Pre- and post-weaning injections of bovine somatotropin to optimize puberty achievement of *Bos indicus* beef heifers[1]

Philipe Moriel,[†,2] Bruno Ieda Cappellozza,[‡] Matheus B. Piccolo,[†] Reinaldo F. Cooke,[‖] Miguel F. Miranda,[$] Luis F. D. Batista,[$] Rafael S. Carvalho,[$] Eduardo A. Colombo,[$] Felipe V. Santili,[$] Ramiro V. O. Filho,[$] Vitor S. M. Ferreira,[$] and Jose L. M. Vasconcelos[$]

[†]University of Florida, IFAS – Range Cattle Research and Education Center, Ona, FL 33865; [‡]Elanco Animal Health, São Paulo 04703-002, Brazil; [‖]Department of Animal Science, Texas A&M University, College Station, TX 77843; and [$]Department of Animal Production, São Paulo State University, Botucatu 18168-000, Brazil

ABSTRACT: The present study evaluated the growth and puberty attainment of *Bos indicus* heifers administered recombinant bovine somatotropin (bST) or saline injections during preweaning and/or postweaning. On day 0, 177 suckling Nellore heifers were stratified by initial age and body weight (BW) (80 ± 10 d; 97 ± 16 kg), and randomly assigned, in a 2 × 2 factorial design (n = 44 to 45 heifers/treatment), to receive s.c. injections of saline (5 mL 0.9% NaCl) or sometribove zinc (Posilac; Elanco, Greenfield, IN; 6.14 mg/kg of $BW^{0.75}$) on days 0 and 10 (PRE) and/or days 167 and 177 (POS). All heifers were managed as a single group in *Brachiaria decumbens* pastures from day 0 until 24 d postweaning (day 191), and then provided a corn silage–based TMR from days 191 to 380 to achieve 65% to 70% of mature BW at the end of the study (day 380). Heifer full BW was collected on days 0, 10, 167, 177, and monthly from days 191 to 380. Transrectal ultrasonography of ovaries was performed on days 1 and 10 of each month from days 229 to 380 to assess the percentage of pubertal heifers. Liver biopsies and blood samples from jugular vein were collected on days 0, 10, 167, 177, and 380. Additional blood samples were collected monthly from days 259 to 380 (n = 10 to 15 heifers/treatment). No interactions among day of the study, PRE, and POS injections of saline or bST were detected ($P \geq 0.11$). Preweaning bST injections increased heifer average daily gain (ADG) from days 0 to 10 and plasma IGF-1 on day 10 ($P \leq 0.03$), did not affect ADG from days 0 to 177, plasma IGF-1 from days 259 to 380, and any liver gene mRNA expression ($P \geq 0.19$), but tended to decrease ADG from days 191 to 380 ($P = 0.07$) and percentage of pubertal heifers on days 349 ($P = 0.07$), 359 ($P = 0.002$), and 380 ($P = 0.0001$) compared with saline injections. Postweaning bST injections increased plasma IGF-1 on day 177 and overall liver mRNA expression of *GHR-1A* ($P \leq 0.05$), decreased plasma IGF-1 from days 259 to 380 ($P = 0.03$), tended to decrease liver mRNA expression of *GHR-1B* on day 177 ($P = 0.08$), but did not affect ADG from days 167 to 177 and 191 to 380, and puberty attainment from days 229 to 380 ($P \geq 0.12$) compared with saline injections. Thus, preweaning and postweaning injections of bST successfully increased heifer plasma IGF-1 concentrations 10 d after first injection. Postweaning injections of bST had no impact on puberty attainment, whereas preweaning bST injections of bST impaired puberty attainment of Nellore beef heifers.

Key words: beef heifers, Bos indicus, insulin-like growth factor 1, metabolic imprinting, puberty, somatotropin

[1]The study was funded by Elanco Animal Health (Brooklin, São Paulo, Brazil).

[2]Corresponding author: pmoriel@ufl.edu

Received October 3, 2018.
Accepted November 15, 2018.

