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Effects of supplements with increasing glucogenic precursor content on reproduction and nutrient utilization in young postpartum range cows ☆

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ABSTRACT

Altering nutrient utilization in young postpartum beef cows from milk production to body weight gain has potential to improve reproductive performance. A 2-year study conducted at the Corona Range and Livestock Research Center from February to July in 2003 (n = 33) and 2004 (n = 26) evaluated responses of 2- and 3-year-old postpartum beef cows grazing native range to 2 supplements with increasing glucogenic potential (GP). Supplements were fed at 1135 g·cow^{-1}·day^{-1} twice weekly for approximately 70 days postpartum and provided 1) 341 g CP, 142 g ruminally undegradable protein, 57 g GP (GP57), or 2) 341 g CP, 151 g RUP + 80 g propionate salt (NutroCAL, Kemin Industries, Inc.), 121 g GP (GP121). Supplement × year interactions were observed for days to first estrus ($P = 0.04$) and 24-h milk production at ~60 days postpartum ($P = 0.04$). Cows fed GP57 took longer to return to estrus in 2004 than in 2003, while cows fed GP121 returned to cyclicity in similar days postpartum regardless of year. Cows fed GP57 produced more milk in 2004 than 2003, but cows fed GP121 produced similar amounts of milk regardless of year. Cows had similar ($P = 0.61$) glucose half-lives after glucose tolerance test at ~55 day postpartum (77 and 68 ± 12 min for GP57 and GP121, respectively). Young cows fed GP121 exhibited consistent milk production and return to estrus regardless of the effects of year.

1. Introduction

Cows grazing dormant native range may benefit from supplementation to reduce impacts of nutrient limitations occurring during gestation and lactation. Reduced fertility of first- and second-calf cows is a source of decreased productivity faced by cow-calf producers in the southwestern US, and may be attributed to the magnitude of negative energy balance before and after parturition (Waterman et al., 2006). Supplementing ruminally undegradable protein (RUP) to increase metabolizable protein supply once ruminally degradable protein (RDP) requirements are met (NRC, 2000) may have effects beyond meeting a protein deficiency, such as altering nutrient use priority for lactation or synthesis of maternal tissues (Hunter and Magner, 1988; Triplett et al., 1995; Waterman et al., 2006). Increased supply of RUP may result in alterations in gluconeogenesis and energy metabolism (Bell and Bauman, 1997; Cronjé et al., 1991; Waterman et al., 2006). Dormant range forage diets are characterized by higher production of acetate as a product of ruminal fermentation (Appeddu-Richards, 1998; McCollum, 1983). As propionate decreases, gluconeogenesis decreases (Aschenbach et al., 2010), which could result in a reduced rate of acetate oxidation (Preston and Leng, 1987). Accumulation of acetate has been

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hypothesized to reduce insulin sensitivity through inhibition caused by post-receptor buildup of ketone and free fatty acids (Boden, 1998; Tardif et al., 2001). Waterman et al. (2006) found that 2-year-old cows grazing dormant range were more sensitive to insulin and returned to estrus 9 days earlier when fed a supplement fortified with glucogenic precursors.

By increasing supplemental glucogenic precursors, we hypothesized that nutrients would be partitioned away from lactation, resulting in decreased days to first estrus and improved pregnancy rates. A two-year experiment was conducted to evaluate metabolic and production responses of 2- and 3-year-old postpartum range cows to increased supplemental glucogenic precursors, with an additional objective to evaluate the year-to-year variation in response to the supplements. To accomplish our objective, we evaluated return to estrus, milk production, weight change responses, insulin sensitivity, and nutrient status of postpartum 2- and 3-year-old range beef cows in response to protein supplements containing increasing glucogenic potential (GP) provided by 0 or 80 g/day of calcium propionate salt.

2. Materials and methods

All animal handling and experimental procedures were conducted in accordance with guidelines of the Institutional Animal Care and Use Committee of New Mexico State University.

2.1. Study site and diet description

The study was conducted during 2003 and 2004 at the Corona Range and Livestock Research Center (average elevation = 1900 m) 13 km east of Corona, NM. Average annual precipitation is 400 mm, most of which occurs in July and August (Forbes and Allred, 2001). Precipitation was below average for most of 2003 and above average in spring 2004 compared to the 10-year average (Fig. 1). Forages in experimental pastures were predominantly blue grama (*Bouteloua gracilis*) and wolftail (*Lycurus phleoides*), with other less dominant grasses and forbs (Forbes and Allred, 2001). Experimental pastures averaged 288 ha in size, were not grazed in the preceding 9 months, and contained 400 ± 99 kg DM/ha of herbaceous forage during the first week of March, which does not account for forage produced during the study (Black, 2005). Forage availability was considered sufficient when measured the first week of March, at 17.1 $kg \cdot cow^{-1} \cdot d^{-1}$ (not accounting for forage produced during the study).

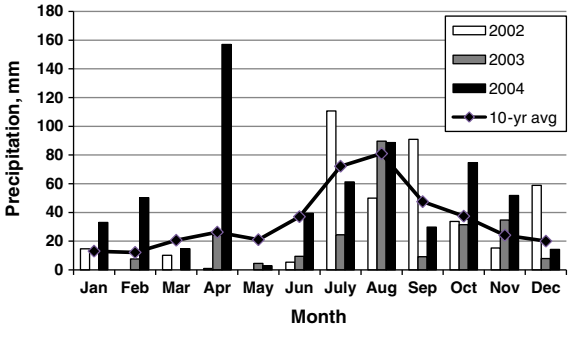

Fig. 1. Bars show annual precipitation by month for 2002 (year preceding study), 2003, and 2004 (years of study). Line shows 10-year average precipitation.

