

# Effects of supplements that contain increasing amounts of metabolizable protein with or without Ca-propionate salt on postpartum interval and nutrient partitioning in young beef cows<sup>1</sup>

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**ABSTRACT:** Cattle grazing winter range forages exhibit interannual variation in response to supplementation. This variation may be mediated by circulating concentrations and subsequent metabolism of glucose, which are influenced by forage quality and availability. A study conducted at the Corona Range and Livestock Research Center during 2 dry years evaluated responses of young postpartum beef cows ( $n = 51$ , initial BW =  $408 \pm 3$  kg, and BCS =  $5.1 \pm 0.04$  in year 1;  $n = 36$ , initial BW =  $393 \pm 4$  kg, and BCS =  $4.5 \pm 0.05$  in year 2) to supplements that met or exceeded metabolizable protein (MP) requirements. Supplements were fed at 908 g/d per cow and provided 327 g of CP, 118 g of ruminally undegradable protein (RUP), and 261 g of MP from RUP (RMP), calculated to meet the MP requirement; 327 g of CP, 175 g of RUP, and 292 g of MP from RUP (RMP<sup>+</sup>), which supplied 31 g of excess MP; or 327 g of CP, 180 g of RUP, 297 g of MP from RUP, and 100 g of propionate salt (NutroCal, Kemin Industries, Inc., Des Moines, IA; RMP<sup>+</sup>P), which supplied 36 g of excess MP. Body weights were recorded once every 2 wk, and blood samples were collected 1x/wk in year 1 and 2x/wk in year 2 for 100 d postpartum. Postpartum anestrus was evaluated by progesterone from weekly

blood samples, and pregnancy was confirmed by rectal palpation at weaning. As MP from RUP with or without propionate increased, a decrease ( $P = 0.03$ ) was observed in postpartum interval; however, differences in pregnancy percentage ( $P = 0.54$ ) were not influenced by treatments. We hypothesized that additional AA from RUP along with propionate would increase supply of glucogenic precursors and, therefore, glucogenic potential of the diet. Therefore, a postpartum glucose tolerance test was conducted near the nadir of cow BW to evaluate the rate of glucose clearance. Glucose tolerance tests showed that RMP<sup>-</sup>- or RMP<sup>+</sup>P-supplemented cows had greater ( $P = 0.03$ ) rates of glucose clearance, which might have influenced the observed abbreviation of the postpartum interval. A glucose tolerance test conducted at the end of supplemental treatments revealed no differences in glucose clearance ( $P = 0.47$ ) among previously supplemented cows. These data suggest that not only vegetative quality, duration of lactation, and season of grazing, but also type of supplementation may play a pivotal role in the young postpartum beef cow's ability to respond and incorporate nutrients into insulin-sensitive tissues.

**Key words:** beef cow, glucose tolerance, insulin sensitivity, propionate, protein supplementation, reproduction

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## INTRODUCTION

Protein is often a limiting nutrient when cows graze mature vegetation (Krysl et al., 1987; Wallace, 1987; Johnson et al., 1998), and protein supplementation can

enhance intake and digestibility of dormant range forages (Owens et al., 1991) and improve cow performance (Miner et al., 1990; Wiley et al., 1991; Serrato-Corona et al., 1997). Feeding ruminally undegradable protein (RUP) once ruminally degradable protein (RDP) requirements are satisfied (NRC, 2000) can encourage repartitioning of nutrients away from nutrient sinks, such as lactation (Lee et al., 1985; Hunter and Magner, 1988; Triplett et al., 1995), or promote synthesis of maternal tissues for maintenance, growth, and reproduction via aspects of improved nutrient use (Miner et al., 1990; Wiley et al., 1991). Some evidence suggests that supplying additional protein in the form of RUP may influence nutrient partitioning via effects on aspects of

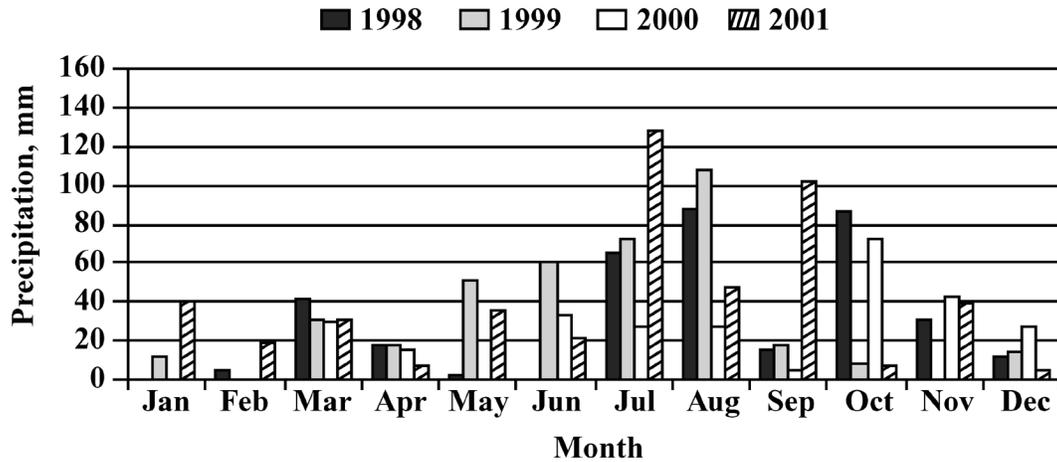
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**Figure 1.** Monthly precipitation in pastures grazed by 2-yr-old postpartum beef cows from January through July for consecutive years prior to study (1998–1999) and during study (2000–2001). Annual precipitation was 363, 391, 280, and 483 mm for 1998, 1999, 2000, and 2001, respectively.

glucose supply and metabolism (Bell and Bauman, 1997).

Range forage diets generally promote high ruminal production of acetate relative to propionate (Cronje et al., 1991). As glucogenic potential (GP) of the diet increases, subsequent improvements in net glucose synthesis occur, which increases the rate of acetate oxidation (Preston and Leng, 1987). Conversely, an accumulation of acetate resulting from an inadequate supply of glucogenic precursors may reduce insulin sensitivity through the production of ketones and free fatty acids (Dresner et al., 1999; Schmitz-Peiffer et al., 1999; Tardif et al., 2001). By increasing GP in supplements, we hypothesized that nutrients could be partitioned toward maternal tissues to optimize animal production (i.e., reproduction) via improved responsiveness of tissue to metabolic signals and away from nutrient sinks (i.e., lactation).

Therefore, our objective was to evaluate metabolic and production responses of young postpartum beef cows to supplements with increasing RUP with or without propionate salt.

## MATERIALS AND METHODS

### Study Area and Forage Quality

Studies were conducted over 2 consecutive years at New Mexico State University's Corona Range and Livestock Research Center, located 13 km east of Corona, New Mexico. The Corona Range and Livestock Research Center has an average elevation of 2,000 m with an average annual precipitation of 380 mm. The majority of precipitation occurs from July through September from convectional thunderstorms. Figure 1 illustrates precipitation patterns for the 2 yr preceding the current study and the 2 yr during which our study took place (1998, 1999, 2000, and 2001, respectively). Predomi-

nant grass species at the study site include blue grama (*Bouteloua gracilis*), threeawns (*Aristida* spp.), and wolftail (*Lycurus phleoides*). Other less dominant perennial grasses and annual forbs were also present in relatively low abundance (Knox, 1998; Forbes, 1999). The average annual forage production at the study site is 1,000 kg/ha (unpublished data). Forage availability was in excess of cattle needs in both years of the study even though the study occurred during a drought.

Two ruminally cannulated cows grazed with supplemented cows throughout the study and were used to collect diet extrusa samples each time cows moved into fresh pastures at the initiation of supplementation and during the breeding season each year (March 11 and May 6 in year 1; March 18 and June 9 in year 2, respectively). These samples were analyzed to estimate the nutritional composition of forages grazed by supplemented cows. Ruminal contents from cannulated cows were completely removed and stored in 208-L plastic tubs, and ruminal walls were sponge-dried to remove any residual moisture from the rumen as described by Lesperance et al. (1960). After removal of ruminal contents, cows were released into experimental pastures and allowed to graze for 45 to 60 min. After the grazing period, an aliquot of extrusa was collected from cannulated cows, and original ruminal contents were replaced. Collected extrusa samples (one for each cow) were placed into a forced-air oven at 55°C for 48 to 72 h with continual turning every 6 h until extrusa samples were free of moisture. After drying, extrusa samples were ground through a Wiley mill (2-mm screen, Arthur H. Thomas Co., Philadelphia, PA) and analyzed for CP using the microKjeldahl protocol (AOAC, 1990) and for NDF (Goering and Van Soest, 1970). Organic matter was determined by ashing ground extrusa samples in a muffle furnace at 600°C for 6 h.

