Oversupplying metabolizable protein in late gestation for beef cattle: effects on prepartum BW, ruminal fermentation, nitrogen balance, and skeletal muscle catabolism¹

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ABSTRACT: The objective of the study was to determine the effect of oversupplying MP during late gestation on maternal BW, ruminal fermentation, nitrogen balance, and skeletal muscle catabolism. Crossbred Hereford heifers ($n = 24$) were assigned to a control treatment designed to meet MP requirements (CON) or a treatment providing 133% of the MP requirement (HMP). Heifers were individually fed their treatment from day -55 ± 3 relative to parturition and DMI was summarized by week. BW was measured on day -55 ± 3 , -41 ± 3 , -27 ± 3 , and -8 ± 3 . Ruminal digesta samples were collected on day -34 ± 5 and -15 ± 4 for short-chain fatty acid and ammonia-N ($\text{NH}_3\text{-N}$) concentration. Plasma was collected the day prior to ruminal digesta samples and analyzed for plasma urea-N. Nitrogen balance was measured over a 6-d period starting on day -34 ± 4 and -15 ± 4 . Following completion of the N balance periods, muscle biopsies were collected from the longissimus dorsi and analyzed for abundance of proteins relating to skeletal muscle catabolism. Data were analyzed as a randomized complete block (date of parturition)

design with repeated measures using the MIXED procedure of SAS. Heifers fed HMP increased conceptus-corrected BW by a greater magnitude than CON at day -8 relative to -55 and -41 (treatment $\times$ day, $P < 0.01$). DMI increased ($P < 0.01$) by 18% on week -2 compared to -8 , but then decreased ($P < 0.01$) by 8.0% for week -1 . N-intake, apparent N digestion, N excretion, and N retention (g/d) were all greater ($P < 0.01$) for HMP heifers than CON but did not differ when expressed as a proportion of N intake. Ruminal $\text{NH}_3\text{-N}$ decreased (treatment $\times$ day, $P < 0.01$) as parturition approached for HMP (10.1 to 8.6 mg/dL); whereas, $\text{NH}_3\text{-N}$ was not affected for CON (1.0 to 1.3 mg/dL). Consequently, plasma urea-N was greater ($P < 0.01$) for HMP heifers (15.0 vs. 7.5 mg/dL). Heifers fed HMP had improved ($P < 0.01$) DM, OM, and NDF digestibility relative to CON heifers. The abundance of calpastatin was greater ($P = 0.03$) and calpain tended to be greater ($P = 0.085$) for CON cows compared to HMP. Feeding greater quantities of MP during late gestation may improve ruminal fermentation, N balance, and improve BW gain prepartum.

Key words: beef cow, metabolizable protein, nitrogen balance, prepartum, ruminal fermentation

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INTRODUCTION

Beef cow nutrient requirements increase dramatically during the last trimester due to

exponential fetal growth (Robinson et al., 1977; Bauman and Currie, 1980), mammary development (Ratnayake et al., 1974), colostrogenesis, and preparation for lactogenesis (NASEM, 2016). In particular, fetal myofiber hypertrophy and hyperplasia require significant quantities of AAs (Du et al., 2010) as muscle protein concentration increases from ~60 to 110 mg/g of muscle tissue between days 160 and 280 of gestation (Prior et al., 1979). Additionally, AAs are estimated to provide 30% to 50% of the oxidized substrates used by the fetus if maternal nutrition is sufficient, and up to 70% of the oxidized substrates when the dam is in a state of prolonged undernutrition (Bauman and Currie, 1980).

Concurrently, fetal growth reduces gastrointestinal capacity, leading to increased digesta passage rate and decreased DMI (Scheaffer et al., 2001; Linden et al., 2014). The resultant constraints on maternal MP intake and digestion may cause a transient AA deficiency for the dam resulting in catabolism of skeletal muscle to meet the fetal AA requirement. Du et al. (2005) and Wood et al. (2013) demonstrated increased protein abundance of ubiquitin in skeletal muscle for gestating beef cows exposed to a mild global nutrient restriction during gestation and loss of skeletal muscle has been reported for periparturient dairy cattle in a negative N balance (Chibisa et al., 2008). However, the occurrence and extent of the negative ME and MP balance for beef cattle, without an imposed feed restriction, is not well characterized.

The hypothesis for this study was that oversupplying MP would mitigate a prepartum maternal nitrogen deficiency thereby improving N balance. The primary objective was to evaluate whether increasing MP supply would improve nitrogen balance and decrease indicators of skeletal muscle catabolism. A secondary objective was to characterize prepartum DMI, ruminal fermentation, and plasma and serum metabolites.

MATERIALS AND METHODS

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

Experimental Design, Cow Husbandry, and Dietary Treatments

This paper is one of two companion papers addressing the effects of increasing MP supply prepartum for beef cattle. The current paper addresses the prepartum responses while the companion

paper (Hare et al., in press) addresses post-partum responses of the dam and progeny.

A total of 24 primiparous 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) and ranged between 3 and 3.5 yr old at calving. The non-cannulated heifers were ranged from 2 to 2.5 yr old at calving. Heifers were bred using fixed-time artificial insemination with sexed semen (90% female) from one Angus sire (Cole Creek Cedar Ridge IV, Reg. No. 1659099 (CAN), Genex Co-operative Inc., Shawano, WI), to minimize variation due to genetics for the offspring. The experiment was arranged as a randomized complete block design with the expected date of parturition used as a blocking factor. Within and among blocks, heifers were assigned to treatments while balancing for the number of cannulated and noncannulated heifers and initial, nonconceptus-corrected BW (measured immediately on two consecutive days 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 day -55 ± 3.7 relative to parturition. At this time, heifers were housed in individual pens (9 m²) with rubber mats. Pens were cleaned and washed daily. Eight days prior to parturition, heifers were moved to pens (18 m²) bedded with straw and maintained there until 7 d following parturition.

Treatments consisted of the control treatment (CON; $n = 12$; noncannulated = 5, cannulated = 7) or a treatment where metabolizable protein was purposely over-fed (HMP; $n = 12$; noncannulated = 5, cannulated = 7). 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 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 isocaloric based on the net energy concentration. 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. The actual date of experiment initiation was calculated based on the expected date of parturition using a 278 d gestation based on the sire's expected progeny

difference for gestation length. The CON and HMP diets consisted of the same barley hay and wheat straw with cows being offered one of two prepartum supplemental pellets based on their respective treatment (Table 1). All diets contained the same forage-to-concentrate ratio (60:40). Nutrient requirements were predicted for a 550-kg heifer (BCS of 5 on a scale of 1 to 9), a 12-mo calving interval, 260 d in gestation (median day of gestation during the period of treatment provision), and a calf birth weight of 36 kg. Diets were fed twice daily at 0900 and 1630 hours targeting ad libitum intake (5% to 10% refusals on an as-fed basis).

Data and Sample Collection

DMI. DMI was determined daily by measuring the weight of the feed offered and the weight of the feed that was refused. The as-fed feed disappearance was then corrected for DM content of the diet and refusals. Throughout the study, samples of forages were collected once weekly and samples of concentrate (CON and HMP pellets) were collected every 2 wk. Samples of refusals were taken daily and composited by week (proportional to the

quantity refused per day on an as-is basis). The feed samples were placed in a forced-air oven at 55 °C until achieving a constant weight to determine DM concentration.

