Effects of energy supplementation frequency and forage quality on performance, reproductive, and physiological responses of replacement beef heifers[1]

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ABSTRACT: The objective of this study was to compare performance, physiological, and reproductive responses of beef heifers consuming forages differing in nutritional quality and offered a low-starch energy supplement at 2 different frequencies. Forty-eight Brahman × British heifers (initial age = 294 ± 3 d) were allocated into 1 of 16 drylot pens (3 heifers/pen) which were randomly assigned to receive, in a 2 × 2 factorial arrangement of treatments: 1) low-quality hay [LQ; stargrass (*Cynodon nlemfuensis*) with 8% CP and 81% NDF, DM basis] and daily supplementation (S7); 2) LQ and supplementation 3 times weekly (S3); 3) medium-quality hay [MQ; bermudagrass (*C. dactylon*) with 12% CP and 74% NDF, DM basis] and S7; and 4) MQ and S3. Throughout the study (d 0 to 120), hay was offered in amounts to ensure ad libitum access, and a supplement based on soybean hulls and wheat middlings was offered at weekly rates of 15.8 and 7.9 kg/heifer (DM basis) for LQ and MQ, respectively. Forage and total DMI were evaluated daily, from d 20 to 26, d 34 to 40, and d 48 to 54. Blood samples were collected weekly for determination of plasma progesterone to evaluate puberty attainment. Blood samples were also collected daily, from d 13 to 16, d 27 to 30, d 41 to 44, and d 55 to 58 for determination of plasma urea nitrogen (PUN), glucose, insulin, IGF-I, and NEFA. On d 60, heifers were reallocated by treatment into 4 paddocks and exposed to Angus bulls (1:12 bull:heifer ratio) until d 120. Date of conception was estimated retrospectively by subtracting gestation length (286 d) from the calving date. Heifers receiving S7 had similar ($P = 0.52$) ADG compared with S3 heifers (0.27 vs. 0.25 kg/d). Heifers provided S7 had less daily variation in hay DMI and plasma concentrations of glucose, NEFA, and IGF-I compared with S3 cohorts (supplementation frequency × day interaction; $P < 0.01$). Similarly, heifers offered MQ and LQ and receiving S7 had less daily variation in total DMI, energy and protein intake, and plasma concentrations of PUN compared with heifers offered MQ and LQ and receiving S3 (hay quality × supplementation frequency × day interaction; $P < 0.01$). Attainment of puberty and pregnancy were hastened in S7 heifers compared with S3 heifers (supplementation frequency × week interaction; $P < 0.02$). Therefore, reproductive development of beef replacement heifers consuming diets based on low- and medium-quality forages are enhanced when low-starch energy supplements are offered daily instead of 3 times weekly.

Key words: beef heifers, energy supplementation frequency, forage quality, performance, reproduction

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INTRODUCTION

Energy intake is the primary nutritional consideration for reproductive development of beef heifers (Mass, 1987). Hence, energy supplementation is often required in cow-calf production systems, particularly those based on low-quality forages (Schillo et al., 1992; Roberts et al., 1997). However, the expenses associated with energy supplementation can significantly increase production costs and become unattractive to cow-calf producers. A typical approach to decrease these expenses is to reduce the frequency of supplementation, such as 3 times weekly instead of daily, to minimize costs associated with labor, fuel, and equipment.

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Our research group recently demonstrated that replacement heifers consuming low-quality forages and receiving a low-starch energy supplement daily had greater growth rates, hastened puberty attainment, and improved pregnancy rates compared with cohorts supplemented 3 times weekly (Cooke et al., 2008). These outcomes were associated with reduced daily variation in circulating concentrations of hormones and metabolites, including insulin and glucose, resulting in enhanced energy utilization by daily-fed heifers. Conversely, Drewnoski et al. (2011) reported that stocker cattle consuming medium-quality hay (**MQ**) and supplemented with low-starch energy feed daily or 3 times weekly had similar growth rates. Therefore, we hypothesized that frequency of energy supplementation can be reduced without impacting performance and reproductive development if replacement heifers are consuming medium-quality forages. Based on this rationale, the objective of this study was to compare concentrations of hormones and metabolites associated with energy metabolism, DMI, growth rates, puberty attainment, and pregnancy rates of replacement heifers consuming low-quality hay (**LQ**; 8% CP, DM basis) or MQ (12% CP, DM basis), and receiving a low-starch energy supplement daily or 3 times weekly.

MATERIALS AND METHODS

Animals were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the University of Florida Institutional Animal Care and Use Committee.

The study was conducted from September through December 2010 at the University of Florida – IFAS, Range Cattle Research and Education Center, Ona. The experiment was divided into a sampling phase (September and October; d 0 to 59) and a breeding phase (November and December; d 60 to 120).