To estimate diet digestibility, ground extrusa samples (3 g) were placed in duplicate polyester bags (10

**Table 1.** Forage characteristics from extrusa samples at the beginning of supplementation and breeding for pastures grazed by 2-yr-old postpartum beef cows

Item	Extrusa collection	
	Begin supplementation	Begin breeding
	(%)	
Year 1 (2000)		
CP <sup>1</sup>	4.2	7.4
NDF <sup>1</sup>	59.3	61.1
24-h OM digestibility	31.3	44.5
48-h OM digestibility	41.1	50.5
96-h OM digestibility	49.9	59.2
Year 2 (2001)		
CP <sup>1</sup>	5.8	9.6
NDF <sup>1</sup>	54.0	53.0
24-h OM digestibility	38.1	57.1
48-h OM digestibility	47.6	62.8
96-h OM digestibility	58.0	66.0

<sup>1</sup>OM basis.

cm × 20 cm; pore size = 53 ± 10 µm; Ankom Technology Corp., Fairport, NY). Duplicate bags containing ground extrusa as well as empty sealed polyester bags (i.e., blanks) were placed into 60-cm × 60-cm zippered laundry bags with an attached cord and incubated in the rumen of each cannulated cow for a 96-h period. Polyester bags (2 per cow) containing ground extrusa samples were removed at 0 h before insertion into the rumen and after ruminal incubation at 24, 48, and 96 h along with a blank bag (one from each cow). Blank polyester bags were used to correct for influx of particles during incubation by subtracting the blank bag residue from each bag collected at the same incubation time. Upon removal from the rumen, bags were subjected to an initial rinse by submerging 3 times in a 19-L bucket filled with cold water. After initial rinsing, bags were frozen (−20°C) until future analysis. This procedure was replicated in both years of the study to estimate forage OM digestibility. A description of forage quality is presented in Table 1.

### Animals and Management

All animal handling and experimental procedures were in accordance with guidelines established by the New Mexico State University Institutional Animal Care and Use Committee. Fifty-one lactating 2-yr-old primiparous cows (initial BW = 408 ± 3 kg; BCS = 5.1 ± 0.04) were used in year 1, and 36 lactating 2-yr-old primiparous cows (initial BW = 393 ± 4 kg; BCS = 4.5 ± 0.05) were used in year 2. Body condition scores were measured using the 9-point scale system (1 = emaciated to 9 = extremely obese). Cows were predominantly Angus (≥75%) with Hereford and Simmental breeding making up the remaining fraction.

Before calving, cows were managed as 2 separate herds: cows <5 d from parturition were managed in a

small paddock that was close to calving facilities, and cows >5 d from parturition grazed pastures adjacent to experimental pastures. At calving, calf BW was recorded, and cow-calf pairs were moved into a common experimental pasture and managed as one herd for the duration of the study (e.g., late February through mid July). In both years, the herd was rotated between a 300- and a 360-ha pasture during the study. A 60-d breeding season was initiated in early May (Julian dates of 125 and 124 for year 1 and 2, respectively), and the herd was moved into the ungrazed pasture when breeding was initiated (Figure 2).

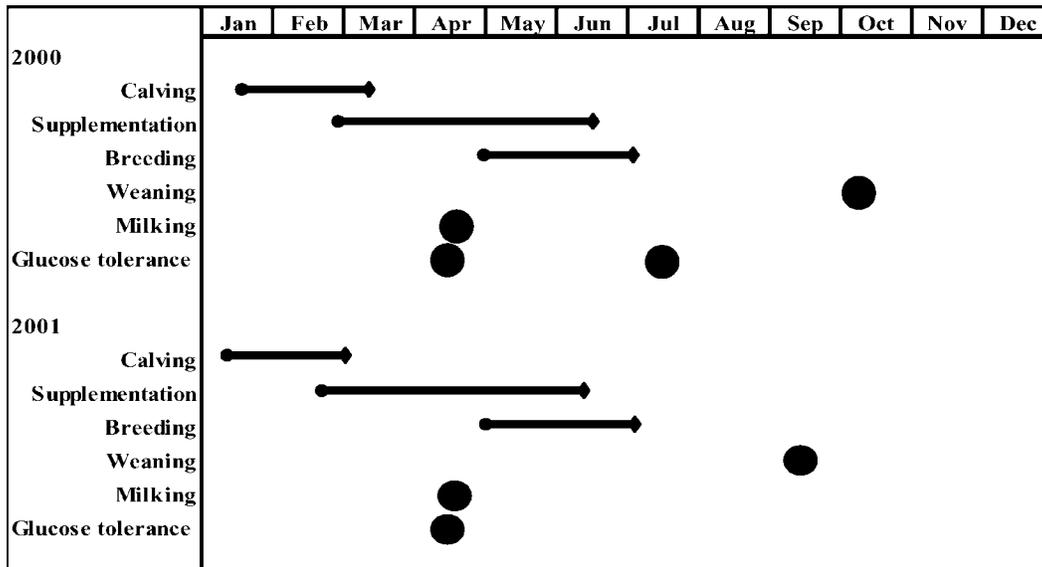
### Supplementation

In both years of the study, cows were stratified by calving date and within strata were randomly assigned to one of 3 supplemental treatments. Supplementation began 10 ± 2 d after parturition to facilitate proper nurturing of the dam with her offspring. Cows were individually fed supplement for approximately 90 d (year 1) or 100 d (year 2), which included the first 36 d (year 1) and 31 d (year 2) of the 60-d breeding seasons (Figure 2). Cows were gathered, and calves were separated from their dams on Monday and Friday throughout the study immediately after their morning grazing bout at approximately 0900.

Three supplements were formulated on an as-fed basis to be isocaloric and isonitrogenous (Table 2) with adequate RDP and MP; NRC, 1996). Supplements (Table 2) were fed on an as-fed basis at a rate of 908 g/d to provide: 626 g of TDN, 327 g of CP, 118 g of RUP, and 261 g of MP from RUP [**RMP**; calculated to meet the MP requirement (NRC, 1996)]; 595 g of TDN, 327 g of CP, 175 g of RUP, and 292 g of MP from RUP (**RMP<sup>+</sup>**; calculated to supply 31 g of MP in excess of the requirement); or 628 g of TDN, 327 g of CP, 180 g of RUP, 297 g of MP from RUP, and 100 g of propionate salt (NutroCal, Kemin Industries, Inc., Des Moines, IA; **RMP<sup>+</sup>P**; calculated to supply 36 g of MP in excess of the requirement plus propionate). All supplements were fortified with macro- and microminerals and vitamin A, and all cows and calves had free access to a loose salt-mineral mix [contained not less than 11% Ca, 8% P, 4% Mg, 4% K, 2,000 ppm of Cu, 4,000 ppm of Zn, 2,500 ppm of Mn, 13 ppm of Se, and 54.4 (1,000 IU/kg) of vitamin A (as-is basis)]. Cow predicted mineral intake met or exceeded NRC requirements (1996).

### Measurements

Measurements in both years of the study were conducted similarly, unless otherwise stated. Fecal output was estimated before the breeding season to characterize relative forage intake. Chromium sesquioxide (Cr<sub>2</sub>O<sub>3</sub>) was administered through slow-release boluses (Cattle Chrome for 200- to 500-kg cattle; Captec, Auckland, New Zealand). Boluses were administered using a bolus applicator gun (Captec) in 24 (year 1; 8 cows



**Figure 2.** Timeline for specific events as they occurred during both years of the study. Activities that encompassed weeks or months are represented with a line representing the start and end of an activity; events that occurred on a specific day are represented by (●) for both years of the study.

per supplement) and 18 (year 2; 6 cows per supplement) cows at approximately 60 d postpartum. To verify the manufacturer's estimated  $\text{Cr}_2\text{O}_3$  release rate, a bolus was weighed and placed into the rumen of each of 2 cannulated cows and recovered on d 19. Manufacturers' specifications were used during both years of the study because calculated release rates (e.g., difference between initial and removal weight divided by days of incubation of  $\text{Cr}_2\text{O}_3$ ) from cannulated cows were not different from manufacturers' estimates. Fecal grab samples were collected 10 and 17 d after bolus application on days when cows were gathered for supplementation. Samples were collected on only 2 d because of logistic constraints and potential negative impact on grazing behavior (i.e., payout interval of  $\text{Cr}_2\text{O}_3$  and ability to gather cows and calves). Fecal grab samples were also collected from nontreated cows and composited for use in making standards and controls following procedures outlined by Williams et al. (1962). Feces were dried in a 55°C forced-air oven for 72 h or until completely dry and ground in a Wiley mill until they passed through a 2-mm screen. Chromium concentration was determined in fecal samples by atomic absorption spectroscopy (Perkin-Elmer Model 3010, Perkin Elmer Life and Analytical Sciences, Inc., Boston, MA) after ashing in a 600°C muffle furnace, and subsequent digestion and resolubilization were determined using methods described by Williams et al. (1962).

