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Ruminant Nutrition

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Supplementation frequency and amount modulate postweaning growth and reproductive performance of *Bos indicus*-influenced beef heifers

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Abstract

This 2-yr study evaluated the growth and puberty attainment of *Bos indicus*-influenced beef heifers offered 2 different postweaning concentrate supplementation amounts and delivery frequencies. On day 0 of each year, 64 Brangus crossbred heifers were stratified by initial body weight (BW) and age (mean = 244 ± 22 kg; 314 ± 17 d) and assigned into 1 of 16 bahiagrass pastures (4 heifers/pasture/yr). Treatments were randomly assigned to pastures in a 2 × 2 factorial design (4 pastures/treatment/yr) and consisted of concentrate dry matter (DM) supplementation at 1.25% or 1.75% of BW which were offered either daily (7×) or 3 times weekly (3×) for 168 d. On day 56 of each year, heifers were assigned to an estrus synchronization protocol consisting of intravaginal controlled internal drug release (CIDR) insertion on day 56, CIDR removal on day 70, i.m. injection of 25 mg of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on day 86, and i.m. injection of 100 µg of gonadotropin-releasing hormone and timed-AI at 66 h after $PGF_{2\alpha}$ injection (day 89). Heifers were exposed to Angus bulls from day 89 to 168 (1 bull/pasture). Pregnancy diagnosis was assessed on day 213 of each year. Supplementation amount × frequency effects were not detected ($P \geq 0.12$) for any variable, except for plasma concentrations of glucose ($P = 0.10$) and urea nitrogen (PUN; $P = 0.01$). Herbage mass, herbage allowance, and nutritive value did not differ ($P \geq 0.12$) among treatments. Increasing supplementation DM amount from 1.25% to 1.75% of BW increased ($P \leq 0.05$) plasma concentrations of insulin-like growth factor 1 (IGF-1), overall average daily gain (ADG), final BW, percentage of pubertal heifers on day 89, pregnancy and calving percentages, and percentage of heifers calving within the first 21 d of the calving season. However, reducing the supplementation frequency from daily to 3× weekly, regardless of supplementation amount, did not impact overall pregnancy and calving percentages ($P \geq 0.42$), but caused ($P \leq 0.05$) fluctuations in plasma concentrations of insulin and IGF-1 and decreased ($P \leq 0.03$) overall ADG, final BW, puberty attainment on days 56, 89, and 168, and percentage of heifers calving during the first 21 d of the calving season. Hence, increasing the supplement DM amount did not prevent the negative effects of reducing the frequency of supplementation (3× vs. 7× weekly) on growth and reproduction of replacement *Bos indicus*-influenced beef heifers.

Key words: beef heifers, *Bos indicus*, frequency, growth, puberty, supplementation

Abbreviations

ADF	acid detergent fiber
ADG	average daily gain
AI	artificial insemination
BW	body weight
CIDR	controlled internal drug release
CP	crude protein
CV	coefficient of variation
DM	dry matter
GnRH	gonadotropin-releasing hormone
IGF-1	insulin-like growth factor 1
IVDOM	in vitro digestible organic matter
LH	luteinizing hormone
NDF	neutral detergent fiber
NEg	net energy for gain
NEm	net energy for maintenance
P4	progesterone
$PGF_{2\alpha}$	prostaglandin F2α
PUN	plasma urea nitrogen
TDN	total digestible nutrients

Introduction

Beef heifers that attained puberty before the initiation of the breeding season had greater overall pregnancy percentage and calving percentage during the first 21 d of the calving season compared with cohorts that attained puberty during the breeding season (Moriel et al., 2017). Beef heifers that calved within the first 21-d period of the calving season also had greater overall pregnancy percentage and calf weaning weights from second until 6th parturitions and remained in the herd longer than females that calved during the 2nd and 3rd 21-d period of calving season (Cushman et al., 2013).

Infrequent concentrate supplementation (i.e., 3 times (3×) weekly) reduced growth and reproductive performance of *Bos indicus*-influenced beef heifers compared with daily concentrate supplementation (Cooke et al., 2008; Moriel et al., 2012). This reduction in performance was partially attributed to the increased oscillations in circulating concentrations of glucose, insulin, and insulin-like growth factor 1 (IGF-1) and delayed puberty attainment of heifers receiving infrequent supplementation (Moriel et al., 2012). Increased growth rate and nutrient intake can be used to positively impact attainment of puberty and pregnancy in beef heifers (Moriel et al., 2017) by also modulating the circulating concentrations of hormones and metabolites associated with energy and protein metabolism (Cooke et al., 2007). We hypothesized that reducing the frequency of postweaning concentrate supplementation from daily to 3× weekly would not impair growth and puberty attainment of replacement beef heifers if concentrate supplementation dry matter (DM) amount simultaneously increased from 1.25% to 1.75% of body weight (BW). Thus, our objectives were to evaluate the combination of 2 different concentrate DM supplementation amount (1.25% vs. 1.75% of BW) and frequency (daily vs. 3× weekly) on growth and puberty attainment of *Bos indicus*-influenced heifers.

Materials and Methods

The 2-yr experiment was conducted at the University of Florida, Institute of Food and Agricultural Sciences—Range Cattle Research and Education Center (IFAS-RCREC), Ona, Florida (27°23′N and 81°56′W) from September 2017 to December 2018 (year 1) and September 2018 to December 2019 (year 2). All practices utilized herein were approved by the IFAS-Animal Research Committee (#201709984).