Experimental Design and Animals

Forty-eight Brahman × British heifers (initial BW = 241 ± 2 kg; initial age = 294 ± 3 d) were used in the study. During the sampling phase (d 0 to 59), heifers were ranked by initial BW and age, and randomly allocated into 16 drylot pens (15 × 5 m; 3 heifers/pen). Pens were assigned to receive, in a 2 × 2 factorial arrangement, 1 of the 4 treatment combinations: 1) LQ [stargrass (*Cynodon nlemfuensis*)] and daily supplementation (**S7**); 2) LQ and supplementation 3 times weekly (**S3**); 3) MQ [bermudagrass (*C. dactylon*)] and S7; and 4) MQ and S3. Pen was considered the experimental unit (4 pens/treatment combination). For the breeding phase (d 60 to 120), heifers were re-allocated by treatment into 4 bahiagrass (*Paspalum notatum*) pastures previously mowed to the shortest possible stubble height (5 cm) to ensure that no forage was available for grazing, and exposed to mature Angus bulls.

Diets

Throughout the study (d 0 to 120), hay was offered in amounts to ensure ad libitum access, and low-starch energy supplement was offered at weekly rates of 15.8 and 7.9 kg/heifer (DM basis) for LQ and MQ, respectively. This supplementation rate was designed to result in weekly iso-caloric and iso-nitrogenous DMI between LQ and MQ heifers based on nutritional quality of hay and supplement, heifer initial BW and age, and predicted intake (NRC, 1996) to support initial ADG of 0.40 kg/heifer daily. Supplement and hay were not mixed. Heifers were offered supplements at 0700 h, daily (**S7**) or on Mondays, Wednesdays, and Fridays (**S3**). Supplement was completely consumed within 1 h by S7 heifers and within 6 h by S3 heifers. A complete commercial mineral/vitamin mix (14% Ca, 9% P, 24% NaCl, 0.20% K, 0.30% Mg, 0.20% S, 0.005% Co, 0.15% Cu, 0.02% I, 0.05% Mn, 0.004% Se, 0.3% Zn, 0.08% F, and 82 IU/g of vitamin A) and water were also offered for ad libitum consumption throughout the experiment. Hay and supplement samples were analyzed for nutrient content by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). All samples were analyzed by wet chemistry procedures for concentrations of CP (method 984.13; AOAC, 2006), ADF (method 973.18 modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp., Fairport, NY; AOAC, 2006), and NDF (method for use in an Ankom 200 fiber analyzer, Ankom Technology Corp.; Van Soest et al., 1991). Calculations of TDN used the equation proposed by Weiss et al. (1992), whereas NE_m and NE_g were calculated with the equations proposed by the NRC (1996). Composition of supplement and nutritional profile of hay and supplement are described in Table 1.

Sampling

Heifers were weighed on 2 consecutive d to determine both full and shrunk (after 16 h of feed and water restriction) BW at the beginning (d 0 and d 1) and end of the experiment (d 120 and 121). Shrunk BW was used to determine heifer ADG during the study. Blood samples were collected weekly (Wednesday) throughout the entire experiment to determine onset of puberty according to plasma progesterone (**P4**) concentrations. Heifers were considered pubertal once plasma P4 concentrations were equal or greater than 1.5 ng/mL for 2 consecutive wk (Cooke and Arthington, 2009).

During the sampling phase, in addition to the weekly collections, blood samples were obtained once per d during 4 consecutive d, every other week, starting at 4

Table 1. Composition of the supplement, low-quality (LQ) and medium-quality (MQ) hay fed to replacement beef heifers throughout the study (d 0 to 120)[1,2]

Nutrient profile, DM basis	Supplement	LQ	MQ
DM, %	94.0	93.2	93.7
TDN,[3] %	70.5	50.5	52.0
CP, %	16.3	8.30	12.7
RDP, % of CP	43.5	65.0	60.5
NE_m,[4] Mcal/kg	1.56	0.826	0.881
NE_g,[4] Mcal/kg	0.968	0.275	0.352
NDF, %	50.9	80.9	74.1
ADF, %	30.2	45.0	44.1
Starch, %	4.90	0.350	1.20
Crude fat, %	3.60	1.050	0.900
Ca, %	0.650	0.320	0.355
P, %	0.525	0.170	0.230

[1] Supplements (as-fed basis) consisted of 49.0% soybean hulls, 30.3% wheat middlings, 12.2% dried distillers grain, 4.50% molasses, 0.800% calcium carbonate and 3.20% canola pellets.

[2] Values obtained from a commercial laboratory wet chemistry analysis (Dairy One Forage Laboratory, Ithaca, NY).

[3] Calculated as described by Weiss et al. (1992).