Prepartum BW was measured approximately 21 (year 1) and 12 (year 2) d before the start of calving. Body weight measurements were obtained immediately after consumption of supplements once every 2 wk beginning with the initiation of supplemental feeding and continuing through termination of the breeding season. Days to BW nadir (i.e., time from parturition to lowest

BW postpartum) were determined from BW measurements. Average daily BW gains were determined for each cow from prepartum BW to BW nadir, BW nadir to the end of breeding, BW nadir to the end of supplementation, end of supplementation to the end of breeding, and end of breeding to weaning. Calf BW was recorded monthly through the end of breeding and at weaning (Figure 2) for calculation of adjusted 205-d weaning weight (Koger and Knox, 1945). Calf 205-d weaning weights were not adjusted by sex of calf or age of dam. Actual ages of calves at weaning were  $221 \pm 1.7$  and  $206 \pm 1.9$  d in year 1 and 2, respectively. Cows were sold after weaning because of inadequate rainfall. Cow BCS was recorded prepartum, at the end of supplementation, and at weaning. Body condition scores were assessed on a scale of 1 to 9 (1 = emaciated to 9 = extremely obese) for each cow by 2 independent technicians using visual assessment and vertebral palpation techniques (Herd and Sprott, 1986).

Serum samples were collected once per week (Friday; year 1) and twice per week (Monday and Friday; year 2) via coccygeal venipuncture (Corvac, Sherwood Medical, St. Louis, MO) beginning at approximately 55 d postpartum. Blood was collected immediately after cows had received and consumed supplement and was allowed to coagulate at ambient temperature for 2 h and centrifuged at  $1,500 \times g$  for 20 min after collection. Serum was decanted into plastic vials, frozen ( $-20^\circ\text{C}$ ), and stored for later analysis.

Progesterone concentrations were used to identify the number of days to first luteal activity (progesterone  $\geq 1$  ng/mL) and days to first estrus ( $\geq 2$  consecutive progesterone concentrations  $\geq 1$  ng/mL) for the postpartum interval. Progesterone concentrations were determined by solid phase RIA (Coat-A-Count, Diagnostic Products

**Table 2.** Composition of protein supplements (all units as fed) increasing in metabolizable protein from ruminally undegradable protein with or without a Ca-propionate salt

Item	Supplement <sup>1</sup>		
	RMP	RMP <sup>+</sup>	RMP <sup>+</sup> P
	————— (%) —————		
<b>Ingredient</b>			
Cottonseed meal	65.6	24.8	33.0
Wheat middlings	14.3	42.5	22.7
Hydrolyzed feather meal	—	20.0	20.0
Soybean meal	8.9	—	—
Molasses	9.0	9.0	9.0
Urea	0.7	0.7	0.7
NutroCal <sup>2</sup> (Ca-propionate)	—	—	11.0
Potassium chloride <sup>3</sup>	0.9	1.7	1.9
Dicalcium phosphate <sup>3</sup>	0.3	1.0	1.5
Vitamin A <sup>3,4</sup>	0.15	0.15	0.15
Copper chloride <sup>3</sup>	0.06	0.03	0.01
Sodium selenite <sup>3</sup>	0.09	0.05	0.06
Zinc oxide <sup>3</sup>	0.03	0.09	0.04
EDDI <sup>5</sup>	0.001	0.001	0.001
<b>Nutrient composition</b>			
DM	87.30	88.90	90.10
Calcium	0.29	0.49	3.15
Phosphorus	1.00	1.12	1.11
Magnesium	0.47	0.39	0.37
Potassium	2.02	2.22	2.20
Sulfur	0.33	0.53	0.52
Sodium	0.07	0.27	0.23
	————— (ppm) —————		
Manganese	35.07	71.35	46.62
Zinc	709.93	786.23	774.83
Iron	163.67	127.89	125.85
Copper	178.25	197.68	196.30
Selenium	1.54	1.71	1.70
Cobalt	0.12	0.10	0.09
Iodine	7.97	8.90	8.78
	————— (1,000 IU/kg) —————		
Vitamin A	66.14	73.46	72.71
	————— (g/d) —————		
TDN	626	595	628
CP	327	327	327
RDP <sup>6</sup>	209	169	163
RUP <sup>6</sup>	118	157	164
Estimated GP <sup>7</sup>	0	12.4	92.4
As fed g/d per head	908	908	908

<sup>1</sup>RMP = required metabolizable protein (NRC, 2000); RMP<sup>+</sup> = excess metabolizable protein above requirement; RMP<sup>+</sup>P = excess metabolizable protein above requirement with additional Ca-propionate added.

<sup>2</sup>Kemin Industries, Inc. (Des Moines, IA)

<sup>3</sup>Used to fortify supplements.

<sup>4</sup>Contains 66,139 IU/kg.

<sup>5</sup>EDDI = ethylene diamine dihydroiodide.

<sup>6</sup>RDP = ruminally degradable protein; RUP = ruminally undegradable protein.

<sup>7</sup>GP = glucogenic potential of supplements; calculated assuming 40% of metabolizable protein from RUP is potentially glucogenic and propionate is nearly 100% glucogenic.

Four serum composites were prepared for each cow by combining 1 mL of serum from each week's serum sample (i.e., Friday samples) to represent the first, second, and final 30 d of supplementation (which included the first 30 d of breeding), and the final 30 d of the breeding season (when supplementation had ceased). The results from these samples were used to evaluate nutrient status postcalving. Composite samples were analyzed using commercially available kits to measure serum urea nitrogen (SUN; Cat. No. 66-20, Sigma Diagnostics, St. Louis, MO; interassay coefficient of variation = 7.2%), serum glucose (Cat. No. 315-100, Sigma Diagnostics; interassay coefficient of variation = 6.7%), serum NEFA (Cat. No. 994-75409, Wako Chemicals USA, Inc., Richmond, VA; interassay coefficient of variation = 5.5%), and serum insulin. Insulin concentrations were measured using commercially available reagents for RIA (Diagnostic Products Inc.) with an interassay coefficient of variation of 8.5% and 100% recovery.

Cows from each treatment were individually milked on the day after supplementation (year 1; 8 cows per supplement) or twice (i.e., on 2 consecutive days after supplementation; year 2; 6 cows/d per supplement) at approximately 57 d postpartum (Figure 2) to estimate peak milk yield (Jenkins and Ferrell, 1992). In brief, on the day of milking, cows were gathered from their pasture, calves were removed, cows were administered an i.m. injection of oxytocin (20 IU; Vedco, Inc., St. Joseph, MO) 5 min before milking to facilitate milk letdown, and cows were milked dry using a portable milking machine until machine pressure could not extract any additional fluid, at which time individual teats were hand-stripped. Milk collected from the initial milking was subsequently discarded. Cows were kept separate from calves for 6 h, and then milked a second time. Milk weight was recorded after the final milking, and an aliquot was retained for analysis of milk protein, lactose, butterfat, and solids nonfat, which was conducted by an independent laboratory (Pioneer Dairy Laboratories, DHIA, Artesia, NM). Final milk weight collected 6 h after initial milking was multiplied by 4 to provide an estimate of 24-h milk production (Sawyer et al., 1999). Daily (24 h) milk constituent excretion (g/d) was calculated by multiplying constituent concentration by daily milk production (Appeddu et al., 1997).

