2]	Treatment	Day	T × D[3]
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: day 6.7 ± 0.9 (mean ± SD); days 11.7 ± 0.9, 27.8 ± 2.9, 32.8 ± 2.9, 70.2 ± 3.2, and 111.9 ± 2.9.

[2]Largest SEM is reported.

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

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

Table 8. Postpartum urine metabolite concentrations 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]					
Item	CON	HMP		Day 7	Day 28		*P*-values		
N	10	11	SEM[2]	20	21	SEM[2]	Treatment	Day	T × D[3]
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 days 6.7 to 11.7 ± 0.9 (mean ± SD) and from days 27.8 to 32.8 ± 2.9 relative to parturition.

[2]Largest SEM shown.

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

Calf growth performance. At birth and day 112, calf BW, chest circumference, wither height, hip height, and body length did not differ ($P \geq 0.21$; Table 11) due to maternal prepartum treatment. Calf ADG (kg/d), calculated by linear regression, did not differ ($P = 0.82$) by treatment.

Calf gene expression. The treatment by day interaction was detected for the expression of PKM ($P = 0.043$; Figure 1) and PPARG ($P = 0.047$; Figure 2) in heifer calf skeletal muscle. The expression of PKM at day 111 for CON calves was twice that of their PKM expression at day 2 while expression did not differ from HMP calves at either day 2 or 111. For HMP calves, the expression of PPARG was 113% greater at day 111 compared to day 2, although the expression of PPARG was not different from that of the CON calves at either day 2 or 111. The interaction between T× D for the expression of GP1 and SDHA was detected ($P = 0.026$ and 0.043, respectively; Table 12), but the means were not separated based on the Tukey's post hoc test. Treatment only tended to increase ($P = 0.097$) the expression of HACL1 for in calves from CON compared to HMP heifers. At day 111, the expressions of IGF-I, IGF-IR, INSR, MBNL1,

Table 9. Postpartum circulating blood metabolites for cows fed diets 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		Day 7	Day 28	Day 70	Day 112				
N	10	11	SEM[2]	21	21	18	21	SEM[2]	Treatment	Day	T × D[3]
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	6.3	6.3	7.5	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 days 6.7 ± 0.9 (mean ± SD), 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.

Table 10. Abundance (AU[1]) of proteins related to energy balance and protein catabolism at day 12.8 ± 0.9 for cows fed diets formulated to provide 100% (**CON**) or 133% (**HMP**) of predicted metabolizable protein requirements during the final 8 wk of gestation

	Treatment			
Item	CON	HMP		
n	10	11	SEM[2]	*P*-value
mTOR	0.87	1.00	0.16	0.56
p-mTOR	0.89	0.68	0.17	0.40
p-mTOR/mTOR	1.05	0.63	0.41	0.46
Akt	1.66	1.13	0.34	0.28
p-Akt	1.02	1.40	0.45	0.54
p-Akt/Akt	0.88	1.02	0.30	0.74
Calpain	0.98	0.49	0.22	0.13
Calpastatin	0.99	0.58	0.33	0.36
Ubiquitin	1.13	1.62	0.24	0.16

[1]Abundance expressed in arbitrary units.

[2]Largest SEM shown.

Table 11. BW and frame measurements at birth and day 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		
N	10	11	SEM[1]	*P*-value
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[2], kg/d	0.74	0.76	0.04	0.82

[1]Largest SEM shown.

[2]ADG calculated by the linear regression of the difference between weaning and birth BW by day postparturition.

MEF2A, PLCB4, and SRF were all greater ($P \leq 0.041$) compared to day 2, whereas the MYF5 expression in skeletal muscle was less ($P = 0.026$) at day 111. Aconitase 2 and MYF6 expression tended to be greater ($P = 0.067$ and 0.057, respectively) at day 111 compared to day 2.