Transl. Anim. Sci. 2019.3:443–455
doi: 10.1093/tas/txy125

INTRODUCTION

Although postweaning injections of bovine somatotropin (bST) hastened puberty attainment of *Bos taurus* heifers (Cooke et al., 2013), less emphasis has been placed on preweaning management strategies despite their greater impact on heifer puberty attainment compared with postweaning management practices (Roberts et al., 2007). This greater impact of preweaning strategies may be attributed to metabolic imprinting, which is the concept that body physiological outcomes to early-life nutritional challenges can persist for long periods, even after the removal of such challenges (Lucas, 1991; Du et al., 2010). Beef heifers early-weaned at 70 d of age and limit-fed a high-concentrate diet for 90 d after weaning had similar body weight (BW), but hastened puberty attainment than heifers weaned at 270 d of age (Moriel et al., 2014). In that study, heifer average daily gain (ADG) and plasma IGF-1 concentrations from 70 to 160 d of age explained approximately 34% of the variability on age at puberty (Moriel et al., 2014). Likewise, Piccolo et al. (2018) reported that three preweaning injections of bST (250 mg of sometribove zinc/heifer), administered every 14 d and starting at 132 d of age, hastened puberty attainment in Angus × Brahman beef heifers.

Bos indicus beef heifers are known for achieving puberty at older ages than *B. taurus*–influenced heifers (Short et al., 1994). However, the impact of preweaning and postweaning injections of bST on attainment of puberty of *B. indicus* beef heifers is unknown. It was hypothesized that bST injections would enhance heifer growth and puberty attainment compared with saline injections, as observed by Piccolo et al. (2018). Moreover, heifer puberty achievement would be enhanced at greater magnitude by preweaning vs. postweaning injections of bST due to metabolic imprinting effects. Thus, this study evaluated the growth and puberty attainment of Nellore heifers administered injections of bST or saline solution during preweaning only, postweaning only, or both phases.

MATERIALS AND METHODS

The experiment described herein utilized heifers that remained at a commercial cow–calf operation (Sítio Boa Vista do Rio Claro, São Manuel, São Paulo Brazil) from February 2016 to March 2017. All animals were cared for by acceptable practices as outlined in the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010).

Animals and Treatments

Preweaning phase (days 0 to 167). On day 0, cow–calf pairs (n = 177; cow BW = 470 ± 57 kg; cow BCS = 2.9 ± 0.2, scale 1 to 9) of Nellore dams and their suckling Nellore heifers were stratified by heifer age and initial heifer BW (age = 80 ± 10 days; heifer BW = 97 ± 16 kg) and randomly assigned to 1 of 4 treatments, in a 2 × 2 factorial design (n = 44 to 45 heifers/treatment). Treatments were administered to heifers only and consisted of two injections of saline or bST (Posilac, Elanco, Greenfield, IN) administered 10 d apart during preweaning phase only (days 0 and 10; PRE), postweaning phase only (days 167 and 177; POS), or during both phases (days 0, 10, 167, and 177). Hence, all treatment combinations included preweaning and postweaning injections of saline (SAL-SAL); preweaning and postweaning injections of bST (BST-BST); preweaning injections of bST and postweaning injections of saline (BST-SAL); and preweaning injections of saline and postweaning injections of bST (SAL-BST). All injections were administered subcutaneously on the right side of the neck. Saline injections consisted of 5 mL of a 0.9% NaCl solution, whereas bST injections were administered at 6.14 mg/kg of $BW^{0.75}$. The bST dosage was selected according to Piccolo et al. (2018) who successfully reported an increase in plasma IGF-1 concentrations after similar dosage of bST without any detrimental effects to growth and physiological parameters of Brangus-crossbred heifers. The number of injections was selected based on two criteria: 1) to increase the plasma IGF-1 concentrations for 20 to 28 days after the first bST injection during (PRE heifers) or not (POS heifers) the age window that heifers were susceptible to nutrition-induced impacts on puberty (70 to 180 days of age; Moriel et al., 2014) and 2) to minimize the number of animal handlings and reduce labor.