[4] Calculated with the equations proposed by the NRC (1988).

h after supplement was offered to determine concentrations of glucose, plasma urea nitrogen (**PUN**), insulin, NEFA, and IGF-I. These samples were collected from d 13 to 16, d 27 to 30, d 41 to 44, and d 55 to 58, which were classified as periods (**PR1**, **PR2**, **PR3** and **PR4**, respectively). Periods began on Monday and ended on Thursday. Hay and total DMI were quantified during the sampling phase, from d 20 to 26, d 34 to 40, and d 48 to 54, which were also classified as periods (**PR-A, PR-B, and PR-C**) that began and ended on Sundays. Hay DMI was evaluated by collecting and weighing orts daily and subtracting from the total hay offered within each pen. Samples of the offered and the refused hay were collected daily from each pen and dried for 48 h at 55°C in forced-air ovens for DM calculation. In addition, samples of offered hay and supplement were collected before the beginning of the study, as well as during DMI evaluation, pooled by day and period, and submitted to the commercial laboratory (Dairy One Laboratory) for nutrient analysis. During the breeding phase, each group was exposed to 1 mature Angus bull (1:12 bull:heifer ratio), and bulls were rotated weekly between groups to account for potential bull effects. All bulls used in this study were submitted to and approved by a breeding soundness evaluation (Chenoweth and Ball, 1980) before the breeding season. Heifer pregnancy status was verified by detecting a fetus with transrectal ultrasonography (5.0 MHz transducer, Aloka 500V, Wallingford, CT) 70 d after the end of the experiment. Date of conception was estimated retrospectively by subtracting gestation length (286 d; Reynolds et al., 1980) from the calving date.

Blood Analysis

Blood samples were collected via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing 158 USP units of freeze-dried sodium heparin, placed on ice immediately, and centrifuged at 2,400 × *g* for 30 min at 4ºC for plasma collection. Plasma was frozen at −20°C on the same day of collection.

Glucose and PUN concentrations were determined using quantitative colorimetric kits G7521 and B7551, respectively (Pointe Scientific, Inc., Canton, MI). Insulin concentrations were determined using a bovine-specific commercial ELISA kit (B1009; Endocrine Technologies Inc., Newark, CA). Concentrations of NEFA were determined using a commercial kit (HR Series NEFA – 2; Wako Pure Chemical Industries Ltd. USA, Richmond, VA) with the modifications described by Pescara et al. (2010). Concentrations of P4 were determined according to procedures described by Galvão et al. (2004). Concentrations of IGF-I were determined using a human-specific commercial ELISA kit (SG100; R&D Systems, Inc., Minneapolis, MN) with 100% cross-reactivity with bovine IGF-I. Nevertheless, this IGF-I procedure was validated for bovine samples using pools of plasma collected from yearling beef heifers 7 d after the administration of saline or sometribove zinc (250 mg subcutaneously), which is known to increase plasma IGF-I concentrations in beef cattle (Buskirk et al., 1996). Both plasma pools (low and high IGF-I) were included into each assay as quality controls. Across all assays, mean IGF-I concentration was 226 ± 3 ng/mL in samples from heifers receiving sometribove zinc, and 138 ± 5 ng/mL in samples from heifers that received saline. The intra- and inter-assay CV were, respectively, 1.9 and 4.9% for glucose, 7.2 and 9.5% for PUN, 8.7 and 8.8% for insulin, 2.3 and 3.4% for NEFA, 3.5 and 5.5% for IGF-I, and 8.2 and 12.8% for P4.

Statistical Analysis

Performance and physiological data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC) and Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. The model statement used for ADG contained the effects of hay quality, supplementation frequency, and the resultant interactions. Data were analyzed using pen(hay quality × supplementation frequency) and heifer(pen) as random variables. The model statement used for DMI and physiological parameters contained

the effects of hay quality, supplementation frequency, period, day, and all resultant interactions. The effect of day included: 1) days when all heifers (S3 and S7) were supplemented (**SUPPALL**), and 2) days that only S7 heifers were supplemented (**S7ONLY**). For DMI analysis, pen(hay quality × supplementation frequency) was included as random variable. For the analysis of physiological variables, pen(hay quality × supplementation frequency) and heifer(pen) were included as random variables. Puberty and pregnancy data were analyzed using the GLIMMIX procedure of SAS with Satterthwaite approximation. The model statement contained the effects of hay quality, supplementation frequency, wk of puberty or pregnancy attainment, and all resultant interactions. Data were analyzed using pen(hay quality × supplementation frequency) and heifer(pen) as random variables. All results are reported as least squares means and were separated using LSD. Significance was set at $P \leq 0.05$, tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to main effects if no interactions were significant, or according to the greatest-order interaction detected.

RESULTS AND DISCUSSION

We have demonstrated recently that reducing supplementation frequency of low-starch energy feeds was detrimental to performance and reproductive development of replacement beef heifers grazing low-quality forages (Cooke et al., 2008). Conversely, reducing supplementation frequency of low-starch energy feeds did not impact performance of stocker cattle receiving diets based on medium-quality hay (Drewnoski et al., 2011). Based on this discrepancy, the present study was designed to directly compare different supplementation frequencies within low-quality and medium-quality forages, but not compare the effects of hay quality on heifer growth, physiological, and reproductive parameters. Therefore, the results reported and discussed herein focus mainly on the effects of supplementation frequency on heifer development across hay qualities, or within hay quality when interactions including hay quality × supplementation frequency are detected ($P < 0.05$).

