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Influence of seasonal forage quality on glucose kinetics of young beef cows[1]

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ABSTRACT: Extensive range livestock production systems in the western United States rely heavily on rangeland forages to meet the nutritional needs of grazing livestock throughout the year. Interannual variation in the quantity and quality of rangeland forage in the Northern Great Plains, as well as throughout much of the western United States, may play a pivotal role in how well grazing ruminants sequester nutrients in their tissues. This variation in forage quality may influence the ability of a beef cow to utilize dietary nutrients via changes in tissue responsiveness to insulin. Identifying specific periods and production states in which this phenomenon is manifested will provide insight into the development and implementation of strategic and targeted supplementation practices that improve nutrient utilization during times of nutritional imbalance and may improve the lifetime productivity of grazing range beef cows. A 2-yr study was conducted to monitor serum metabolites, glucose kinetics during glucose tolerance tests, and forage chemical composition every 90 d in young cows (2 to 4 yr of age; n = 28). In yr 1 and 2, cows were managed on 4 pastures varying in size from 36 to 76 ha in yr 1 and 49 to 78 ha in yr 2. Regardless of year, cow age, or cow physiological status, the main factor influencing glucose half-life was season of the year ($P = 0.02$). Effects of season on glucose half-life closely followed assessments describing forage quality, with glucose half-lives of 46, 39, 43, and 51 ± 3.9 min for May, August, December, and March, respectively. Elevated glucose half-life during seasons in which forage quality is of lower nutritive value indicated that tissue responsiveness to the actions of insulin followed seasonal changes in forage quality. Glucose half-life tended ($P = 0.09$) to decrease between May and August, increased ($P = 0.04$) between December and March, and showed a tendency ($P = 0.10$) to decrease in seasons of greater nutrient density (May and August) compared with seasons of lower nutrient density (December and March). Seasonal changes in serum metabolites were also observed and corresponded with changes in forage quality. The results support our hypothesis that as the season progresses and forage quality declines, maternal tissues become less responsive to insulin, indicating that targeted supplementation with glucogenic precursors during these seasons of nutritional stress may improve cow performance.

Key words: beef cow, forage quality, gestation, glucose kinetics, serum metabolite

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INTRODUCTION

Production demands for domestic range livestock are such that reproductive-age females are removed from the herd when they fail to or are unlikely to conceive. To achieve optimal levels of production, harvested and purchased feedstuffs are often fed to domestic stock to augment periods of nutritional stress, and thus become one of the largest input costs a producer will encounter. For extensive western livestock production systems, the timing and amount of precipitation are crucial in late spring and early summer for the establishment of cool-season grasses (Heitschmidt et al., 1995, 1999; Grings et al., 2005b). Furthermore, as summer progresses, temperatures increase while precipitation decreases, followed by decreases in forage quality that continue through fall and winter, negatively impacting livestock production (Adams and Short, 1988).

Waterman et al. (2006) demonstrated that postpartum range cows consuming supplements containing varying concentrations of glucogenic precursors parti-

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tion nutrients differently. As a result, rates of glucose sequestration into maternal tissues can be modified. If nutritional stresses that render maternal tissues less responsive to the actions of insulin can be identified, then metabolically targeted and seasonally strategic supplementation regimes may minimize this effect. Our hypothesis was that seasonal changes in forage quality manipulate the ability of range beef cows to effectively utilize nutrients provided by range forages.

Therefore, our objectives were 1) to determine whether seasonal changes in forage quality influence the range cow's ability to incorporate nutrients into body tissues, and 2) to provide insight into whether physiological status interacts with the tissue response to forage quality. To accomplish these objectives, glucose kinetics were determined following a bolus dose of glucose [i.e., glucose tolerance tests (**GTT**)].

MATERIALS AND METHODS

The Fort Keogh Livestock and Range Research Laboratory (**LARRL**) Institutional Animal Care and Use Committee approved all animal handling and experimental procedures used in the current study.

Study Area and Forage Quality

This study was conducted from May 2004 through March 2006 at the Fort Keogh LARRL, located approximately 1.6 km west of Miles City, Montana (46°22′N 105°5′W). The LARRL encompasses 22,500 ha and has an average elevation of 730 m, which includes rolling hills and barren land set apart by roughly eroded ridges, peaks, and mesas, with small intersecting streams that seasonally drain into large permanent rivers meandering through broad, nearly level valleys. Soils on the site are dominated by Sonnett loams (fine, smectitic, frigid Aridic Haplustalfs) and include a complex of Kobase silty clay loams (fine, smectitic, frigid Torrertic Haplustepts) and Gerdrum clay loams (fine, smectitic, frigid Torrertic Natrustalfs) on approximately 15% of the area. All soils are deep, well-drained, and formed from alluvium. Average daily temperatures range from –10°C in January to 24°C in July, with daily maximum temperatures occasionally exceeding 37°C during summer and daily minimums occasionally dropping below –40°C during winter. Average annual precipitation is 340 mm, with the majority of precipitation occurring from April through September from convectional thunderstorms. Figure 1 illustrates precipitation patterns for the 2-yr period preceding the current study and the 2.5-yr period during which the current study took place (2002 through 2006). Predominant grass genera at the study sites include grama (*Bouteloua*), needlegrass (*Hesperostipa*), and wheatgrass (*Pascopyrum*) within a mixed-grass-dominated rangeland (Küchler, 1964). The average annual forage standing crop at the study site was 870 ± 14 kg/ha (Grings et al., 2005b). The quantity of forage available was in excess of cattle needs (low stocking rate) in both years of the study, even though the study occurred during a period of extended drought.

Rumen extrusa samples were analyzed to estimate and describe the nutritional chemical composition of forages grazed by the experimental cows. Diet extrusa samples were collected on May 19, August 18, and November 23, 2004, and on February 23, 2005; and also on May 18, August 17, and November 23, 2005, and on February 22, 2006. Two ruminally cannulated cows grazed with the experimental cows throughout the study. On the day of extrusa sampling, ruminal contents from the cannulated cows were completely removed and stored in 208-L plastic tubs, and the ruminal walls were sponge-dried to remove any residual moisture, as described by Lesperance et al. (1960). After removal of ruminal contents, the cows were released into the experimental pastures and allowed to graze for 45 to 60 min. After the grazing period, extrusa was removed from the rumen and thoroughly mixed. An aliquot was saved for analysis, and the original ruminal contents were replaced.

Collected extrusa samples (1 from each cow) were frozen at –20°C, lyophilized, ground to pass a 2-mm screen, and stored until analysis for DM, OM (AOAC, 1990), and NDF (Goering and Van Soest, 1970). Subsamples of ground extrusa were placed in glass, square-bottomed jars with metal-rod inserts and dried in a 60°C oven for 12 h. Upon removal from the drying oven, ground extrusa samples were put into jars capped with lids and subsequently placed on a roller grinder for 24 h (Mortenson, 2003). Nitrogen was determined by combustion techniques using a C-N analyzer (CE Elantech, Inc., Lakewood, NJ). Nitrogen values were multiplied by 6.25 to obtain CP, which was expressed on an OM basis.

To estimate diet digestibility, ground extrusa samples (5 g) were placed in duplicate Dacron bags (10 × 20 cm; pore size = 53 ± 10 μm; Ankom Technology Corp., Fairport, NY). On d 26 after extrusa collection, duplicate bags containing ground extrusa, as well as empty, sealed Dacron bags (i.e., blanks) were placed into 60 × 60-cm zippered laundry bags with an attached cord. Dacron bags (4/cow) containing ground extrusa samples and a blank bag (1/cow) were placed into the rumen at specific times to allow for 96, 48, 24, 12, 6, 4, 2, and 0 h of incubation. The amount of residue in the blank Dacron bag was subtracted from each sample bag collected at the same incubation time to correct for influx of particles during incubation. Upon removal from the rumen, at 0 h, the bags were subjected to an initial rinse by submerging them 3 times in a 19-L bucket. The 19-L bucket was filled with cold water to stop fermentation (0-h bags were not inserted into the rumen but were subjected to the rinsing in the 19-L bucket). The bags were stored in plastic zippered bags before being frozen at –20°C until further analysis.