During the preweaning phase, all cow–calf pairs were managed as a single group, rotated monthly

among six pastures of *Brachiaria decumbens* (54 ha/pasture), and provided free choice access to commercial trace mineral salt (Fosbovi Reprodução, DSM Produtos Nutricionais, São Paulo, Brazil; DM basis: 11.1% Ca, 9.0% P, 1.8% S, 14.1% Na, 60 mg/kg Co, 1,500 mg/kg Cu, 1,800 mg/kg Fe, 75 mg/kg I, 1,800 mg/kg Mn, 17 mg/kg Se, and 4,500 mg/kg Zn) from days 0 to 167.

Postweaning phase (days 167 to 380). Heifers were transferred into a single 40-ha *B. decumbens* pasture (50.0% TDN, 7.5% CP; DM basis) immediately after weaning (day 167). From days 167 to 191, heifers were provided free choice access to water, ground mombaça (*Panicum maximum*) grass silage (Table 1), and 1 g/kg of BW (as-fed) of a commercial mixture of trace minerals and vitamins (Fosbovinho Proteico ADE, DSM Produtos Nutricionais; 26.6% CP, 60.2% TDN, 4.9% Ca, 3.4% P, 1.7% S, 1.3% Na, 2.6 mg/kg Co, 250 mg/kg Cu, 20 mg/kg I, 500 mg/kg Mn, 2.5 mg/kg Se, 750 mg/kg Zn, 31,000, IU/kg vitamin A, 4,000 IU/kg vitamin D_3, and 525 IU/kg vitamin E).

On day 191, heifers were transferred to a 6-ha *B. decumbens* pasture with free choice access to water. A TMR was formulated using NRC (2016) and provided in amounts to ensure that all heifers would achieve between 65% and 70% of mature BW on day 380 (assuming a mature BW of 450 kg). Hence, heifers were limit-fed a corn silage–based TMR at 2.39% of BW (DM basis) from days 191 to 380. The TMR consisted of 68.7% corn silage and 31.3% soybean meal (DM basis). Heifers were provided free choice access to a commercial trace mineral and vitamin mixture (Bovipac Plus, MCassab, São Paulo, Brazil; DM basis: 19.1% Ca, 8.9% P, 2.5% S, 10.5% Na, 1,094 mg/kg Cu, 5,855 mg/kg Fe, 39 mg/kg I, 1,003 mg/kg Mn, 19 mg/kg Se, 2,625 mg/kg Zn, 206 IU/kg vitamin A, 58,800 IU/kg vitamin D_3, and 784 mg/kg vitamin E) from days 191 to 380.

Sample and Data Collection

Individual full BW was collected from all heifers at 0800 h on days 0, 10, 167, and 177, whereas individual BW and BCS of all cows were collected on days 0 and 167. Full postweaning BW and BCS of heifers were obtained on days 229, 259, 289, 320, 349, and 380. From days 229 to 380, transrectal ultrasonography of ovaries (Probe UST 5561–7.5 MHz linear array transducer, Aloka Prosound 2, Corimetrics Medical Systems, Inc., Wallingford, CT) was performed in all heifers twice monthly (days 1 and 10 of each month), by the same trained veterinarian, to determine the percentage of pubertal heifers. Age at puberty was set as the day when corpus luteum (CL) was detected. Body weight at puberty attainment was determined using the monthly ADG (calculated using BW obtained on day 1 of each month), and initial and final 30-d BW measurements of the respective month when CL was detected [BW at puberty = initial BW of the respective month + (ADG of the respective month × number of days between the day at CL detection and initial BW collection)].

A subgroup of heifers were randomly selected on day 0 and used for collections of blood (n = 15

Table 1. Average nutritional composition (DM basis) of pastures, grass silage, and corn silage-based TMR provided to heifers throughout the study

Item	Preweaning pasture[a]	Postweaning pasture[a]	Grass silage[b]	TMR[c]
DM, %	39.0	22.7	31.0	50.8
CP, %	9.8	15.8	5.9	20.9
NDF, %	68.3	56.6	78.0	37.1
ADF, %	40.8	29.3	48.6	23.3
TDN[d], %	54.6	60.6	45.9	70.0
NEm[e], Mcal/kg	1.13	1.38	0.76	1.69
NEg[e], Mcal/kg	0.57	0.80	0.22	1.08
Ca, %	0.32	0.62	0.49	0.29
K, %	1.65	2.76	1.05	1.25
Mg, %	0.25	0.37	0.27	0.24
P, %	0.25	0.35	0.15	0.36
S, %	0.17	0.24	0.19	0.40

[a]Samples of brachiaria pastures collected monthly during preweaning (days 0 to 167) and postweaning phases (days 192 to 380).