Diet samples were collected via ruminal evacuation techniques (Lesperance et al., 1960) at the beginning of supplementation (late March) and beginning of breeding (early May) in 2003 and at the beginning of supplementation (early April) in 2004 to evaluate diet quality (Table 1). Three postpartum ruminally-cannulated cows that grazed in common with experimental cows were used for all diet sample collections.

2.2. Forage disappearance and fecal output

Ruminal forage disappearance (Table 2) was estimated (in 2003 only) by in situ techniques as described by Waterman et al. (2006). Briefly, ground extrusa samples (3 g) were placed in duplicate polyester bags (10 cm × 20 cm; pore size 53 ± 10 μm; Ankom Technology Corp., Fairport, NY). Bags were incubated in ruminally cannulated cows (n = 6) for 0 (no incubation), 8, 20, 32, 44, 72, and 96 h. Blank bags were used to correct for influx of particles during incubation. Upon removal from the rumen and initial rinsing, bags were frozen at −20 °C until future analysis of NDF (Van Soest et al., 1991) and OM (AOAC, 2000). Disappearance rate was determined using the NLIN procedure of SAS (SAS Inst., Inc., Cary, NC).

To further characterize diet quality, fecal output was estimated at approximately 60 days postpartum (Table 2). Chromium sesquioxide (Cr_2O_3) slow-release boluses (Cattle Chrome for 200- to 500-kg cattle; Captec, Auckland, New Zealand) were administered orally with a bolus applicator gun (Captec) into the rumen of 4 cows (n = 2 per year, 1 from each treatment). Fecal grab samples were collected 14 and 17 days after bolus application on days when cows were gathered for supplementation. Samples were dried in a 55 °C forced-air oven for 72 h or until completely dry and ground in a Wiley mill to pass a 2-mm screen. Chromium concentrations were determined at an independent laboratory (SDK Laboratories, Hutchinson, KS).

2.3. Animals and supplementation

Two- and 3-year-old cows of predominantly Angus breeding with slight Hereford and Simmental influences were used (n = 33 in 2003 [age 2: n = 9; age 3: n = 24], n = 26 in 2004 [age 2: n = 12; age 3: n = 14], n = 59 total). Cows in these age classes are commonly managed as one group (separate from older cows) on extensively-managed ranches in the western

Table 1
Crude protein and NDF concentration of rumen extrusa samples at the start of supplementation (2003 and 2004) and start of breeding (2003) from experimental pastures.

Item	Extrusa collection	
	Supplementation start	Breeding start
2003		
CP%, OM basis	13.8 ± 0.60	15.4 ± 0.02
NDF%, OM basis	80.0 ± 0.60	86.5 ± 1.10
2004		
CP%, OM basis	11.3 ± 0.04	ND
NDF%, OM basis	80.0 ± 0.50	ND

ND, not determined.

Table 2
Organic matter and NDF in situ disappearance of extrusa samples collected at supplementation start and at breeding start from experimental pastures in 2003 and fecal output at 60 day postpartum from 2003 and 2004.

Item	Extrusa collection	
	Supplementation start	Breeding start
OM disappearance		
96-h	63.9 ± 1.0	76.9 ± 1.0
Rate, %/h	4.7 ± 0.35	4.6 ± 0.40
NDF disappearance		
96-h	61.4 ± 0.9	76.2 ± 0.9
Rate, %/h	3.8 ± 0.48	5.8 ± 0.56
	Year	
	2003	2004
Fecal output, kg OM/day	6.1 ± 0.42	5.0 ± 0.42
Fecal output, % of BW	1.53 ± 0.11	1.37 ± 0.11

United States. On each supplementation day (Fridays and Mondays), cattle were gathered after the morning grazing bout and calves were sorted from their dams at the supplementation facility. Weighed amounts of experimental supplements were provided in a rubber tub to each cow in an individual stall, and cows consumed supplement within 30 min of offering. On pasture, cows were allowed free access to water and a loose trace mineral salt at all times. Supplements were fed at a rate of 1135 g·cow^{-1}·day^{-1}, cubed and milled at Hi-Pro Feeds, Friona, TX and provided 1) 341 g CP, 142 g RUP, 57 g GP (GP57), or 2) 341 g CP, 151 g RUP + 80 g propionate salt (NutroCal, Kemin Industries, Inc.), 121 g GP (GP121). Glucogenic potential was calculated by the equation of Preston and Leng (1987), where 40% of the RUP is considered to be glucogenic (Overton et al., 1999; Vanhatalo et al., 2003). NutroCal contains 80% propionate, which was assumed to be 95% glucogenic (Steinhour and Bauman, 1988). All supplements were based on wheat middlings, cottonseed meal, and feather meal, with or without propionate salt to achieve desired supplies of glucogenic precursors. Supplements were formulated so that CP approached 50% RDP and 50% RUP, and were fortified with minerals and vitamin A (Table 3).

Cows grazed pastures adjacent to experimental pastures before calving: in 2003, cows were managed within age group; in 2004, 2-year-old cows were managed separately and 3-year-old cows were managed with 4-year-old cows in an adjacent pasture (Fig. 2). This slight difference in pre-study conditions was applied for ease of research center management and was assumed to have no impact on cow responses to postpartum nutritional treatments. All cow/calf pairs were moved to a common pasture within 10 days post-calving and began receiving supplement the subsequent supplementation day. Cows were assigned to supplement treatment by calving date and age (2 = first parity, 3 = second parity) so that similar days postpartum were reflected in each treatment group. Mean calving dates were 10 March 2003 and 5 March 2004. Individually, cows were strategically fed for 73 ± 2 days postpartum in 2003 and 65 ± 1 day postpartum in 2004. Supplementation termination coincided with the first 21 days of the breeding season (6 June) in 2003, but ceased before breeding start (15 May) in 2004. The determination to cease supplementation was based upon the onset of positive BW change. Supplementation was expected to be most effective during weight loss, underpinning the decision to cease supplementation after mean cow weight change was positive. When comparing differences in duration of supplementation, days were reduced in 2004 due to improved forage conditions created by above average April precipitation (Fig. 1) and earlier establishment of BW nadir. In both years, cows were moved to an adjacent ungrazed pasture 2 weeks before breeding started. Four reproductively sound bulls were turned out with cows on May 15 of both years for a 57-day (2003) or a 55-day (2004) breeding season.