Heifers receiving S7 had similar ($P = 0.52$; data not shown) ADG compared with S3 heifers (0.27 vs. 0.25 kg/d; SEM = 0.02). These results differ from studies reporting increased ADG of cattle fed low-quality forages and offered low-starch energy supplements daily instead of 3 times weekly (Cooke et al., 2007a, 2008), but agree with studies reporting similar ADG of cattle fed greater-quality forages and offered energy supplements daily or infrequently (La Manna, 2002; Loy et al., 2008; Drewnoski et al., 2011). Nevertheless, attainment of puberty (Figure 1) and pregnancy (Figure 2) were hastened

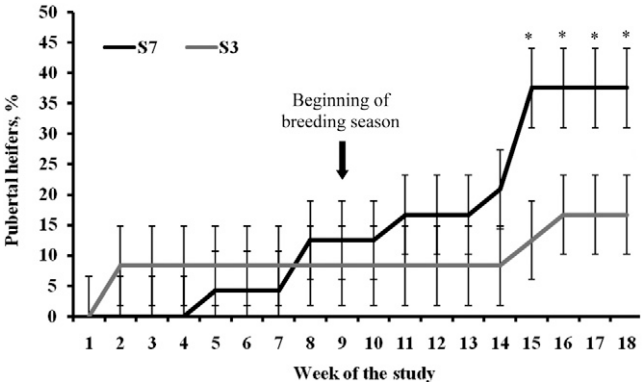

Figure 1. Puberty attainment of forage-fed replacement beef heifers offered a low-starch energy supplement daily (S7) or 3 times weekly (S3). Heifers were considered pubertal once plasma progesterone concentrations were greater than 1.5 ng/mL for 2 consecutive wk, and puberty attainment was declared at the first wk of elevated progesterone. Heifers were exposed to bull breeding (1:12 bull:heifer ratio) beginning on wk 9 of the study. A supplementation frequency × wk interaction was detected ($P = 0.01$). Treatment comparison within weeks: * $P = 0.05$.

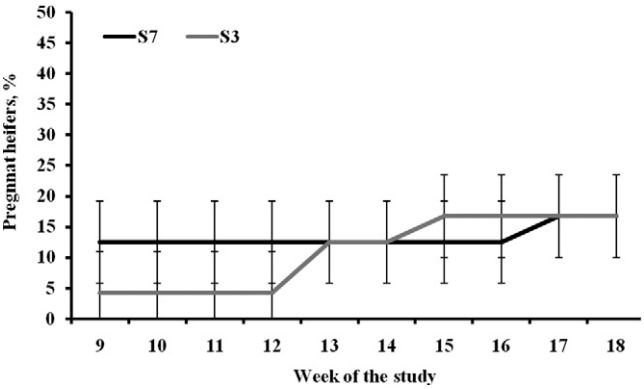

Figure 2. Pregnancy attainment of forage-fed replacement beef heifers offered a low-starch energy supplement daily (S7) or 3 times weekly (S3). Date of conception was estimated retrospectively by subtracting gestation length (286 d; Reynolds et al., 1980) from the calving date. A supplementation frequency × wk interaction was detected ($P = 0.02$) because S7 heifers became pregnant early in the breeding season (week effect; $P = 0.99$) whereas S3 heifers became pregnant later in the breeding season (week effect; $P < 0.01$).

(supplementation frequency × wk interaction; $P < 0.01$ and $P = 0.02$, respectively) in S7 heifers compared with S3 heifers, concurring with our previous research reporting that reproductive development and performance of replacement beef heifers are enhanced when low-starch energy supplements are offered daily instead of 3 times weekly (Cooke et al., 2008). However, caution should be adopted when interpreting treatment effects on pregnancy outcomes given that final pregnancy rates were lower than expected for S3 and S7 heifers (16.6 % of pregnant heifers/total heifers for both groups; $P = 0.99$; SEM = 7.6) according to previous work from our research group (Cooke et al., 2007b, 2008), and this matter is discussed later within this manuscript.

A supplementation frequency × day interaction was detected for hay DMI ($P < 0.01$; Table 2) because hay in-

Table 2. Estimated hay DMI and plasma concentrations of glucose, NEFA, and IGF-I of replacement beef heifers offered low-starch energy supplements daily (S7) or 3 times weekly (S3)[1]

Item [2]	S3	S7	SEM	*P*[3]
Hay DMI, kg/d				
SUPPALL	2.55	3.36	0.09	< 0.01
S7ONLY	3.15	3.38	0.09	0.12
P[4]	< 0.01	0.84		
Glucose, mg/dL				
SUPPALL	70.5	76.3	2.9	0.16
S7ONLY	80.5	76.7	2.9	0.31
P[4]	< 0.01	0.76		
NEFA, mEq/L				
SUPPALL	0.151	0.157	0.011	0.72
S7ONLY	0.176	0.158	0.011	0.32
P[4]	< 0.01	0.81		
IGF-I, ng/mL				
SUPPALL	64.1	82.7	5.8	0.04
S7ONLY	75.6	79.0	5.8	0.70
P[4]	< 0.01	0.12		

[1] Hay DMI was evaluated from each pen (3 heifers/pen) from d 20 to 26, d 34 to 40, and d 48 to 54 of the study (d 0 to 120). Blood samples were collected and harvested for plasma on d 13 to 16, d 27 to 30, d 41 to 44, and d 55 to 58 of the study.