[b]Samples of mombaça grass silage collected from days 167 to 191.

[c]Samples of TMR collected monthly from days 191 to 380. Total mixed ration consisted of 68.7% corn silage and 31.3% soybean meal (DM basis).

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

[e]Calculated using the equations proposed by the NRC (2000).

heifers/treatment) and liver samples (n = 10 heifers/treatment) on days 0, 10, 167, 177, and 380. Additional blood samples were collected from the same heifers on days 259, 289, 320, and 350. Blood samples were collected (10 mL) via jugular venipuncture in sodium-heparin (158 USP)–containing tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ), placed on ice immediately after collection, and then centrifuged at 1,200 × g for 25 min at 4°C. Plasma was stored frozen at −20°C until later analysis of plasma concentrations of IGF-1.

All liver samples were collected via needle biopsy, following the procedure described by Arthington and Corah (1995). Immediately following collection, 100 mg of wet liver tissue/heifer was stored into 1.5 mL of RNA stabilization solution (RNAlater, Ambion Inc., Austin, TX), kept on ice for 8 h, and stored at −20°C until later analyses of mRNA expression of *cyclophilin*, *GH receptor 1A* and *1B* (*GHR-1A* and *GHR-1B*), *IGF-1*, *IGF binding protein 3* (*IGFBP-3*), and *40S ribosomal protein S9* (*RSP9*).

Laboratory Analyses

Hand-plucked samples of pastures, grass silage, trace mineral and vitamin mixtures, and TMR were collected monthly, and sent in duplicate to a commercial laboratory (3R LAB, Lavras, Minas Gerais, Brazil) for concentrations of DM (method 930.15; AOAC, 2006), CP (method 984.13; AOAC, 2006), TDN (Weiss et al., 1992), NEm, and NEg (NRC, 2000). Concentrations of NDF and ADF were determined using the method of Van Soest et al. (1991) adapted for an ANKOM 200 Fiber Analyzer (ANKOM Technology, Macedon, NY). Nutritional composition of pasture, grass silage, and TMR samples is shown in Table 1.

Plasma 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 and previously validated for bovine samples (Moriel et al., 2012). Interassay and intra-assay CV for IGF-1 assay were 1.31% and 2.65%, respectively.

A detailed description of procedures for mRNA isolation and tissue gene expression was described by Cappellozza et al. (2014). Briefly, total RNA was extracted from liver tissue samples using the TRIzol Plus RNA Purification Kit (Invitrogen, Carlsbad, CA). Extracted RNA was quantified via UV absorbance (UV Mini 1240; Shimadzu Scientific Instruments, Inc., Columbia, MD) at 260 nm, incubated (2.5 µg) at 37°C for 30 min in the presence of RNase-free (DNase; New England Biolabs, Inc., Ipswich, MA), and reverse transcribed using the High Capacity cDNA Reverse Transcription Kit with random hexamers (Applied Biosystems, Foster City, CA). Real-time PCR was completed using the SYBR Green PCR Master Mix (Applied Biosystems) and gene-specific primers (20 pM each) with the StepOne Real-time PCR system (Applied Biosystems). At the end of each real-time PCR, amplified products were subjected to a dissociation gradient (95°C for 15 s, 60°C for 30 s, and 95°C for 15 s) to verify the amplification of a single product by denaturation at the anticipated temperature. The amplified products were purified with the QIAquick PCR purification kit (Qiagen Inc., Valencia, CA) and sequenced at the Department of Animal Science from University of Florida to verify the specificity of amplification. All amplified products represented only the genes of interest. Primer sequence of target genes is shown in Table 2 and was validated by previous studies, except for *GHR-1B*, which was designed based on the bovine gene sequences deposited in the National Center for Biotechnology Information and using the Primer Express v.3.0.1 software (Applied Biosystems, Foster City, CA). Responses were quantified based on the threshold cycle (CT) and were normalized to the geometrical mean CT values of *cyclophilin* and *RSP9* (ΔCT) examined in the same sample and assessed at the same time as the targets. Within each target gene, results are expressed as relative fold change ($2^{-\Delta\Delta CT}$) using the average ΔCT of all samples (Ocón-Grove et al., 2008). Interassay and intra-assay CV for mRNA expression of *IGF-1*, *IGFBP-3*, *GHR-1A*, and *GHR-1B* were 2.77% and 3.77%, 2.94% and 3.11%, 2.33% and 4.27%, and 2.59% and 3.89%, respectively (Bustin et al., 2009).