DISCUSSION

The current study was designed to evaluate the effect of oversupplying MP to beef heifers during late gestation and whether carry-over effects would persist in the postpartum period. Nutrient supply was predicted using the CNCPS 6.5 model and retrospective analysis predicted that CON and HMP heifers were provided with 831 and 1,137 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 (%DM) for the HMP diet was 154.8% of that in the CON diet. The prepartum responses have been previously reported in a companion paper (Hare et al., submitted). During the postpartum period, heifers were fed a common ration that was adequate in ME and MP supply (126% and 103% of ME and MP, respectively; CNCPS 6.5). As such, the treatments imposed allowed us to evaluate carry-over effects

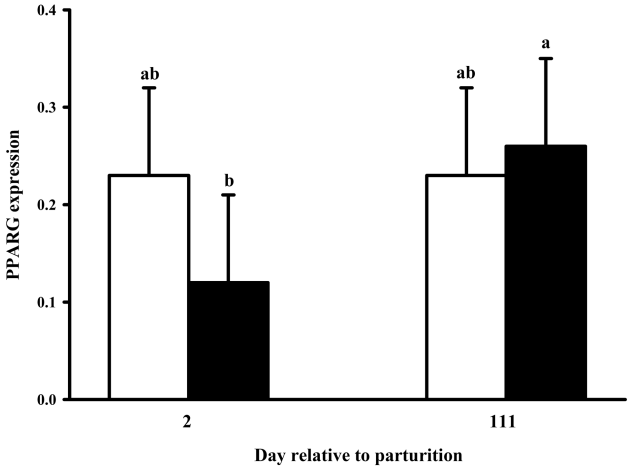

Figure 1. The interaction of treatment and day on heifer calf expression of pyruvate kinase (**PKM**; $P = 0.043$). Calves were born to dams that were fed a diet designed to meet 100% of the MP requirement (**CON**, white columns) or a high MP diet that was designed to supply 133% of the MP requirement (**HMP**, black columns). The *P*-values for the main effects of treatment and day for expression of PKM were 0.31 and 0.050, respectively. Expression of PKM in skeletal muscle tissue was greater for CON calves at day 111 ± 2.9 (mean ± SD) compared to CON at day 2 while not differing from HMP calves at either day 2 or 111 ± 2.9. Expression of PKM at days 2 and 111 ± 2.9 was not different for HMP calves. Means within a dependent variable that have uncommon letters differ.

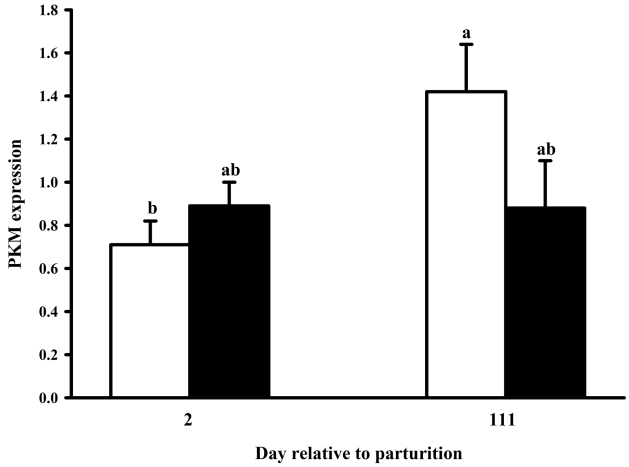

Figure 2. The interaction of treatment and day on heifer calf expression of peroxisome proliferator activated receptor gamma (**PPARG**; $P = 0.047$). Calves were born to dams that were fed a diet designed to meet 100% of the MP requirement (**CON**, white columns) or a high MP diet that was designed to supply 133% of the MP requirement (**HMP**, black columns). The *P*-values for the main effects of treatment and day for expression of PPARG were 0.30 and 0.035, respectively. Expression of PPARG in skeletal muscle tissue was greater for HMP calves at day 111 ± 2.9 (mean ± SD) compared to day 2 while not differing from CON calves at day 2 or 111 ± 2.9. CON calves' expression of PPARG was not different at day 2 compared to day 111 ± 2.9. Means within a dependent variable that have uncommon letters differ.