[2] SUPPALL = days when all S3 and S7 heifers were supplemented; S7ONLY = days that only S7 heifers were supplemented.

[3] Day comparison within each supplementation frequency.

[4] Supplementation frequency comparison within each day.

take was greater for S7 heifers compared with S3 heifers on SUPPALL days ($P < 0.01$), but similar on S7ONLY days ($P = 0.12$). In addition, hay intake was similar (day effect; $P = 0.84$) across SUPPALL and S7ONLY days in S7 heifers, but reduced (day effect; $P < 0.01$) during SUPPALL compared with S7ONLY days in S3 heifers (Table 2). Supporting our results, other studies reported that forage DMI is associated negatively with intake of low-starch energy supplements due to substitution effect (Caton and Dhuyvetter, 1997; Kunkle et al., 2000; Bodine and Purvis, 2003), and feeding a low-starch energy supplement daily instead of 3 times weekly reduced the daily oscillation in forage intake (Cooke et al., 2007a). However, Loy et al. (2007) and Cooke et al. (2007a) reported that forage intake of infrequently supplemented cattle was reduced when energy supplements were offered, but greater on the remaining days compared with cattle supplemented daily. In the present study, forage DMI during S7ONLY days was similar between S7 and S3 heifers; therefore, mean hay DMI was greater ($P < 0.01$; data not shown) for S7 compared with S3 heifers (3.4 vs. 2.9 kg of DM daily, respectively; SEM = 0.09). Similarly, Loy et al. (2008) and Drewnoski et al. (2011) reported that cattle offered low-starch energy supplements 3 times weekly had reduced overall forage DMI compared with cohorts supplemented daily, and attributed this outcome to improved ruminal function and metabolism in daily-fed cattle. In the present study, however, ruminal parameters to support and elucidate the differences detected in hay DMI between S7 and S3 heifers were not evaluated.

A hay quality × supplementation frequency × day interaction was detected ($P < 0.01$) for total DMI (Table 3). For both LQ and MQ, total intake was greater ($P < 0.01$) for S3 heifers on SUPPALL days, but reduced ($P < 0.01$) on S7ONLY days compared with S7 heifers. Total DMI of S7 heifers was also similar (day effect; $P \geq 0.40$) across SUPPALL and S7ONLY days, but greater (day effect; $P < 0.01$) during SUPPALL compared with S7ONLY days in S3 heifers (Table 3), indicating that daily supplementation of low-starch feeds also prevented daily oscillations in total DMI in heifers receiving LQ or MQ. In addition, mean total DMI tended ($P = 0.07$; data not shown) to be greater for S7 compared with S3 heifers (5.07 vs. 4.60 kg of DM daily, respectively; SEM = 0.10). However, differences detected in mean hay and total DMI between S7 and S3 heifers were not substantial enough to impact heifer ADG. Based on hay and total DMI of each pen, estimated mean NE_g intake was greater ($P = 0.02$; data not shown) for S7 vs. S3 heifers (2.85 vs. 2.75 Mcal/d, respectively; SEM = 0.03), whereas estimated mean CP intake was similar ($P = 0.46$; data not shown) between both groups (0.63 vs. 0.64 kg/d, respectively; SEM = 0.01). These results suggest that similar mean CP intake between S7 and S3 heifers may have limited the benefits of greater NE_g intake on ADG of S7 heifers.

Conversely, the greater NE_g intake of S7 heifers may have contributed to their enhanced reproductive development compared with S3 heifers (Mass, 1987). Our previous research (Cooke et al., 2008) also suggested that daily supplementation of low-starch energy feeds enhanced dietary energy utilization and reproductive function of replacement heifers by reducing oscillation in nutrient intake and circulating concentrations of hormones and metabolites such as insulin and glucose. Indeed, in the present study, NE_m, NE_g, and CP intake of S3 heifers were greater during SUPPALL compared with S7ONLY days (day effect, $P < 0.01$), but did not vary (day effect; $P > 0.42$) within S7 heifers, independently if heifers received LQ and MQ (hay quality × supplementation frequency × day interaction, $P < 0.01$; Table 3). Accordingly, NE_m, NE_g, and CP intake were greater ($P < 0.01$) for S3 heifers on SUPPALL days, but reduced ($P < 0.01$) on S7ONLY days compared with S7 heifers (Table 3). Supporting these results and our rationale, supplementation frequency × day interactions were detected ($P < 0.01$) for plasma concentrations of glucose, NEFA, and IGF-I (Table 2), whereas a hay quality × sup-

Table 3. Estimated total DMI, nutrient intake, and plasma urea nitrogen (PUN) concentrations of replacement beef heifers consuming low-quality (LQ) or medium-quality (MQ) hay, and offered low-starch energy supplements daily (S7) or 3 times weekly (S3)[1]