Statistical Analyses

All data were analyzed as a 2 × 2 factorial design using SAS (SAS Institute, Inc., Cary, NC, version 9.4) with Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. Heifer was the experimental unit, whereas heifer (PRE × POS) was included as random effect in all analyses. Growth performance data were analyzed using the MIXED procedure, whereas puberty attainment was analyzed using the GLIMMIX procedure. Heifer ADG, BW, and age at puberty, and mature BW on day 380 were tested for fixed effects of preweaning injections (PRE), postweaning injections (POS), and all resulting interactions. Heifer BW, BCS, plasma IGF-1, liver mRNA expression, and puberty attainment were analyzed as repeated measures and tested for fixed

Table 2. Primer sequences and accession number for all gene transcripts analyzed by quantitative real-time PCR

Target gene	Primer sequence	Accession no.
Cyclophilin		
Forward	5′-GGTACTGGTGGCAAGTCCAT-3′	NM_178320.2
Reverse	5′-GCCATCCAACCACTCAGTCT-3′	
IGF-1		
Forward	5′-CTCCTCGCATCTCTTCTATCT-3′	NM_001077828
Reverse	5′-ACTCATCCACGATTCCTGTCT-3′	
IGFBP-3		
Forward	5′-AATGGCAGTGAGTCGGAAGA-3′	NM_174556.1
Reverse	5′-AAGTTCTGGGTGTCTGTGCT-3′	
GHR-1A		
Forward	5′-CCAGCCTCTGTTTCAGGAGTGT-3′	AY748827
Reverse	5′-TGCCACTGCCAAGGTCAAC-3′	
GHR-1B		
Forward	5′-AGCCTGGAGGAACCATACGA-3′	–
Reverse	5′-TAGCCCCATCTGTCCAGTGA-3′	
RSP9		
Forward	5′-CCTCGACCAAGAGCTGAAG-3′	DT860044
Reverse	5′-CCTCCAGACCTCACGTTTGTTC-3′	

Primer sequence for IGF-1, IGFBP-3, and GHR-1A were obtained from Coyne et al. (2011), whereas those for cyclophilin and RSP9 were obtained from Cooke et al. (2008) and Janovick-Guretzky et al. (2007), respectively. Primer sequence for GHR-1B was designed based on the bovine gene sequences deposited in the National Center for Biotechnology Information and using the Primer Express v. 3.0.1 software (Applied Biosystems, Foster City, CA).

effects of PRE, POS, day of the study, and all resulting interactions using heifer (PRE × POS) as the subject. Heifer BW and age on day 0 were included as covariates in all analyses, but removed from the model if $P > 0.10$. Proper covariance structure for each statistical analysis was selected based on the lowest Akaike information criterion. Compound symmetry covariance structure was used for statistical analyses of preweaning and postweaning heifer BW, cow BW and BCS, postweaning heifer BCS, and liver mRNA expression of *IGF-1* and *IGFBP-3*. Autoregressive 1 covariance structure was used for the statistical analyses of liver mRNA expression of *GHR-1A* and *-1B*, and puberty attainment. All results are reported as least-squares means. Data were separated using PDIFF if a significant *F*-test was detected. Significance was set at $P \leq 0.05$, and tendencies at $P > 0.05$ and ≤ 0.10.

RESULTS

Effects of PRE × POS × day and PRE × POS were not detected for any variable in the study ($P \geq 0.11$), and hence, effects of PRE and POS treatments were reported separately.