Table 3
Composition of protein supplements (all units as fed) containing increasing amounts of glucogenic precursors.

Item	GP57	GP121
	%	
Ingredient		
Wheat middlings	59.53	46.64
Hydrolyzed feather meal	16.50	17.10
Cottonseed meal	10.05	14.20
NutroCal	–	7.10
Molasses	9.00	9.00
Urea	0.70	0.70
Potassium chloride	2.75	2.90
Monocalcium phosphate	0.65	0.95
Calcium carbonate	0.45	1.10
Vitamin A premix	0.10	0.10
Trace mineral premix	0.11	0.06
Sodium selenite	0.08	0.08
Manganese sulfate	0.05	0.06
Copper sulfate	0.02	0.01
Cobalt carbonate	< 0.01	<0.01
EDDI	–	<0.01
Nutrient Composition		
DM	86.98	88.18
Calcium	0.52	2.16
Phosphorus	0.90	0.90
Magnesium	0.32	0.30
Potassium	2.49	2.51
Sulfur	0.43	0.42
Sodium	0.25	0.23
	ppm	
Manganese	250.01	250.02
Zinc	360.59	349.98
Iron	157.74	175.32
Copper	100.00	100.00
Selenium	2.00	2.00
Cobalt	3.00	3.00
Iodine	6.83	5.91
	1000 IU/kg	
Vitamin A	44.00	44.00
	g/day	
TDN	732	727
CP	341	341
RDP	199	190
RUP	142	151
Estimated glucogenic potential	57	121
As fed g/day per head	1135	1135

2.4. Sampling and analysis

Serum samples were collected twice weekly on supplementation days (Monday and Friday) via coccygeal venipuncture

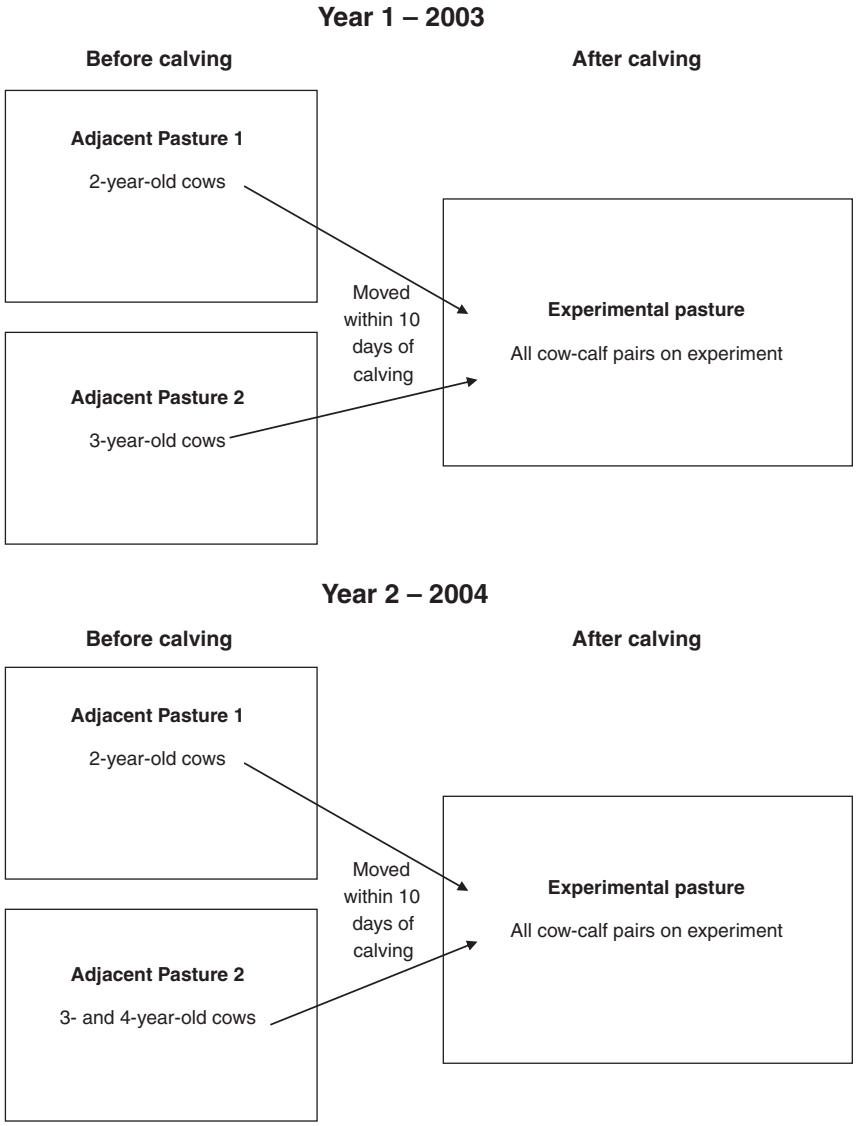

Fig. 2. Movement and management of cows and cow-calf pairs before and after calving during each year of the study.

(Corvac, Sherwood Medical, St. Louis, MO) beginning 40 days postpartum for the analysis of progesterone to determine days to first estrus. When 2 consecutive concentrations >1 ng/mL were recorded, the first date was designated as return to estrus. Samples were analyzed for progesterone by solid-phase radioimmunoassay (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA) as described by Schneider and Hallford (1996). Cows were diagnosed for pregnancy via rectal palpation at weaning (26 September 2003, 24 September 2004). Calving interval was calculated for each cow diagnosed as pregnant.