BW and rib and rump fat thickness. Heifers were weighed on two consecutive days at the start of the study (days –56 and –55 relative to expected parturition) and every 2 wk thereafter until and including the day of parturition. Heifer BW was corrected for conceptus weight using equations in NASEM (2016). 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) on day -55 ± 3.7 and -13 ± 4.6 relative to parturition.

Ruminal fermentation, apparent total tract digestibility, nitrogen balance, 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. Samples were collected at 1300 hours on days -34 ± 3.9 and -15 ± 4.4 relative to parturition.

Table 1. Ingredient and chemical composition of high metabolizable protein (HMP = formulated to 133% of requirements) and control (CON = formulated to be 100% of requirements) diets fed during the final 8 wk of gestation

Item	Prepartum diets	
	CON	HMP
Ingredient composition, % DM		
Whole plant barley hay	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

¹High 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 supplement³; chemical composition: 9.244 kIU/kg of vitamin A, 0.843 kIU/kg of vitamin D₃, 0.185 kIU/kg of vitamin E, 708 ppm of Cu, 192 ppm of Fe, and 987 ppm of Zn.

²Control and HMP supplements contained 24.03% oat hulls, 27.73% molasses beet, 40.67% ground barley grain, and 7.56% custom mineral and vitamin supplement³; chemical composition: 9.245 kIU/kg of vitamin A, 0.843 kIU/kg of vitamin D₃, 0.185 kIU/kg of vitamin E, 709 ppm of Cu, 154 ppm of Fe, and 972 ppm of Zn.

³Diet contained 0.65% Ca, 0.31% P, 0.43% Mg, 1.85% K, 0.29% Na, 0.23% S, 487 ppm Zn, and 0.15 ppm Se.

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

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

Digesta was strained through two layers of cheese-cloth 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% sulfuric acid and was stored frozen (-20°C) until being used for ammonia-N concentration (Fawcett and Scott, 1960).

Ruminal pH was measured every 5 min in cannulated cows from day -35 until parturition using the Lethbridge Research Center ruminal pH measurement system (LRCpH; Penner et al., 2006). The LRCpH was standardized and subsequently inserted through the ruminal cannula into the ventral sac of the rumen. Two 1-kg weights were attached to maintain the LRCpH in the ventral sac. Each week, 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 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 millivolt recordings to pH using beginning and ending linear regressions with the assumption of linear drift over time (Penner et al., 2006).

Digestibility and N balance were determined in two separate periods with the first period starting on day -34 ± 3.9 and the second starting on day -15 ± 4.4 relative to parturition. Samples of each feed ingredient and feed refusals were collected daily and composited (proportionally by the quantity refused per day on an as-is basis), and analyzed for DM and nutrient concentration (described below). Over the 6-d measurement period, total fecal output and urine output were collected, weighed, and recorded. 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 placed in a container, mixed thoroughly, and 5% of the total fecal weight was collected and used to prepare a composite sample (6-d composite for each heifer for each period). Total urine collection was conducted using a Foley catheter (Bard Medical, Covington, GA) that was inserted the day prior to 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 days -34 to -29 ± 3.9 and days -15 to -10 ± 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 urine sample was collected each day and stored at -20°C until analysis (described below).

The DM content of the offered feed, feed refusal, and fecal samples was determined by drying the composite sample in a forced-air oven (55°C) 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, UK) and sent to Cumberland Valley Analytical Services (Abbotsford, BC, Canada) for analysis of DM, OM, CP, EE, aNDF_{OM} (amylase treated NDF corrected for ash content), ADF, and starch (Rosser et al., 2013). Apparent total tract digestibility of DM, OM, CP, and ether extract and total tract digestibility of aNDF_{OM} , ADF, and starch were calculated as the difference between nutrient intake and fecal nutrient 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). Crude protein content was analyzed according to the AOAC (1994) method for Kjeldahl determination of CP using an N to CP conversion of 6.25. 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 prior to ruminal samples at 1000 hours to coincide with urinary catheterization (days -34 ± 5.6 and -16 ± 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). Blood was placed on ice and immediately centrifuged ($2,000 \times g$ at 4°C for 15 min) to separate plasma while blood samples for serum were allowed to sit at room temperature for 10 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 from Wako (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 intraplate assay variation were (mean $\pm$ SD) $4.5 \pm 3.4\%$ and $2.9 \pm 0.4\%$ for plasma insulin.

Muscle biopsies and protein abundance. Approximately 400 mg of muscle tissue from the longissimus lumborum (caudal to 13th rib, ~10 cm lateral from spine) was collected using a disposable biopsy punch (Acu-Punch, Acuderm, Ft. Lauderdale, FL) from cows on days -28 ± 4.0 and -9 ± 4.5 relative to parturition. Tissues were snap frozen in liquid N_2 and stored at $-80^\circ C$ until analysis. Abundance of proteins involved in muscle synthesis and degradation were determined according to Wood et al. (2013) and Nichols et al. (2017). Briefly, samples were homogenized in ice cold radioimmuno-precipitation assay buffer (1% Triton X-100, 0.1% SDS, 50 mM Tris-HCl, pH 8.0, 150 mM, NaCl, and 0.5% sodium deoxycholate) containing protease and phosphatase inhibitors (Thermo Scientific, Nepean, ON, Canada). Concentrations of total protein in the homogenate were determined using a commercially available bicinchoninic acid kit (Pierce BCA Protein Assay Kit, Pierce Biotechnology) using BSA as a standard. Protein concentrations were standardized across samples to 1 mg/ μ L with Laemmli sample buffer (Bio-Rad Laboratories Ltd, Mississauga, ON, 5.8 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 was 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) with a 0.2- μ m 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 $4^\circ C$. The next morning, the membranes were triple washed in Tris-buffered saline with 1% Tween-20 (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 in Tris-buffered saline with 1% Tween-20. 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 the value was subtracted from the band intensity measurement. Band intensities are expressed as corrected arbitrary units (AUs) and expressed relative to the average of the control treatment.

Statistical Analysis

All data were analyzed as a randomized complete block design using the PROC MIXED of SAS 9.4 (SAS Institute, Cary, NC). 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 (week or day) 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.

RESULTS

Cow BW and Rib and Rump Fat Depth

A treatment by day interaction was observed for BW ($P = 0.007$; Figure 1). BW did not differ between treatments, but HMP heifers increased BW by 16.5 and 33.8 kg on day -8 relative to days -41 and -56, respectively; whereas CON heifers only increased BW by 13.5 kg from days -56 to -8. Prepartum rib and rump fat depth did not differ ($P \geq 0.31$; Table 2) by treatment, day, or the interaction of day and treatment.

Intake and Apparent Digestibility

When measured on a weekly basis, DMI as a percentage of BW or kilogram per day summarized from week -8 to parturition was not affected ($P \geq 0.36$; Table 3) by treatment or the interaction of treatment and week (data not shown). DMI (% BW) was greater ($P < 0.001$) during weeks -3 and -2 compared to weeks -8 by 16.2% and 17.7%, respectively. From weeks -2 to -1, DMI (% of BW) decreased by 8.0%.