Animals and diets

All heifers utilized herein were originated from the RCREC and were previously weaned at 260 to 280 d of age. From weaning until the start of the study (day 0 of each year), heifers remained on a single bahiagrass (*Paspalum notatum*) pasture (10 ha) and received concentrate supplementation at 1% of BW (DM basis; Table 1). On day 0 of each year, Brangus crossbred heifers (n = 64 heifers/yr) were stratified by BW (244 ± 28 kg in year 1; 244 ± 17 kg in year 2) and age (311 ± 18 d in year 1; 317 ± 16 d in year 2), and randomly allocated into 1 of 16 bahiagrass pastures (0.93 ha and 4 heifers/pasture). Treatments were randomly assigned to pastures (4 pastures/treatment/yr), in a 2 × 2 factorial arrangement of treatments, and consisted of concentrate DM supplementation (Table 1) provided at 1.25% or 1.75% of BW from days 0 to 168. Within each concentrate supplementation amount, pastures were then assigned to receive similar weekly concentrate amount which was offered either daily (7×) or 3 times weekly (Monday, Wednesday and Friday) at 0800 hours from days 0 to 168. Due to the combination of relatively high nutrient requirements of growing beef heifers and specific nutritive value of pastures and environmental conditions at the RCREC, concentrate supplementation amounts described above were selected according to our previous study

Table 1. Average nutritional composition[1] of concentrate (days 0 to 168) and stargrass hay (days 89 to 168)

	Concentrate[2]		Hay	
Item	Year 1	Year 2	Year 1	Year 2
DM, %	87.2	94.1	95.4	92.3
CP, %	22.5	21.8	5.60	9.00
Crude fat, %	—	—	—	—
ADF, %	27.3	30.4	43.4	45.8
NDF, %	42.5	44.4	79.7	75.1
TDN[3], %	74.0	73.0	52.0	55.0
NEm[4], Mcal/kg	1.74	1.71	0.90	1.01
NEg[4], Mcal/kg	1.12	1.10	0.35	0.46
Ca, %	0.96	0.91	0.19	0.25
P, %	0.59	0.46	0.14	0.19
Mg, %	0.25	0.25	0.31	0.14
K, %	1.65	1.38	0.55	1.16
Na, %	0.07	0.06	0.04	0.02
S, %	0.35	0.30	0.12	0.13
Fe, mg/kg	211	210	74.0	35.0
Zn, mg/kg	47.0	48.0	17.0	30.0
Cu, mg/kg	12.0	9.00	6.00	3.00
Mn, mg/kg	30.0	31.0	20.0	35.0
Mo, mg/kg	2.30	1.60	0.30	0.30

[1]Samples of concentrate and hay were collected every 28 d from days 0 to 168 and 89 to 168, respectively. All samples were pooled within each year and sent in duplicates to a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY) for wet chemistry analyses.
[2]As-fed basis: 23% soybean hulls, 22% soybean meal, 22% dried distillers grains, 15% cottonseed hulls pellets, 15% cracked corn, 2% Ca carbonate, and 1% sugarcane molasses (United Feed Company, Okeechobee, FL 34972).
[3]Calculated as described by Weiss et al. (1992).
[4]Calculated using the equations proposed by NRC (2000).

demonstrating greater growth and puberty attainment of beef heifers supplemented with concentrate at amounts >1.0% of BW compared with cohorts supplemented at <1.0% of BW (Moriel et al., 2017). Weekly concentrate DM amount was periodically adjusted using the average shrunk BW of all heifers in each pasture on days 0, 42, 86, 114, and 140 (1.25% or 1.75% of BW multiplied by 7 d). Throughout the study, heifers were provided free-choice access to water and trace mineral supplement (University of Florida Cattle Research Winter Mineral; Vigortone, Brookville, Ohio, USA; 16.8%, 1.0%, 20.7%, and 4.0% of Ca, Mg, NaCl, and P, respectively, and 60, 1750, 350, 60, and 5,000 mg/kg of Co, Cu, I, Se, and Zn, respectively). Free-choice access to stargrass (*Cynodon nlemfuensis*) hay was offered to heifers from days 89 to 168 (Table 1).

On day 56 of each year, all heifers were assigned to an estrus synchronization protocol (Mallory et al., 2011). The protocol consisted of intravaginal controlled internal drug release (CIDR; 1.38 g P4; Zoetis Animal Health, Florham Park, NJ) insertion on day 56, CIDR removal on day 70, i.m. injection of 25 mg of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 5 mL Lutalyse; Zoetis Animal Health) on day 86, and i.m. injection of 100 µg of gonadotropin-releasing hormone (2 mL Factrel; Zoetis Animal Health) and timed-artificial insemination (AI) at 66 h after $PGF_{2\alpha}$ injection (day 89). Semen utilized at AI were obtained from a single Angus bull. All heifers were exposed to yearling Angus bulls from days 89 to 168 (1 bull/pasture). Bulls were randomly assigned to pastures at the start of the breeding season, and then rotated among pastures every 14 d from days 89 to 168 to remove any potential bull effect. All bulls successfully passed a breeding soundness exam 60 d before the start of the breeding season.

Sample collection

Concentrate disappearance time was calculated for each pasture by visiting the feed bunk every 30 min on days 56, 70, and 84 of each year, and recording the number of hours needed for complete disappearance of the respective concentrate amount offered at 0800 hours. Individual shrunk BW was measured on days 0, 42, 86, 114, 140, and 168 of each year, following 12 h of feed and water withdrawal. Blood samples (10 mL) from jugular vein were collected into tubes containing no additives (Vacutainer, Becton Dickinson) for serum harvest on days 0, 7, 49, 56, 82, 89, 161, and 168 of each year to determine the serum concentrations of progesterone (P4). Heifers were considered pubertal when plasma P4 concentrations were ≥1.5 ng/mL in 2 consecutive 7-d apart blood collections (Cooke and Arthington, 2009). Percentage of pregnant heifers were determined via rectal palpation by a trained veterinarian 45 d after the end of the breeding season (day 213 of years 1 and 2). Heifers were checked twice daily for calving, and calving date was determined using Julian date.

Additional blood samples (10 mL) were collected via jugular venipuncture into sodium-heparin (158 USP) containing tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) for plasma harvest on days 69 and 70 of each year to determine the plasma concentrations of glucose, insulin, IGF-1, and plasma urea nitrogen (PUN). All blood samples were collected 4 h after morning supplementation to correspond to the peak of ruminal fermentation and end products release after concentrate intake (Moriel et al., 2012; Artioli et al., 2015; Silva et al., 2018), and to match with a day that all heifers received concentrate supplementation (Monday; day 69) and a day that only 7× heifers, regardless of supplement amount, received concentrate supplementation (Tuesday; day 70). All blood samples were placed on ice immediately after collection. Blood samples for serum harvest were kept overnight at 4 °C, whereas blood samples for plasma harvest were centrifuged within 20 min of blood collection. All blood samples were centrifuged at 1,200 × *g* for 25 min at 4 °C. Serum and plasma samples were stored at −20 °C until later laboratory analysis.