Milk production was measured to assess nutrient utilization effects of supplementation. A subsample of cows (n = 17 in 2003 [GP57: n = 8; GP121: n = 9]; n = 13 in 2004 [GP57: n = 7; GP121: n = 6) were milked with a portable milking machine (Coburn Co., Inc., Whitewater, WI; powered by a gasoline generator) approximately 56 ± 1 day postpartum on a day after supplementation using a modified weigh-suckle-weigh technique (Appeddu-Richards, 1998). Each year, cows were selected by calving date so the group would reflect ~60 days postpartum at milking. Briefly, cows were milked, penned away from their calves with ad libitum access to water for a recorded period of time (approximately 5 h), then milked again. Oxytocin (1 mL, 20 USP units/mL) was administered intramuscularly to induce milk letdown for both milkings. Cows were milked to a consistent endpoint with the milking machine, then each teat was hand-stripped. Time between each milking and milk weight from the second milking were used to estimate 24-h milk yield. Milk subsamples were collected into preservative-coated vials for later analysis of protein, lactose, butterfat, and solids non-fat by an independent laboratory (Pioneer Dairy Labs, DHIA, Artesia, NM).

After calving, cows were weighed weekly until the termination of the breeding season, and at weaning. Days from calving to BW nadir were determined from lowest weight obtained postpartum. Intervals of BW change were calculated and

included beginning of supplementation to BW nadir, BW nadir to end of supplementation, BW nadir to end of breeding, end of supplementation to end of breeding, and end of breeding to weaning. Body condition scores (BCS; 1 = emaciated, 9 = obese) were assigned by two experienced technicians as described by Waterman et al. (2006).

Calves were weighed at birth, branding (1 May 2003; 17 April 2004) and weaning. Branding weights were adjusted for average age at branding (47 days). Average calf age at branding was 52 ± 2.6 days in 2003 and 42 ± 1.7 days in 2004. Adjusted 205-day weaning weights were used as a measure of calf growth with no adjustment for sex of calf or age of dam. Actual calf age at weaning was 200 ± 2.6 days in 2003 and 204 ± 1.7 days in 2004.

To determine whether glucose clearance rates were altered by supplementation, a glucose tolerance test (GTT) was conducted 52 ± 2 days postpartum in 2003 and 63 ± 2 days postpartum in 2004 on the same subsample of cows used for milk production estimates. The GTT was implemented 24 h after supplementation as described by Waterman et al. (2006). Briefly, 50% dextrose was infused at 0.5 mL/kg of BW through an indwelling jugular catheter. Blood samples (10 mL) were collected at −1, 0, 3, 6, 9, 12, 15, 20, 40, 60, 80, 120, 140, 160, and 180 min relative to infusion, centrifuged for serum collection, and stored at −20 °C. Glucose was analyzed with a commercial kit ([Trinder] method, Cat. No. 315-100, Sigma Diagnostics, St. Louis, MO or enzymatic endpoint method, Cat. No. TR12421, Thermo Electron Corp., Waltham, MA). Insulin was analyzed by solid-phase radioimmunoassay (DCP kit, Diagnostic Products Corp., Los Angeles, CA) as validated by Reimers et al. (1982). Serum glucose and insulin areas under the curve (AUC) were calculated using trapezoidal summation. Glucose half-life was estimated by determining the time required for a 50% decrease in peak serum glucose concentration. Insulin:glucose ratio, an indicator of insulin sensitivity, was calculated by dividing insulin AUC by glucose AUC (Subiyatno et al., 1996).

In 2004, an acetate tolerance test (ATT; adapted from Cronjé et al., 1991) was conducted on a subsample of cows (n = 8; n = 4 from each supplement group) 79 ± 1 day postpartum to assess the glucogenic quality of the diet. A 20% acetic acid solution was infused at 1.25 mL/kg BW via indwelling jugular catheter. Blood samples (10 mL) were collected at –1, 0, 1, 3, 5, 7, 10, 15, 30, 60, and 90 min relative to infusion, centrifuged for serum collection, and stored at −20 °C. Serum was filtered with a centrifugal filter device for 2 h at 5000 × *g* for deproteinization (Millipore Centricon® YM-10 centrifugal device, Millipore Corp., Burlington, MA). Filtered serum was analyzed for acetate concentration using gas chromatography (Varian 3400, Walnut Creek, CA). Sample preparation was adapted from Goetsch and Galyean (1983). Acetate half-life was calculated as the time required for a 50% decrease in peak serum acetate concentration.

As a chute-side measure of energy dysfunction, whole-blood β-hydroxybutyrate levels were measured with a hand-held ketone sensor (MediSense/Abbott Laboratories, Abingdon, UK, validated by Byrne et al. (2000)) two times during the experiment: once during the supplementation period (27 ± 2 days postpartum) and once after supplementation had ceased (130 ± 2 days postpartum). Serum samples were analyzed at Texas Veterinary Medical Laboratory (Amarillo, TX) for ketone sensor validation. In 2003, all cows were sampled, while in 2004, a subsample of cows was used (n = 8 total, n = 4 from each supplement group). Subsamples (~1 drop) of progesterone blood samples were used for ketone analysis.

To further evaluate nutrient status and effectiveness of supplements, three serum composites were prepared for each cow by pooling 0.5 mL of serum from samples collected during the periods of supplementation until bulls were turned in, supplementation during the breeding season, and after supplementation had ceased. Composite samples were analyzed using commercially available kits for NEFA (Cat. No. 994 75409, Wako Chemicals USA, Inc., Richmond, VA), serum urea N (SUN; Cat. No. TR15421, Thermo Electron Corp., Waltham, MA), and glucose (Cat. No. 12421, Thermo Electron Corp., Waltham, MA). Insulin concentrations were analyzed as previously described.