During the N balance periods, DMI (kg/d) was not affected ($P = 0.21$; Table 4) by treatment or the interaction of treatment and day but increased by 1 kg/d ($P < 0.001$) for measurements initiated on day -15 relative to day -34. Corresponding to the increase for DMI on day -15, the intake of OM, ADF, NDF, and starch increased ($P < 0.001$). An interaction for treatment and day was detected for

CP intake ($P = 0.005$; data not shown), with HMP having greater overall CP intake and an increase in CP intake from days -34 to -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 were greater ($P \leq 0.002$) for HMP heifers than CON heifers. Starch digestibility decreased ($P = 0.024$) by 5.5 percentage units from days -34 to -15 relative to parturition.

Ruminal Fermentation

Treatment and the interaction of treatment and week did not affect ($P \geq 0.14$; Table 5) minimum, mean, and maximum ruminal pH. However, minimum pH tended to be greater during week -1 relative to week -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 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 days -34 to -15, no differences were detected for the molar proportion of SCFA based on day of measurement prepartum. The ruminal ammonia-N concentration for HMP was greater (treatment $\times$ day; $P = 0.002$) than CON on days -34 and -15, despite a decrease in concentration from days -34 to -15 for HMP heifers (Figure 2).

Urine Output and Metabolite Excretion

Heifers consuming HMP had 2.6 kg/d greater urine output than CON heifers ($P = 0.025$; Table 7). Regardless of treatment, urine output tended to increase ($P = 0.080$) from days -34 to -15 relative to parturition. Urinary creatinine and 3-MH concentrations did not differ ($P \geq 0.19$) by treatment, day, or the interaction of treatment and day. Despite greater urine output, the urinary urea concentration was 78.6% greater for HMP heifers than CON (567.0 and 317.5 mg/dL; $P < 0.001$). 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 days -34 to -15 ($P = 0.022$).

Figure 1. The interaction of treatment and day ($P = 0.007$) for CON (100% of MP requirement, white columns) and HMP (133% of MP requirement, black columns) heifers where HMP heifers gained 16.5 and 33.8 kg on day -8 ± 3.9 (mean $\pm$ SD) relative to days -41 ± 3.7 and -55 ± 3.7 , whereas CON heifers only gained 13.5 kg from day -55 ± 3.7 to -8 ± 3.9 . Means within a dependent variable that have uncommon letters differ ($P < 0.05$).

Table 2. Parturition 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

Item	Treatment			Day ¹				SEM ²	P-values		
	CON	HMP	SEM ²	Day -55	Day -41	Day -27	day -8		Treatment	Day	T × D ³
<i>n</i>	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 ($P < 0.05$).

¹Day relative to parturition: day -55 ± 3.7; day -41 ± 3.7; day -27 ± 3.6; day -8 ± 3.9 (mean ± SD).

²Largest SEM shown.

³T × D = treatment and day interaction.

⁴Interaction data described elsewhere.

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

Table 3. Parturition DMI 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								SEM ¹	P-value		
	CON	HMP	SEM	-8	-7	-6	-5	-4	-3	-2	-1		Treatment	Week	T × W ²
<i>n</i>	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

¹Largest SEM shown.

²T × W = treatment by week interaction.

Table 4. Parturition 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

Item	Treatment		SEM ²	Day ¹		SEM ²	P-values		
	CON	HMP		day −34	day −15		Treatment	Day	T × D ³
<i>Intake, kg/d</i>									
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
<i>Apparent total tract digestibility, % DM</i>									
N	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
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 ($P < 0.05$).

¹Collections occurred from day -34.4 to -29.4 ± 3.9 and from day -14.9 to -9.9 ± 4.4 (mean ± SD) relative to parturition.

²Largest SEM is reported.

³T × D = treatment and day interaction.

⁴Interaction data described elsewhere.

Table 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

Item	Treatment			Week relative to parturition						<i>P</i> -values		
	CON	HMP	SEM ¹	-5	-4	-3	-2	-1	SEM ¹	Treatment	Week	T × W ²
<i>n</i>	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 ($P < 0.05$).

¹Largest SEM shown.

²T × W = treatment by week interaction.

Table 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

Item	Treatment			Day ¹			<i>P</i> -values		
	CON	HMP	SEM ²	day -34	day -15	SEM ²	Treatment	Day	T × D ³
<i>N</i>	6	7		13	13				
Total SCFA, mM	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	0.0	0.7	0.7	0.0	<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	0.0	0.8	0.7	0.0	<0.001	0.79	0.79
Valeric acid	1.0	1.2	0.0	1.1	1.1	0.0	<0.001	0.29	0.41
Caproic acid	0.4	0.3	0.0	0.4	0.4	0.0	0.15	0.42	0.32
Ruminal ammonia-N ⁴ , mg/dL	1.1	9.3	0.5	5.5	4.9	0.3	<0.001	0.027	0.002

¹Collections occurred on days -34.4 ± 3.9 and -14.9 ± 4.4 (mean $\pm$ SD) relative to parturition.

²Largest SEM is reported.

³T × D = treatment and day interaction.

⁴Interaction data are described elsewhere.

Figure 2. The interaction of treatment and day ($P = 0.003$) for CON (100% of MP requirement, white columns) and HMP (133% of MP requirement, black columns) heifers where ruminal ammonia-N concentrations were greater for HMP heifers compared to CON, but concentration decreased at day -14.9 ± 4.4 (mean $\pm$ SD) relative to -34.4 ± 3.9 for the HMP heifers. Means within a dependent variable that have uncommon letters differ ($P < 0.05$).

Blood Metabolites and Insulin

Plasma glucose, serum NEFA, and serum BHBA were not affected ($P \geq 0.19$; Table 8) by treatment, day, or the interaction of treatment and day. Plasma insulin tended (interaction, $P = 0.067$) to be greater for HMP relative to CON on day -16 while CON and HMP did not differ on day -34 . Plasma urea-N was greater for HMP heifers compared to CON heifers (15.0 vs. 7.5 mg/dL; $P < 0.001$).

Nitrogen Balance

The interaction of treatment and day affected nitrogen intake ($P = 0.007$; Figure 3). The interaction was a result of HMP heifers consuming 65% more N at day -34 and 78% more N at day -15 than CON heifers. Fecal N output tended to be greater for HMP than CON ($P = 0.054$; Table 9) and was greater on day -15 than day -34 relative

Table 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

Item	Treatment		SEM ²	Day ¹		SEM ²	P-values		
	CON	HMP		day -34	day -15		Treatment	Day	T × D ³
<i>n</i>	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
<i>Urine metabolite concentrations</i>									
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
<i>Urine metabolite output</i>									
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

¹Collections occurred from days -34.4 to -29.4 ± 3.9 and from days -14.9 to -9.9 ± 4.4 (mean ± SD) relative to parturition.

²Largest SEM is reported.

³T × D = treatment and day interaction.

Table 8. Prepartum circulating plasma and serum 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

Item	Treatment		SEM ²	Day ¹		SEM ²	P-values		
	CON	HMP		day -34	day -16		Treatment	Day	T × D ³
<i>n</i>	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 β-hydroxybutyrate, 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

¹Collections occurred on day -33.9 ± 5.6 and from day -16.2 ± 4.0 (mean ± SD) relative to parturition.

²Largest SEM is reported.

³T × D = treatment and day interaction.

to parturition. The quantity of nitrogen excreted in urine was affected by a treatment × day interaction ($P = 0.016$; Figure 4). When fecal- and urinary-N were combined, heifers consuming HMP rations excreted 70% and 96% more nitrogen on measurements initiated on days -34 and -15 than heifers consuming CON ($P = 0.011$; Figure 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 day -34 to 134% on day -15 ($P = 0.004$; Figure 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 overall 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 ($P = 0.45$).