To determine whether glucose clearance rates were altered by supplementation, glucose tolerance tests (GTT) were conducted on 18 postpartum cows (6 cows per supplement) twice in year 1 (mid April and mid July, d 65 and 155 postpartum, respectively) and once in year 2 (mid April; d 65 postpartum; Figure 2). A 50% dextrose solution was infused through an indwelling jugular catheter at either 0.25 mL/kg of BW (year 1, mid April; 3 cows per supplement) or 0.50 mL/kg BW [year 1, mid April (3 cows per supplement); year 1, mid July; and year 2, mid April]. Blood samples were collected via the jugular catheter at -1, 0, 3, 6, 9, 12, 15, 20, 40, 60, 80, 100, 120, 140, 160, and 180 min

Corp., Los Angeles, CA) as described by Schneider and Hallford (1996), with an interassay coefficient of variation of 5.6% and 106% recovery.

relative to glucose infusion. Blood samples were allowed to coagulate for 30 min, and then centrifuged at  $1,500 \times g$  for 20 min. Serum was decanted and stored at  $-20^{\circ}\text{C}$  for future analysis.

Glucose and insulin analyses were conducted using the methods already described. The interassay coefficient of variation for insulin was 3.3% with 101% recovery. Baseline glucose and insulin concentrations were determined using preinfusion concentrations from time  $-1$  and  $0$  min. Glucose half-life was estimated for each animal by regressing the logarithmically transformed glucose concentrations over time (Kaneko, 1989). Area under the curve was determined for insulin and glucose concentrations using the trapezoidal summation method.

### Statistical Analysis

Data were analyzed as a completely randomized design using the MIXED procedure (SAS Inst., Inc., Cary, NC) with cow as the experimental unit. Continuous variables were analyzed as a  $2 \times 3$  factorial arrangement using the main effects of year and supplement and their interaction in the model. Covariates used in the model were calving date, calf sex, days supplemented, and prepartum BW and BCS. Two preplanned contrast statements were constructed to separate least squares means when no interaction was detected: 1) RMP vs. RMP<sup>+</sup> + RMP<sup>+</sup>P to evaluate supplements differing in MP from RUP with and without propionate; 2) RMP<sup>+</sup> vs. RMP<sup>+</sup>P to evaluate supplement with and without propionate. The REPEATED statement was used in the analysis for composited serum samples, which tested the fixed effects of treatment, date of composite, year, and their interaction; animal within treatment  $\times$  year was used in the RANDOM statement, and compound symmetry was used as the covariance structure. A test for normality on days to first luteal activity and days to first estrus was conducted using univariate procedures of SAS. Categorical data (i.e., treatment pregnancy rates) were analyzed using the CATMOD procedure of SAS. Results were considered significant if  $P \leq 0.10$ .

## RESULTS AND DISCUSSION

Supplements used in this experiment were formulated to include increasing levels of MP from RUP with or without the inclusion of a Ca-propionate salt. We hypothesized that increasing MP and propionate would increase the GP of these supplements. Preston and Leng (1987) suggested that the GP for AA presented to the small intestine could be determined by assuming that 40% of the MP from RUP is potentially glucogenic. In support of this suggestion, Reilly and Ford (1971) showed that glucose production was correlated positively with daily protein intake and that the rate of glucose production from AA was correlated with AA entry rate. Use of AA for gluconeogenesis was suggested

to be dependent on their supply to the liver. In addition, Seal and Parker (1996) showed that 98% of propionate intraruminally infused was converted to glucose in the liver in chronically catheterized steers infused with 1 mol of radiolabeled propionic acid/d. We fed a similar amount of propionate (approximately 1.05 mol/d) in the present experiment.

According to Model 1 of the Beef Cattle NRC (2000), MP balance for RMP, RMP<sup>+</sup>, and RMP<sup>+</sup>P was  $-5$ ,  $25$ , and  $32$  g/d, respectively. Because no excess MP was available in the RMP supplement, the added GP for that diet was set to zero, and the differences in MP from RUP for RMP<sup>+</sup> and RMP<sup>+</sup>P were calculated ( $31$  and  $36$  g of excess MP from RUP/d for RMP<sup>+</sup> and RMP<sup>+</sup>P, respectively). If one accepts the aforementioned assumptions, the GP can be calculated by multiplying the excess MP from RUP by 40% and adding 78 g of propionate/d to the RMP<sup>+</sup>P supplement. The calculated GP of the 3 supplements used in this experiment were  $0$ ,  $12.4$ , and  $92.4$  g/d for RMP, RMP<sup>+</sup>, and RMP<sup>+</sup>P, respectively. Forage characteristics from the present experiment were used to describe the nutrient profile of the dormant range for use in the model, along with the nutrient composition provided by the supplements.

Postpartum beef cows grazing dormant winter range often lose substantial BW immediately after parturition (Appeddu et al., 1997). Body weight losses can be exacerbated by environmental conditions and forage quantity and quality. Our study was conducted during 2 consecutive drought years (Figure 1), and, therefore, the lack of nutrient-rich vegetation further restricted nutrient availability to the young lactating beef cows. Krysl et al. (1987) described how CP concentrations decline during forage dormancy and that supplemental nutrients are often required to achieve a desired level of production. Although nutritional quality of the range forages improved over the course of the study in both years and overall forage quality was greater in year 2 (Table 1), supplemental nutrients were required to achieve optimal production (i.e., reproductive competency and longevity). Early spring precipitation (Figure 1) and the establishment of annual forbs most likely influenced forage quality improvements in year 2.

Inclusion of RDP into range supplements often enhances intake and digestibility of dormant range forage (Owens et al., 1991). Estimated fecal output, an indirect measure of forage and subsequent supplement intake, revealed no differences ( $P = 0.31$ ) in relation to cows supplemented with increasing concentrations of MP from RUP with or without propionate salt (Table 3). Our results indicate that RDP concentration (average =  $180.5 \pm 14.3$  g/d) in supplements provided to young postpartum beef cows might have equally influenced forage intake and that altering RUP or MP from RUP with or without propionate did not exert any long-term effects on forage intake. Hunter and Siebert (1987) showed that when RDP concentrations were adequate, minimal influences on intake of poor quality forages occurred when additional RUP was provided.

**Table 3.** Estimated fecal output for 2-yr-old postpartum cows fed supplements increasing in metabolizable protein from ruminally undegradable protein with or without Ca-propionate salt

Item <sup>3</sup>	Year		SEM	<i>P</i> -value	Supplement <sup>1</sup>			SEM	Contrast <sup>2</sup>	
	2000	2001			RMP	RMP <sup>+</sup>	RMP <sup>+</sup> P		1	2
	(n = 22)	(n = 15)			(n = 11)	(n = 13)	(n = 13)			
Fecal output, kg of OM/d	5.6	3.4	0.40	0.01	4.9	4.1	4.4	0.50	0.31	0.62
Fecal output, % of BW	1.69	0.97	0.15	0.01	1.49	1.25	1.25	0.18	0.28	0.97

<sup>1</sup>RMP = required metabolizable protein (NRC, 2000), RMP<sup>+</sup> = excess metabolizable protein above requirement, and RMP<sup>+</sup>P = excess metabolizable protein above requirement and additional propionate.

<sup>2</sup>Contrasts: 1 = RMP vs. RMP<sup>+</sup> + RMP<sup>+</sup>P; 2 = RMP<sup>+</sup> vs. RMP<sup>+</sup>P.

<sup>3</sup>Supplement × year interaction; *P* = 0.60 to 0.70.

Estimated fecal output was less (*P* = 0.01) in year 2, which may be indicative of forage quality and subsequent digestion when compared with year 1 (Table 3). Cochran et al. (1987) cautioned the use of intensive handling of grazing livestock as it might interfere with grazing behavior; therefore, we collected fecal samples only on days that were previously allocated for gathering (i.e., days in which supplement was offered) and that were within the sampling period suggested by the manufacture (i.e., d 6 to d 20 after bolus administration). Furthermore, our standard errors for fecal output were well within the range of values found in the literature (Kattinig et al., 1993; Hollingsworth et al., 1995; Huston et al., 1999).

Body weight at the start of supplementation was lower in year 2 (*P* = 0.04) but not statistically separable across supplements (Table 4). This suggests that after parturition, cow BW were equally represented across all supplements at the start of supplementation. Cows had lower BCS at BW nadir (*P* = 0.01) in year 2 as would be expected because they started in poorer condition. A supplement response (*P* = 0.03) was observed for the nadir of BCS with a slight improvement for cows supplemented with higher concentrations of MP from RUP with or without propionate. A supplement × year interaction was observed for prepartum BCS (*P* = 0.03) and BW (*P* = 0.04), which was a direct effect of year and change in ranking occurring in year 2 (Table 5). Because of this known source of variation, both prepartum BCS and BW were used as covariates in the statistical analysis when appropriate.