2.5. Statistical analysis

Data were analyzed as a completely randomized design by analysis of variance using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) with cow as the experimental unit, using the Kenward–Roger degrees of freedom method. The model included fixed effects of supplement, year, and their interaction. Covariates were used when $P \leq 0.10$ and included cow age, calving date, and days on supplement. Body weight at calving was used as a covariate for statistical analyses of BCS. Some cows were used in both study years, thus carryover effects were tested as covariates as described by Milliken and Johnson (1984) and were found not significant ($P \geq 0.14$). Data from the 2004 ATT was analyzed with supplement, cow age, and their interaction in the model. Blood ketone and serum metabolite concentrations were analyzed with period as the repeated factor and cow as the subject with compound symmetry covariance structure. The model included supplement, year, period of measurement, and associated interactions. Pregnancy data were analyzed using the GENMOD procedure of SAS, with a model that included supplement, year, and their interactions. Significance was determined at $P \leq 0.10$ and LS means are shown throughout.

3. Results

3.1. Reproduction, milk production, and calf weight

A supplement × year interaction ($P = 0.07$) was observed for days to first estrus (Table 4). Cows fed GP121 in this study consistently returned to estrus in the same time frame over the 2 years than cows fed GP57, who required more days after calving to return to estrus in 2004 than they did in 2003. Pregnancy rates at weaning were 100% for both supplement groups and during both years after a breeding season of 57 days or less (Tables 5 and 6). Calving intervals were similar for both supplement groups ($P = 0.53$), but was 9 days longer ($P = 0.09$) for cows in 2004 than 2003.

A supplement × year interaction ($P = 0.03$) was observed for 24-h milk production (Table 4). Cows fed GP57 produced 34% more milk in 2004 than in 2003, while GP121-fed cows produced similar amounts of milk in both years. Likewise, supplement × year interactions mimicking the milk production trend were observed for lactose ($P = 0.08$) and solids

Table 4
Supplement × year interactions ($P \leq 0.10$) for days to first estrus, 24-h milk production, lactose, solids non-fat, and cow BCS at weaning for young postpartum cows grazing native range and fed supplements with increasing glucogenic precursors in 2003 and 2004. Supplements were 50:50 ruminally degradable and ruminally undegradable protein (RUP) plus 0 or 80 g/day calcium propionate (GP57, GP121).

	Year		
	2003	2004	SEM
Days to first estrus			
GP57	58[a]	78[b]	5
GP121	61[a]	65[a]	5
24-h milk production, g/day			
GP57	7100[a]	9528[b]	431
GP121	7153[a]	7615[a]	460
Milk lactose, g/day			
GP57	360[a]	477[b]	22
GP121	355[a]	390[a]	26
Milk solids non-fat, g/day			
GP57	621[a]	811[b]	38
GP121	610[a]	674[a]	45
Days from BW nadir to estrus			
GP57	9[a]	35[b]	6
GP121	17[a]	19[a]	5
Cow BCS at weaning			
GP57	5.1[a]	4.4[c]	0.12
GP121	4.8[b]	4.7[b]	0.12

[a,b,c] $P \leq 0.10$.

non-fat ($P = 0.10$). On the other hand, both butterfat and protein were similar ($P \geq 0.14$) regardless of supplement (Table 5). Cows produced similar amounts of butterfat in both years ($P = 0.50$), but milk protein production was higher ($P = 0.01$) in 2004 than 2003 (Table 6).

Dam supplement treatment did not impact calf weights at branding or weaning ($P \geq 0.28$). Even though calf branding weights were adjusted to calf age at branding (47 days of age), calves weighed less at branding in 2004 than in 2003 ($P < 0.01$), but had similar weights at weaning ($P = 0.61$) in both years.

3.2. Cow weight and body condition

Cow BW and BW change were similar for both supplement groups at all measurement points and intervals ($P \geq 0.14$). Cows were lighter ($P \leq 0.02$) in 2004 than 2003 at all measurement points until end of breeding, but were similar ($P \geq 0.16$) in BW at the end of breeding and weaning. Cows lost more weight ($P < 0.01$) from beginning of supplementation to BW nadir in 2004 than in 2003, but gained similar amounts of weight from BW nadir to end of supplementation in both years ($P = 0.59$). These results also influenced weight change from beginning to end of supplementation, which was positive in 2003, but negative in 2004 ($P < 0.01$). Cows in 2004 gained 70 kg more from BW nadir to end of breeding than in 2003 ($P < 0.01$). In 2004, cows continued to gain weight from the end of supplementation to the end of breeding, while cows lost weight in 2003 ($P < 0.01$). Cows gained similar amounts of weight regardless of year from the end of breeding to weaning ($P = 0.86$), and were of similar weight at weaning.

Cows reached BW nadir at similar ($P \geq 0.50$) days postpartum in both supplement groups and during both years. A supplement × year ($P = 0.03$) interaction was observed for days from BW nadir to first estrus (Table 4). Cows fed GP121 returned to cyclicity in similar days after BW nadir in both years, while cows fed GP57 took 26 days longer to return to estrus after BW nadir in 2004 than 2003.

Cow BCS were similar for both supplement groups ($P \geq 0.54$) at all measurement points except weaning, where a supplement × year interaction occurred (Tables 4, 5, and 6). Cows fed GP57 had a higher BCS at weaning in 2003 than 2004, while cows fed GP121 had similar BCS in both

Table 5
Supplement effects on reproduction, milk constituents, calf and cow body weight, cow body condition, glucose tolerance test responses, serum metabolites, and blood ketones for young postpartum cows grazing native range and fed supplements with increasing glucogenic precursors in 2003 and 2004.