Heifer BW and age on day 0 did not differ among treatments ($P \geq 0.75$). However, heifer BW and age on day 0 were included as covariates in the analyses of heifer BW and ADG from days 0 to 177 ($P \leq 0.001$), except for ADG from days 0 and 177 that was covariate-adjusted to age on day 0 only ($P = 0.04$). Effects of PRE × day of the study were ($P = 0.02$) detected for heifer BW from days 0 to 177. However, BW on days 10, 167, and 177 did not differ between heifers administered preweaning injections of bST or saline ($P \geq 0.12$; Table 3). Effects of POS × day of the study tended ($P = 0.07$) to be detected for heifer BW from days 0 to 177, which did not differ between saline and bST heifers on days 10 and 167 ($P \geq 0.20$), but tended to be greater on day 177 following postweaning injections of bST vs. saline ($P = 0.10$; Table 3).

Preweaning injections of saline or bST did not affect heifer ADG from days 0 to 177 and 167 to 177 ($P \geq 0.22$). However, heifer ADG from days 0 to 10 was greater for those administered preweaning injections of bST vs. saline ($P = 0.03$), whereas ADG from days 10 to 167 was greater for heifers administered preweaning injections of saline vs. bST ($P = 0.05$; Table 3). Heifer preweaning or postweaning injections of saline and bST did not affect cow BW, BCS, and BW and BCS change from days 0 to 167 ($P \geq 0.17$; Table 3). Postweaning injections of saline and bST did not affect heifer ADG from days 167 to 177, and overall ADG from days 0 to 177 ($P \geq 0.12$; Table 3). Effects of PRE and POS were not detected ($P \geq 0.31$) for heifer ADG from days 177 to 191 (0.27, 0.26, 0.23, and 0.29 ± 0.057 kg/d for

Table 3. Growth performance (days 0 to 177) of Nellore heifers administered, in a 2 × 2 factorial design, injections of saline solution, or BST during preweaning (days 0 and 10) or postweaning phases (days 167 and 177)

	Preweaning injections				*P*-value	Postweaning injections				*P*-value
Item	Saline	BST	SEM	*P*-value[a]	PRE × day	Saline	BST	SEM	*P*-value[a]	POS × day
Heifer BW[b], kg										
Day 10	109	111	1.35	0.21	0.02	110	109	1.36	0.48	0.07
Day 167	187	185	1.35	0.24		185	187	1.36	0.20	
Day 177	187	184	1.35	0.12		184	187	1.36	0.10	
Heifer ADG[b], kg/d										
Days 0 to 10	0.48	0.65	0.061	0.03		0.62	0.51	0.061	0.14	
Days 10 to 167	0.47	0.45	0.008	0.05		0.45	0.47	0.009	0.12	
Days 167 to 177	−0.01	−0.07	0.068	0.54		−0.06	−0.01	0.069	0.59	
Days 0 to 177	0.47	0.45	0.009	0.24		0.45	0.46	0.009	0.28	
Cow BW, kg										
Day 0	487	482	5.68	0.61	0.22	483	486	5.7	0.63	0.41
Day 167	465	456	5.68	0.25		460	461	5.7	0.95	
Cow BCS										
Day 0	2.89	2.87	0.020	0.47	0.80	2.88	2.88	0.020	0.89	0.95
Day 167	2.83	2.82	0.020	0.63		2.83	2.82	0.020	0.93	
BW change, kg	−21.5	−27.1	2.84	0.17		−22.9	−25.6	2.84	0.50	
BCS change	−0.06	−0.05	0.019	0.87		−0.06	−0.05	0.019	0.84	

The study began on day 0 and heifer calves were weaned on day 167. Injections of saline (5 mL 0.9% NaCl) or bST (6.14 mg/kg of $BW^{0.75}$) were administered subcutaneously in the neck and 10 d apart during preweaning (days 0 and 10) and/or postweaning (days 167 and 177).

[a]*P*-value for comparison of treatment within day.

[b]Covariate-adjusted to BW and age on day 0 ($P \leq 0.001$), except for ADG from days 0 and 177 that was covariate-adjusted to age on day 0 only ($P = 0.04$).

PRE-bST, PRE-saline, POS-bST, and POS-saline heifers, respectively).