Herbage mass and allowance were assessed on days 0 and 89 of each year. Herbage mass was calculated using the double sampling technique (Gonzalez et al., 1990). Herbage allowance was determined by dividing the average herbage mass of each pasture by the respective average total heifer BW on each respective pasture (Sollenberger et al., 2005). Hand-plucked samples of pastures were obtained on days 0 and 89, whereas samples of concentrate and hay were collected every 28 d from days 0 to 168 and day 89 to 168, respectively. Pasture, concentrate, and hay samples were dried at 56 °C for 72 hr using a forced-air oven. Thereafter, forage and concentrate samples were ground to pass a 4-mm and 1-mm stainless steel screens, respectively, using a Wiley mill (Model 4, Thomas-Wiley Laboratory Mill, Thomas Scientific, Swedesboro, NJ). Concentrate and hay samples were sent in duplicate to a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY) and analyzed for concentrations of crude protein (CP; method 984.13; AOAC, 2006), total digestible nutrients (TDN; Weiss et al., 1992), net energy for maintenance (NEm) and gain (NEg; NRC, 2000). Pasture samples were sent to the University of Florida Forage Evaluation Support Laboratory (Gainesville, FL) to determine the CP concentrations using the micro-Kjeldahl technique for N (Gallaher et al., 1975) and in vitro organic matter digestibility (IVOMD) using the 2-stage technique (Moore and Mott, 1974).

Laboratory analyses

Plasma concentrations of P4 and insulin were assessed in duplicate samples using a solid-phase, chemiluminescent enzyme immunoassay (Immulite 1000, Diagnostics Products Corp.) that was previously validated for bovine samples (Artioli et al., 2015; Moriel et al., 2017). The lowest detectable concentrations of P4 and insulin analyses were 0.2 ng/mL for P4 and 2 µIU/mL for insulin, whereas intra-assay coefficient of variation (CV) of P4 and insulin analyses were 3.22% and 4.14%, respectively.

Plasma IGF-1 concentrations were measured using a commercial ELISA kit (SG100; R&D Systems, Inc., Minneapolis, MN) validated for bovine samples (Moriel et al., 2012). Plasma concentrations of glucose and PUN were assessed by using commercial quantitative colorimetric kits (#G7521 and B7551, respectively; Pointe Scientific Inc., Canton, MI). Intra- and inter-assay CV for IGF-1, glucose, and PUN assays were 1.88% and 2.12%, 3.04% and 3.11%, and 2.98% and 4.01%, respectively.

Statistical analyses

Pasture was considered the experimental unit for all statistical analyses. Data were analyzed as completely randomized design using a 2 × 2 factorial arrangement of treatments and using SAS (SAS Institute Inc., Cary, NC, USA, version 9.4) with Satterthwaite approximation to adjust the denominator degrees of freedom for the test of fixed effects. Initial BW and age were included as covariates into all statistical analyses but removed from the model if $P > 0.10$. Heifer(pasture) and pasture(supplementation amount × supplementation frequency) were included as random effects in all statistical analyses. Heifer BW, concentrate disappearance time, plasma and herbage data were analyzed as repeated measures using the MIXED procedure and heifer(pasture) as subject, and tested for fixed effects of supplementation frequency, supplementation amount, day of the study, year, and all resulting interactions. Puberty attainment and calving distribution were analyzed as repeated measures using the GLIMMIX procedure and heifer(pasture) as subject, and tested for fixed effects of supplementation frequency, supplementation amount, day of the study, year, and all resulting interactions. Compound symmetry was the covariance structure used in all repeated measures analyses as it generated the lowest Akaike information criteria. Pregnancy percentage, calving percentage, and calving date were analyzed using the GLIMMIX procedure and tested for fixed effects of supplementation frequency, supplementation amount, year, and all resulting interactions. Heifers were sorted into prepubertal or pubertal based on their puberty status at the initiation of breeding season. Pregnancy and calving percentages of prepubertal vs. pubertal heifers was tested for fixed effects of puberty status, supplementation amount, supplementation frequency and all resulting interactions, using pasture(supplementation frequency × supplementation amount × year) and heifer(pasture) as random effects. All results are reported as LS means. Data were separated using PDIFF when a significant *F*-test was detected. Significance was set at $P \leq 0.05$, and tendencies if $P > 0.05$ and ≤ 0.10.

Results

Except for plasma concentrations of glucose and PUN, effects of supplementation amount × supplementation frequency × day of the study × year, supplementation amount × supplementation frequency × year, supplementation amount × year, supplementation frequency × year, and supplementation amount × supplementation frequency were not detected ($P \geq 0.12$) for any variable analyzed in the study.

Pasture evaluation

Effects of supplementation amount × day of the study, supplementation frequency × day of the study, supplementation amount, and supplementation frequency were not detected ($P \geq 0.12$) for herbage mass, herbage allowance, and IVOMD and CP, which all decreased ($P < 0.0001$) from day 0 to 89 (Table 2).

Concentrate disappearance time

Effects of supplementation amount × supplementation frequency were detected ($P = 0.005$) for concentrate disappearance. Heifers supplemented 3× weekly at 1.75% of BW required the greatest ($P < 0.0001$) number of hours to achieve 100% of concentrate disappearance (17.5 ± 0.97 h), followed by those supplemented 3× weekly at 1.25% of BW (11.1 ± 0.97 h). Heifers supplemented daily at 1.75% and 1.25% of BW required the least ($P < 0.0001$) number of hours (2.8 ± 0.97 h vs. 2.3 ± 0.97 h; $P = 0.70$) to achieve complete concentrate disappearance.

Plasma measurements

Effects of supplementation amount × day of the study were detected ($P = 0.05$) for plasma concentrations of IGF-1, but not for plasma concentrations of insulin ($P = 0.46$; Table 3). Plasma concentrations of IGF-1 were greater ($P = 0.04$) for heifers supplemented at 1.75% vs. 1.25% of BW on days that all heifers received concentrate supplementation (Monday) but did not differ ($P = 0.35$) on days that only heifers assigned to daily supplementation received their supplement (Tuesday). Effects of supplementation frequency × day of the study tended ($P = 0.06$) to be detected for plasma concentrations of IGF-1 and were detected ($P = 0.04$) for plasma concentrations of insulin (Table 3). Plasma concentrations of IGF-1 and insulin of 7× heifers, regardless of supplementation amount, did not differ ($P \geq 0.12$) between days that all heifers received supplementation and days that only 7× heifers received supplementation. In contrast, 3× heifers had greater ($P < 0.0001$) plasma concentrations of IGF-1 and insulin on days that all heifers received concentrate supplementation compared with days that only 7× heifers received concentrate supplementation (Table 3).