Response	Supplement[a]				*P*-value
	GP57	SEM	GP121	SEM	
Pregnancy rate, %	100	–	100	–	–
Ratio	28/28	–	31/31	–	–
Calving interval, days	363	4	360	3	0.53
Milk constituents, g/day					
Butterfat	287	18	274	19	0.62
Protein	222	9	202	10	0.14
Calf BW, kg					
Branding	70	2	70	2	0.92
205-days	245	4	239	4	0.28
Cow BW, kg					
Begin supp	396	8	398	8	0.82
Nadir	367	8	365	7	0.86
End supp	400	8	398	8	0.91
Begin breed	395	8	396	8	0.94
End breed	418	9	423	9	0.69
Wean	469	9	474	9	0.68
Cow BW change, kg					
Begin supp–Nadir	−29	3	−33	3	0.40
Nadir–end supp	32	3	33	3	0.82
Begin supp–end supp	−3	5	2	5	0.57
Nadir–end breed	52	3	57	3	0.14
End supp–end breed	19	4	24	4	0.37
End breed–wean	50	4	51	4	0.75
Days to BW nadir	46	3	45	3	0.90
Cow BCS					
Begin supplementation	4.1	0.07	4.1	0.07	0.81
Begin breeding	4.1	0.07	4.1	0.07	0.86
End supplementation	4.4	0.07	4.4	0.07	0.87
End breeding	4.4	0.07	4.5	0.07	0.54
Cow BCS change					
Begin supp–begin breed	−0.03	0.06	−0.07	0.06	0.69
Begin supp–end supp	0.26	0.07	0.25	0.07	0.89
End supp–end breed	0.05	0.07	0.09	0.07	0.70
End breed–wean	0.40	0.10	0.27	0.10	0.39
GTT response					
Glucose half-life, min	77	12	68	12	0.61
Glucose AUC	8923	1063	8291	1111	0.68
Insulin AUC	206	19	186	20	0.47
Insulin:glucose ratio	0.029	0.005	0.029	0.005	0.99
Acetate half-life, min[b]	28.2	4.7	23.6	4.7	0.52
Blood ketones, mmol/L					
Whole-blood β-hydroxybutyrate	0.27	0.026	0.25	0.025	0.54
Serum metabolites					
Glucose, mg/100 mL	54	2	52	2	0.56
NEFA, μmol/L	372	15	345	15	0.21
Serum urea N, mg/100 mL	12.5	0.45	12.0	0.44	0.47

[a] Supplements were 50:50 ruminally degradable and ruminally undegradable protein (RUP) plus 0 or 80 g/day calcium propionate (GP57, GP121).
[b] Acetate tolerance test conducted on subsample of cows (n = 8) during 2004 only.

Table 6
Year effects on reproduction, milk constituents, calf and cow body weight, cow body condition, glucose tolerance test responses, serum metabolites, and blood ketones for young postpartum cows grazing native range and fed supplements with increasing glucogenic precursors in 2003 and 2004.

Response	Year				*P*-value
	2003	SEM	2004	SEM	
Pregnancy rate,%	100	–	100	–	–
Ratio	33/33	–	26/26	–	–
Calving interval, d	357	3	366	4	0.09
Milk constituents, g/day					
Butterfat	271	17	290	21	0.50
Protein	194	9	231	10	0.01
Calf BW, kg					
Branding	76	2	65	2	< 0.01
205-days	241	4	244	4	0.61
Cow BW, kg					
Begin supp	411	8	383	9	0.02
Nadir	394	7	338	8	< 0.01
End supp	425	7	373	8	< 0.01
Begin breed	417	8	374	9	< 0.01
End breed	413	8	428	9	0.22
Wean	462	9	481	10	0.16
Cow BW change, kg					
Begin supp–nadir	−17	3	−46	3	< 0.01
Nadir–end supp	34	3	31	3	0.59
Begin supp–end supp	18	6	−19	6	< 0.01
Nadir–end breed	20	3	89	3	< 0.01
End supp–end breed	−14	3	57	4	< 0.01
End breed–wean	50	3	51	4	0.86
Days to BW nadir	47	3	44	3	0.50
Cow BCS					
Begin supp	4.2	0.07	4.1	0.07	0.42
Begin breed	3.9	0.06	4.3	0.07	< 0.01
End supp	4.5	0.07	4.3	0.08	0.16
End breed	4.3	0.07	4.6	0.08	0.006
Cow BCS change					
Begin supp–begin breed	−0.25	0.06	0.15	0.07	< 0.01
Begin supp–end supp	0.34	0.06	0.18	0.07	0.12
End supp–end breed	−0.20	0.07	0.33	0.08	< 0.01
End breed–wean	0.59	0.09	0.08	0.10	< 0.01
GTT response					
Glucose half-life, min	77	11	68	13	0.61
Glucose AUC	10,506	996	6708	1176	0.02
Insulin AUC	220	18	173	21	0.10
Insulin:glucose ratio	0.027	0.005	0.031	0.005	0.50
Blood ketones, mmol/L					
Whole-blood β-hydroxybutyrate	0.25	0.018	0.26	0.031	0.83
Serum metabolites					
NEFA, μmol/L	395	14	321	16	0.001

years. Cow BCS change was similar for both supplements for all measurement intervals ($P \geq 0.39$). Cows were in similar ($P = 0.42$) body condition at the beginning of supplementation in both years. Cows lost one-quarter of a condition score from the beginning of supplementation to breeding start in 2003, but gained condition (0.15 condition scores) in 2004 ($P < 0.01$), which was reflected in lower cow BCS at the beginning of breeding in 2003 ($P < 0.01$). Cows gained condition during the supplementation period during both years and were in similar ($P = 0.16$) body condition at the end of supplementation. Cows lost condition from the end of supplementation to the end of breeding in 2003, but gained condition over this time period in 2004 ($P < 0.01$), which was reflected in lower body condition at the end of breeding in 2003 ($P = 0.006$).

3.3. Glucose and acetate tolerance tests

Cows had similar responses to GTT regardless of supplement group ($P \geq 0.47$). Glucose AUC and insulin AUC were smaller ($P \leq 0.10$) in 2004 than 2003, which may suggest improved insulin responsiveness in 2004; however, glucose half-life and insulin:glucose ratio were similar in both years ($P \geq 0.50$). Similar acetate half-lives were observed for cows in both supplement groups ($P = 0.52$).