	LQ				MQ			
Item[2]	S3	S7	SEM	P[3]	S3	S7	SEM	P[3]
Total DMI, kg/d								
SUPPALL	7.31	5.05	0.14	< 0.01	5.72	5.07	0.14	< 0.01
S7ONLY	2.82	5.14	0.14	< 0.01	3.48	5.03	0.14	< 0.01
P[4]	< 0.01	0.40			< 0.01	0.70		
NE_m intake, Mcal/d								
SUPPALL	10.03	5.88	0.12	< 0.01	6.92	5.30	0.12	< 0.01
S7ONLY	2.34	5.96	0.12	< 0.01	3.10	5.26	0.12	< 0.01
P[4]	< 0.01	0.42			< 0.01	0.69		
NE_g intake, Mcal/d								
SUPPALL	5.74	2.99	0.04	< 0.01	3.67	2.49	0.04	< 0.01
S7ONLY	0.79	3.02	0.04	< 0.01	1.22	2.47	0.04	< 0.01
P[4]	< 0.01	0.47			< 0.01	0.67		
CP intake, kg/d								
SUPPALL	1.03	0.60	0.01	< 0.01	0.82	0.68	0.01	< 0.01
S7ONLY	0.23	0.61	0.01	< 0.01	0.44	0.68	0.01	< 0.01
P[4]	< 0.01	0.53			< 0.01	0.66		
PUN, mg/dL								
SUPPALL	17.6	20.0	1.1	0.15	23.8	23.6	1.1	0.89
S7ONLY	11.8	19.3	1.1	< 0.01	20.9	23.0	1.1	0.19
P[4]	< 0.01	0.16			< 0.01	0.27		

[1] Hay and total DMI were evaluated from each pen (3 heifers/pen) from d 20 to 26, d 34 to 40, and d 48 to 54 of the study (d 0 to 120). Individual nutrient intake was calculated based on pen feed intake and nutritional content. Blood samples were collected and harvested for plasma on d 13 to 16, d 27 to 30, d 41 to 44, and d 55 to 58 of the study.

[2] SUPPALL = days when all S3 and S7 heifers were supplemented; S7ONLY = days that only S7 heifers were supplemented.

[3] Supplementation frequency comparison within each day.

[4] Day comparison within each supplementation frequency.

plementation frequency × day interaction was detected for plasma concentrations of PUN ($P < 0.01$; Table 3)

Plasma glucose concentrations in S7 heifers were similar (day effect; $P = 0.76$) across SUPPALL and S7ONLY days, but greater (day effect; $P < 0.01$) during S7ONLY compared with SUPPALL days in S3 heifers (Table 2). Similarly, our previous research (Cooke et al., 2008) also reported that plasma glucose concentrations in heifers supplemented infrequently were increased during non-supplementation days, and attributed this outcome to the time required for synthesis and activation of gluconeogenic enzymes to substantially change the magnitude of glucose synthesis and release by the liver. In addition, other studies also reported that plasma glucose concentrations of forage-fed developing heifers (Cooke et al., 2007b) and yearling steers (Cooke et al., 2007a) offered supplements based on low-starch energy byproducts 3 times weekly were greater at 28 vs. 4 h after supplementation. However, no supplementation frequency effects were detected ($P = 0.48$; data not shown) for plasma insulin concentrations (1.01 vs. 1.56 ng/mL for S3 and S7 heifers, respectively; SEM = 0.55), and this outcome was unexpected because insulin is directly influenced by nutrient intake and circulating glucose concentrations (Vizcarra et al., 1998) and is altered by supplementation frequency (Cooke et al., 2007a; Cooke et al., 2008).

Plasma NEFA concentrations in S7 heifers were similar (day effect; $P = 0.81$) across SUPPALL and S7ONLY days, but greater (day effect; $P < 0.01$) during S7ONLY compared with SUPPALL days in S3 heifers (Table 2). These outcomes indicate that fat tissue mobilization was stimulated in S3 heifers during non-supplementation days (Ellenberger et al., 1989). In fact, NE_m intake during S7ONLY in S3 heifers receiving LQ and MQ (Table 3) were below their requirements (4.93 Mcal/d; NRC, 1996). The increased NEFA concentrations in S3 heifers during S7ONLY may also have contributed to differences detected in plasma glucose concentrations as well as reproductive performance between S7 and S3 heifers. Circulating NEFA are nutritional modulators of cattle reproduction and may directly impair synthesis and release of gonadotropins (DiCostanzo et al., 1999; Hess et al., 2005). In addition, NEFA may increase expression of gluconeogenic enzymes and decrease the uptake of glucose by body tis-

sues (Kammula, 1976; White et al., 2011), which may explain the concurrent increases of plasma NEFA and glucose concentrations in S3 heifers during S7ONLY d.