Effects of supplementation amount × supplementation frequency × day of the study tended ($P = 0.10$) to be detected for plasma concentrations of glucose (Figure 1a) and were detected ($P = 0.01$) for plasma concentrations of PUN (Figure 1b). Heifers supplemented 3× weekly at 1.25% and 1.75% of BW had greater ($P \leq 0.03$) plasma glucose concentrations on days that only 7× heifers received supplementation compared to days that all heifers received supplementation. Plasma glucose concentrations of 7× heifers, regardless of concentrate amount, did not differ ($P \geq 0.11$) between days that all heifers received supplementation and days that only 7× heifers received supplementation (Figure 1a). Heifers supplemented 3× weekly at 1.25% of BW had greater ($P < 0.0001$) plasma concentrations of PUN on days that only 7× heifers received supplementation compared to days that all heifers received supplementation and did not differ ($P \geq 0.11$) among all remaining treatments (Figure 1b).

Growth performance

Effects of supplementation frequency × day of the study and supplementation amount × day of the study were detected for heifer BW ($P < 0.0001$; Table 4). Heifer BW from days 0 to 86 did not differ ($P \geq 0.48$) between heifers supplemented at 1.25% and

Table 2. Average herbage mass and allowance, IVDOM, and CP of bahiagrass pastures (4 pastures/treatment/yr; 0.93 ha and 4 heifers/pasture)

	Day of the study			P-value		
Item[1]	0	89	SEM	Day	Amount × day	Freq. × day
Herbage mass, kg DM/ha	5220	2574	71.8	<0.0001	0.88	0.30
Herbage allowance, kg DM/kg BW	5.38	2.15	0.098	<0.0001	0.85	0.30
IVOMD, %	49.0	44.6	0.63	<0.0001	0.52	0.12
CP, % of DM	11.21	8.60	0.219	<0.0001	0.82	0.50

[1]Herbage mass and allowance were determined on days 0 and 89 of each year. Herbage mass was calculated using the double sampling technique (Gonzalez et al., 1990). Herbage allowance was determined by dividing the average herbage mass of each pasture by the respective average total heifer BW on each respective pasture (Sollenberger et al., 2005). Heifers were provided, in a 2 × 2 factorial design, concentrate DM supplementation at 1.25% or 1.75% of BW offered either daily or 3X weekly from day 0 to 168 (2 yr; 64 heifers/yr; 4 pastures/treatment combination/yr).

Table 3. Plasma concentrations of IGF-1 and insulin of heifers grazing bahiagrass pastures and randomly assigned, in a 2 × 2 factorial arrangement of treatments, to receive concentrate DM supplementation at 1.25% or 1.75% of BW offered either daily (7×) or 3X weekly (3×; Monday, Wednesday, and Friday) from days 0 to 168 (2 yr; 64 heifers/yr; 4 pastures/treatment combination/yr)

	Supplementation amount				P-value[3]	Supplementation frequency				P-value[3]
Item[1]	1.25%	1.75%	P-value[2]	SEM	Amount × day	3×	7×	P-value[2]	SEM	Frequency × day
Plasma IGF-1, ng/mL										
Days all heifers supplemented	102.3	115.2	0.04	4.54	0.05	115.0	102.6	0.05	4.52	0.06
Days only 7× supplemented	75.9	81.9	0.35	4.54		80.4	92.4	0.64	4.52	
P-value[4]	<0.0001	<0.0001				<0.0001	0.12			
Plasma insulin, μIU/mL										
Days all heifers supplemented	10.96	9.52	0.20	0.84	0.46	10.13	10.34	0.85	0.84	0.04
Days only 7× supplemented	8.58	7.85	0.49	0.77		7.10	9.33	0.04	0.77	
P-value[4]	0.002	0.007				<0.0001	0.18			

[1]Blood samples were collected via jugular venipuncture for plasma harvest 4 h after morning supplementation to correspond to the peak of ruminal fermentation and end products release after concentrate intake, and to match with a day that all heifers received concentrate supplementation (Monday; day 69) and a day that only 7× heifers, regardless of supplement amount, received concentrate supplementation (Tuesday; day 70).
[2]P-value for the comparison of treatments within day of the study.
[3]P-value for the effects of supplementation amount × day of the study and supplementation frequency × day of the study.
[4]P-value for the comparison of days within each respective treatment.

1.75% of BW, but heifers supplemented at 1.75% of BW were heavier ($P \leq 0.04$) on days 114, 140, and 168 compared with those supplemented at 1.25% of BW. Heifer BW from days 0 to 42 did not differ ($P \geq 0.22$) between 7× and 3× heifers, but 7× heifers were heavier ($P \leq 0.007$) on days 86, 114, 140, and 168 compared with 3× heifers.

Effects of supplementation frequency × supplementation amount were not detected ($P \geq 0.76$) for heifer average daily gain (ADG) from days 0 to 86, 86 to 168, and 0 to 168. Heifer ADG from days 0 to 86 did not differ ($P = 0.42$) between heifers supplemented at 1.25% and 1.75% of BW. However, heifer ADG from days 86 to 168 and 0 to 168 was greater ($P \leq 0.02$) for heifers supplemented at 1.75% vs. 1.25% of BW (Table 5). Heifer ADG from days 86 to 168 did not differ ($P = 0.28$) between 3× and 7× heifers, whereas ADG from days 0 to 86 and 0 to 168 was greater ($P \leq 0.01$) for 7× vs. 3× heifers (Table 5).

Reproductive parameters

Effects of supplementation frequency × supplementation amount were not detected ($P \geq 0.22$) for puberty attainment, pregnancy and calving percentages, and calving date. Effects of supplementation amount × day of the study ($P = 0.02$) and supplementation frequency × day of the study ($P = 0.006$) were detected for puberty attainment (Table 5). Concentrate supplementation at 1.75% of BW increased ($P = 0.05$) the percentage of pubertal heifers on day 89, but not on days 56 and 168 ($P \geq 0.18$), compared with supplementation at 1.25% of BW. Daily supplementation of concentrate increased ($P = 0.03$) the percentage of pubertal heifers on days 56, 89, and 168 (Table 5). Pregnancy and calving percentages were greater ($P \leq 0.05$) for heifers supplemented at 1.75% vs. 1.25% of BW and did not differ ($P \geq 0.11$) between 3× and 7× heifers (Table 5). Calving date decreased ($P = 0.05$) for heifers supplemented at 1.75% vs. 1.25% of BW and tended ($P = 0.07$) to decrease for 7× vs. 3× heifers (Table 5).