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 (Hare et al., submitted). Although the experimental design did not allow for direct measurement of N balance during the postpartum period as we were unable to perform total urinary and fecal collections, the lack of difference in the postpartum abundance of calpain and ubiquitin indicates that prepartum treatment did not influence skeletal muscle catabolism during the postpartum period (Goll et al., 2008). The 3-MH:creatinine ratio was also not different among CON and HMP cows. Moreover, milk yield data and calf growth were not affected. These urine, muscle protein abundance, cow and calf BW data, and milk yield all indicate that improvements for prepartum N-balance likely have little effect on postpartum performance; at least when cows are in a positive N-balance. 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. We are not aware of other studies assessing N-balance in beef cattle in early lactation.

Oversupplying MP prepartum reduced colostrum milk fat concentration and tended to reduce the colostrum net energy content. This response is consistent with previous studies where colostrum and milk fat yield have been reported to be reduced when pregnant ewes were fed a diet supplying 40% more than the late gestation requirements relative to 100% of the predicted requirement (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 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 16 carbons, respectively) and incorporation of long-chain fatty acids (≥16 carbons) from peripheral supply (Bauman and Griinari, 2003). Without knowing the fatty acid composition of the colostrum, we cannot determine which mechanisms drive the reduction in colostrum fat when MP was oversupplied during late gestation.

Table 12. Gene expression of heifer calves born to primiparous heifers that were fed rations formulated to supply 100% (CON) or 133% (HMP) of their predicted metabolizable protein requirement during the final 8 wk of gestation

	Treatment			Day[1]					
Gene	CON	HMP		2	111		*P*-value		
n[4,5]	9	8	SEM[2]	15	17	SEM[2]	Treatment	Day	T × D[3]
IGF-I	0.63	0.67	0.07	0.46	0.84	0.07	0.70	<0.001	0.22
IGF-IR	1.10	1.11	0.06	0.85	1.36	0.09	0.84	<0.001	0.54
IGF-II	0.89	0.79	0.07	0.84	0.84	0.07	0.30	0.98	0.48
IGF-IIR	1.19	1.01	0.08	1.11	1.08	0.10	0.12	0.82	0.51
ACO2	1.17	1.07	0.11	0.97	1.28	0.13	0.52	0.067	0.73
DLK1	0.70	0.63	0.09	0.62	0.71	0.09	0.59	0.52	0.22
EGR3	0.70	0.62	0.14	0.70	0.62	0.14	0.70	0.67	0.47
GPD1[6]	0.95	0.85	0.09	0.82	0.98	0.09	0.47	0.23	0.026
HACL1	0.68	0.51	0.07	0.50	0.69	0.09	0.097	0.11	0.34
INSR	0.97	0.96	0.08	0.76	1.17	0.10	0.93	0.005	0.79
MBNL1	1.18	1.11	0.11	0.92	1.37	0.14	0.68	0.006	0.52
MEF2A	1.23	1.10	0.13	1.02	1.32	0.15	0.51	0.041	0.43
MYF5	0.74	0.61	0.11	0.80	0.55	0.11	0.43	0.026	0.94
MYF6	1.01	1.09	0.12	0.89	1.22	0.14	0.60	0.057	0.78
MYOD1	0.86	0.77	0.10	0.91	0.71	0.09	0.51	0.14	0.26
MYOG	0.86	0.80	0.10	0.80	0.86	0.10	0.64	0.64	0.10
PFKM	0.96	0.87	0.10	0.87	0.96	0.12	0.50	0.54	0.38
PKM	1.06	0.88	0.12	0.80	1.15	0.15	0.31	0.050	0.043
PLCB4	1.13	1.09	0.10	0.90	1.33	0.10	0.82	<0.001	0.83
PPARG	0.23	0.19	0.09	0.18	0.25	0.06	0.73	0.035	0.047
PYGM	0.84	0.76	0.10	0.70	0.90	0.10	0.57	0.17	0.31
SDHA[6]	0.94	0.82	0.09	0.87	0.89	0.11	0.39	0.91	0.043
SFRP1	1.08	1.03	0.12	1.00	1.11	0.13	0.78	0.34	0.88
SRF	1.03	0.99	0.14	0.80	1.22	0.13	0.86	0.002	0.36
TTN	0.95	0.89	0.11	0.81	1.04	0.12	0.69	0.10	0.73

[1]Day relative to parturition

[2]Largest SEM shown.