3.4. Blood ketones and serum metabolites

Cows in both supplement groups had similar blood β-hydroxybutyrate concentrations over the course of the experiment ($P = 0.54$). However, whole-blood β-hydroxybutyrate concentrations nearly doubled after supplementation ceased, which was during a period of active vegetation growth ($P < 0.01$; 0.18 vs 0.33 ± 0.02 mmol/L for during vs after supplementation, respectively). Similar blood β-hydroxybutyrate concentrations were observed for both years ($P = 0.83$).

Main effects of supplement and year for serum metabolites are presented in Tables 5 and 6. Serum glucose, NEFA, and urea N concentrations were similar ($P \geq 0.21$) for both supplement groups. Serum NEFA concentrations were lower ($P = 0.001$) in 2004 than in 2003. A supplement × year × period interaction ($P = 0.03$) was observed for serum insulin (Table 7). Overall, insulin concentrations were lower in 2004 than 2003. In 2003, insulin concentrations were similar for both supplement groups during the first two measurement periods, then declined after supplementation ceased. A similar pattern was observed for cows fed GP121 in 2004, but cows fed GP57 had similar insulin concentrations during all three measurement periods. Year × period interactions ($P < 0.01$) were observed for serum glucose and serum urea nitrogen (Table 7). Glucose concentrations were similar in all measurement periods in 2003, but were higher initially and declined over time in 2004. Overall, serum urea nitrogen concentrations were lower in 2003 than 2004. In both years, SUN concentrations were similar during the first and last measurement period. Concentrations declined during the middle period in 2003, but increased during the middle period in 2004.

4. Discussion

4.1. Reproduction, milk production, and calf weight

Within the managerial, environmental, and nutritional conditions of this study, there were no limitations affecting pregnancy at weaning, as all cows were pregnant. Both supplements ameliorated nutritional restrictions on fall pregnancy rate that would have occurred without supplementation. Overall, cows in this experiment returned to estrus more quickly than cows in previous studies conducted in the same pastures at the Corona Range and Livestock Research Center (Endecott, 2003). Waterman et al. (2006) found that cows fed supplements containing increased glucogenic precursors, whether from RUP or from RUP plus propionate, cycled sooner than cows fed a cottonseed meal-based supplement with no additional glucogenic precursors. However, these authors did not observe a difference for days to first estrus between the increased glucogenic precursor groups as was observed in

Table 7
Supplement × year × period ($P = 0.03$) interaction for serum insulin, and year × period ($P < 0.01$) interactions for serum glucose and serum urea nitrogen for young postpartum cows grazing native range and fed supplements with increasing glucogenic precursors in 2003 and 2004.

	Year					
Serum insulin, ng/mL	2003			2004		
Supp × year × period, $P = 0.03$	Supplement[1]					
Measurement period	GP57	GP121	SEM	GP57	GP121	SEM
Supplementation before breeding	1.62[c]	1.61[c]	0.11	0.74[a]	1.16[b]	0.12
Supplementation during breeding	1.79[c]	1.60[c]	0.10	0.69[a]	0.65[a]	0.11
After supplementation ceased	1.45[b]	1.29[b]	0.10	0.68[a]	0.64[a]	0.11

Serum glucose, mg/100 mL

Year × period, $P < 0.01$	Year		SEM
Measurement period	2003	2004	
Supplementation before breeding	49.6[ab]	60.1[c]	3.1
Supplementation during breeding	50.5[ab]	52.9[b]	2.9
After supplementation ceased	50.3[ab]	44.9[a]	2.9

Serum urea nitrogen, mg/100 mL

Year × period, $P < 0.01$	Year		SEM
Measurement period	2003	2004	
Supplementation before breeding	10.32[b]	13.24[c]	0.64
Supplementation during breeding	7.16[a]	19.52[d]	0.60
After supplementation ceased	10.26[b]	13.02[c]	0.60

[1]Supplements were 50:50 ruminally degradable and ruminally undegradable protein (RUP) plus 0 or 80 g/day calcium propionate (GP57, GP121).
[a,b,c,d] $P \leq 0.10$.

1 year of the present experiment with quicker return to cyclicity for GP121 cows in 2004. Calving intervals for all cows were near or less than 365 days, which allowed the cows to move up in the calving cycle, improving their chances to remain in the herd.

Similar responses during both years for both milk production and return to estrus for GP121-fed cows suggests that the combination of glucogenic precursors (metabolizable protein from RUP plus propionate salt) may have shifted nutrient utilization, particularly in 2004 when pasture conditions were more favorable. All cows had smaller glucose and insulin AUC in 2004, but cows fed GP121 may have been more responsive to insulin, and were able to take advantage of better pasture conditions by shifting nutrients toward reproduction and away from milk production. Waterman et al. (2006) found a 9% decrease in milk production when cows were fed a supplement that contained 100 g/day propionate salt compared with a supplement similar to GP57 during two drought years. Even though pregnancy rates were perfect, greater supply of GP altered milk production and reduced time required to initiate reproductive events in 2004.

4.2. Cow weight and body condition

Increased days to first estrus and days from BW nadir to first estrus for GP57 cows in 2004 may have been contributed to by increased overall weight loss for cows in 2004 compared to 2003. Cows fed GP121 had a consistent response for both variables, regardless of year.