Effects of supplementation amount × day of the study and supplementation frequency × day of the study were detected ($P \leq 0.05$) for calving distribution (Table 6). Calving percentage increased ($P \leq 0.05$) from days 363 to 384 for heifers supplemented at 1.75% vs. 1.25% of BW. Calving percentage was greater ($P \leq 0.05$) from days 370 to 391 for 7× vs. 3× heifers (Table 6).

Effects of supplementation amount × puberty status, but not supplementation frequency × puberty status ($P \geq 0.52$), tended to be detected for pregnancy ($P = 0.07$) and calving percentages ($P = 0.10$; Table 7). Among pubertal heifers, pregnancy and calving percentages did not differ ($P \geq 0.18$) between those supplemented at 1.25% and 1.75% of BW. For heifers that were prepubertal at the start of the breeding season, pregnancy and calving percentages increased ($P \leq 0.05$) when supplementation amount increased from 1.25% and 1.75% of BW (Table 7).

Discussion

An interaction between supplementation amount and frequency was observed for concentrate disappearance time. Heifers supplemented daily (regardless of the supplementation amount) required on average 2.6 ± 0.97 hr to achieve complete supplement disappearance. However, when supplementation frequency was reduced from daily to 3× weekly, concentrate disappearance time increased by nearly 4.4- and 6.9-fold for heifers offered concentrate DM supplementation at 1.25% and 1.75% of BW, respectively, which are in agreement with previous studies (Moriel et al., 2016) and partially explain the results observed for plasma concentrations of glucose and PUN (further discussion provided below). Contrary to our hypothesis, however, interaction effects between supplementation amount × frequency were not detected for any additional variable analyzed herein. In the current study, increasing concentrate supplementation amount from 1.25% to 1.75% of BW did not overcome the negative consequences of reducing the frequency of concentrate supplementation on growth and reproductive performance of beef heifers. These results are in agreement with our previous study exploring the interaction between supplementation amount and frequency on postvaccination growth and immune responses of preconditioning beef steers

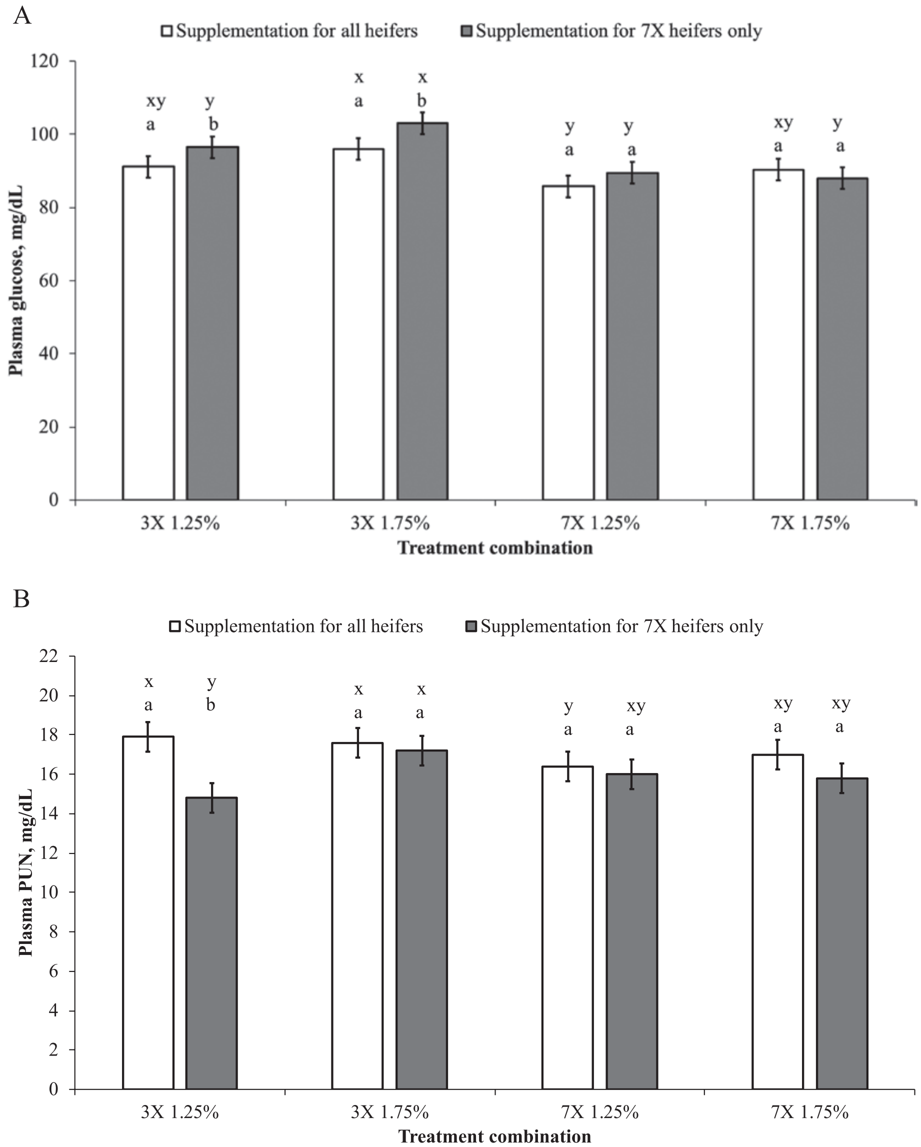

Figure 1. Plasma concentrations of glucose (a) and PUN (b) of heifers grazing bahiagrass pastures and randomly assigned, in a 2 × 2 factorial arrangement of treatments, to receive concentrate DM supplementation at 1.25% or 1.75% of BW offered either daily (7×) or 3× weekly (3×; Monday, Wednesday, and Friday) from days 0 to 168. Blood samples were collected 4 h after morning supplementation and to correspond with a day that all heifers received concentrate supplementation (Monday; day 69) and a day that only 7× heifers received concentrate supplementation (Tuesday; day 70). [a,b]Within each treatment combination, means without a common superscript differ ($P \le 0.05$). [x,y]Within day, means without a common superscript differ ($P \le 0.05$).