Body weight nadir represents the magnitude of postpartum cow BW loss caused by negative energy balance. The time required to attain BW nadir after parturition along with the rate of BW loss provides a measure of negative energy balance. Body weight nadir also represents a transition from a negative to a positive energy balance and is closely associated with the resumption of reproductive competence in dairy cattle (Zurek et al., 1995; Beam and Butler, 1997, 1999).

Days from prepartum or parturition to BW nadir (approximately 89 or 48 d, respectively) were similar during both years of the study (*P* ≥ 0.12; Table 4), which suggests that interannual variation did not impact energy balance. However, in year 2, overall BCS was lower

(*P* = 0.01) than in year 1 at BW nadir, which was expected because cows started out at a lower BCS in year 2 compared with year 1. Alternatively, no differences in magnitude of BW nadir (*P* = 0.25) occurred in cows that received supplements containing increasing concentrations of MP from RUP with or without propionate. Differences in BCS at nadir might indicate that cows that receive higher concentrations of MP from RUP with or without propionate (i.e., increasing GP) may retain better body condition (*P* = 0.03). However, the rate of BW loss (kg/d) from prepartum BW to BW nadir was not influenced by year (*P* = 0.90) nor by supplement type (*P* = 0.45). Supplementation and interannual variation did not influence cow BCS at the end of supplementation for year (*P* = 0.89) or supplement (*P* = 0.88). A year effect (*P* < 0.01) was observed for ADG from the end of supplementation to the end of breeding; cows in year 2 exhibited higher rates of BW gain. Year effects observed for the second year of this study were most likely associated with differences in forage quality (Table 1).

The supplement × year interaction was significant (*P* = 0.05) for ADG from BW nadir to end of supplementation (*P* = 0.05) and from BW nadir to end of breeding (*P* = 0.01), which indicates that the response of cows to supplements was closely related to forage quality (Table 5). By increasing supplement MP from RUP with propionate (RMP<sup>+</sup>P), observed improvements in ADG occurred (*P* = 0.01) from end of supplementation to the end of breeding compared with RMP and RMP<sup>+</sup> (Table 4). Cows that started out at lighter BW in year 2 (start of supplementation) were heavier at weaning (*P* = 0.01) and had a greater ADG from the end of breeding to weaning (*P* = 0.06).

Adjusted calf weaning weight was approximately 17% less (*P* = 0.01) in year 1 compared with year 2 (Table 4). The lighter weaning weights were most likely caused by drought conditions, which lowered forage nutrient density and subsequently influenced milk production. A supplement response (*P* = 0.05) was observed for adjusted 205-d weaning weight, which indicates that cow supplement form might alter calf performance. Overall, weaning weights were approximately 50 kg lower in this study than those previously reported for similar breed and aged cows grazing the same pastures

**Table 4.** Effects of year and supplementation with increasing metabolizable protein from ruminally undegradable protein with or without Ca-propionate salt on production variables for 2-yr-old beef cows grazing low-quality native forage

Item <sup>3</sup>	Year		SEM	P-value	Supplement <sup>1</sup>			Contrast <sup>2</sup>		
	2000	2001			RMP	RMP <sup>+</sup>	RMP <sup>+</sup> P	SEM	1	2
BCS <sup>4</sup>	(n = 48)	(n = 36)			(n = 28)	(n = 29)	(n = 27)			
Nadir	3.9	3.4	0.06	0.01	3.6	3.8	3.7	0.07	0.03	0.65
End of supplementation	4.5	4.6	0.07	0.89	4.6	4.6	4.5	0.06	0.77	0.67
Weaning	4.5	4.7	0.06	0.05	4.6	4.6	4.5	0.06	0.19	0.24
BW	(n = 43)	(n = 36)			(n = 26)	(n = 27)	(n = 26)			
Start supplementation, kg	341.8	332.8	3.12	0.04	339.4	338.3	334.3	3.59	0.47	0.42
Nadir, kg	318.3	314.3	3.10	0.36	320.0	313.6	315.6	3.56	0.25	0.68
End supplementation, kg	359.4	369.1	3.79	0.07	367.5	364.0	361.4	4.35	0.37	0.69
Weaning, kg	390.7	417.5	5.11	0.01	404.5	401.2	406.5	6.03	0.93	0.53
Prepartum to BW nadir, d	87.1	90.6	2.40	0.28	87.1	90.6	88.8	2.83	0.45	0.66
Parturition to BW nadir, d	45.1	50.6	2.50	0.12	45.7	50.0	47.9	2.90	0.86	0.29
Magnitude of BW loss, kg	-82.2	-86.1	3.10	0.36	-80.7	-86.8	-84.8	3.56	0.25	0.68
Prepartum BW – BW nadir, kg/d	-0.97	-0.98	0.05	0.90	-0.96	-0.98	-0.98	0.06	0.74	0.94
End supplementation – end breeding, kg/d	0.54	1.21	0.12	0.01	0.77	0.74	1.11	0.10	0.22	0.01
End breeding – weaning, kg/d	0.11	0.27	0.05	0.06	0.21	0.20	0.16	0.05	0.56	0.52
Weaning weight (WW), kg	(n = 45)	(n = 36)			(n = 26)	(n = 28)	(n = 27)			
Adj. 205-d WW	161.2	193.7	2.1	0.01	175.1	181.9	175.2	2.4	0.26	0.05
Milk, g/d	(n = 23)	(n = 36)			(n = 20)	(n = 20)	(n = 19)			
24-h milk production	5,581.0	6,382.8	347.6	0.08	5,477.0	6,494.7	5,974.1	395.3	0.10	0.37
Protein	139.4	176.7	10.3	0.01	146.8	169.6	157.8	11.8	0.22	0.50
Lactose	282.9	318.2	17.2	0.12	276.8	327.3	297.4	19.6	0.09	0.45
Butterfat	204.2	279.0	18.6	0.01	206.5	287.6	230.7	21.2	0.01	0.41
Solids-not-fat	468.0	552.2	30.8	0.04	466.2	555.2	508.9	35.0	0.11	0.38
Reproduction	(n = 47)	(n = 36)			(n = 27)	(n = 29)	(n = 27)			
Days to first luteal activity	81.7	111.8	1.8	0.01	99.3	95.5	95.4	2.0	0.18	0.99
Days to first estrus	(n = 40)	(n = 33)			(n = 24)	(n = 26)	(n = 23)			
Pregnancy rate, %	89.1	86.1	0.4	0.68	88.9	89.3	85.2	0.4	—	—
Ratio <sup>5</sup>	(41/46)	(31/36)	—	—	(25/28)	(24/27)	(23/27)	—	—	—

<sup>1</sup>RMP = required metabolizable protein (NRC, 2000), RMP<sup>+</sup> = excess metabolizable protein above requirement, and RMP<sup>+</sup>P = excess metabolizable protein above requirement and additional propionate.

<sup>2</sup>Contrasts: 1 = RMP vs. RMP<sup>+</sup> + RMP<sup>+</sup>P; 2 = RMP<sup>+</sup> vs. RMP<sup>+</sup>P.

<sup>3</sup>Supplement × year interaction;  $P = 0.14$  to  $0.87$ .

<sup>4</sup>Body condition score on a 9-point scale (1 = emaciated to 9 = extremely obese).

<sup>5</sup>Number of cows bred divided by number of cows exposed.

and receiving comparable supplements (Sawyer, 2000) under drought conditions.

Trends in calf weaning weight paralleled those of cow milk production. Year effects were significant for milk production ( $P = 0.08$ ) and tended toward significance for all constituents ( $P \leq 0.12$ ; Table 4), most likely because the drought in year 1 influenced forage quality. A supplement response ( $P = 0.10$ ) occurred for milk production and paralleled calf weaning BW; the RMP<sup>+</sup>-supplemented cows numerically produced the highest quantity of milk and weaned the heaviest calves. These data agree with other findings that RUP-supplemented cows produce more milk (Appeddu et al., 1997; Sawyer, 2000). Supplement differences were observed ( $P \leq 0.10$ ) or tended to be observed ( $P \leq 0.11$ ) for all milk constituents except milk protein ( $P = 0.22$ ) for cows receiving increasing MP from RUP with or without propionate compared with RMP.