[3]T × D = treatment by day interaction.

[4]At day 111, $n = 16$ for DLK1, INSR, and MYOD1, whereas $n = 17$ for the remaining genes at day 111. For HMP heifers, $n = 7$ for DLK1, INSR, and MYOD1 and $n = 8$ for the remaining genes.

[5]Samples of longissimus lumborum were only collected and analyzed from heifer calves to reduce bias due to gender.

[6]Means for the treatment by day interaction did not separate using Tukey's post hoc test mean separation test.

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 (Hatfield et al., 1995; 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 days 7, 13, 28, and 33, relative to more advanced DIM. 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. Milk yields observed in the current study are within the range reported by Freetly et al. (2006). However, the pattern of milk yield observed in the present study differs from that in NASEM (2016) with an expected peak milk yield occurring around 8.5 wk postpartum, the decline in milk production observed was likely regulated by calf demand. Quantitatively, the estimated 24-h milk production represented 16.6%, 11.7%, 6.6%, and 4.2% of the calf BW at 7, 28, 70, and 111 d of age, respectively. The observed consumption of milk in the present study, at times when calves relied solely on milk (until day 33) and when reported as a percentage of BW, are near maximum values 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 by d 60 as suggested by Boggs et al. (1980) coincides with

the observed decrease in milk yield at days 70 and 112 in the present study. Supporting the notion that calves reduced reliance on milk following day 33, calf ADG was 0.75 kg/d between days 7 and 28, 0.68 kg/d between days 28 and 70, and 1.12 kg/d between days 70 and 111. The growth response for the calves suggests that nutrient density of the diet consumed decreased or quantity decreased between days 28 and 70 supporting reduced reliance on milk as part of their diet. The increase in ADG between days 70 and 111 is suggestive of an improved digestive efficiency due to physiological maturation of the digestive tract as the calves transitioned to greater reliance on DM to meet their nutrient requirements. Simultaneously, increased mRNA expression in muscle for IGF-I, IGF-IR, INSR, SRF, and MBLN1 and downregulated expression of MYF5 are all representative of the shift from embryonic and fetal stimulation of myogenesis and myofiber growth (Liu et al., 1993; Mavalli et al., 2010; Paradis et al. 2017) to post-natal regulation of muscle fiber hypertrophy (Nielsen, 1992; Micke et al., 2011).

There were very few differences for the mRNA expression of genes in muscle tissue of calves between treatments. Nevertheless, the observed difference in progeny gene expression for PKM and PPARG at day 111 relative to day 2 between CON and HMP heifers, respectively, align with the hypothesis of fetal programming (Barker et al., 2002; Du et al. 2010). Pyruvate kinase, the final enzymatic step in glycolysis that converts phosphoenolpyruvate to pyruvate (Pilkis and Granner, 1992) has recently been implicated, using proteomic profiling, to contribute to increased lean tissue growth in animals experiencing hypertrophic muscle growth (Hamelin et al., 2006), accelerated muscle accumulation (Lametsch et al., 2006; Lehnert et al., 2006), and being differentially expressed between progeny of sires with high and low EPD for carcass weight, with the former having increased abundance of PKM2 relative to progeny of low EPD sires (Keady et al., 2013). Of course, within these studies, PKM is not the sole glycolytic enzyme linked to greater potential for muscle growth, but current results suggest that increases in glycolytic capacity by upregulation of a variety of glycolytic enzymes may be necessary to support lean tissue accumulation. While we cannot confirm, the increased mRNA expression for PKM at day 111 in CON progeny may suggest greater glycolytic capability of muscle tissue when compared to HMP. This may be due to greater abundance of type II muscle fibers as previous research has indicated that nutrient restriction increases the proportion of type IIx fibers (Zhu et al., 2006) and type II muscle fibers have a greater reliance on glycolytic activity (Maltin, 2008). However, as we did not evaluate muscle fiber type, we cannot confirm this speculation.