Upon thawing, bags were individually rinsed in cold tap water until the effluent was clear, after which the bags were frozen (–20°C), lyophilized, and weighed.

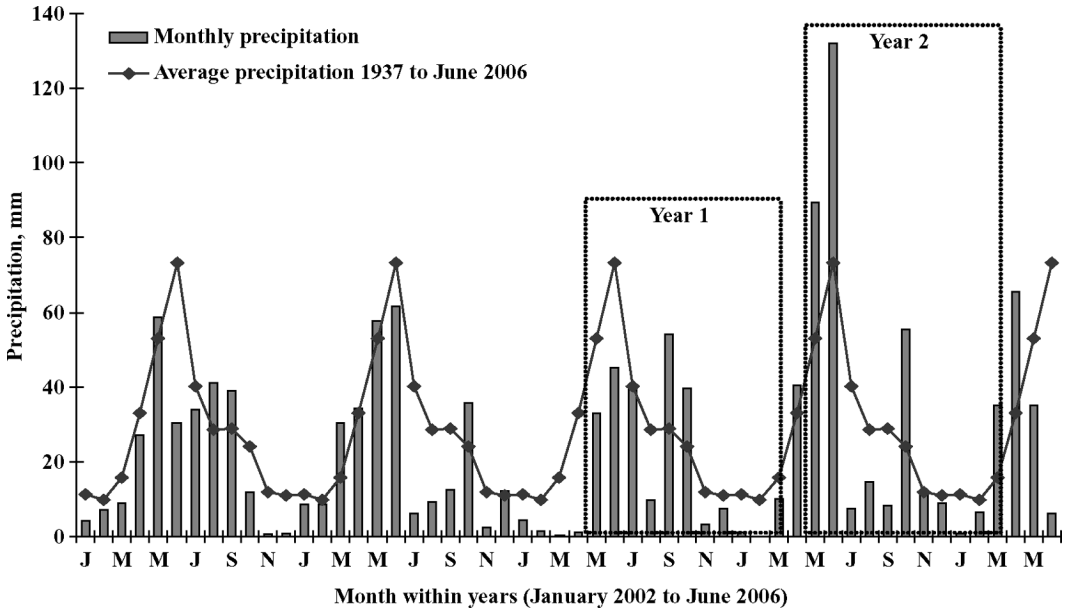

Figure 1. Monthly precipitation from January 2002 to June 2006 (bars) and 69-yr average (line) for Miles City, Montana. Annual precipitation was 264, 280, 240, 380, and 108 mm for 2002, 2003, 2004, 2005, and the first half of 2006, respectively with a 69-yr average annual precipitation of 342 mm. The 2-yr study began in May of 2004 and was terminated in March of 2006. This information was obtained from the Western Regional Climate Center (WRCC, 2006).

Residue remaining in the bag was analyzed for DM, OM, and NDF, and NDF disappearance was calculated. To estimate ME of the diets consumed, 48-h in situ OM digestibility (ISOMD) was used to calculate ME. Conversion of ISOMD to DE was accomplished by using the formula of Rittenhouse et al. (1971), as follows:

$$\text{DE (Mcal/kg)} = [0.039 \times (\%\ \text{ISOMD})] - 0.10,$$

and DE was converted to ME by using the relationship provided by the NRC (2000):

$$\text{ME (Mcal/kg)} = \text{DE (Mcal/kg)} \times 0.82.$$

Herd Management

Twenty-eight cows (21 lactating and 7 nonlactating cows, with no duplication of cows in yr 2) ranging from 2 to 4 yr of age (7 lactating, 2-yr-old; 7 lactating, 3-yr-old; 7 lactating, 4-yr-old; and 7 mixed aged, nonlactating cows) were used each year and managed as a single herd. Cows were predominantly Angus (≥75%), with Hereford, Red Angus, Charolais, and Tarentaise making up the remainder. Cows were mated by natural service in a 32-d breeding season that included an injection of $PGF_{2\alpha}$ (25 mg i.m.; Pharmacia Animal Health, Kalamazoo, MI) 7 d after bulls were turned in with the cows. Breeding occurred from June 7 to July 9 in yr 1 and June 13 to July 15 in yr 2, resulting in cows calving during mid-March to mid-April. All cows (n = 30, including the 2 ruminally cannulated cows) were managed as 1 herd, except during the breeding season, when the 7 nongestating, nonlactating cows were removed and isolated from the bulls. At the termination of the breeding season, all cows were grouped together for the remainder of the study. In both years of the study, the cows were managed on 4 pastures varying in size from 36 to 76 ha in yr 1 and 49 to 78 ha in yr 2. Moderate-quality harvested forage (91.6% OM as a percentage of DM, 15.7% CP, and 59.7% NDF on an OM basis) was provided during the winter of yr 1 and not in yr 2 based on forage availability, weather conditions, and the physiological state of the cows. Calves were weaned on August 31 in both years of the study.

Animal Data Collection

Milk yield of lactating cows was determined at approximately 70 ± 7 d postpartum in both years (Jenkins and Ferrell, 1992) by using a modified weigh-suckle-weigh technique (Wiley et al., 1991; Triplett et al., 1995; Waterman et al., 2006). Beal et al. (1990) indicated that use of a milking machine provides a more repeatable method than multiple weights on a calf before and after a suckling event. Milk yields were determined on June 2 in yr 1 and June 1 in yr 2. In brief, on the day of milking, the cows were gathered from their pasture, the calves were removed from their dam, and the cows were administered an i.m. injection of oxytocin (20 IU; Vedco Inc., St. Joseph, MO) 5 min before milking to facilitate milk letdown. The time interval from oxytocin administration to milk collection was recorded (Beal et al., 1990). Cows were then milked dry by using a portable milking machine (SuperKart, Coburn Company

Inc., Whitewater, WI) until the machine pressure could not extract any additional fluid, at which time individual teats were hand stripped. Milk collected from the initial milking was discarded. Cows were kept separate from the calves and then milked a second time by using the same procedures. Milk weight was recorded after the final milking, and an aliquot was retained for analysis of milk protein, lactose, butterfat, solids-not-fat, and milk urea-N (**MUN**) by the Rocky Mountain Dairy Herd Improvement Association (Logan, UT). Final milk weight collected 6 h after the initial milking in yr 1 and at 7 h in yr 2 (due to a mechanical malfunction of the milking machine) was then multiplied by an appropriate factor to provide an estimate of 24-h milk production (Appeddu et al., 1997). Daily (24-h) milk constituent secretion (g/d) was calculated by multiplying the constituent concentration by daily milk production (Appeddu et al., 1997; Waterman et al., 2006).

To determine whether glucose clearance kinetics were altered by seasonal changes in forage quality, GTT were conducted on the 28 cows (21 lactating and 7 nonlactating). Glucose tolerance tests were administered during 4 production stages (midlactation nongestation, late lactation early gestation, nonlactating midgestation, and nonlactating late gestation, respectively) and seasons of the year (on May 26, August 25, and December 1, 2004, and March 1, 2005; and on May 25, August 24, and December 1, 2005, and March 1, 2006). A 50% (wt/vol) dextrose solution was infused through an indwelling jugular cannula at 0.50 mL/kg of BW (0.25 g of glucose/kg of BW) by using 60-mL syringes inserted into a modified, industrial-sized, caulking gun. Blood samples were collected via jugular indwelling catheter at –1, 0, 3, 6, 9, 12, 15, 20, 40, 60, 80, 100, 120, 140, 160, and 180 min relative to glucose infusion. Blood samples were allowed to coagulate, and were then centrifuged at 1,500 × *g* for 30 min. Serum was decanted and stored at –20°C until analysis.