When supplements with increasing GP were fed (RMP<sup>+</sup> and RMP<sup>+</sup>P), glucose availability was likely increased, which could play a role in altering tissue re-

sponsiveness to insulin as evident by the increase in glucose disappearance ( $P = 0.11$ ) as well as the rate acetate was oxidized (Preston and Leng, 1987). The most important factor for fluid milk production is the availability of glucose. Glucose is converted to lactose in mammary tissue and becomes the main osmotic factor in determining fluid milk produced (Vilote, 2002). Lactose was greater ( $P = 0.09$ ) for RMP<sup>+</sup>- and RMP<sup>+</sup>P-supplemented cows compared with RMP-supplemented cows. The numerically lower milk production (8.7%;  $P = 0.37$ ) and butterfat secretion (24.7%;  $P = 0.41$ ) in milk of the RMP<sup>+</sup>P-supplemented cows compared with RMP<sup>+</sup>-fed animals may be indicative of differential nutrient partitioning. If the RMP<sup>+</sup>P cows were more sensitive to insulin, they would partition nutrients away from milk production. The RMP<sup>+</sup> and RMP<sup>+</sup>P supplements were similar in nutrient composition, differing primarily in added Ca-propionate.

An important outcome of shifting nutrient partitioning in postpartum cows would be improved reproductive efficiency. A test of normality failed to reject

**Table 5.** Supplement  $\times$  year interactions for 2-yr-old beef cows fed supplements increasing in metabolizable protein from ruminally undegradable protein with or without Ca-propionate salt

Item	Supplement <sup>1</sup>			SEM	Supplement $\times$ year
	RMP	RMP <sup>+</sup>	RMP <sup>+</sup> P		
Year 1 (2000)					
BCS <sup>2</sup>	(n = 17)	(n = 17)	(n = 17)		
Prepartum	4.5	4.7	5.0	0.09	0.03
BW	(n = 17)	(n = 17)	(n = 17)		
Prepartum, kg	396.6	405.3	419.1	6.40	0.04
	(n = 14)	(n = 15)	(n = 14)		
BW nadir-end supplementation, kg/d	0.30	0.44	0.53	0.12	0.05
BW nadir-end breeding, kg/d	0.31	0.38	0.56	0.08	0.01
Year 2 (2001)					
BCS <sup>2</sup>	(n = 12)	(n = 12)	(n = 12)		
Prepartum	4.3	4.5	4.7	0.09	0.03
BW	(n = 12)	(n = 12)	(n = 12)		
Prepartum, kg	397.1	393.0	387.4	6.40	0.04
BW nadir-end supplementation, kg/d	1.04	1.00	0.71	0.12	0.05
BW nadir-end breeding, kg/d	1.05	1.08	0.91	0.08	0.01

<sup>1</sup>RMP = required metabolizable protein (NRC, 2000), RMP<sup>+</sup> = excess metabolizable protein above requirement, and RMP<sup>+</sup>P = excess metabolizable protein above requirement and additional propionate.

<sup>2</sup>Body condition score on a 9-point scale (1 = emaciated to 9 = extremely obese).

the hypothesis that days to first luteal activity or days to first estrus is normally distributed ( $P = 0.24$  or  $P = 0.51$ , respectively). This observation has also been described by Azzam et al. (1991). Days between calving and first luteal activity did not differ ( $P = 0.29$ ) among supplemented cows (Table 4). However, luteal activity was delayed in year 2 ( $P < 0.01$ ) compared with year 1, even though cow gains were greater. This might have been caused by greater milk production in year 2. Identifying luteal activity that does not result in an estrous event was important because it indicates that nutritional restrictions were being alleviated.

Compared with cows fed RMP, postpartum interval (i.e., days to first estrus) decreased ( $P = 0.03$ ) with increasing MP from RUP with or without propionate (i.e., increased GP) in postpartum cow supplements. When RMP<sup>+</sup> and RMP<sup>+</sup>P were fed, more nutrients were available to support milk production and improve reproduction. Our data suggest that increasing GP resulted in greater milk production and stimulated resumption of estrus.

Overall pregnancy rates for supplemented cows were not influenced by year ( $P = 0.54$ ; Table 4) or supplement ( $P = 0.80$ ; Table 4). However, an earlier return to estrus increases the probability that conception will occur more frequently (Randel, 1990; Short et al., 1990; Lents et al., 2003). Calving data of the cows for the following year were not recorded because cows were removed from the herd after weaning because of drought.

Mean glucose concentration for the duration of both years of the study was greater ( $P = 0.01$ ) for cows supplemented with RMP<sup>+</sup>P compared with RMP<sup>+</sup> (Table 6). An increase in serum glucose concentrations was unexpected because glucose is tightly regulated and dependent upon a variety of factors that influence equilibrium

between glucose entry and clearance by tissues (Kaneko, 1989). Therefore, the supplement-associated increase in glucose concentrations may indicate that more glucose was available for entry when RMP<sup>+</sup>P was fed. We speculate that as the glucose supply increases, a proportionally greater amount of nutrients are utilized by nonmammary tissues. The consequences of these changes might have been manifested by an increase in ADG from the end of supplementation through breeding and a decrease in days to first estrus for cows supplemented with increasing concentrations of MP from RUP with propionate.

Serum urea nitrogen concentrations were greater ( $P = 0.04$ ) when RMP<sup>+</sup> and RMP<sup>+</sup>P were fed compared with RMP and were greater ( $P = 0.07$ ) when RMP<sup>+</sup> vs. RMP<sup>+</sup>P was fed. This suggests that excess AA from MP without propionate were removed by the liver, deaminated, and the amine groups were converted to urea. Drackley et al. (2001) indicated that excess AA are catabolized by the liver and excreted as urea. Our data support this and further support our hypothesis that by increasing MP from RUP with propionate (i.e., highest supplement concentration of GP), AA are spared from catabolism because of an improved glucose supply being satisfied by supplemental propionate, which may allow excess AA to be partitioned toward other protein needs. Typically, SUN concentrations of 10 to 12 mg/100 mL are considered to be optimal (Hammond et al., 1993; Stateler et al., 1995). All supplemented cows in the present experiment had SUN concentrations within this range, which suggests that supplemental protein was most likely provided at an appropriate level.

An additional measure used to evaluate nutrient status was serum NEFA concentrations. Mean NEFA concentrations were similar ( $P = 0.14$ ) for all supplemented

**Table 6.** Effects of supplementation with increasing metabolizable protein from ruminally undegradable protein with or without Ca-propionate salt on serum metabolites and on glucose and insulin kinetics after a glucose tolerance test in 2-yr-old beef cows grazing low-quality native forage around postpartum body weight nadir

Item <sup>3</sup>	Year		SEM	P-value	Supplement <sup>1</sup>			SEM	Contrast <sup>2</sup>	
	2000	2001			RMP	RMP <sup>+</sup>	RMP <sup>+</sup> P		1	2
Metabolite concentration <sup>4</sup>					(n = 27)	(n = 28)	(n = 27)			
Glucose, mg/100 mL	—	—	—	—	49.4	48.3	51.9	0.6	0.40	0.01
Serum urea nitrogen, mg/100 mL	—	—	—	—	10.5	11.2	10.7	0.2	0.04	0.07
NEFA, $\mu$ mol/L	—	—	—	—	219.1	235.9	226.1	6.6	0.14	0.29
Glucose tolerance test	(n = 18)	(n = 18)			(n = 12)	(n = 12)	(n = 12)			
Baseline glucose, mg/100 mL	81.5	73.7	4.75	0.24	85.3	79.4	68.1	5.9	0.11	0.18
Peak glucose, mg/100 mL	189.2	199.6	6.6	0.27	197.4	193.6	192.2	8.1	0.65	0.90
Peak time, min	3.5	3.5	0.3	1.00	3.8	3.0	3.8	0.4	0.48	0.22
Baseline insulin, ng/mL	1.01	0.93	0.21	0.80	1.24	1.13	0.56	0.27	0.22	0.13
Peak insulin, ng/mL	15.64	14.83	2.38	0.81	13.64	13.71	18.36	2.91	0.51	0.27
Peak time, min	10.7	6.3	0.7	0.01	7.5	7.8	10.3	0.82	0.15	0.04
Glucose half-life, min	53.29	97.13	11.39	0.01	100.34	69.31	55.98	13.96	0.03	0.50
Glucose disappearance, %/min	1.42	0.89	0.09	0.01	0.97	1.12	1.37	0.10	0.03	0.11
Glucose area, (mg/100 mL) min	14,217.6	15,021.8	846.7	0.48	16,054.7	14,615.5	13,188.0	1,004.1	0.09	0.30
Insulin area, (ng/mL) min	277.6	322.1	41.7	0.43	357.6	277.6	264.3	51.4	0.17	0.84

<sup>1</sup>RMP = required metabolizable protein (NRC, 2000), RMP<sup>+</sup> = excess metabolizable protein above requirement, and RMP<sup>+</sup>P = excess metabolizable protein above requirement and additional propionate.