Abundance of Proteins Involved in Protein Catabolism

The relative abundance of calpastatin was ($P = 0.029$) greater for CON compared to HMP heifers; whereas the relative abundance of calpain for CON heifers only tended ($P = 0.085$) to be greater than HMP. Treatment did not affect ($P \geq 0.22$) the abundance of mTOR, p-mTOR, p-mTOR/mTOR, Akt, p-Akt, p-Akt/Akt, and ubiquitin. Similarly, the relative abundance of all proteins was not affected by the interaction of treatment and day ($P \geq 0.17$) or day ($P \geq 0.13$), apart from p-Akt ($P = 0.085$), and the p-Akt/Akt ratio ($P = 0.075$) that tended to

decrease by 0.35 and 0.39 AU, respectively, at day -9 relative to day -28.

DISCUSSION

Effect of Increasing Prepartum MP Supply

The current study was designed to evaluate the effect of oversupplying MP to beef heifers during

Figure 3. The interaction of treatment and day ($P = 0.005$) for CON (100% of MP requirement, white columns) and HMP (133% of MP requirement, black columns) heifers on N intake where HMP heifers had greater CP intake relative to CON heifers, and HMP CP intake was greater at day -14.9 ± 4.4 (mean $\pm$ SD) relative to -34.4 ± 3.9 . Means within a dependent variable that have uncommon letters differ ($P < 0.05$).

late gestation on nutrient intake, ruminal fermentation, and nitrogen balance. The retrospectively calculated nutrient supply predicted using CNCPS 6.5 indicated that the CON and HMP heifers were provided 831 and 1137 g MP/d at a measured consumption of 11.4 and 12.3 kg DM/d. As such, diets differed in MP content 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 oversupplying MP during late gestation to beef heifers relative to a CON treatment that was near predicted requirements.

Supplementation of CP during late gestation has been reported to increase BW prior to parturition for beef cows when compared diets that are marginally adequate for CP (Martin et al., 2007; Larson et al., 2009) and greater MP supply during the last trimester of gestation linearly increased 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 improved CP supply. The improvement in BW observed in the current study when feeding HMP is therefore partially supported by the previously mentioned studies. However, it is not clear which body components account for the added conceptus corrected BW

Table 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

Item	Treatment		SEM ²	Day ¹		SEM ²	P-values		
	CON	HMP		day -34	day -15		Treatment	Day	T $\times$ D ³
<i>n</i>	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
<i>Nitrogen excretion, g/d</i>									
Feces	72.0	82.3	3.6	71.7	82.6	3.9	0.054	0.043	0.70
Urine	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
<i>Nitrogen excretion, % N intake</i>									
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
<i>Apparent N digestion</i>									
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
<i>N retained</i>									
g/d	59.4	97.1	4.8	73.4	83.1	5.1	<0.001	0.15	0.60
% of N intake	35.5	33.5	1.8	34.5	34.5	1.9	0.45	0.99	0.65

¹Collections occurred from days -34.4 to -29.4 $\pm$ 3.9 and from days -14.9 to -9.9 $\pm$ 4.0 (mean $\pm$ SD) relative to parturition.

²Largest SEM is reported.

³T $\times$ D = treatment and day interaction.

⁴Interaction data are described elsewhere.

Figure 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 diet designed to meet 100% of the MP requirement and HMP (black columns) were fed a prepartum diet designed to supply 133% of the 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 day -14.9 ± 4.0 (mean $\pm$ SD) relative to -34.4 ± 3.9 for the HMP heifers. Means within a dependent variable that have uncommon letters differ.

Figure 5. The effect of the interaction of treatment and day ($P = 0.011$) on total N excretion where HMP (133% of MP requirement, black columns) heifers had greater nitrogen excretion than CON (100% of MP requirement, white columns) heifers on day -14.9 ± 4.4 (mean $\pm$ SD) compared to day -34.4 ± 3.9 but had equal nitrogen excretion on day -34.4 ± 3.9 as CON heifers on day -14.9 ± 4.4 . Means within a dependent variable that have uncommon letters differ ($P < 0.05$).

with increased MP supply. Moreover, although we attempted to restrict dietary changes to MP supply, we did observe greater total tract digestibility for HMP relative to CON and as such, we cannot solely or directly attribute the improvement in BW gain to greater 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 nitrogen digestion, and retained nitrogen were

Figure 6. The effect of the interaction of treatment and day ($P = 0.004$) on apparent N digested for CON (100% of MP requirement, white columns) and HMP (133% of MP requirement, black columns) heifers, whereby HMP had greater apparent N digestion than CON heifers on day -14.9 ± 4.4 (mean $\pm$ SD) and day -34.4 ± 3.9 . Means within a dependent variable that have uncommon letters differ ($P < 0.05$).

greater for HMP heifers than CON heifers. Thus, our hypothesis was confirmed: increasing dietary MP in late gestation improves N balance prepartum; however, as describe above we are unable to solely link the responses to MP. However, both the CON and HMP heifers had a positive N balance as the measured N retention values were 59.4 and 97.1 g N/d, respectively. However, N balance experiments commonly underestimate nitrogen losses (Owens, 1967; MacRae et al., 1993; Spanghero and Kowalski, 1997). In order to validate our N retention data, N accretion during late gestation was estimated using a factorial approach that considered the accretion and efficiency of conversion of MP to net protein (NP) in the respective maternal and fetal tissues. Heifer BW gain was corrected for conceptus weight (NASEM, 2016) and all BW gain was assumed to be skeletal muscle accretion. Efficiency of conversion of MP to NP for BW gain was predicted to be 0.50 (NASEM, 2016), and the 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 days -55 and -8 was then calculated as 15.6 and 36.4 g N/d, respectively. Conceptus weight was predicted for uterine (Koong et al., 1975), fluid, membrane, and fetal weights (based on day 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 days -55 and -8 was predicted to be 2.05% and 2.40% (Ferrell et al., 1976). Fluid N composition at days -55 and -8 was predicted to be 0.39% and 0.46% (Ferrell et al., 1976) while

Table 10. Prepartum abundance¹ of proteins related to energy balance and protein catabolism for heifers fed diets formulated to provide 100 (CON) or 133% (HMP) of predicted metabolizable protein requirements during the final 8 wk of gestation

Item	Treatment		SEM ³	Day relative to parturition ²		SEM ³	P-value		
	CON	HMP		-28	-9		Treatment	Day	T × D ⁴
<i>n</i> ^{5,6}	10	11		21	21				
mTOR	0.94	0.81	0.12	0.88	0.87	0.10	0.40	0.96	0.32
p-mTOR	0.87	0.75	0.11	0.88	0.74	0.09	0.47	0.13	0.22
p-mTOR/mTOR	1.23	0.80	0.47	0.93	1.10	0.48	0.52	0.62	0.40
Akt	1.01	0.93	0.14	0.98	0.96	0.11	0.69	0.84	0.69
p-Akt	0.73	1.13	0.30	1.11	0.76	0.23	0.34	0.084	0.52
p-Akt/Akt	0.74	1.17	0.36	1.15	0.76	0.33	0.39	0.075	0.99
Calpain	1.20	0.60	0.23	0.98	0.81	0.18	0.085	0.3	0.17
Calpastatin	0.95	0.48	0.15	0.82	0.62	0.15	0.029	0.33	0.62
Ubiquitin	1.05	1.37	0.19	1.19	1.22	0.15	0.22	0.87	0.71

¹Abundance of proteins are expressed as arbitrary units (AUs).