Serum metabolite concentrations were analyzed in duplicate by using commercially available kits to measure glucose via the glucose oxidase method (kit TR15321, Thermo DMA, Louisville, CO; intraassay CV of 3.5% and interassay CV of 8.2%), urea-N via the urease method (kit TR12321, Thermo DMA; intraassay CV of 2.7% and interassay CV of 5.3%), and NEFA [acyl-CoA synthetase-acyl-CoA oxidase (ACS-ACOD) method; cat. no. 994-75409, Wako Chemicals USA Inc., Richmond, VA; intraassay CV of 2.3% and interassay CV of 5.4%]. Serum insulin concentrations were measured in duplicate by using solid-phase ^{125}I-insulin RIA (Coat-A-Count kit, Diagnostic Products Inc., Los Angeles, CA). The insulin assay had an intraassay CV of 4.4% and an interassay CV of 10.1%, with 99% recovery.

Serum baseline metabolite and hormone concentrations were measured by using preinfusion concentrations from time –1 and 0 min. Glucose half-life was estimated for each animal by regressing the logarithmically transformed glucose concentrations against time (Kaneko, 1989; Regnault et al., 2004). Area under the curve (**AUC**) was determined for insulin and glucose concentrations by using trapezoidal summation.

Cow BW and BCS were recorded on the morning of each GTT. Body condition scores (1 = emaciated to 9 = extremely obese) were assigned by 2 experienced technicians, as described by Herd and Sprott (1986) and Wagner et al. (1988).

In both years, 1 cow failed to become pregnant and was removed from the study (a 3-yr-old cow in yr 1 and a 2-yr-old in yr 2). Furthermore, a 4-yr-old nonpregnant cow succumbed to hardware disease before the December GTT in yr 2, and 1 pregnant 3-yr-old cow aborted after being diagnosed as pregnant at the August GTT, and her data were subsequently removed from the analysis in December and March of yr 2.

Statistical Analysis

Diet quality data were analyzed by using PROC MIXED (SAS Inst. Inc., Cary, NC) with cow as the experimental unit. Rate of in situ NDF disappearance (**ISNDFD**) was determined by using first-order kinetics (Smith et al., 1971). The REPEATED statement was used to account for extrusa sampling before each GTT by using animal within season (**S**) × year (**Y**) as the subject and compound symmetry as the covariance structure. Orthogonal linear functions were used to partition variation associated with the season in which the diet quality measurements occurred. Estimates were considered significant if $P \leq 0.05$.

Milk yield data were analyzed by using PROC MIXED of SAS (SAS Institute Inc.) with cow as the experimental unit. Main effects of Y and cow age (**A**), along with their interactions replicated in 2 yr, were included in the model. Partitioned, single df orthogonal polynomial functions were used to test the effect of cow age when there was no Y × A interaction. Statistical significance was set at $P \leq 0.05$.

Data obtained at the time of GTT (i.e., animal BW and BCS, baseline serum metabolites, and metabolites measured during the GTT) were also analyzed by using PROC MIXED of SAS, with cow as the experimental unit for determining effects of Y, A, and physiological state (**P**). Effects of season when GTT was administered were determined within cows. Because all 4-yr-old cows were pregnant in yr 1, orthogonal linear functions (Table 1) of 11 Y-A-P subclasses were used to test for effects by using the random effect of cow within subclass as the error term. Three additional orthogonal linear functions were used to partition the variation associated with the season in which the GTT occurred (Table 2). Estimates were considered significant if $P \leq 0.05$.

RESULTS AND DISCUSSION

Diet Quality

Interannual variation in the timing and amount of precipitation received during seasonal precipitation

Table 1. Single df orthogonal functions used to test the effect of treatment (year, age, and pregnancy status)

	Treatment[1]										
Item[2]	120	121	130	131	141	220	221	230	231	240	241
n	n = 5	n = 7	n = 2	n = 6	n = 7	n = 3	n = 6	n = 1	n = 7	n = 2	n = 7
Year											
Y	–0.2	–0.2	–0.2	–0.2	–0.2	0.2	0.2	0.2	0.2	0.0	0.2
Age											
A1	–0.5	–0.5	0.5	0.5	0.0	–0.5	–0.5	0.5	0.5	0.0	0.0
A2	0.0	–0.5	0.0	–0.5	1.0	–0.25	–0.25	–0.25	–0.25	0.5	0.5
Y × A1	0.1	0.1	–0.1	–0.1	0.0	–0.1	–0.1	0.1	0.1	0.0	0.0
Y × A2	0.0	0.1	0.0	0.1	–0.2	–0.05	–0.05	–0.05	–0.05	0.0	0.2
Pregnancy											
P	–0.5	0.5	–0.5	0.5	0.0	–0.5	0.5	–0.5	0.5	0.0	0.0
Y × P	0.1	–0.1	0.1	–0.1	0.0	–0.1	0.1	–0.1	0.1	0.0	0.0
A1 × P	0.25	–0.25	–0.25	0.25	0.0	0.25	–0.25	–0.25	0.25	0.0	0.0

[1]Treatments are represented by a 3-digit number, with the first digit representing the year, the second digit representing the cow age, and the last digit representing the pregnancy status (0 = not pregnant and 1 = pregnant).
[2]Y = Year; A1 = 2- vs. 3-yr-old cows; A2 = 2- + 3- vs. 4-yr-old cows; P = pregnant vs. nonpregnant.

events, accompanied by subsequent ambient temperatures, greatly influence forage production (Sims and Singh, 1978a,b; Grings et al., 2005b). Total monthly precipitation during the spring forage production season was 4, 62, and 62% of the 69-yr average for April, May, and June in yr 1, respectively, and 123, 157, and 180% of the 69-yr average for April, May, and June in yr 2, respectively (Figure 1). The importance of precipitation during this seasonal period in the Northern Great Plains and throughout much of the western United States represents the period when the majority of range forage is being produced (Heitschmidt and Vermeire, 2005). Table 3 provides the main effects of S, Y, S × Y, and seasons [May vs. August (**S1**), December vs. March (**S2**), and May and August vs. December and March (**S3**)]. Data for main effects are discussed only when no significant ($P < 0.05$) S × Y interaction was observed.

Diet quality, as defined by extrusa OM, CP, NDF, and ISNDFD, was episodic within and among both years of this study and agreed with the findings of others (Adams and Short, 1988; Johnson et al., 1998; Grings et al., 2005b). Extrusa OM concentrations were greater in yr 1 than in yr 2 ($P < 0.01$) and decreased with advancing season, as evidenced by the tendency for decline between December and March ($P = 0.06$) and the decline from May and August vs. December and March ($P = 0.04$); however, no interaction for S × Y was observed ($P = 0.35$), indicating that the observed changes in OM followed similar trends for both years (Table 3). A S × Y interaction ($P < 0.01$) was observed for extrusa CP (Table 3). Extrusa CP concentrations in yr 1 followed a typical pattern of elevated CP concentrations in early summer (May), decreasing through late summer (August) and winter (December), and with a substantial increase in spring at the start of the primary production season (March; Table 3), which is consistent with observations reported by others (Johnson et al., 1998; Grings et al., 2005b). Crude protein concentration in December of yr 2 was higher than that observed in yr 1 (Table 3). Precipitation received in late fall of yr 2 most likely allowed for an increase in fall forage production, which contributed to the higher CP concentrations observed in December of yr 2. Short et al. (1996) observed similar increases in December extrusa CP concentrations in years with above-average fall precipitation. The May vs. August × Y interaction was not influenced by a change in rank, but rather a change in magnitude, which was mainly influenced by precipitation (Figure 1). Crude protein concentrations declined more rapidly between May and August in yr 2 because of the typical senescence of forages observed in Northern Great Plains, whereas in yr 1 precipitation was received more frequently throughout the summer, extending the growing season and allowing the nutritive value of forage to remain higher. The December vs. March × Y interaction resulted from lower CP concentrations in December of yr 1 resulting from minimal fall moisture compared with yr 2, when fall precipitation was above normal, allowing for an increase in fall forage production and consequently increased CP concentrations in December.