<sup>2</sup>Contrasts: 1 = RMP vs. RMP<sup>+</sup> + RMP<sup>+</sup>P; 2 = RMP<sup>+</sup> vs. RMP<sup>+</sup>P.

<sup>3</sup>Supplement  $\times$  year interaction;  $P = 0.30$  to  $0.80$ .

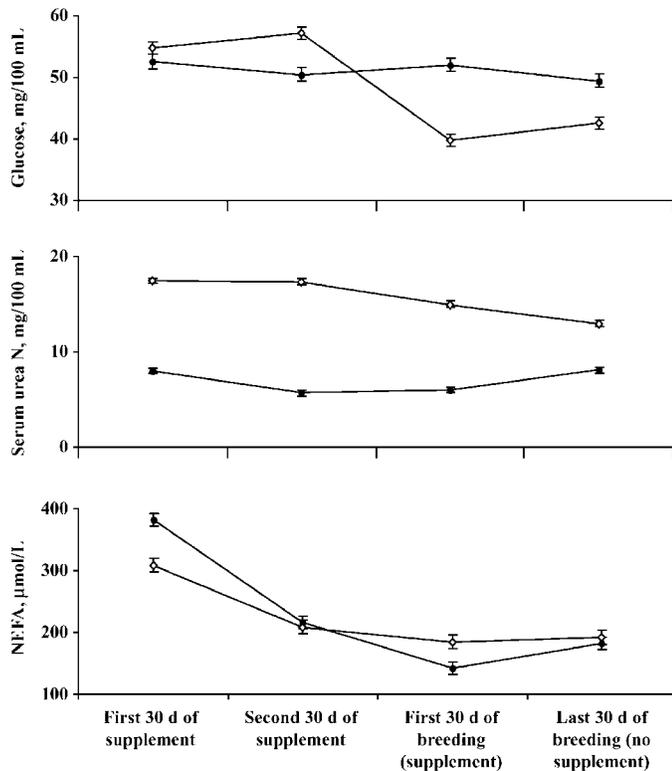
<sup>4</sup>Data presented in Figure 3 show year  $\times$  sampling interval interactions;  $P < 0.01$ .

cows and were substantially less than concentrations in previous studies that were conducted in the same pastures with similar supplemental treatments (Sawyer, 2000). The lower concentrations observed for NEFA in the present experiment may indicate improved fatty acid use during periods of nutritional stress or exhaustion of labile fat depots. The low BCS measured at BW nadir in this study supports the latter. In contrast to previous studies, in this study serum glucose concentrations were not lowered with the inclusion of RUP (Rusche et al., 1993; Sawyer, 2000) nor was NEFA diminished (Sletmoen-Olson et al., 2000).

Metabolites in serum samples composited over 30-d periods are presented in Figure 3. In year 1, serum glucose concentrations tended to decline as the forage growing season progressed, which suggests that more glucose was incorporated into tissues when forage quality improved (Brockman, 1985) and might have contributed to improved tissue sensitivity to insulin (Brockman, 1986). This is supported by our insulin data, which show elevated insulin concentrations during the early periods compared with an observed decline in the last period (Figure 4). Serum urea nitrogen concentrations were significantly greater ( $P < 0.01$ ) in year 2 than in year 1. These elevated SUN concentrations might have resulted from greater protein content in year 2 forages (Table 1). In general, SUN and NEFA concentrations declined over the course of the study, which indicates that forage quality and nutrient status improved. These trends are consistent with increasing glucose pool size, resulting in reduced deamination of proteins for glucose synthesis, reduced rates of adipose catabolism, and increased efficiency of fatty acid metabolism as time postpartum progressed.

A supplement  $\times$  year  $\times$  composite sampling period interaction was observed for serum insulin concentrations ( $P < 0.01$ ; Figure 4); insulin concentrations were lower in year 1 than year 2. Serum insulin concentrations during the first 30 d of supplementation were lower in year 1 than those observed in year 2, which coincided with the poorer quality vegetation consumption as well as the overall nutritional status of the animals. After supplementation ceased (last 30 d of breeding), all cows in both years of the study exhibited lower serum insulin concentrations. The interaction resulted not from a change in rank or direction but from a change in the magnitude of the insulin response. In year 1, insulin concentrations increased with advancing season, whereas in year 2, insulin was less variable, which may be a function of improved forage conditions. In both years, RMP<sup>+</sup>P-supplemented cows had greater serum insulin concentrations, which were most likely in response to the additional propionate. Interestingly, our data showed that RMP<sup>+</sup>P-supplemented cows had elevated serum insulin concentrations immediately before the last 30 d of breeding when no supplementation was offered. This period of elevated serum insulin corresponded with faster rates of BW gain for cows receiving the RMP<sup>+</sup>P supplement, which suggests that cows supplemented with the RMP<sup>+</sup>P were predisposed to take advantage of better quality vegetation after the supplementation period.

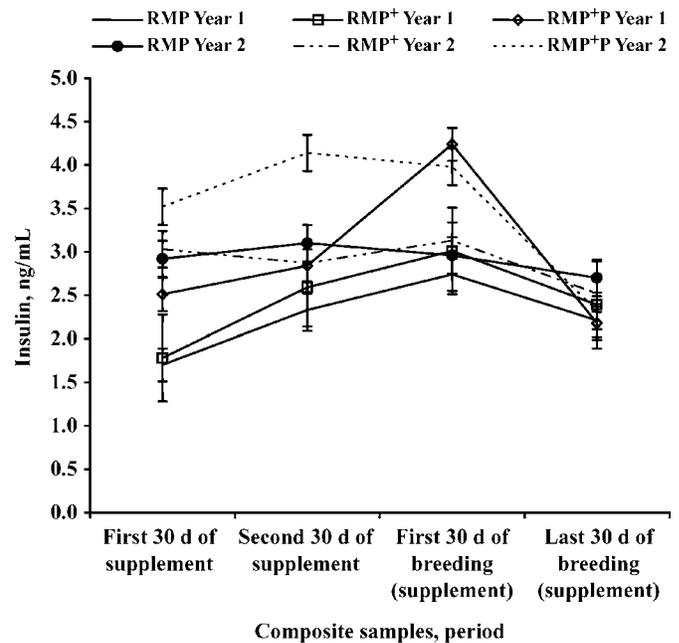
Evolutionary success of ruminants has relied on their ability to efficiently utilize structural carbohydrates such as cellulose (Bell and Bauman, 1997). However, nonstructural carbohydrates (e.g., starches and sugars) that ferment rapidly in the rumen are subject to microbial degradation and are, therefore, not available to



**Figure 3.** Compositing mean serum glucose, urea N (mg/100 mL), and NEFA ( $\mu\text{mol/L}$ ) concentrations from compositing samples collected over successive 30-d intervals from cows receiving increasing metabolizable protein from ruminally undegradable protein with or without Ca-propionate salt in 2 consecutive years [2000 ( $\bullet$ ;  $n = 46$ ) and 2001 ( $\diamond$ ;  $n = 36$ )]. The year  $\times$  sampling interval interaction ( $P < 0.01$ ) was significant for glucose, serum urea N, and NEFA.

cows. As a result, minimal amyloytic carbohydrate digestion and absorption occur in the small intestine when low quality forages are consumed. Ruminants rely almost exclusively on gluconeogenesis by the liver and, less importantly, the kidney to fulfill tissue glucose requirements. The principle precursor for glucose is propionate (Bell and Bauman, 1997), an end product of ruminal fermentation. Consumption of dormant range forages shift acetate:propionate, which may encourage unfavorable alterations in energy metabolism. Reduced hepatic supply of propionate substantially increases the requirement and subsequent use of other substrates for gluconeogenesis (i.e., glucogenic amino acids, glycerol, and lactate) to meet tissue glucose demands. Furthermore, tissue resistance to the actions of insulin may increase as a mechanism to conserve glucose for specific, noninsulin-dependent functions (i.e., cerebral tissue) at the expense of other important production parameters (e.g., lactation vs. growth or reproduction).