²Day relative to parturition: -28 ± 4.0 and -9 ± 4.5 (mean ± SD).

³Largest SEM shown.

⁴T × D = treatment by day interaction.

⁵CON *n* = 10, apart from calpain and calpastatin where *n* = 8; HMP *n* = 11, apart from calpain and calpastatin when *n* = 7 and 8, respectively.

⁶Day -28 *n* = 21, apart from p-Akt, Akt, calpain, and calpastatin when *n* = 19, 19, 16, and 17, respectively. Day -9 *n* = 21, apart from calpain and calpastatin when *n* = 15 and 16, respectively.

membrane N composition at days -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) 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. Finally, fetal N accretion between days -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 was assumed to be similar to the value estimated by Bell (1995) for a 35 kg Holstein fetus at day 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. We were unable to account for the N accretion within colostrum as yield was not measured. Collectively, N accretion due to 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 between measured and 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 a lack of accuracy in estimating N accretion while using constant efficiencies of conversion for MP to NP. For example, using 0.32 as the efficiency of conversion 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. Additionally, Lapierre et al. (2007) highlighted the importance in utilizing individual efficiencies of conversion for MP to NP corresponding to differing levels of nutrient consumption. For lactation, efficiency of conversion of MP to NP is shown to respond to excess or deficient MP provision (Lapierre et al., 2007). Similarly, Castillo et al. (2000) and Peyraud et al. (1995) have found that the efficiency of nitrogen utilization decreases when protein is in surplus of requirements, yet the responsiveness of various

tissues (i.e., BW gain, fetal growth, or 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 relative to CON heifers as represented by decreased abundance of proteins relating to muscle degradation. Approximately 80% to 90% of the protein catabolism in skeletal muscle results from the enzymatic activity of ubiquitin though the system must function synergistically with the calpain proteolytic system (Goll et al., 2008). Calpain cleaves peptide chains from the myofibril but cannot catalyze the release of AA from the peptide chains (Tan et al., 1988; Goll et al., 2003; Smith and Dodd, 2007), whereas the proteasome can complete the catabolic release of AA from the peptide chain and yet, is unable to release myofilaments from the myofibril (Koochmaraie, 1992; Solomon and Goldberg, 1996). The lack of difference in the relative abundance of ubiquitin between treatments indicates that oversupply MP prepartum does not affect the net release of AA to peripheral supply by the ubiquitin proteasome.

Despite the lack of difference in ubiquitin abundance between treatments, CON heifers tended to have greater abundance of calpain. Upregulation of calpain could suggest that CON heifers were in a greater catabolic state relative to the HMP heifers, but as calpain is inhibited by the activity of calpastatin (Campbell and Davies, 2012), the greater abundance of calpastatin for CON heifers opposes the notion that CON experienced a greater degree of muscle catabolism. Increases in calpastatin activity are positively associated with both hypertrophic muscle growth (Koochmaraie et al., 1995) and accumulation of muscle mass in response to administration of β -adrenergic agonist (Bardsley et al., 1992; Parr et al., 1992). Yet, in the current study, both the CON and HMP heifers gain BW regardless of the differing calpastatin response. As previously discussed, the magnitude of gain was greater for HMP heifers and it may be related to the relative abundance of calpain and calpastatin in comparison to the CON. Heifers fed HMP had reduced abundance of both proteins compared to the CON and BW change by day -8 for HMP heifers was 2.5 times greater than CON heifers. The effect of the relative abundance of both proteins between treatments and their impact on rate of BW gain is speculative but supported by some evidence in the literature. For example, Li et al. (2008) determined in neonatal pigs that the rate of skeletal

muscle accumulation was negatively correlated with mRNA expression of calpain and calpastatin. The physiological responses between a neonatal and mature animal are likely to differ, but greater density or overexpression of calpastatin in vitro in mature cells results in a 50% reduction in cyclic cell activity where cells were maintained in a quiescent but reversible phase of the cell cycle (Stifanese et al., 2008). Therefore, it may be that the greater abundance of calpain and calpastatin indicates a modulation of growth rate rather than increased skeletal muscle catabolism in response to a lesser N balance for CON heifers.

As an additional approach to evaluate 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) approximately 90% of bound 3-MH is in skeletal muscle, (2) 3-MH is not used for protein synthesis, and (3) 3-MH is excreted rapidly in urine (Harris and Milne, 1981). The excretion of 3-MH by HMP heifers was greater than CON, suggesting increased muscle turnover. Linkage among the findings for N balance, abundance of calpain and calpastatin, 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 protein synthesis. Moreover, given HMP heifers had a greater BW, it could be postulated that body protein mass was also greater. That said, there was no difference in the urinary 3-MH:creatinine ratio for HMP and CON heifers, further suggesting that there were no differences in protein catabolism per unit muscle mass between treatments. Thus, the data suggest that, as a result of their positive N balance, oversupply MP in late gestation did not reduce skeletal muscle catabolism in late gestation and given the increase in conceptus-corrected BW, it is likely that beef cattle do not rely extensively on skeletal muscle as a source of AA in late gestation.

Heifers in this study were fed a basal diet containing a low-quality forage 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 heifers relative to CON. Often forage-based rations are deficient in rumen degradable protein (RDP) and supplementation is provided

to improve NDF degradability (Paterson et al., 1994) by meeting microbial N-demands. Indeed, we observed that heifers fed HMP had greater ruminal ammonia-N concentrations compared to CON. Based on the rumen ammonia-N concentrations, the RDP supply appeared to be over-estimated during dietary formulation as ruminal ammonia-N averaged 1.1 mg/dL for the CON heifers compared to 9.3 mg/dL for the HMP heifers. The ruminal ammonia concentration for the CON heifers was below the saturation constant for several fibrolytic bacterial species in culture and likely limited microbial growth and potentially fiber digestion (Schaefer et al., 1980). The overestimation of RDP supply could be due to several factors including the use of tabular values for RDP or model inaccuracy when for diets containing a high proportion of forage with low forage quality. Nevertheless, while the predicted MP supply was deemed adequate, we cannot overlook that RDP was likely limiting. 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 h after feeding, suggesting that the sample may have been collected near peak ammonia-N demand. For low-quality forages, ammonia-N is typically first-limiting nutrient (Horn and McCollum, 1987) and CP supplementation frequently increases diet digestibility (Wallace, 1988; DelCurto et al., 1990; Beatty et al., 1994). As such, the increased rumen ammonia-N coupled with the improved total tract digestibility and increased branch-chain SCFA for HMP than CON supports the notion that RDP may have been limiting for CON (Schaefer et al., 1980; Andries et al., 1987). Thus, the improvements for OM and NDF digestibility for HMP over CON were likely mediated through increased proliferation or activity of cellulolytic microbial species (and therefore, the 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, concentrations of SCFA have been contested for their validity as an estimate of treatment effects on rumen fermentation in vivo (Hall, 2013; Hall et al., 2015) due to unaccounted variation in other factors such as ruminal absorption, fluid passage rate, fluid dilution, and ruminal volume (Hall et al., 2015). It follows logically that

the same factors plus urea entry into the rumen may influence ruminal ammonia-N concentration. Increased water inflow and the resultant 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 day -15 relative to day -34 also coinciding with greater urine output as an indicator for increased water intake and potentially ruminal liquid dilution. Additionally, the time of ruminal fluid collection (4 h postfeeding) in the present study occurred when ruminal fluid fill is expected to be greater and therefore the low ammonia-N for CON heifers may be the result of increased fluid dilution rather than insufficient RDP.