Table 2. Single df orthogonal functions for glucose tolerance tests conducted during different seasons and stages of production

	Season			
Item[1]	May	August	December	March
S1	–1.0	1.0	0.0	0.0
S2	0.0	0.0	–1.0	1.0
S3	–0.5	–0.5	0.5	0.5

[1]S1 = May vs. August; S2 = December vs. March; S3 = May and August vs. December and March.

Table 3. Seasonal influences on chemical composition of lyophilized extrusa samples from cows grazing rangelands in the Northern Great Plains

	Month of year					*P*-value[1]								
Item	May	Aug	Dec	March	SEM	S	Y	S × Y	S1	S2	S3	S1 × Y	S2 × Y	S3 × Y
Year 1														
OM, % of DM	90.1	90.3	87.8	88.3	1.1	0.06	<0.01	0.35	0.62	0.06	0.04	0.72	0.50	0.11
CP, % of OM	10.9	8.4	6.7	10.2	0.5	<0.01	0.58	<0.01	<0.01	0.61	<0.01	<0.01	<0.01	0.09
NDF, % of OM	66.4	67.9	71.8	71.1	2.0	<0.01	0.47	0.04	<0.01	0.29	<0.01	0.02	0.17	0.09
48-h ISNDFD[2]	74.7	68.6	67.8	55.3	4.8	0.02	0.02	0.15	0.02	0.55	0.01	0.11	0.08	0.84
48-h ISOMD[3]	67.3	62.7	49.2	50.2	3.6	<0.01	0.79	0.09	<0.01	0.51	<0.01	0.02	0.70	0.82
96-h ISNDFD[2]	81.1	76.1	74.0	61.9	3.9	<0.01	0.19	0.03	0.02	0.68	<0.01	0.13	<0.01	0.45
ME, Mcal/kg	2.1	1.9	1.5	1.5	0.1	<0.01	0.79	0.09	<0.01	0.51	<0.01	0.02	0.70	0.82
Year 2														
OM, % of DM	84.3	85.2	85.7	82.7	1.1	0.06	<0.01	0.35	0.62	0.06	0.04	0.72	0.50	0.11
CP, % of OM	13.2	6.8	9.1	6.0	0.5	<0.01	0.58	<0.01	<0.01	0.61	<0.01	<0.01	<0.01	0.09
NDF, % of OM	59.2	71.9	72.5	77.7	2.0	<0.01	0.47	0.04	<0.01	0.29	<0.01	0.02	0.17	0.09
48-h ISNDFD[2]	73.6	50.2	47.1	53.7	4.8	0.02	0.02	0.15	0.02	0.55	0.01	0.11	0.08	0.84
48-h ISOMD[3]	78.0	51.7	49.5	50.4	3.6	<0.01	0.79	0.09	<0.01	0.51	<0.01	0.02	0.70	0.82
96-h ISNDFD[2]	85.9	67.8	54.1	69.5	3.9	<0.01	0.19	0.03	0.02	0.68	<0.01	0.13	<0.01	0.15
ME, Mcal/kg	2.4	1.6	1.4	1.5	0.1	<0.01	0.79	0.09	<0.01	0.51	<0.01	0.02	0.70	0.82

[1]S = season; Y = year; S1 = May diet collection vs. August diet collection; S2 = December diet collection vs. March diet collection; S3 = May and August diet collections vs. December and March diet collections.
[2]In situ NDF disappearance, % of OM.
[3]In situ OM digestibility, % of OM.

An S × Y interaction (P = 0.01) was observed for extrusa NDF between May and August (Table 3). Although the pattern of change was similar between years, the magnitude of change in extrusa NDF was greater in yr 2. In essence, the lower concentrations of NDF observed in May of yr 2 were accompanied by a more rapid increase in fiber content with advancing season than in yr 1. This interaction was likely due to differences in precipitation between the 2 yr. Substantially less precipitation occurred during April to June in yr 1 than in yr 2 (Figure 1), but precipitation declined to a minute amount by July 1 in yr 2, which most likely caused actively growing plants to senesce, thereby yielding greater concentrations of plant fiber. These responses in extrusa NDF are consistent with previous observations on similar pastures during summer grazing studies (Grings et al., 2004). Furthermore, responses observed with increasing concentrations of NDF in the current study as the season progressed are consistent with observations reported by others (Funk et al., 1987; Brandyberry et al., 1991; Johnson et al., 1998). December extrusa NDF concentrations were similar in both years; however, in the December to March interval, an S × Y interaction for 96-h ISNDFD showed greater (P = 0.03) extrusa ISNDFD in March for yr 2 than in yr 1, indicating that energy availability most likely was more limiting during this sampling period in yr 1. Rate of ISNDFD was not affected by S (P = 0.89) or S × treatment (P = 0.63; data not shown), but a Y effect (P < 0.01) was observed, with greater rates of ISNDFD in yr 1 than in yr 2 (4.81 and 4.32 ± 0.09%/h, respectively).

The 48-h ISNDFD decreased (P = 0.01) with advancing season from August to December, began to improve by March of the following year, and was greater in yr 1 than in yr 2 (P = 0.02; Table 3). An interaction for S × Y tended to exist (P = 0.15) for 48-h ISNDFD, and again was evident (P = 0.03; Table 3) for 96-h ISNDFD. These interactions followed trends similar to that described for extrusa NDF concentrations, showing that an increase in fiber associated with plant senescesence corresponded to a decrease in rumen degradability. A decline in the disappearance of NDF as forages senesce is expected (Johnson et al., 1998). The ME content of the forage decreased (P < 0.01) in response to advancing season and was not different between years (P = 0.79; Table 3).

The interannual changes in forage quality described above appear to explain differences observed in animal performance as well as in basal metabolism throughout different seasons of the year. These relationships provide evidence that seasonal changes in nutritional environments influence the ability of range beef cows to utilize dietary nutrients provided by range forages.

Milk Yield

In the current study, estimates for milk yield were obtained approximately 70 ± 7 d postpartum, on June 1 and 2 for yr 1 and 2, respectively. Relative differences among cows in milk yield are expected to reflect differences in nutrient intake or partitioning at the initial GTT in both years of the study.

An A × Y interaction for 24-h milk yield was observed (P = 0.05; Table 4), which resulted from a change in rank among differently aged cows and a change in magnitude of the milk yield between the 2 yr of the study. Milk yields in this study were less than those of nonsup-

Table 4. Least squares means for age × year interactions for milk production and milk constituents collected approximately 70 d after parturition on June 1 in yr 1 and on June 2 in yr 2

Item	Year		SEM	*P*-value, age × year
	2004	2005		
2-yr-old cows	n = 6	n = 7		
Milk yield, kg/d	5.08	6.49	0.59	0.05
Protein, g/d	160	193	17	0.06
Lactose, g/d	258	337	29	0.04
Butterfat, g/d	219	299	26	0.07
Solids-not-fat, g/d	469	592	51	0.05
Milk urea-N, mg/100 mL	14.6	16.2	0.8	0.13
3-yr-old cows	n = 7	n = 7		
Milk yield, kg/d	7.64	6.88	0.56	0.05
Protein, g/d	255	209	17	0.06
Lactose, g/d	372	355	29	0.04
Butterfat, g/d	338	301	26	0.07
Solids-not-fat, g/d	701	630	48	0.05
Milk urea-N, mg/100 mL	13.0	15.6	0.7	0.13
4-yr-old cows	n = 7	n = 7		
Milk yield, kg/d	7.63	6.31	0.57	0.05
Protein, g/d	226	200	17	0.06
Lactose, g/d	389	322	29	0.04
Butterfat, g/d	304	285	27	0.07
Solids-not-fat, g/d	690	582	49	0.05
Milk urea-N, mg/100 mL	11.3	15.9	0.7	0.13

plemented 2-yr-old cows grazing pastures similar to those in the current study (Grings et al., 2005a). Lower milk yield may have occurred because cows were not supplemented, but it could also be related to the fact that peak milk yield had already transpired when milk yield was assessed. Grings et al. (2005a) indicated that peak milk yield for similar spring-calving 2-yr-old cows occurs at approximately d 61 of lactation. As might be expected, yields of all milk constituents were proportional to milk yield (Table 4). There was a tendency ($P = 0.06$) for cows of different ages to have dissimilar MUN concentrations; concentrations of MUN decreased linearly ($P = 0.02$) with increasing cow age (15.4, 14.3, and 13.6 ± 0.51 mg/100 mL for 2-, 3-, and 4-yr-old cows, respectively). Cows in yr 1 had lower concentrations of MUN than in yr 2 (13.0 vs. 16.0 ± 0.42 mg/100 mL; $P = 0.01$), which likely reflects the lower CP concentrations provided in the May forage in yr 1 compared with yr 2 (Table 3).