The results from each year of the study varied from one another. Precipitation was below average for most of 2003 and above average in spring 2004 compared to the 10-year average (Fig. 1). In particular, there was a 6.5-fold increase in April precipitation in 2004 compared to 2003, and a 6-fold increase compared to 10-year average April precipitation. In a semi-arid environment, this precipitation pattern was a significant change from normal. Even though more precipitation was received in the spring months of 2004, cows experienced greater weight loss. However, when average calving date and days to BW nadir are evaluated, cows in 2004 reached BW nadir in mid-April, thus the majority of their weight loss occurred before the precipitation was received. The dramatic increase in forage quality after the higher-than-average late April precipitation is reflected by the cows 2004 gaining 80 kg more from BW nadir to end of breeding than in 2003. The year differences observed may be partially explained by a 6.5-fold increase in April precipitation in 2004 compared to 2003, and a 6-fold increase compared to 10-year average April precipitation (Fig. 1). The majority of precipitation at this location generally occurs in July and August, so a subsequent increase in forage quality is anticipated following spring precipitation (Krysl et al., 1987). This is not reflected in Table 1 because the 2004 extrusa sample was collected before April precipitation occurred, but is reflected in larger weight gains for cows in 2004 after supplementation ceased.

4.3. Glucose and acetate tolerance tests

Glucose tolerance tests were conducted to evaluate glucose clearance and nutrient utilization. Shorter glucose half-lives would be expected for cows shifting nutrients away from milk production and toward body weight gain. Glucose half-lives for all cows were in the range of those reported by Waterman et al. (2006) and were considered insulin-resistant, as glucose half-lives were at least two-fold greater than the normal value of 35 min described by Kaneko (1997). Both supplements in the current experiment were similar to two protein-containing supplements fed by Waterman et al. (2006) and were very similar to each other, which may help to explain lack of differences for glucose clearance in the current experiment. Waterman et al. (2006) reported differences in glucose clearance, but the supplements used in that study had greater variation in GP.

Acetate clearance can be an indication of diet glucogenic potential (Cronjé et al., 1991). Increased glucogenic potential in the diet should allow for faster clearance of acetate. Acetate half-lives for cows in this experiment were comparable to those observed in sheep fed low quality forage diets such as wheat straw and oat hay (range 21 to 29 min; Cronjé et al., 1991; Egan, 1965; Jarrett and Filsell, 1960; Reid, 1958; Weston, 1966). Shorter acetate half-lives have been observed for animals consuming higher quality forage and mixed diets (ad libitum green pasture: 9 min, Jarrett and Filsell, 1960; lucerne hay + maize + peanut meal: 13 min; Weston, 1966). Basal diets in this experiment were probably relatively low in glucogenic potential, which may have resulted in slow acetate clearance. Even with a 64 g difference in glucose precursor

supplementation, the diets were not different enough to elicit distinct acetate clearances.

4.4. Blood ketones and serum metabolites

During negative energy balance, increased blood ketone body concentration may occur, which may be indicative of reduced gluconeogenesis, delayed acetate oxidation, and enhanced fat mobilization and ketogenesis (Chagas et al., 2009). One particular ketone body, β-hydroxybutyrate, has been shown to promote insulin resistance in rat cardiomyocytes (Tardif et al., 2001). If this same relationship exists in lactating cows grazing dormant forage, insulin resistance could result in part from a low-quality diet. Whole blood β-hydroxybutyrate concentrations were measured during and after the supplementation period in both years, and doubled after supplementation ceased, which was during a period of active vegetation growth. Levels of β-hydroxybutyrate did not approach 1.2 mmol/L, an indication of subclinical ketosis in dairy cows (Akers, 2002).

Typically, SUN concentrations of 10 to 12 mg/100 mL are considered to be optimal (Hammond et al., 1993; Stateler et al., 1995), and concentrations were just under this range in 2003, and slightly higher than this range in 2004. Higher SUN (from increased ruminal N) and lower serum NEFA concentrations (suggesting less mobilization of fat) in 2004 may reflect the perceived increase in forage quality after higher than average spring precipitation.

4.5. Summary

Conclusions can be drawn from this experiment concerning effects of environment (year), and increasing glucogenic precursors in supplements fed to young postpartum range cows. In 2004, high April precipitation probably yielded improved forage quality (and most likely lower ruminal acetate to propionate ratio) during the spring and early summer after the 2004 extrusa sample was collected. Cows produced more milk with relatively less butterfat in 2004 than 2003, suggesting reduced adipose tissue mobilization and less endogenous lipid incorporation into milk, which is supported by decreased NEFA concentrations in 2004. This was also supported by decreased insulin and glucose AUC in 2004 compared to 2003, suggesting increased insulin sensitivity.

Cows exhibited exceptional reproductive performance as all cows were pregnant after a breeding season of 57 days or less. Supplements used in this study differed only in the presence or absence of a small quantity of propionate salt, yet increasing supplemental glucogenic precursors resulted in a more consistent response in both years in days to first estrus for cows fed GP121. Furthermore, milk production for cows fed GP121 was consistent regardless of year, while cows fed GP57 produced 30% more milk in 2004 compared to 2003. Cows in both supplement groups weaned calves of similar weights and had similar weight change, and whole blood β-hydroxybutyrate responses. After supplementation ceased, whole blood β-hydroxybutyrate concentrations doubled, suggesting supplementation with glucogenic precursors improved fatty acid metabolism.

Improvements in forage quality due to increased spring precipitation allowed for earlier cessation of supplementation and yielded similar responses to a longer supplementation period in a year with less spring precipitation. Strategic supplementation with a combination of glucogenic precursors may shift nutrient utilization in young postpartum range cows grazing dormant forage.

5. Conclusions

All cows were pregnant at weaning, yet some differences between supplement groups were observed. When native forages were of higher quality, cows supplemented with a combination of glucogenic precursors from RUP and propionate partitioned nutrients toward reproduction (fewer days to first estrus) and away from milk production (decreased yield). The effectiveness of propionate as a range protein supplement ingredient resulted in small changes.

Conflict of interest

Rachel Endecott, Shad Cox, Christina Rubio, Clint Löest, Dean Hawkins, and Mark Petersen have no financial interest and received no compensation from Hi-Pro Feeds or Kemin Industries, Inc.

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