In contrast, PPARG is well studied and has been strongly linked to greater adipogenesis and intramuscular fat deposition (as reviewed by Baik et al., 2017), despite that, the expression of PPARG in muscle tissue is minor compared to its expression in adipose tissue (Braissant et al., 1996; Loviscach et al., 2000). Further support is the growing body of research reporting that positive impacts on adipose-related carcass characteristics are attainable in progeny born to dams that are supplemented protein during gestation (Larson et al., 2009; Underwood et al., 2010; Long et al., 2012; Mulliniks et al., 2012; Shoup et al., 2015; Summers et al., 2015; Wilson et al., 2016), and it is possible that the changes in adipogenesis observed in these studies might have been mediated through programmed expression of PPARG in offspring from supplemented dams. However, it is not possible to determine whether the observed carcass responses in the previously mentioned studies were due to changes in protein supply or the inherent increase in energy provision as both level of provision (Yan et al., 2011) and source of protein (Radunz et al., 2012) are known to impact carcass composition in offspring. Unfortunately, calves in the present study were not fed until slaughter and hence we are unable to determine whether the changes in gene expression in muscle tissue that we observed would translate to altered carcass characteristics.

The Postpartum Transition Period for Beef Cattle

Characterization of the postpartum portion of the transition period for beef cattle is limited (NASEM, 2016). Data from the current study indicate that DMI decreased from week 1 to that observed during weeks 2 and 3 and then increased for week 4 relative to parturition. Although the cause is likely multifactorial, it is unclear as to why DMI decreased in weeks 2 and 3 following parturition. Ruminal fill and hepatic oxidation are primary theories explaining regulation of DMI for cattle (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 8 and 22 d after calving (n = 4) and concluded that distention is likely not the sole factor regulating feed intake in the periparturient period. Despite the suggestion that distension may not limit DMI, diets in the present study were purposely dilute to avoid overfeeding of nutrients postpartum and, as a consequence, contained 23% straw (DM basis). The low digestibility of straw coupled with the potential decrease in passage rate after parturition may have partially limited DMI postpartum. Additionally, Allen et al. (2009) suggested that feed intake is metabolically controlled by hepatic oxidation. In dairy cattle, elevated NEFA concentrations 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 Andersen, 2006). As serum NEFA concentrations were observed to be greatest on days 7 and 28 in our study, the reduction in DMI during weeks 2 and 3 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 days 7 and 28 than at days 70 and 112 and rump fat, but not rib fat thickness was less at day 112 compared to days 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 in dairy cattle (Leblanc, 2010; Roberts et al., 2012).

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 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 results in the present study are in contrast to that of Freetly et al. (2006) where 47 of the 60 cows observed lost tissue energy in the first 53 d of lactation indicating high likelihood for a negative energy balance. In the present study, the energy requirement for uterine involution and reparation of the reproductive tract was not considered in the calculation 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 two-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 weeks 1 to 2 and 3 and remained low during week 4. The magnitude of change varied from a maximum ruminal pH of 7.1 at week 1 to 6.7 at week 4 and minimum ruminal pH decreased from 6.2 at week 1 to 5.8 in week 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 weeks 2, 3, and 4 while the supply of fermentable substrates was less as a result of reduced DMI. Penner et al. (2007, 2009) also reported risk for low ruminal pH during the postpartum phase of the transition period; however, those studies were conducted with Holstein heifers (Penner et al., 2007) and cows (Penner and Oba, 2009) and the cattle were fed a diet with much greater fermentability than the cows in the present study. Causative factors for the low ruminal pH occurring concurrent with low DMI may be due to highly variable DMI observed among days (data not shown). Past research has demonstrated that short-term exposure to low feed intake has been identified as a risk factor for low pH even with diets that would not be expected to induce low ruminal pH (Zhang et al., 2013; Aschenbach et al., 2018). Future research is needed to confirm this theory.

In conclusion, supplying MP greater than requirements by 33% during late gestation had limited carry-over effects on the dam postpartum but altered expression of genes related to protein and fat metabolism in the calf. Our data also suggest that the lactation curve may not reflect that represented in current models such as the NASEM (2016). Additionally, DMI and ruminal pH were not coupled in the first 4 wk postpartum, suggesting that postpartum beef cows may be at risk of low ruminal pH even with relatively low DMI. 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.

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