Improved forage quality observed in the spring of yr 2 (Table 3) was not directly evident in measurements of milk yield and milk constituents, which may suggest that dietary nutrients from more nutrient-dense forages may be partitioned away from milk production and toward other maternal needs, at least for multiparous cows in this study. However, for primaparous cows, the exact opposite appeared to be true, as shown in Table 4. These data suggest that cows of similar genetic makeup respond differently, depending on the year and the forage quality being consumed at the time when these milk production measurements were collected. Milk lactose, the main osmotic factor for milk yield, was lower in yr 2 for multiparous cows when forage quality was high (Table 4). Glucose influences milk yield through its conversion to lactose in mammary tissue, where it becomes the main osmotic factor determining fluid milk production (Vilotte, 2002). Although the relationships between milk yield, glucose availability, and cow age cannot be fully elucidated from this study, they indicate that some type of differential nutrient partitioning may be occurring when forages of different quality are consumed.

Animal Performance and Metabolism

The focus of the ensuing discussion is on the nature of observed interactions derived by single df estimable functions. Tables 5, 6, and 7 provide main effect estimates for treatments (i.e., Y, A, and P) and seasons (S1, S2, and S3). Data for main effects are discussed only when no significant ($P < 0.05$) S × treatment interaction was observed.

In the May to August interval of yr 1, there was little precipitation (Figure 1), but CP concentrations remained above cow requirements (NRC, 2000) through August. In yr 2 more precipitation was received through June, but forage CP declined below cow requirements by August because of earlier senescence of forage in yr 2. This phenomenon most likely occurred in response to inadequate moisture and elevated summer temperatures in yr 2. Other forage quality indicators followed trends similar to that observed for CP (Table 3). These

Table 5. Estimates of single df effects (estimate ± SEM) for treatments and seasons for BW, BCS, and serum metabolites before administration of the glucose tolerance tests

Item[1]	BW,[2] kg	BCS[3]	Glucose,[4] mg/100 mL	Insulin,[5] ng/mL	Urea-N,[6] mg/100 mL	NEFA,[7] μ*M*
Treatment						
Y	11 ± 15	0.02 ± 0.15	–1.5 ± 5.2	–1.43 ± 0.12*	–2.99 ± 0.29*	155 ± 48*
A1	79 ± 18*	0.73 ± 0.17*	4.2 ± 6.2	–0.10 ± 0.15	0.28 ± 0.34	68 ± 57
A2	73 ± 15*	0.30 ± 0.14*	4.0 ± 5.1	–0.12 ± 0.12	–0.06 ± 0.28	55 ± 48
Y × A1	–21 ± 18	0.47 ± 0.17*	–4.3 ± 6.2	0.11 ± 0.15	0.06 ± 0.34	101 ± 57
Y × A2	–8 ± 15	–0.45 ± 0.14*	2.8 ± 5.2	0.20 ± 0.12	0.15 ± 0.28	104 ± 48*
P	–63 ± 18*	–1.72 ± 0.17*	–3.2 ± 6.2	–0.45 ± 0.15*	–0.43 ± 0.34	112 ± 57
Y × P	25 ± 18	–0.30 ± 0.17	–0.2 ± 6.2	0.10 ± 0.15	–0.09 ± 0.34	–16 ± 57
A1 × P	–17 ± 18	–0.21 ± 0.17	1.2 ± 6.2	–0.16 ± 0.15	–0.64 ± 0.34	–40 ± 57
Seasons						
S1	47 ± 5.3*	0.67 ± 0.10*	–9.4 ± 2.1*	0.62 ± 0.15*	–6.69 ± 0.31*	–2 ± 48
S2	35 ± 5.4*	–0.37 ± 0.10*	–3.9 ± 2.2	–0.35 ± 0.15*	7.54 ± 0.31*	305 ± 50*
S3	29 ± 3.8*	0.13 ± 0.07	3.3 ± 1.6*	0.08 ± 0.11	–1.39 ± 0.22*	470 ± 35*

[1]Y = year; A1 = 2- vs. 3-yr-old cows; A2 = 2- + 3- vs. 4-yr-old cows; P = pregnant vs. nonpregnant; S1 = May glucose tolerance test vs. August glucose tolerance test; S2 = December glucose tolerance test vs. March glucose tolerance test; S3 = May and August glucose tolerance test vs. December and March glucose tolerance test.
[2]Overall mean = 547.7 ± 7.3.
[3]Scale from 1 to 9 (1 = emaciated to 9 = extremely obese). Overall mean = 5.8 ± 0.1.
[4]Overall mean = 75.7 ± 2.5.
[5]Overall mean = 1.3 ± 0.01.
[6]Overall mean = 7.4 ± 0.1.
[7]Overall mean = 564 ± 23.
*$P \leq 0.05$.

differences between years in forage quality manifested themselves as S × Y interactions (S1 × Y; Table 8) affecting BW and BCS. In yr 1, cows gained less weight but gained more BCS from May to August than in yr 2, when weight gain was greater than in yr 1 but BCS remained constant during the May to August period. The increase in cow BCS was more evident in yr 1, when cows began the study at a lower BW (Table 8). The beginning of study BW and BCS for all cows were 473 kg with a 4.7 BCS in yr 1, and 513 kg with a 5.3 BCS in yr 2. This may indicate that BCS is more sensitive to weight gain in thinner (lighter weight) animals than

Table 6. Estimates of single df interaction effects (estimate ± SEM) for treatments and seasons for glucose responses from glucose tolerance tests

Item[1]	Glucose peak,[2] mg/100 mL	Glucose peak time,[3] min	Glucose clearance rate,[4] mL/min	Glucose half-life,[5] min	Glucose area under the curve,[6] (mg/100 mL)/min
Treatment					
Y	–20 ± 8.5*	0.46 ± 0.27	0.01 ± 0.17	–0.5 ± 5.8	–270 ± 1,407
A1	13 ± 10.1	0.10 ± 0.32	–0.31 ± 0.20	7.3 ± 6.9	1,508 ± 1,670
A2	2 ± 8.4	0.04 ± 0.26	–0.09 ± 0.17	1.9 ± 5.7	1,243 ± 1,368
Y × A1	–11 ± 10.1	0.22 ± 0.32	–0.22 ± 0.20	2.7 ± 6.9	–342 ± 1,670
Y × A2	1 ± 8.5	0.09 ± 0.27	–0.08 ± 0.17	1.0 ± 5.8	303 ± 1,401
P	–15 ± 10.1	–0.09 ± 0.32	–0.18 ± 0.20	7.9 ± 6.9	–893 ± 1,670
Y × P	5 ± 10.1	–0.51 ± 0.32	0.07 ± 0.20	–1.9 ± 6.9	–733 ± 1,670
A1 × P	3 ± 10.1	–0.12 ± 0.32	0.12 ± 0.20	–2.1 ± 6.9	–381 ± 1,670
Seasons					
S1	26 ± 8.8*	–0.47 ± 0.33	0.32 ± 0.12*	–7.1 ± 4.1	–557 ± 482
S2	–30 ± 9.1*	0.06 ± 0.34	–0.43 ± 0.13*	8.8 ± 4.2*	–730 ± 496
S3	34 ± 6.3*	–0.06 ± 0.24	–0.24 ± 0.09*	4.9 ± 3.0	2,401 ± 350*

[1]Y = year; A1 = 2- vs. 3-yr-old cows; A2 = 2- + 3- vs. 4-yr-old cows; P = pregnant vs. nonpregnant; S1 = May glucose tolerance test vs. August glucose tolerance test; S2 = December glucose tolerance test vs. March glucose tolerance test; S3 = May and August glucose tolerance test vs. December and March glucose tolerance test.
[2]Overall mean = 242.1 ± 4.1.
[3]Overall mean = 3.6 ± 0.1.
[4]Overall mean = 1.7 ± 0.1.
[5]Overall mean = 44.6 ± 2.8.
[6]Overall mean = 16,966 ± 677.
*$P \leq 0.05$.