The Prepartum Transition Period for Beef Cattle

For dairy cattle, the transition period has been identified as the 3 wk prior to and 3 wk following parturition (Drackley, 1999). The transition period for dairy cattle is accompanied by significant changes 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 week -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. (2014) 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 (Hayirli 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. Previous research has largely focused on energy dense rations fed to close-up dairy cattle as a strategy 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 as parturition approaches (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 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 increase observed for ruminal pH. Other studies have reported increased passage rate (Gunter et al., 1990; Hanks et al., 1993; Linden et al., 2014) that may also contribute to greater ruminal pH through reduced SCFA production and potentially greater proton outflow (Allen, 1997).

Results of the current study indicate that provision of MP in excess of predicted requirements improved N balance, nutrient digestion, and prepartum BW gain for HMP heifers relative to CON. In addition, feeding HMP improved ruminal fermentation as indicated by greater concentrations of ruminal ammonia-N and NDF digestibility. Despite decreased DMI (%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, relative abundance of calpain and calpastatin, and the lack of difference in urinary 3-MH and 3-MH:creatinine between treatments.

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LITERATURE CITED

- Adams, D. C., R. T. Clark, T. J. Klopfenstein, and J. D. Volesky. 1996. Matching the cow with forage sources. *Rangelands* 18:57–62.
- Allen, M. S. 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. *J. Dairy Sci.* 80:1447–1462. doi:10.3168/jds.S0022-0302(97)76074-0.
- Anderson, R. V., R. J. Rasby, T. J. Klopfenstein, and R. T. Clark. 2005. An evaluation of production and economic efficiency of two beef systems from calving to slaughter. *J. Anim. Sci.* 83:694–704. doi:10.2527/2005.833694x.
- Andries, J. I., F. X. Buysse, D. L. de Brabander, and B. G. Cottyn. 1987. Isoacids in ruminant nutrition: Their role in ruminal fermentation and intermediary metabolism and possible influences on performances – a review. *Anim. Feed Sci. Technol.* 18:169–180. doi:10.1016/0377-8401(87)90069-1.
- AOAC. 1994. Association of official analytical chemists. Official methods of analysis. Washington, DC.
- Bardsley, R. G., S. M. Allcock, J. M. Dawson, N. W. Dumelow, J. A. Higgins, Y. V. Lasslett, A. K. Lockley, T. Parr, and P. J. Buttery. 1992. Effect of beta-agonists on expression of calpain and calpastatin activity in skeletal muscle. *Biochimie* 74:267–273. doi:10.1016/0300-9084(92)90125-X.
- Bauman, D. E., and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. *J. Dairy Sci.* 63:1514–1529. doi:10.3168/jds.S0022-0302(80)83111-0.
- Beatty, J. L., R. C. Cochran, B. A. Lintzenich, E. S. Vanzant, J. L. Morrill, R. T. Brandt, Jr, and D. E. Johnson. 1994. Effect of frequency of supplementation and protein concentration in supplements on performance and digestion characteristics of beef cattle consuming low-quality forages. *J. Anim. Sci.* 72:2475–2486. doi:10.2527/1994.7292475x.
- Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *J. Anim. Sci.* 73:2804–2819. doi:10.2527/1995.7392804x.
- Broring, N., W. J. Wilton, and P. E. Colucci. 2003. Body condition score and its relationship to ultrasound backfat measurements in beef cows. *C. J. Anim. Sci.* 83:593–596. doi:10.4141/A01-002.
- Campbell, R. L., and P. L. Davies. 2012. Structure-function relationships in calpains. *Biochem. J.* 447:335–351. doi:10.1042/BJ20120921.
- Castillo, A. R., E. Kebreab, D. E. Beever, and J. France. 2000. A review of efficiency of nitrogen utilization in lactating dairy cows and its relationship with environmental pollution. *J. Anim. Feed Sci.* 9:1–32. doi:10.22358/jafs/68025/2000.
- Chibisa, G. E., G. N. Gozho, A. G. Van Kessel, A. A. Olkowski, and T. Mutsavangwa. 2008. Effects of peripartum propylene glycol supplementation on nitrogen metabolism, body composition, and gene expression for the major protein degradation pathways in skeletal muscle in dairy cows. *J. Dairy Sci.* 91:3512–3527. doi:10.3168/jds.2007-0920.
- Dann, H. M., M. P. Carter, K. W. Cotanch, C. S. Ballard, T. Takano, and R. J. Grant. 2007. Effect of partial replacement of forage neutral detergent fiber with by-product neutral detergent fiber in close-up diets on periparturient performance of dairy cows. *J. Dairy Sci.* 90:1789–1801. doi:10.3168/jds.2006-692.
- DelCurto, T., R. C. Cochran, D. L. Harmon, A. A. Beharka, K. A. Jacques, G. Towne, and E. S. Vanzant. 1990. Supplementation of dormant tallgrass-prairie forage: I. Influence of varying supplemental protein and (or) energy levels on forage utilization characteristics of