Within LQ heifers, those receiving S7 had greater ($P < 0.01$) PUN concentrations compared with S3 heifers during S7ONLY days, but similar ($P = 0.15$) during SUPPALL days. Within MQ heifers, those receiving S7 and S3 had similar PUN concentrations during SUPPALL ($P = 0.89$) and S7ONLY days ($P = 0.19$). However, independently of hay quality, S3 heifers had greater (day effect; $P < 0.01$) PUN concentrations during SUPPALL compared with S7ONLY days, whereas PUN was similar (day effect; $P > 0.16$) across SUPPALL and S7ONLY days in S7 heifers. Concentrations of PUN are positively associated with intake of CP, RDP, and concentrations of ruminal ammonia (Hammond, 1997). Therefore, the reduced PUN concentrations in S3 heifers during S7ONLY compared with SUPPALL days can be attributed to decreased CP intake given that heifers were only offered hay. Although CP intake during S7ONLY days was reduced ($P < 0.01$; Table 3) for S3 heifers compared with S7 heifers independently of hay quality, S3 heifers receiving LQ consumed 37% of the CP intake of S7 cohorts (0.23 vs. 0.61 kg of CP, respectively), whereas S3 heifers receiving MQ consumed 68% of the CP intake S7 cohorts (0.44 vs. 0.68 kg of CP, respectively) during S7ONLY days. Therefore, this decrease in CP intake of S3 heifers receiving MQ compared with S7 cohorts during S7ONLY days may have contributed to the lack of concurrent differences ($P = 0.19$) in PUN concentrations. In addition, mean PUN concentrations were greater ($P = 0.01$; data not show) for S7 heifers compared with S3 heifers (21.5 vs. 18.5 mg/dL; SEM = 0.8) irrespective of hay quality. Optimal PUN concentration in beef heifers range between 11 and 15 mg/dL (Byers and Moxon, 1980), indicating that all heifers in the present study were consuming CP and RDP in excess. Given that energy is also required to metabolize ruminal ammonia into urea by the liver (Reynolds, 1992), the lack of differences on ADG between S3 and S7 heifers may also be associated with a greater amount of energy being partitioned towards N recycling instead of growth in S7 heifers.

Plasma IGF-I concentrations were greater ($P = 0.04$) for S7 heifers compared with S3 heifers during SUPPALL days, but similar during S7ONLY days (Table 2). In addition, plasma IGF-I concentrations in S3 heifers were greater during S7ONLY compared with SUPPALL days (day effect; $P < 0.01$), but similar (day effect; $P = 0.12$) across SUPPALL and S7ONLY days in S7 heifers (Table 2). Concurring with these findings, our previous research (Cooke et al., 2008) also reported that plasma IGF-I concentrations were greater for S7 vs. S3 heifers when supplement was provided to all heifers, whereas plasma IGF-I concentrations were greater at 28 vs. 4 h after supplementation in forage-fed developing heifers offered low-starch energy supplements 3 times weekly (Cooke et al., 2007b, 2008). Circulating IGF-I concentrations are positively associated with nutrient intake in cattle (Ellenberger et al., 1989; Bossis et al., 1999). Hence, results reported herein further support the significant daily variation of nutrient intake in S3 heifers, and the greater nutrient intake of S7 heifers during S7ONLY days (Table 3). Moreover, IGF-I enhances the responsiveness of ovarian cells to gonadotropins (Spicer and Stewart, 1996; Armstrong et al., 2001), increases the success of ovulation of the dominant follicle (Roche, 2006), and promotes embryonic development and consequently establishment and maintenance of early pregnancy in cattle (Thatcher et al., 2001; Bilby et al., 2006). Therefore, increased plasma IGF-I concentrations of S7 heifers during SUPPALL days compared with S3 heifers may have also contributed to differences detected herein for reproductive parameters (Figures 1 and 2).

Reproductive function of beef heifers are highly associated with nutritional status, growth rates, and circulating concentrations hormones and metabolites associated with energy metabolism (Roberts et al., 1997; Wettemann and Bossis, 2000; Diskin et al., 2003). In the present study, however, S7 heifers had hastened puberty and pregnancy attainment compared with S3 heifers despite their similar ADG, concurring with previous research from our group demonstrating that heifer reproductive development and performance may not be entirely dependent on growth rates (Cooke et al., 2007b, 2009). In addition, acute oscillations in circulating glucose and IGF-I due to infrequent energy supplementation influenced puberty and pregnancy attainment in replacement beef heifers consuming low-quality forages, perhaps by impairing efficiency in energy use (Cooke et al., 2008). Based on this rationale, we theorized that infrequent energy supplementation would not impair growth and reproductive development of heifers receiving MQ due to reduced need for supplement intake, and consequent decreased variation in DMI and circulating hormones and metabolites associated with nutrient metabolism. However, if heifers received LQ or MQ, nutrient intake and plasma concentrations of glucose, NEFA, PUN, and IGF-I varied significantly in S3 heifers but remained constant across sampling days for S7 heifers, resulting in hastened puberty and pregnancy attainment. Therefore, reproductive development and performance of beef replacement heifers consuming diets based on low- and medium-quality forages are enhanced when low-starch energy supplements are offered daily instead of 3 times weekly.