(Moriel et al., 2016). Together, both studies demonstrate that a reduced supplementation frequency elicits similar detrimental impacts to immunity of beef steers (Moriel et al., 2016) and also reproductive performance of beef heifers (current study) irrespective of concentrate amount offered.

Herbage mass and nutritive value of pastures modulate the growth performance of beef animals (Inyang et al., 2010). Herbage mass, herbage allowance, and nutritive value of pastures were not impacted by concentrate supplementation amount and frequency, but all variables decreased from days 0 to 89 (onset of the breeding season) as a consequence of forage intake and seasonal-induced effects on forage production and composition (Arthington and Brown, 2005; Vendramini et al., 2015). However, supplementation was designed to address the fluctuations in nutritive value of pastures and meet the daily energy and protein requirements of beef heifers gaining >0.50 kg of BW daily (NASEM, 2016). In addition, herbage allowance on day 89 was above the minimal threshold necessary to prevent limitations to forage intake and growth of cattle grazing bahiagrass (1.4 kg DM/kg BW; Inyang et al., 2010), and all heifers were provided free choice access to hay after the onset of the breeding season. Consequently, forage availability and nutritive value were not limiting factors to growth and reproductive performance of heifers utilized herein.

Supplementation amount

Moriel et al. (2017) supplemented beef heifers at 1.05% and 1.52% of BW (DM basis) for 168 d and observed an increase in overall ADG (0.41 vs. 0.51 kg/d, respectively), but no effects on puberty attainment at the start of the breeding season (38% vs. 40% of pubertal heifers, respectively), overall pregnancy (70% vs. 70%, respectively), and calving percentages (56.7% vs. 61.7%,

Table 4. BW of heifers grazing bahiagrass pastures and randomly assigned, in a 2 × 2 factorial arrangement of treatments, to receive concentrate DM supplementation at 1.25% or 1.75% of BW offered either daily (7×) or 3× weekly (3×; Monday, Wednesday, and Friday) from days 0 to 168 (2 yr; 64 heifers/yr; 4 pastures/treatment combination/yr)

	Day of the study							
BW[1], kg	0	42	86	114	140	167	SEM	P-value[2]
Supplementation amount								
1.25%	244	272	306	324	341	353	1.97	<0.0001
1.75%	244	272	308	330	348	362	1.96	
P-value[3]	1.00	0.83	0.48	0.04	0.01	0.004		
Supplementation frequency								
3×	244	270	303	323	340	353	1.95	<0.0001
7×	244	274	310	332	349	363	1.96	
P-value[3]	1.00	0.22	0.007	0.002	0.003	0.0009		

[1]Shrunk BW obtained after 12 h of feed and water withdrawn and covariate adjusted for heifer BW on day 0 ($P < 0.0001$).
[2]P-value for the effects of supplementation amount × day of the study and supplementation frequency × day of the study.
[3]P-value for the comparison of treatments within day of the study.

Table 5. ADG and reproductive performance of heifers grazing bahiagrass pastures and randomly assigned, in a 2 × 2 factorial arrangement of treatments, to receive concentrate DM supplementation at 1.25% or 1.75% of BW offered either daily (7×) or 3× weekly (3×; Monday, Wednesday, and Friday) from days 0 to 168 (2 yr; 64 heifers/yr; 4 pastures/treatment combination/yr)

	Supplementation amount				Supplementation frequency			
Item[1]	1.25%	1.75%	SEM	P-value [2]	3×	7×	SEM	P-value [2]
ADG, kg/d								
Days 0 to 86	0.72	0.74	0.021	0.42	0.68	0.77	0.020	0.01
Days 86 to 168	0.59	0.66	0.018	0.003	0.61	0.64	0.018	0.28
Days 0 to 168	0.65	0.71	0.015	0.02	0.65	0.71	0.015	0.007
Pubertal heifers [3], % of total								
Day 0	0.0	0.0	4.02	0.99	0.0	0.0	4.09	0.85
Day 56	47.2	55.0	4.02	0.18	44.6	57.6	4.09	0.03
Day 89	81.1	92.0	4.02	0.05	80.0	93.0	4.09	0.03
Day 168	90.5	95.1	4.02	0.42	86.3	99.3	4.09	0.03
Pregnant heifers day 213 [4], % of total	64.8	83.0	5.25	0.02	72.4	75.3	5.21	0.70
Calving first offspring, % of total	58.6	72.5	5.63	0.05	59.5	71.9	5.59	0.11
Calving date, day of the study	390	382	3.41	0.05	391	382	3.8	0.07

[1]Heifers were assigned to an estrus synchronization protocol which consisted of CIDR insertion on day 56, CIDR removal on day 70, $PGF_{2\alpha}$ injection on day 86, and GnRH injection and timed-AI on day 89. Heifers were exposed to yearling Angus bulls from days 89 to 168. Bulls were rotated among pastures every 14 d.
[2]P-value for the comparison of treatments within day of the study.
[3]Blood samples collected on days 0, 7, 49, 56, 82, 89, 160, and 168 to determine serum concentrations of progesterone. Heifers were considered pubertal when plasma progesterone concentrations were ≥1.5 ng/mL in 2 consecutive 7-d apart blood collections (Cooke and Arthington, 2009).
[4]Overall pregnancy percentage (AI + bulls) determined via rectal palpation 45 d after the end of the breeding season.

respectively), and calving distribution as supplementation DM amount increased from 1.05% to 1.52% of BW. In contrast, overall ADG and percentage of pubertal heifers at the start of the breeding season as well as pregnancy and calving percentages increased with supplementation amount in the current study. Likewise, a greater percentage of pregnant heifers in the current study calved within the first 21-d of the calving season as supplementation amount increased from 1.25% to 1.75% of BW. Herbage mass was not reported in the previous study, but it is likely the main factor explaining the discrepancy between the 2 studies on supplementation amount-induced effects on heifer growth and reproductive performance. Supporting this rationale, overall heifer ADG in Moriel et al. (2017) was less than the overall ADG reported herein.