- beef steers in confinement. *J. Anim. Sci.* 68:515–531. doi:10.2527/1990.682515x
- Drackley, J. K. 1999. ADSA foundation scholar award. Biology of dairy cows during the transition period: the final frontier? *J. Dairy Sci.* 82:2259–2273. doi:10.3168/jds.S0022-0302(99)75474-3
- Du, M., J. Tong, J. Zhao, K. R. Underwood, M. Zhu, S. P. Ford, and P. W. Nathanielsz. 2010. Fetal programming of skeletal muscle development in ruminant animals. *J. Anim. Sci.* 88(13 Suppl.):E51–E60. doi:10.2527/jas.2009-2311
- Du, M., M. J. Zhu, W. J. Means, B. W. Hess, and S. P. Ford. 2005. Nutrient restriction differentially modulates the mammalian target of rapamycin signaling and the ubiquitin-proteasome system in skeletal muscle of cows and their fetuses. *J. Anim. Sci.* 83:117–123. doi:10.2527/2005.831117x
- Fawcett, J. K., and J. E. Scott. 1960. A rapid and precise method for the determination of urea. *J. Clin. Pathol.* 13:156–159. doi:10.1136/jcp.13.2.156
- Ferrell, C. L., W. N. Garrett, and N. Hinman. 1976. Growth, development and composition of the udder and gravid uterus of beef heifers during pregnancy. *J. Anim. Sci.* 42:1477–1489. doi:10.2527/jas1976.4261477x
- Goll, D. E., G. Neti, S. W. Mares, and V. F. Thompson. 2008. Myofibrillar protein turnover: the proteasome and the calpains. *J. Anim. Sci.* 86(14 Suppl.):E19–E35. doi:10.2527/jas.2007-0395
- Goll, D. E., V. F. Thompson, H. Li, W. Wei, and J. Cong. 2003. The calpain system. *Physiol. Rev.* 83:731–801. doi:10.1152/physrev.00029.2002
- Gunter, S. A., M. B. Judkins, L. J. Krysl, J. T. Broesder, R. K. Barton, B. R. Rueda, D. M. Hallford, and D. W. Holcombe. 1990. Digesta kinetics, ruminal fermentation characteristics and serum metabolites of pregnant and lactating ewes fed chopped alfalfa hay. *J. Anim. Sci.* 68:3821–3831. doi:10.2527/1990.68113821x
- Hall, M. B. 2013. Dietary starch source and protein degradability in diets containing sucrose: Effects on ruminal measures and proposed mechanisms. *J. Dairy Sci.* 93:311–322. doi:10.3168/jds.2012-5663
- Hall, M. B., T. D. Nennich, P. H. Doane, and G. E. Brink. 2015. Total volatile fatty acid concentrations are unreliable estimators of treatment effects on ruminal fermentation in vivo. *J. Dairy Sci.* 98:3988–3999. doi:10.3168/jds.2014-8854
- Hanks, D. R., M. B. Judkins, B. A. McCracken, D. W. Holcombe, L. J. Krysl, and K. K. Park. 1993. Effects of pregnancy on digesta kinetics and ruminal fermentation in beef cows. *J. Anim. Sci.* 71:2809–2814. doi:10.2527/1993.71102809x
- Hare, K. S., K. M. Wood, C. Fitzsimmons, and G. B. Penner. 2018. Over-supplying metabolizable protein in late gestation for beef cattle: Effects on post-partum ruminal fermentation, blood metabolites, skeletal muscle catabolism, colostrum composition, milk yield and composition, and calf growth. *J. Anim. Sci.* 97:407–423.
- Harris, C. I., and G. Milne. 1981. The urinary excretion of N-methyl histidine by cattle: validation as an index of muscle breakdown. *Br. J. Nutr.* 44:129–140. doi:10.1079/BJN19810116
- Hayirli, A., R. R. Grummer, E. V. Nordheim, and P. M. Crump. 2003. Models for predicting dry matter intake of holsteins during the prefresh transition period. *J. Dairy Sci.* 86:1771–1779. doi:10.3168/jds.S0022-0302(03)73762-X
- Horn, G. W., and F. T. McCollum. 1987. Energy supplementation of grazing ruminants. In: *Proceeding of Grazing Livestock Nutrition Conference*, Jackson, WY. p. 125–136.
- Khorasani, G. R., E. K. Okine, and J. J. Kennelly. 1996. Forage source alters nutrient supply to the intestine without influencing milk yield. *J. Dairy Sci.* 79:862–872. doi:10.3168/jds.S0022-0302(96)76435-4
- Kohn, R. A., M. M. Dinneen, and E. Russek-Cohen. 2005. Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats. *J. Anim. Sci.* 83:879–889. doi:10.2527/2005.834879x
- Koohmaraie, M. 1992. Ovine skeletal muscle multicatalytic proteinase complex (proteasome): purification, characterization, and comparison of its effects on myofibrils with mu-calpains. *J. Anim. Sci.* 70:3697–3708. doi:10.2527/1992.70103071x
- Koohmaraie, M., S. D. Shackelford, T. L. Wheeler, S. M. Lonergan, and M. E. Doumit. 1995. A muscle hypertrophy condition in lamb (callipyge): characterization of effects on muscle growth and meat quality traits. *J. Anim. Sci.* 73:3596–3607. doi:10.2527/1995.73123596x
- Koong, L. J., W. N. Garrett, and P. V. Rattray. 1975. A description of the dynamics of fetal growth in sheep. *J. Anim. Sci.* 41:1065–1068. doi:10.2527/jas1975.4141065x
- Lapierre, H., G. E. Lobley, D. R. Ouellet, L. Doepel, and D. Pacheco. 2007. Amino acid requirements for lactating dairy cow: reconciling predictive models and biology. *Proceedings of Cornell Nutrition Conference for Feed Manufacturers* East Syracuse, NY. Cornell University, Ithaca, NY. p. 39–59.
- Larson, D. M., J. L. Martin, D. C. Adams, and R. N. Funston. 2009. Winter grazing system and supplementation during late gestation influence performance of beef cows and steer progeny. *J. Anim. Sci.* 87:1147–1155. doi:10.2527/jas.2008-1323
- Li, S., C. Binghai, B. Zhao, X. Yang, M. Z. Fan, and J. Yang. 2008. Decreased expression of calpain and calpastatin mRNA during development is highly correlated with muscle protein accumulation in neonatal pigs. *Comp. Biochem. Physiol.* 152:498–593. doi:10.1016/j.cbpa.2008.12.004
- Linden, D. R., E. C. Titgemeyer, K. C. Olson, and D. E. Anderson. 2014. Effects of gestation and lactation on forage intake, digestion, and passage rates of primiparous beef heifers and multiparous beef cows. *J. Anim. Sci.* 92:2141–2151. doi:10.2527/jas.2013-6813
- MacRae, J. C., A. Walker, D. Brown, and G. E. Lobley. 1993. Accretion of total protein and individual amino acids by organs and tissues of growing lambs and the ability of nitrogen balance techniques to quantitate protein retention. *Anim. Sci.* 57:237–245. doi:10.1017/S000335610000684X
- Marquardt, J. P., R. L. Horst, and N. A. Jorgensen. 1977. Effect of parity on dry matter intake at parturition in dairy cattle. *J. Dairy Sci.* 60:929–934. doi:10.3168/jds.S0022-0302(77)83965-9
- Martin, J. L., K. A. Vonnahme, D. C. Adams, G. P. Lardy, and R. N. Funston. 2007. Effects of dam nutrition on growth and reproductive performance of heifer calves. *J. Anim. Sci.* 85:841–847. doi:10.2527/jas.2006-337
- McCarthy, F. D., W. G. Bergen, and D. R. Hawkins. 1984. Muscle protein turnover in cattle of different genetic backgrounds as measured by urinary N'-methylhistidine excretion. *J. Nutr.* 113:2455–2463. doi:10.1093/jn/113.12.2455
- NASEM. 2016. *Nutrient requirements of beef cattle*. 8th revised ed. Washington, DC: National Academic Press.
- Nichols, K., J. Doelman, J. J. M. Kim, M. Carson, J. A. Metcalf, and J. P. Cant. 2017. Exogenous essential amino acids stimulate an adaptive unfolded protein response in the mammary glands of lactating cows. *J. Dairy Sci.* 100:5909–5921. doi:10.3168/jds.2016-12387