Nevertheless, it is important to note that overall pregnancy rates detected in the present experiment were less than expected according to previous work from our

research group (Cooke et al., 2007b, 2008), and such outcome can be attributed to several factors including heifer PUN concentrations or timing of puberty attainment. Butler et al. (1996) reported that increased PUN concentrations (> 19 mg/dL) are detrimental to pregnancy rates in lactating dairy cows, whereas in the present study PUN concentrations were often greater than 19 mg/dL across all treatment combinations (Table 3). Moreover, fertility in developing heifers is often reduced in the pubertal estrus compared with the second or third estrus (Byerley et al., 1987), whereas in the present study, the majority of heifers attained puberty during the last 4 wk of the breeding season (Figure 1). Hence, elevated PUN concentrations and late puberty attainment likely contributed to reduced overall pregnancy rates detected in the present study; thus, differences detected in pregnancy outcomes between S3 and S7 should be interpreted with caution.

In regards to hay quality effects on heifer performance, physiological, and reproductive responses (Table 4), heifers receiving LQ had greater ($P < 0.01$) ADG compared with MQ heifers. Hay intake was greater ($P < 0.01$) for MQ heifers compared with LQ given that supplementation rate was reduced for MQ vs. LQ heifers (15.8 and 7.9 kg of DM, respectively). However, LQ heifers had greater mean total DMI ($P = 0.05$) as well as NE_m and NE_g intake ($P < 0.01$), but reduced ($P = 0.03$) CP intake compared with MQ heifers (Table 4). Accordingly, LQ heifers had greater mean plasma concentrations of glucose ($P = 0.01$) and reduced mean PUN concentrations ($P < 0.01$) compared with MQ heifers. Although supplementation rates were initially designed to result in weekly iso-caloric and iso-nitrogenous DMI between LQ and MQ heifers, total DMI and energy intake were unexpectedly reduced for MQ heifers compared with LQ. Therefore, the reduced energy intake, translated into reduced plasma concentrations of glucose, combined with the excessive PUN concentrations may have contributed to the reduced ADG of MQ heifers compared with LQ heifers (Reynolds, 1992; Vizcarra et al., 1998). However, no differences between MQ and LQ heifers were detected for puberty ($P = 0.31$) and pregnancy ($P = 0.84$) attainment (Table 4), indicating that the greater ADG of LQ heifers was not sufficient to enhance their reproductive development (Cooke et al., 2007b). It is also important to note that differences detected for performance and physiological variables between LQ and MQ heifers did not impact the major goal of the present study, given that all the effects associated with supplementation frequency reported herein were similar among LQ and MQ heifers.

In summary, offering a low-starch energy supplement daily instead of 3 times weekly to beef heifers consuming LQ or MQ reduced daily variation in nutrient intake and plasma concentrations of PUN, glucose, NEFA, and IGF-I, resulting in hastened attainment of puberty and pregnancy. Therefore, replacement beef heifers receiving diets based on low-quality and medium-quality forages should receive low-starch energy supplements daily to enhance their reproductive development.

Table 4. Performance, physiological, and reproductive parameters of replacement beef heifers consuming low-quality (LQ) or medium-quality (MQ) hay, and offered low-starch energy supplements [1]

Item	LQ	MQ	SEM	*P*
Performance variables[2]				
Hay DMI, kg/d	2.65	3.61	0.09	< 0.01
Total DMI, kg/d	4.92	4.74	0.09	0.05
NE_m intake, Mcal/d	5.78	5.00	0.14	< 0.01
NE_g intake, Mcal/d	2.96	2.38	0.11	< 0.01
CP intake, kg/d	0.59	0.64	0.02	0.03
ADG, kg/d	0.34	0.19	0.02	< 0.01
Plasma variables[3]				
Glucose, mg/dL	81.0	71.2	2.8	0.01
Plasma urea nitrogen, mg/dL	17.2	22.8	0.8	< 0.01
NEFA, mEq/L	0.149	0.172	0.011	0.18
Insulin, ng/mL	1.89	0.70	0.55	0.13
IGF-I, mg/dL	81.8	69.0	5.7	0.14
Reproductive variables				
Puberty rate,[4] %	33.3	20.8	6.5	0.18
Pregnancy rate,[5] %	20.8	12.5	7.6	0.13

[1] Low-starch energy supplement was offered at weekly rates of 15.8 and 7.9 kg (DM basis) for LQ and MQ heifers, respectively.

[2] Hay and total DMI were evaluated from each pen (3 heifers/pen) from d 20 to 26, d 34 to 40, and d 48 to 54 of the study (d 0 to 120). Individual nutrient intake was calculated based on pen feed intake and nutritional content. Heifer ADG was calculated using initial (d 1) and final (d 121) shrunk BW.

[3] Blood samples were collected and harvested for plasma on d 13 to 16, d 27 to 30, d 41 to 44, and d 55 to 58 of the study.

[4] Estrous cycling heifers/total heifers during the study (d 0 to 121).

[5] Pregnant heifers/total heifers during the breeding phase of the study (d 60 to 121).

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