Reproductive success of beef heifers is positively correlated with growth rates (Moriel et al., 2017), energy intake (Schillo et al., 1992) and circulating concentrations of hormones and metabolites associated with energy metabolism, such as glucose, insulin and IGF-1 (Cooke et al., 2007; Moriel et al., 2012). These metabolites and hormones modulate the interaction between nutritional status and reproduction by influencing hypothalamic-hypophyseal activity, hypophyseal secretion of luteinizing hormone (LH; Schillo et al., 1992) and also amplifying LH effects on ovarian follicular cells (Spicer and Echternkamp, 1995). A simultaneous increase in circulating concentrations of glucose, insulin, and IGF-I was expected with greater energy intake (Cappellozza et al., 2014). Plasma concentrations of glucose increased as supplementation amount increased from 1.25% to 1.75% of BW, but only for those supplemented 3× weekly. This response occurred likely because of the larger supplement amount offered to 3× heifers in a single day leading to greater hepatic synthesis of glucose compared with heifers

Table 6. Calving distribution (% of heifers that calved) of heifers grazing bahiagrass pastures and randomly assigned, in a 2 × 2 factorial arrangement of treatments, to receive concentrate DM supplementation at 1.25% or 1.75% of BW offered either daily (7×) or 3× weekly (3×; Monday, Wednesday, and Friday) from days 0 to 168 (2 yr; 64 heifers/yr; 4 pastures/treatment combination/yr)

	Day of the study												
Calving distribution, % of heifers that calved	363	370	377	384	391	398	405	412	419	426	433	SEM	P-value
Supplementation amount													
1.25% of BW	5.6	11.1	27.8	51.4	75.7	81.9	88.2	88.2	93.8	93.8	100.0	5.92	0.05
1.75% of BW	22.5	30.8	53.3	67.5	81.7	85.8	95.8	100.0	100.0	100.0	100.0	5.92	
P-value	0.05	0.04	0.02	0.05	0.57	0.71	0.47	0.26	0.55	0.55	1.00		
Supplementation frequency													
3×	10.0	10.0	20.0	42.5	71.3	77.5	93.8	93.8	93.8	93.8	100.0	5.73	0.04
7×	18.1	31.9	61.1	76.4	86.1	90.3	90.3	94.4	100.0	100.0	100.0	5.73	
P-value	0.45	0.04	0.001	0.002	0.05	0.23	0.74	0.95	0.55	0.55	1.00		

[1]Effects of supplementation amount × day of the study (P = 0.05) and supplementation frequency × day of the study (P = 0.04) were detected for calving distribution.
[2]P-value for the comparison of treatments within day of the study.

Table 7. Pregnancy and calving percentages of heifers that achieved puberty before the start of the breeding season (pubertal) and heifers that were prepubertal at the start of the breeding season but achieved puberty before the end of the breeding season

	Puberty status at the start of the breeding season[1]				P-value	
Item[1]	Non-pubertal	Pubertal	P-value[2]	SEM	Supp. amount × puberty status	Supp. frequency × puberty status
Pregnant heifers, % of total						
Supplement at 1.25% of BW	10.3	76.0	<0.0001	12.4	0.07	0.52
Supplement at 1.75% of BW	58.8	87.7	0.07	12.4		
P-value[3]	0.015	0.18				
Calving, % of heifers that calved						
Supplement at 1.25% of BW	6.17	65.8	0.001	15.8	0.10	0.87
Supplement at 1.75% of BW	56.4	73.5	0.22	15.8		
P-value[3]	0.05	0.53				

[1]Heifers grazed bahiagrass pastures and were randomly assigned, in a 2 × 2 factorial design, to receive concentrate DM supplementation at 1.25% or 1.75% of BW offered either daily or 3× weekly (Monday, Wednesday, and Friday) from days 0 to 168 (2 yr; 64 heifers/yr; 4 pastures/treatment combination/yr). At the start of the breeding season, 12 and 7 heifers were considered prepubertal in years 1 and 2, whereas 52 and 57 heifers attained puberty before the start of the breeding season in years 1 and 2, respectively.
[2]P-value for the comparison of puberty status within supplementation amount.
[3]P-value for the comparison of supplementation amount within puberty status.

supplemented daily. Among all hormones and metabolites, IGF-1 seems to be the main factor correlated with reproductive performance of beef heifers because IGF-I enhances ovarian responsiveness to gonadotropins (Armstrong et al., 2001) and promotes embryonic establishment and maintenance of early pregnancy in cattle (Bilby et al., 2006). Heifers that achieved puberty and became pregnant during the breeding season had similar ADG and plasma concentrations of glucose and insulin, but greater plasma concentrations of IGF-1 compared with nonpubertal and nonpregnant heifers (Cooke et al., 2007). In agreement, plasma concentrations of insulin did not differ between heifers supplemented at 1.25% and 1.75% of BW, whereas plasma concentrations of IGF-1 on days that all heifers received supplementation increased as supplementation amount increased from 1.25% to 1.75% of BW. The lack of differences on plasma concentrations of insulin between heifers supplemented at 1.25% and 1.75% of BW occurred perhaps because of the timing of blood collection (4 h after supplementation) occurring after the peak of pancreatic release of insulin (1 to 2 h after feeding; Moriel et al., 2008). Although plasma concentrations of IGF-1 were not analyzed throughout the entire study, it is plausible that the greater plasma concentrations of IGF-1 as supplementation amount increased significantly contributed to the enhanced growth and reproduction of heifers supplemented at 1.75% vs. 1.25% of BW.

It was observed by Moriel et al. (2017) that heifers classified as cyclic at the start of breeding season had greater overall pregnancy and calving percentages compared with cohorts that were prepubertal at the start of breeding season. Similar results were observed herein but the magnitude of increase in pregnancy and calving percentages depended on the concentrate supplementation amount offered to heifers. Pregnancy and calving percentages were, respectively, 7.4- and 10.7-fold greater for pubertal vs. prepubertal heifers at the start of the breeding season if concentrate supplementation was provided at 1.25% of BW. However, when supplementation was offered to heifers at 1.75% of BW, pregnancy and calving percentages enhanced by only 1.5- and 1.3-fold for pubertal vs. prepubertal heifers at the start of the breeding season, respectively. These results occurred likely because greater supplementation amount increased BW growth and elicited positive physiological signals associated with greater reproductive success. Together, these results indicate that increasing the supplementation amount can be used as a strategy to minimize the effects of prebreeding puberty status on final reproductive performance of *Bos indicus*-influenced heifers.