- NRC. 2000. Nutrient requirements of beef cattle. 7th revised ed. Washington, DC: National Academic Press.
- Oba, M., and M. S. Allen. 2003. Effects of corn grain conservation method on feeding behavior and productivity of lactating dairy cows at two dietary starch concentrations. *J. Dairy Sci.* 86:174–183. doi:10.3168/jds.S0022-0302(03)73598-X
- Owens, E. C. 1967. Nitrogen balances. *Proc. Nutr. Soc.* 26:116–124.
- Parr, T., R. G. Bardsley, R. S. Gilmour, and P. J. Buttery. 1992. Changes in calpain and calpastatin mRNA induced by beta-adrenergic stimulation of bovine skeletal muscle. *Eur. J. Biochem.* 208:333–339. doi:10.1111/j.1432-1033.1992.tb17191.x
- Paterson, J. A., R. L. Belyea, J. P. Bowman, M. S. Kerley, and J. E. Williams. 1994. The impact of forage quality and supplementation regimen on ruminant animal intake and performance. In: G. C. Fahey, editor, *Forage quality, evaluation and utilization*. The American Society of Agronomy, the Crop Science Society of America, and Soil Science Society of America, Madison, WI. p. 59–114.
- Penner, G. B., K. A. Beauchemin, and T. Mutsvangwa. 2006. An evaluation of the accuracy and precision of a stand-alone submersible continuous ruminal pH measurement system. *J. Dairy Sci.* 89:2132–2140. doi:10.3168/jds.S0022-0302(06)72284-6
- Penner, G. B., K. A. Beauchemin, and T. Mutsvangwa. 2007. Severity of ruminal acidosis in primiparous holstein cows during the periparturient period. *J. Dairy Sci.* 90:365–375. doi:10.3168/jds.S0022-0302(07)72638-3
- Peyraud, J. L., R. Verite, and L. Delaby. 1995. Nitrogen excretion by dairy cows: effect of the diet and of the level of production. *Fourrages*. 142:131–144.
- Prior, R. L., R. A. Scott, D. B. Laster, and D. R. Campion. 1979. Maternal energy status and development of liver and muscle in the bovine fetus. *J. Anim. Sci.* 48:1538–1545. doi:10.2527/jas1979.4861538x
- Rabelo, E., R. L. Rezende, S. J. Bertics, and R. R. Grummer. 2003. Effects of transition diets varying in dietary energy density on lactation performance and ruminal parameters of dairy cows. *J. Dairy Sci.* 86:916–925. doi:10.3168/jds.S0022-0302(03)73674-1
- Rathmacher, J. A., G. A. Link, P. J. Flakoll, and S. L. Nissen. 1992. Gas chromatographic/mass spectrometric analysis of stable isotopes of 3-methylhistidine in biological fluids: application to plasma kinetics in vivo. *Biol. Mass Spectrom.* 21:560–566. doi:10.1002/bms.1200211107
- Ratnayake, P. V., W. N. Garrett, N. E. East, and N. Hinman. 1974. Growth, development and composition of the ovine conceptus and mammary gland during pregnancy. *J. Anim. Sci.* 38:613–626. doi:10.2527/jas1974.383613x
- Robinson, J. J., I. McDonald, C. Fraser, and I. McHattie. 1977. Studies on reproduction in prolific ewes. I. Growth of the products of conception. *J. Agri. Sci.* 88:539–552. doi:10.1017/S0021859600037229
- Rosser, C. L., P. Górka, A. D. Beattie, H. C. Block, J. J. McKinnon, H. A. Lardner, and G. B. Penner. 2013. Effect of maturity at harvest on yield, chemical composition, and in situ degradability for annual cereals used for swath grazing. *J. Anim. Sci.* 91:3815–3826. doi:10.2527/jas.2012-5677
- Russell, J. B., and R. B. Hespell. 1981. Microbial rumen fermentation. *J. Dairy Sci.* 64:1153–1169. doi:10.3168/jds.S0022-0302(81)82694-X
- Russell, J. B., J. D. O'Connor, D. G. Fox, P. J. Van Soest, and C. J. Sniffen. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. *J. Anim. Sci.* 70:3551–3561. doi:10.3168/jds.S0022-0302(84)81397-1
- Russell, J. B. and C. J. Sniffen. 1984. Effect of carbon-4 and carbon-5 volatile fatty acids on growth of mixed rumen bacteria in vitro. *J. Dairy Sci.* 67:987–994. doi:10.3168/jds.S0022-0302(84)81397-1
- Scheaffer, A. N., J. S. Caton, M. L. Bauer, and L. P. Reynolds. 2001. Influence of pregnancy on body weight, ruminal characteristics, and visceral organ mass in beef heifers. *J. Anim. Sci.* 79:2481–2490. doi:10.2527/2001.7992481x
- Scheafer, D. M., C. L. Davis, and M. P. Bryant. 1980. Ammonia saturation constants for predominant species of rumen bacteria. *J. Dairy Sci.* 63:1248–1263. doi:10.3168/jds.S0022-0302(80)83076-1.
- Slot, C. 1965. Plasma creatinine determination: a new and specific Jaffe reaction method. *Scand. J. Clin. Lab. Invest.* 17:381–387. doi:10.3109/00365516509077065
- Smith, I. J., and S. L. Dodd. 2007. Calpain activation causes a proteasome-dependent increase in protein degradation and inhibits the akt signalling pathway in rat diaphragm muscle. *Exp. Physiol.* 92:561–573. doi:10.1113/expphysiol.2006.035790
- Solomon, V., and A. L. Goldberg. 1996. Importance of the ATP-ubiquitin-proteasome pathway in the degradation of soluble and myofibrillar proteins in rabbit muscle extracts. *J. Biol. Chem.* 271:26690–26697. doi:10.1074/jbc.271.43.26690
- Spanghero, M., and Z. M. Kowalski. 1997. Critical analysis of N balance experiments with lactating cows. *Livest. Prod. Sci.* 53:113–122. doi:10.1016/S0301-6226(97)00138-3
- Stifanese, R., M. Aversa, R. De Tullio, F. Salamino, C. Cantoni, M. C. Mingari, C. Prato, S. Pontremoli, and E. Melloni. 2008. Role of the calpain-calpastatin system in the density-dependent growth arrest. *Arch. Biochem. Biophys.* 479:145–152. doi:10.1016/j.abb.2008.09.002
- Tan, F. C., D. E. Goll, and Y. Otsuka. 1988. Some properties of the millimolar Ca²⁺-dependent proteinase from bovine cardiac muscle. *J. Mol. Cell. Cardiol.* 20:983–997. doi:10.1016/0022-2828(88)90576-7
- Tedeschi, L. O., D. G. Fox, M. A. Fonseca, L. Francis, and L. Cavalcanti. 2015. Invited review: models of protein and amino acid requirements for cattle. *R. Bras. Zootec.* 44:109–132. doi:10.1590/S1806-92902015000300005
- Van Emon, M. L., C. S. Schauer, R. S. Eckerman, K. R. Maddock Carlin, and K. A. Vonnahme. 2015. Supplementing metabolizable protein to ewes during late gestation: II. Effects on ewe lamb performance and reproductive efficiency. *J. Anim. Sci.* 93:1332–1339. doi:10.2527/jas2014-7609
- Van Emon, M. L., C. S. Schauer, L. A. Lekatz, S. R. Eckerman, K. Maddock-Carlin, and K. A. Vonnahme. 2014. Supplementing metabolizable protein to ewes during late gestation: I. Effects on ewe performance and offspring performance from birth to weaning. *J. Anim. Sci.* 92:339–348. doi:10.2527/jas.2013-6851
- Wallace, J. D. 1988. Supplemental feeding options to improve livestock efficiency on rangelands. In: R. S. White and R. E. Short, editors, *Achieving efficient use of rangeland resources*. Bozeman, MT: Montana Agricultural Experiment Station. p. 192–100.
- Williamson, D. H., J. Mellanby, and H. A. Krebs. 1962. Enzymic determination of D(-)-beta-hydroxybutyric acid and acetoacetic acid in blood. *Biochem. J.* 82:90–96.
- Wood, K. M., B. J. Awda, C. Fitzsimmons, S. P. Miller, B. W. McBride, and K. C. Swanson. 2013. Influence of pregnancy in mid-to-late gestation on circulating metabolites, visceral organ mass, and abundance of proteins relating to energy metabolism in mature beef cows. *J. Anim. Sci.* 91:5775–5784. doi:10.2527/jas.2013-6589