Table 7. Estimates of single df interaction effects (estimate ± SEM) for treatments and seasons for insulin responses derived from glucose tolerance tests

Item[1]	Insulin peak,[2] ng/mL	Insulin peak time,[3] min	Insulin area under the curve,[4] (ng/mL)/min
Treatment			
Y	–6.9 ± 1.3*	0.48 ± 0.68	–472 ± 42*
A1	–3.4 ± 1.5*	0.27 ± 0.81	–57 ± 50
A2	–1.4 ± 1.37	–0.01 ± 0.68	–8 ± 42
Y × A1	2.3 ± 1.5	–0.39 ± 0.81	92 ± 50
Y × A2	0.4 ± 1.3	–0.39 ± 0.68	12 ± 42
P	–2.8 ± 1.5	–0.40 ± 0.81	–144 ± 50*
Y × P	0.6 ± 1.5	1.14 ± 0.81	27 ± 50
A1 × P	0.2 ± 1.5	–1.04 ± 0.81	–11 ± 50
Season			
S1	2.6 ± 0.60*	0.20 ± 0.92	181 ± 37*
S2	–3.7 ± 0.63*	–1.42 ± 0.95	–220 ± 38*
S3	0.9 ± 0.44*	3.45 ± 0.66*	132 ± 27*

[1]Y = year; A1 = 2- vs. 3-yr-old cows; A2 = 2- + 3- vs. 4-yr-old cows; P = pregnant vs. nonpregnant; S1 = May glucose tolerance test vs. August glucose tolerance test; S2 = December glucose tolerance test vs. March glucose tolerance test; S3 = May and August glucose tolerance test vs. December and March glucose tolerance test.
[2]Overall mean = 8.2 ± 0.6.
[3]Overall mean = 9.0 ± 0.3.
[4]Overall mean = 417 ± 20.
*$P \leq 0.05$.

in animals of heavier weights. This phenomenon is consistent with data presented by the NRC (2000) that demonstrate that lighter weight animals, of a similar mature weight class, require less BW gain to achieve the next BCS than do heavier weight animals of the same mature BW classification. On average, milk yield was greater in yr 1 than in yr 2 (Table 4), which reflects changes in forage quality as well as in BW and BCS measurements.

Changes in baseline concentrations (measurements before glucose challenge) of serum metabolites between May and August were different for the 2 yr (S1 × Y interaction; Table 8). Serum glucose concentrations are tightly regulated and dependent on a variety of factors that influence equilibrium between glucose entry and clearance by tissues (Kaneko, 1989). Baseline glucose concentrations declined from May to August in both years, but the magnitude of decrease in glucose concentrations was greater in yr 1 than yr 2. On the other hand, serum insulin concentrations, which are largely responsible for the regulation of circulating glucose concentrations, increased in both years between May and August, with a larger increase in yr 1 than in yr 2. The increase in baseline insulin and decrease in baseline concentrations of glucose that occurred between samples collected in May and August suggest that nonmammary tissues are responsive to the actions of insulin and sequestering nutrients, thereby promoting increases in BW and BCS.

The S1 × Y interaction was also observed for serum urea-N concentrations. In the interval from May to August, serum urea-N concentrations declined ($P < 0.01$) as the season progressed, in concert with declines observed in forage extrusa CP (Table 3). The interaction resulted from a more substantial decline in serum urea-N concentrations for cows in yr 2 than in yr 1. These results demonstrate the impact of inadequate summer precipitation on range forages, causing forages to senesce, and on the subsequent metabolic changes encountered by grazing ruminants when consuming these senescent forages (Table 8).

An additional measure used to evaluate seasonal nutrient status for range livestock was serum NEFA concentrations. Again, an S1 × Y interaction was observed for serum NEFA concentrations. This interaction also followed trends in forage quality (Table 3), in which cows in yr 1 experienced a decline in serum NEFA concentrations but cows in yr 2 experienced an increase in serum NEFA concentrations from May to August (Table 8).

Before the December to March interval of yr 1, there was more late summer and early fall precipitation than in yr 2. However, extrusa CP concentrations in yr 1 were below cow requirements in December and increased to adequate concentrations by March (NRC, 2000), whereas the fall precipitation received in yr 2 allowed for adequate CP concentrations that met cow requirements in December and then declined below requirements for range beef cows by March (NRC, 2000). Changes in cow BW and BCS between December and March differed between years (S2 × Y; Table 8). In yr 1, cows gained more weight than in yr 2 and experienced a slight decrease in BCS from December to March, whereas in yr 2, weight gain was less than in yr 1 and BCS remained constant during the December to March period.

The S2 × Y interaction also affected baseline serum metabolite concentrations (Table 8). Serum insulin concentrations were greater in yr 1 and decreased from December to March, whereas in yr 2 serum insulin concentrations did not change from December to March. The physiological significance of this interaction is not clear because no differences in glucose concentrations were observed for the December to March period. Serum urea-N and NEFA concentrations were also affected by the S2 × Y interaction (Table 8). In yr 1, cows experienced greater increases in serum urea-N and NEFA concentrations from December to March than in yr 2 (Table 8). These changes in serum metabolites reflect changes in forage quality (Table 3).

The S2 seasonal comparison (May and August vs. December and March) compares a season when forage is generally actively growing and of greater nutritive value with a season when forages are senescent and commonly considered inadequate or marginal in nutritive value. These differences between seasons and years manifested themselves as an S × Y interaction (S2 × Y; Table 8), affecting BW and BCS. In yr 1, cows gained more weight than in yr 2 and increased in BCS from May and August to December and March, whereas in

Table 8. Significant estimates of single df interactions between treatments and seasons (estimate ± SEM) for BW, BCS, and serum metabolites before administration of glucose tolerance test

Item[1]	BW,[2] kg	BCS[3]	Glucose,[4] mg/100 mL	Insulin,[5] ng/mL	Urea-N,[6] mg/100 mL	NEFA,[7] μM
S1 × Y	11 ± 5.5*	–0.46 ± 0.10*	6.7 ± 2.2*	–0.36 ± 0.15*	–2.35 ± 0.33*	154 ± 50*
S2 × Y	–15 ± 5.5*	0.22 ± 0.10*	–4.3 ± 2.2	0.57 ± 0.16*	–6.09 ± 0.32*	–271 ± 51*
S3 × Y	–21 ± 3.9*	–0.42 ± 0.07*	1.4 ± 1.6	–0.04 ± 0.10	–3.75 ± 0.23*	173 ± 36*
S2 × A1	–2 ± 6.6	–0.04 ± 0.12	3.7 ± 2.7	–0.19 ± 0.18	–1.15 ± 0.38*	–40 ± 60
S3 × A1	–9 ± 4.7	–0.01 ± 0.09	–2.9 ± 1.9	0.34 ± 0.13*	–0.20 ± 0.27	57 ± 43
S2 × A2	–7 ± 5.6	–0.14 ± 0.10	2.6 ± 2.3	0.31 ± 0.16*	0.19 ± 0.32	37 ± 51
S3 × A2	–5 ± 4.0	–0.03 ± 0.07	3.7 ± 1.6*	–0.00 ± 0.11	0.32 ± 0.22	48 ± 36
S1 × (Y × A1)	6 ± 6.5	–0.12 ± 0.12	5.6 ± 2.7*	0.07 ± 0.18	0.12 ± 0.39	55 ± 60
S3 × (Y × A1)	–3 ± 4.7	–0.13 ± 0.09	0.8 ± 1.9	–0.19 ± 0.13	–0.25 ± 0.27	118 ± 43*
S1 × P	–15 ± 6.5*	–0.51 ± 0.12*	–1.4 ± 2.7	–0.00 ± 0.18	–0.60 ± 0.39	–18 ± 60
S2 × P	15 ± 6.6*	0.11 ± 0.12	–4.6 ± 2.7	–0.05 ± 0.18	0.98 ± 0.38*	313 ± 60*
S3 × P	6 ± 4.7	0.14 ± 0.09	–2.6 ± 1.9	–0.17 ± 0.13	0.85 ± 0.27*	99 ± 43*
S1 × (Y × P)	8 ± 6.5	0.35 ± 0.12*	2.5 ± 2.7	–0.12 ± 0.18	0.21 ± 0.39	3 ± 60
S2 × (Y × P)	1 ± 6.6	–0.14 ± 0.12	2.9 ± 2.7	0.19 ± 0.18	–0.78 ± 0.38*	–78 ± 60
S3 × (Y × P)	10 ± 4.7*	0.13 ± 0.09	1.3 ± 1.9	0.19 ± 0.13	0.01 ± 0.27	–34 ± 43
S2 × (A1 × P)	2 ± 6.6	0.03 ± 0.12	–1.8 ± 2.7	0.54 ± 0.18*	0.73 ± 0.38	25 ± 60