To ascertain whether animals are insulin insensitive, certain metabolic challenges can be conducted. In this study, we conducted a GTT to evaluate the aforemen-



**Figure 4.** Serum insulin (ng/mL) concentration from compositing samples collected from cows over successive 30-d intervals from cows receiving increasing metabolizable protein from ruminally undegradable protein with or without Ca-propionate salt in 2 consecutive years (2000;  $n = 46$ ) and (2001;  $n = 36$ ). The supplement  $\times$  year  $\times$  sampling period interaction was significant ( $P < 0.01$ ) for serum insulin. Treatments were required metabolizable protein (RMP; NRC, 2000), excess metabolizable protein above requirement (RMP<sup>+</sup>), and excess metabolizable protein above requirement and additional propionate (RMP<sup>+</sup>P).

tioned insulin sensitivity issue with cows receiving increasing concentration of glucogenic precursors. There were no glucose dose (i.e., April in year 1 when 2 levels of glucose were infused)  $\times$  treatment ( $P = 0.37$  to  $0.50$ ) or dose  $\times$  year ( $P = 0.30$  to  $0.80$ ) interactions for the responses measured; therefore, only year and supplement effects are reported (Table 6).

The ability of supplemented cows to clear glucose with increasing MP from RUP with or without propionate (i.e., increased GP) indicates their capability to incorporate nutrients into insulin-responsive tissues. A numerical ( $P = 0.11$ ) decrease in baseline glucose concentrations occurred as MP from RUP increased in supplements with or without propionate (i.e., increased GP; Table 6). It is misleading to compare the baseline glucose concentration to the aforementioned composite samples. The composite samples were collected immediately after supplementation, but the baseline samples for the GTT were collected without an offering of supplement, which may influence metabolite concentrations. Furthermore, glucose concentrations observed during the April GTT were during the period of time when energy balance and forage quality would both be considered poor. Peak glucose concentrations and peak time

**Table 7.** Effects of supplementation with increasing metabolizable protein from ruminally undegradable protein with or without Ca-propionate salt on glucose and insulin kinetics after a glucose tolerance test once supplementation had ceased in 2-yr-old beef cows grazing low quality native forage

Item	Supplement <sup>1</sup>			SEM	Contrast <sup>2</sup>	
	RMP	RMP <sup>+</sup>	RMP <sup>+</sup> P		1	2
	(n = 6)	(n = 6)	(n = 6)			
Baseline glucose, mg/100 mL	55.73	59.44	51.49	4.27	0.96	0.21
Peak glucose, mg/100 mL	214.96	218.88	215.15	10.89	0.88	0.81
Peak time, min	3.5	5.0	3.5	0.65	0.54	0.04
Glucose half-life, min	69.43	63.05	49.46	11.43	0.25	0.70
Glucose disappearance, %/min	1.21	1.20	1.51	0.17	0.18	0.94
Glucose area, (mg/100 mL) min	12,992.3	13,284.3	11,178.0	1,256.4	0.60	0.24
Baseline insulin, ng/mL	0.41	0.61	0.66	0.17	0.31	0.85
Peak insulin, ng/mL	13.0	15.2	18.4	4.16	0.92	0.37
Peak time, min	10.5	10.0	12.5	1.33	0.65	0.20
Insulin area, (ng/mL) min	199.3	255.3	205.0	29.4	0.41	0.24

<sup>1</sup>RMP = required metabolizable protein (NRC, 2000), RMP<sup>+</sup> = excess metabolizable protein above requirement, and RMP<sup>+</sup>P = excess metabolizable protein above requirement and additional propionate.

<sup>2</sup>Contrasts: 1 = RMP vs. RMP<sup>+</sup> + RMP<sup>+</sup>P; 2 = RMP<sup>+</sup> vs. RMP<sup>+</sup>P.

postinfusion were similar ( $P = 0.37$ ) among treatment groups, which indicate consistent testing and infusion procedures.

An increase ( $P = 0.04$ ) in the timing of the insulin peak after glucose infusion was observed, and cows receiving the RMP<sup>+</sup>P supplement took approximately 2.7 min longer to attain their insulin peak compared with RMP- and RMP<sup>+</sup>-supplemented cows. Pancreatic release of insulin in response to a bolus dose of glucose is a function of pancreatic pool size and not de novo insulin synthesis (Kaneko, 1989).

Baseline and peak serum glucose ( $P = 0.80$  and  $0.27$ , respectively) and insulin ( $P = 0.25$  and  $0.81$ , respectively) did not differ between years (Table 6). Serum glucose also reached its peak concentration at the same time in both years; however, timing of the serum insulin peak was 4 min earlier ( $P < 0.01$ ) for cows in year 2. These observed differences in duration to insulin peak after a bolus dose of glucose may be reflective of insulin pool size, release rate, and/or the responsiveness of tissues.

An increase in the glucose disappearance rate ( $P = 0.03$ ), decrease in glucose half-life ( $P = 0.03$ ), and decline in glucose area under the curve ( $P = 0.09$ ) were observed in cows supplemented with increasing MP from RUP with or without propionate (i.e., increasing GP; Table 6). These observations imply that cows that consume greater amounts of MP from RUP with or without propionate are more capable of clearing a glucose load via insulin responsiveness. No differences ( $P = 0.38$ ) were observed in serum insulin area under the curve, indicating that, in response to a glucose load, all cows secreted the same amount of insulin from the pancreas. Therefore, RMP<sup>+</sup>- and RMP<sup>+</sup>P-supplemented cows tended ( $P = 0.11$ ) to clear the same amount of glucose more rapidly with the same concentration of insulin compared with RMP-supplemented cows and, therefore,

were more responsive to an insulin stimulus. However, in year 2, measured glucose half-life ( $P = 0.01$ ) and rate of disappearance ( $P < 0.01$ ) were slower than in year 1 (Table 6). A longer interval for glucose clearance corresponded with higher milk production for cows in year 2. Increased milk production in year 2 coincided with an overall decreased ability to clear glucose, which might have allowed more nutrients to accumulate in the serum and to be taken up by the mammary gland for milk production rather than insulin-sensitive tissues.

A second GTT was administered in July of year 1 to evaluate insulin sensitivity changes after supplementation ceased with improved forage quality (season), which coincided with increased summer rainfall. Measurements from the second GTT are presented in Table 7. As the growing season progressed, forage quality improved slightly, and all cows responded similarly to the July GTT. Although numeric differences were observed, glucose half-life did not differ ( $P = 0.23$ ) for all postpartum cows among treatments in July after supplementation had ceased.

Kaneko (1989) indicated that a “normal” glucose half-life is approximately 35 min. A glucose half-life approaching this value might suggest that insulin responsiveness at the tissue level has been improved. Richards et al. (1989) suggested that insulin sensitivity can decrease as nutritional restrictions increase, but can be restored when limiting nutrients are provided. Data from the April and July GTT support the hypothesis that insulin sensitivity, or the responsiveness of tissues to insulin, may be partially related to diet quality, which can include forage and supplement.

We propose that as forage quality improved (Table 1), cows that received the RMP<sup>+</sup>P supplement were predisposed to gain BW faster because of their enhanced ability to utilize glucose in insulin-responsive tissues. This concept is supported by the observed increase ( $P =$

0.01) in ADG from the end of supplementation to the end of breeding for the RMP<sup>+</sup>P-supplemented cows (Table 4). For animals to gain BW, they must secrete, and tissues must respond to, insulin. As forage quality improved from early to late spring, more nutrients were partitioned toward BW gain. An improvement in insulin sensitivity may extend beyond the supplementation period, continuing to effect nutrient partitioning.

The monetary cost of supplementing postpartum cows with increasing concentrations of GP depends on availability, source, and amount of glucogenic precursors that are incorporated into the supplement; costs in this experiment were \$21.58, \$21.32, and \$38.58 per cow for RMP, RMP<sup>+</sup>, and RMP<sup>+</sup>P, respectively. We conclude that increasing the GP of range supplements increases cow sensitivity to insulin. Improved insulin sensitivity and increased glucose supply resulted in increased fluid milk and milk fat production and a reduced postpartum interval to estrus (i.e., a 9-d difference or one-half of an estrous cycle). Furthermore, the reduction in postpartum interval increases the probability that calves will be born earlier in subsequent years. These changes occurred independently of measurable changes in BW dynamics, which suggests that efficiency of nutrient use was improved.

## IMPLICATIONS

Formulating range supplements for young postpartum beef cows using ruminally degradable and undegradable protein with or without propionate salt may provide a method to more consistently alter metabolic function and decrease duration of postpartum anestrus. Discovery and implementation of strategic and targeted supplementation regimens may enhance the sustainability of extensive Western range livestock production enterprises.

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