Supplementation frequency

Decreasing the frequency of energy supplementation from daily to 3× weekly either reduced ADG of beef steers and heifers by 10% to 21% (Cooke et al., 2008; Loy et al., 2008; Artioli et al., 2015) or had no effects on ADG of beef steers and heifers (Moriel et al., 2012, 2016). In the current study, heifer ADG during the breeding season did not differ between 3× vs. 7× heifers, whereas ADG before the start of the breeding season and throughout the entire study decreased by 11.6% and 8.5%, respectively, when supplementation frequency decreased from daily to 3× weekly. Several factors may explain the discrepancy among those studies such as differences in forage allowance and nutritive values, as well as supplement composition, breed, gender, location, and resulting interactions among these factors. In agreement, supplementation frequency in the current study did not impact heifers ADG during periods of low- vs. medium-quality forages (during the breeding season vs. before the start of the breeding season).

Reducing the frequency of supplementation decreased heifer ADG and BW before the start of the breeding season, partially explaining the delayed puberty attainment of 3× vs. 7× heifers. Although lighter pre-breeding BW and growth rates contributed to delayed puberty attainment, our previous research demonstrated that negative effects of reduced supplementation frequency on puberty attainment also occurred despite the lack of differences on growth performance between 3× and 7× heifers (Cooke et al., 2007, 2008; Moriel et al., 2012). Thus, factors beyond growth performance explain the reduced puberty attainment caused by infrequent concentrate supplementation. Despite the differences observed for puberty attainment, pregnancy and calving percentages did not differ between 3× and 7× heifers. Pregnancy percentage has been shown to either decrease (Cooke et al., 2008) or not be affected (Moriel et al., 2012) by infrequent concentrate supplementation. Calving distribution was not reported previously (Cooke et al., 2008; Moriel et al., 2012), but was negatively impacted by infrequent supplementation in the current study. Less heifers calved within the first 21 d of the breeding season when concentrate supplementation was provided 3× weekly vs. daily. Late calving has been associated with a reduced lifetime productivity of heifers (Cushman et al., 2013) and reflects the later puberty attainment as supplementation frequency decreased. Together, all studies described above indicate that most variables analyzed to evaluate the reproductive success of *Bos indicus*-influenced beef heifers were impaired by decreasing the postweaning frequency of concentrate supplementation, regardless of supplementation amount.

Infrequent concentrate supplementation leads to fluctuations in daily nutrient intake of beef steers and heifers (Cooke et al., 2008; Moriel et al., 2012, 2016; Artioli et al., 2015). Compared to steers offered daily supplementation, hay DM intake of 3× steers decreased by 53% on days that all steers received supplementation and increased by 10% on days that only 7× steers received supplementation (Artioli et al., 2015). Despite the low concentration of starch in the supplements utilized herein and in previous studies described above, fluctuations in daily forage intake are expected because supplementation decrease forage DM intake when TDN:CP ratio is <7 and supplemental TDN is >0.7% of BW (Moore et al., 1999).

Fluctuations in nutrient intake following infrequent supplementation also impact the synthesis and release of multiple hormones and metabolites. As previously observed (Cooke et al., 2008; Moriel et al., 2012, 2016; Artioli et al., 2015), plasma concentrations of IGF-1 and insulin of 7× heifers remained constant across blood collection days, regardless of concentrate amount. Plasma concentrations of IGF-1 and insulin, however, were greater for 3× heifers on days that all heifers received supplementation compared with days that only 7× heifers received supplementation, regardless of supplementation amount.

In contrast to previously observed for plasma insulin and IGF-1, the impacts of reduced frequency of supplementation on plasma concentrations of glucose and PUN reported herein depended on the amount of concentrate supplementation provided to heifers. In agreement with Cooke et al. (2008) and Moriel et al. (2012), plasma concentrations of glucose and PUN remained constant for 7× heifers supplemented at 1.25% and 1.75% between days that all heifers received supplementation vs. days that only 7× heifers were supplemented. Plasma glucose concentrations of 3× heifers varied between blood collection days and were greater on days that only 7× received supplementation compared with days that all heifers received supplementation. These results reflect the pattern of nutrient intake of each treatment as circulating concentrations of glucose are positively modulated by nutrient intake (Cappellozza et al., 2014) and to the time required for synthesis and activation of gluconeogenic enzymes to markedly increase glucose synthesis and release (Cooke et al., 2008; Artioli et al., 2015). As expected, the magnitude of increase in plasma glucose concentrations within heifers supplemented 3× weekly was greater for those supplemented at 1.75% vs. 1.25% of BW, explaining the detection of supplementation amount × supplementation frequency effects observed for plasma glucose concentrations.

Plasma PUN concentrations of heifers supplemented 3× weekly at 1.25% of BW were greater on days that all heifers received supplementation compared with days that only 7× heifers received supplementation, which was expected as plasma PUN concentrations are positively correlated with CP intake and ruminal ammonia concentration (Broderick and Clayton, 1997). Unexpectedly, plasma PUN concentrations of heifers supplemented 3× weekly at 1.75% of BW remained constant across days. These results are probably a result of heifers supplemented 3× weekly at 1.75% of BW having a longer concentrate disappearance time, which may have extended the period of greater circulating PUN concentrations following supplementation. Although plasma concentrations of glucose and PUN differed according to supplementation amount and frequency, plasma PUN concentrations were always within the optimal PUN concentrations for growing beef heifers (15 to 19 mg/dL; Hammond, 1997). In addition, such treatment differences observed for plasma PUN and glucose concentrations had minimal or no biological significance and did not elicit carryover effects on heifer growth and reproductive performance.

In summary, increasing the supplementation amount (1.75% vs. 1.25% of BW) of a low-starch energy-based concentrate positively modulated the circulating concentrations of hormones and metabolites associated with energy and protein metabolism and enhanced growth and reproductive success of beef heifers consuming warm-season forages. However, contrary to our hypothesis, increasing concentrate supplementation did not overcome the negative consequences of reducing the frequency of concentrate supplementation on daily variation in plasma concentrations of hormones and metabolites associated with energy and protein metabolism, and led to impaired growth, delayed attainment of puberty, and reduced pregnancy of beef heifers. Therefore, replacement *Bos indicus*-influenced beef heifers consuming low- and medium-quality forages should be offered daily energy supplementation, regardless of the concentrate amount, in order to enhance their growth and reproductive success.

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Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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