[1]Y = year; A1 = 2- vs. 3-yr-old cows; A2 = 2- + 3- vs. 4-yr-old cows; P = pregnant vs. nonpregnant; S1 = May glucose tolerance test vs. August glucose tolerance test; S2 = December glucose tolerance test vs. March glucose tolerance test; S3 = May and August glucose tolerance test vs. December and March glucose tolerance test.
[2]Overall mean of 548 ± 7.3.
[3]Scale from 1 to 9 (1 = emaciated to 9 = extremely obese). Overall mean = 5.8 ± 0.1.
[4]Overall mean = 75.7 ± 2.5.
[5]Overall mean = 1.3 ± 0.01.
[6]Overall mean = 7.4 ± 0.1.
[7]Overall mean = 564 ± 23.
*$P \leq 0.05$.

yr 2, weight gain was less than in yr 1 and BCS did not change across this seasonal comparison.

The S3 × Y interaction also affected baseline serum urea-N and NEFA concentrations (Table 8). In yr 1, cows experienced an increase in serum urea-N and NEFA concentrations from May and August to December and March, whereas in yr 2, serum urea-N decreased and NEFA concentrations increased more than in yr 1 during the May and August to December and March period. These data on BW, BCS, serum metabolite, and insulin collectively indicate that interannual variation in these phenotypic measurements occur and are directly influenced by forage quality.

In addition to consistent S × Y interactions for phenotypic measurements, the S × physiological status interaction (S1 × P; Table 8) identified consistent BW and BCS changes. Nonpregnant cows gained more weight and increased in BCS from May to August, whereas pregnant cows gained less weight and BCS remained constant during the May to August period.

In the December to March interval, when pregnant cows were in the last 2 trimesters of pregnancy and consuming low-quality range forage (Table 3), available nutrients provided by senescent forage were often inadequate to meet requirements for the dam and developing fetus, resulting in catabolism of maternal tissue to accommodate the demands of pregnancy. In the current study, changes in BW from December to March differed because of pregnancy status (S2 × P; Table 8). Nonpregnant cows gained less weight than pregnant cows from December to March, whereas pregnant cows gained weight with no observed differences in BCS, which indicates that the observed weight gain for pregnant cows most likely reflected gain of the growing fetus.

Pregnancy status also affected changes in baseline concentrations of urea-N and NEFA in serum samples between December and March (S2 × P; Table 8). Nonpregnant cows experienced both an increase in serum urea-N and a decrease in NEFA concentrations from December to March, whereas pregnant cows expressed a greater increase in serum urea-N and an increase in NEFA concentrations during this sampling period.

Pregnancy and lactation status also influenced the changes observed for serum urea-N and NEFA concentrations in the seasonal comparison of May and August vs. December and March (S3 × P; Table 8). Nonpregnant, nonlactating cows experienced a greater decrease in serum urea-N than did pregnant cows, accompanied by a decrease in NEFA concentrations from May and August vs. December and March, whereas pregnant cows expressed a smaller decrease in serum urea-N and an increase in NEFA concentrations during May and August vs. December and March. These data on BW, BCS, and serum metabolites indicate that physiological state and quality of forage consumed influence cow performance.

Table 9. Significant estimates of single df interaction effects between treatments and seasons (estimate ± SEM) for glucose responses derived from glucose tolerance tests

Item[1]	Glucose peak,[2] mg/100 mL	Glucose peak time,[3] min	Glucose clearance rate,[4] mL/min	Glucose half-life,[5] min	Glucose area under the curve,[6] (mg/100 mL)/min
S1 × Y	19 ± 9.2*	–0.78 ± 0.35	0.31 ± 0.13*	–3.5 ± 4.3	1,155 ± 502*
S3 × Y	–19 ± 6.5*	0.04 ± 0.25	–0.10 ± 0.09	4.7 ± 3.0	279 ± 356
S3 × A1	2 ± 7.7	0.23 ± 0.29	0.28 ± 0.11*	–7.1 ± 3.6	–469 ± 423
S2 × A2	–8 ± 9.3	–0.86 ± 0.35	–0.10 ± 0.13	1.9 ± 4.4	299 ± 510
S3 × (Y × A2)	–3 ± 6.5	–0.12 ± 0.24	0.07 ± 0.09	–2.9 ± 3.0	–810 ± 354*
S2 × P	25 ± 11.0*	–0.79 ± 0.42	0.38 ± 0.15*	–12.3 ± 5.1	–1,330 ± 599*
S3 × P	–18 ± 7.7*	0.00 ± 0.29	–0.07 ± 0.11	0.8 ± 3.6	–496 ± 423
S2 × (Y × P)	–34 ± 11.0*	–0.96 ± 0.42	–0.19 ± 0.15	–0.3 ± 5.1	–120 ± 599
S3 × (Y × P)	1 ± 7.7	–0.16 ± 0.29	–0.15 ± 0.11	7.9 ± 3.6	725 ± 423

[1]Y = year; A1 = 2- vs. 3-yr-old cows; A2 = 2- + 3- vs. 4-yr-old cows; P = pregnant vs. nonpregnant; S1 = May glucose tolerance test vs. August glucose tolerance test; S2 = December glucose tolerance test vs. March glucose tolerance test; S3 = May and August glucose tolerance test vs. December and March glucose tolerance test.
[2]Overall mean = 242 ± 4.1.
[3]Overall mean = 3.6 ± 0.1.
[4]Overall mean = 1.7 ± 0.1.
[5]Overall mean = 44.6 ± 2.8.
[6]Overall mean = 16,966 ± 677.
*$P \leq 0.05$.

Glucose Tolerance Test

To ascertain whether cows grazing rangelands in the Northern Great Plains experience periodic nutrient stresses that render maternal tissue less responsive to the actions of insulin, metabolic challenges can be administered. In the current study, a series of seasonal GTT were conducted to measure insulin sensitivity. The ability of range cows to clear or sequester glucose into tissues following a physiological dose of glucose directly reflects the degree or extent of a metabolic imbalance that a cow may be experiencing (Kaneko, 1989).

A significant interaction (S1 × Y) for peak glucose and time required to reach peak glucose concentration revealed that cows in yr 1 had similar peak glucose concentrations and an extended time to reach peak glucose between May and August, whereas in yr 2, peak glucose concentrations increased and peak time was reduced during this sampling period (Table 9). In addition, an S1 × Y interaction for glucose AUC revealed that in yr 1, glucose AUC decreased from May to August, whereas in yr 2, glucose AUC increased during this sampling period. No S1 × Y interactions were observed for insulin peak concentration, insulin peak time, or insulin AUC, indicating that regardless of season (May vs. August) and year, cow insulin responses to the GTT were similar (Table 10).

Two critical and related measurements associated with a GTT are glucose clearance rate (mL/min) and glucose half-life (min). These measurements identify metabolic imbalances that may result from the diet quality and physiological status of the cows. An interaction (S1 × Y; Table 9) for glucose clearance rate resulted from cows in yr 1 experiencing greater decreases in clearance rates between May and August than cows in yr 2. No treatment effect ($P = 0.58$; Table 6) or S × treatment interaction ($P = 0.16$) was observed for glucose half-life, but an S effect was present ($P = 0.02$; Table 6). The significant S effect for glucose half-life demonstrates that seasonal changes in forage quality may create nutritional metabolic imbalances in range cows that alter energy metabolism. Glucose half-lives for May, August, December, and March were 46, 39, 43, and 51 ± 3.9 min, respectively. Seasonal effects revealed a tendency ($P = 0.09$) for glucose half-life to decrease between May and August, but to increase ($P = 0.04$) between December and March. A tendency ($P = 0.09$) for increased glucose half-life between the combined seasons of May and August (high forage nutrient density; summer) vs. December and March (low forage nutrient density; winter; Table 6) was also revealed. Kaneko (1989) indicated that a "normal" glucose half-life is approximately 35 min, and Richards et al. (1989)

Table 10. Significant estimates of single df interactions between treatments and seasons (estimate ± SEM) for insulin responses derived from glucose tolerance tests

Item[1]	Insulin peak,[2] ng/mL	Insulin peak time,[3] min	Insulin area under the curve,[4] (ng/mL)/min
S2 × Y	3.4 ± 0.63*	1.1 ± 0.97	307 ± 39*
S3 × Y	–1.3 ± 0.45*	0.3 ± 0.68	–111 ± 27*
S2 × (A1 × P)	1.6 ± 0.76*	–1.2 ± 1.15	99 ± 46*

[1]Y = year; A1 = 2- vs. 3-yr-old cows; A2 = 2- + 3- vs. 4-yr-old cows; P = pregnant vs. nonpregnant; S1 = May glucose tolerance test vs. August glucose tolerance test; S2 = December glucose tolerance test vs. March glucose tolerance test; S3 = May and August glucose tolerance test vs. December and March glucose tolerance test.
[2]Overall mean = 8.2 ± 0.6.
[3]Overall mean = 9.0 ± 0.3.
[4]Overall mean = 417 ± 20.
*$P \leq 0.05$.

suggests that tissue responsiveness to insulin can decrease as nutritional restrictions increase, but that responsiveness can be restored by providing the limiting nutrients.

In the current study, glucose half-life increased from August to March, indicating a decrease in tissue sensitivity to insulin even as forage quality improved from December to March (Table 3). The shorter glucose half-life in December (poorer forage quality) than in March (better forage quality) is likely related to stage of gestation (i.e., late third trimester in March) because of the nutritional demands of the fetus and of approaching parturition. In addition, March in the Northern Great Plains is the start of the growing season and the nutritional quality of forages would have begun increasing only shortly before the March GTT. Waterman et al. (2006) demonstrated that increasing the MP supply in supplements provided to postpartum spring-calving range cows decreased glucose half-life and days to resumption of estrus. Collectively, these data support the idea that developing and implementing strategic supplementation regimes in autumn through spring may help augment nutritional imbalances experienced by range cows grazing senescent forages.

The principle glucogenic precursor in ruminants is propionate (Bell and Bauman, 1997), an end product of ruminal fermentation. Cows grazing senescent range forages typically experience VFA fermentation, which yields a large acetate:propionate ratio. A low supply of propionate can lead to catabolism of other substrates for gluconeogenesis (i.e., glucogenic AA, glycerol, and lactate), which must be provided by the diet or from catabolism of maternal tissues to meet cow requirements. Consequently, tissue resistance to insulin may be a mechanism to conserve glucose for specific, non-insulin-dependent functions (Huntington and Richards, 2005). Therefore, supplementation regimes that include glucogenic precursors may minimize this effect of insulin-insensitive tissues during seasons known to induce nutritional stresses.

An interaction ($P < 0.01$; S2 × Y) for peak insulin concentrations and insulin AUC revealed that cows in yr 1 experienced decreases in peak insulin concentrations and insulin AUC from December to March, whereas cows in yr 2 experienced a smaller decrease in peak insulin concentration and an increase in insulin AUC from December to March (Table 10). Again, forage quality measurements (Table 3) helped explain the differences between years. Late fall precipitation in yr 2 permitted some fall forage production to occur, which resulted in better forage quality than in yr 1. Improved nutrient availability of forages during December in yr 2 may have improved the metabolic balance and increased tissue responsiveness to insulin compared with cows in December of yr 1. Furthermore, these results for the December to March period for peak insulin concentrations and insulin AUC occurred independently from what was observed for peak glucose concentration, glucose clearance rate, glucose half-life, or glucose AUC (Table 9).

Time to achieve the peak insulin response did not differ by treatment ($P = 0.66$; Table 7), nor was there an effect of S × treatment ($P = 0.32$; Table 10). However, a seasonal difference was detected ($P = 0.01$; Table 7) for May, August, December, and March (7.2, 7.4, 11.4, and 10.0 ± 0.7 min, respectively), with a difference ($P = 0.01$) occurring between May and August (high forage nutrient density) vs. December and March (low forage nutrient density; Table 7). 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). Furthermore, our insulin data coincide with the forage quality measurements observed (see Table 3) and demonstrate that tissues may respond differently to insulin, depending on the season and quality of forage being consumed.

In conclusion, this study documents that rangelands of the Northern Great Plains experience seasonal variation in forage quality and that this variation is directly associated with the timing and amount of precipitation received. Furthermore, as seasons progress from spring (period of predominant forage production; April through June) to fall and gestation progresses, forage quality declines, creating nutritional imbalances in range beef cows. As a result of these nutritional imbalances, range beef cows exhibit seasonal metabolic imbalances and tissues become less responsive to the actions of insulin, which results in a longer glucose half-life. The increase in glucose half-life might exacerbate the nutritional imbalance by starving tissues of metabolic energy (glucose) as well as other essential dietary nutrients.

It is well documented in the scientific literature that protein is often the limiting nutrient in mature vegetation (Krysl et al., 1987; Wallace, 1987) and that protein supplementation improves intake and subsequent digestibility of mature vegetation (Owens et al., 1991). The objectives of the current study were not to ignore these well-established facts, but rather to identify seasonal or production stages when range cows would begin to experience metabolic imbalances and to use endocrine and metabolic responses to evaluate these imbalances when they occurred. Forage diets promote high ruminal acetate production relative to propionate (Cronje et al., 1991), and acetate does not contribute to gluconeogenesis. However, as the proportion of glucogenic precursors from the diet increase, net glucose synthesis may increase and allow higher rates of acetate oxidation (Preston and Leng, 1987). In contrast, acetate accumulation resulting from an inadequate supply of glucogenic precursors to the tricarboxylic acid cycle consequently results in the production of ketones and FFA (Dresner et al., 1999; Schmitz-Peiffer et al., 1999; Tardif et al., 2001), exacerbating the metabolic imbalance. Therefore, compensation for nutritional limitations that range cows experience in the Northern Great Plains and throughout the western United States

may require the development and implementation of strategic supplementation regimes that supply glucogenic precursors, thereby improving range beef cow performance by minimizing the catabolism of maternal tissues during these periods of nutritional stress.

Seasonal alterations in glucose metabolism, as influenced by tissue responsiveness to insulin, in beef cows grazing rangelands in the Northern Great Plains, occur in relation to forage quality. Altered metabolism may ultimately affect physiological performance and reproductive function in range beef cows. Therefore, formulating range supplements for range beef cows, which target known periods of nutritional imbalance with glucogenic precursors, may provide a mechanism to more consistently alter metabolic functions and improve cow performance.

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