Heifer BW from days 191 to 380 was covariate-adjusted to heifer BW and age on day 0 ($P \leq 0.002$), whereas overall ADG from days 191 to 380 was covariate-adjusted to heifer BW on day 0 only ($P = 0.02$). Body weight and age on day 0 were included as covariates in the analyses of BW and age at puberty ($P \leq 0.06$). Effects of PRE × day of the study and POS × day of the study were detected ($P \leq 0.03$) for heifer postweaning BW. Heifers administered preweaning injections of bST were lighter on days 349 and 380 ($P \leq 0.04$), and tended to be lighter on day 259 ($P = 0.06$) compared with heifers administered preweaning injections of saline (Table 4). Heifer BCS did not differ following preweaning or postweaning injections of saline and bST ($P \geq 0.11$). Overall heifer ADG from days 191 to 380 tended to be greater ($P = 0.07$) for heifers administered preweaning injections of saline vs. bST, and did not differ ($P = 0.12$) between heifers administered postweaning injections of saline vs. bST (Table 4). Heifers administered preweaning injections of saline had greater ($P = 0.03$) mature BW on day 380, but similar BW and age at puberty ($P \geq 0.11$) compared with heifers administered preweaning injections of bST (Table 4). Mature BW on day 380, and BW and age at puberty did not differ between heifers given postweaning injections of bST or saline ($P \geq 0.39$; Table 4).

Effects of PRE × day of the study and POS × day of the study were detected ($P \leq 0.01$) for plasma concentrations of IGF-1 collected from days 0 to 177. Heifers administered preweaning injections of bST had greater plasma IGF-1 concentrations on day 10 compared with heifers administered preweaning injections of saline ($P = 0.0001$; Table 5). Heifers given postweaning injections of bST had greater plasma IGF-1 concentrations on day 177 compared with saline ($P = 0.005$; Table 5). Interactions among day of the study, PRE, and POS injections were not detected ($P \geq 0.19$) for plasma concentrations of IGF-1 collected from days 259 to 380, except for main effects of POS and day of the study ($P \leq 0.03$). Preweaning injections of bST or saline did not affect overall plasma IGF-1 concentrations ($P = 0.19$), whereas heifers administered postweaning injections of bST had less overall

Table 4. Postweaning growth performance (days 191 to 380) of Nellore heifers administered, in a 2 × 2 factorial design, injections of saline solution or BST during preweaning (days 0 and 10) or postweaning phases (days 167 and 177)

	Preweaning injections				*P*-value	Postweaning injections				*P*-value
Item	Saline	BST	SEM	*P*-value[a]	PRE × day	Saline	BST	SEM	*P*-value[a]	POS × day
Heifer BW[b], kg										
Day 191	190	188	3.06	0.56	0.02	188	190	3.11	0.91	0.03
Day 229	197	192	3.06	0.25		194	195	3.11	0.99	
Day 259	220	212	3.06	0.06		217	215	3.11	0.46	
Day 289	240	235	3.06	0.15		240	235	3.11	0.20	
Day 320	266	260	3.06	0.13		265	262	3.11	0.33	
Day 349	291	283	3.06	0.03		289	286	3.11	0.45	
Day 380	317	309	3.06	0.04		316	310	3.11	0.24	
Heifer BCS[b,c]										
Day 229	5.12	5.08	0.046	0.51	0.19	5.12	5.10	0.046	0.88	0.27
Day 259	5.10	5.02	0.046	0.20		5.08	5.02	0.046	0.48	
Day 289	5.46	5.50	0.046	0.45		5.54	5.40	0.046	0.23	
Day 320	5.86	5.90	0.046	0.66		5.92	5.84	0.046	0.15	
Day 349	5.96	5.86	0.046	0.16		5.92	5.90	0.046	0.62	
Day 380	6.32	6.26	0.046	0.42		6.36	6.24	0.046	0.08	
ADG days 191 to 380[b], kg/d	0.83	0.80	0.014	0.07	–	0.83	0.80	0.014	0.12	–
Mature BW on day 380[d], %	70.7	68.8	0.63	0.03	–	70.1	69.4	0.65	0.48	–
BW at puberty[e], kg	311	306	3.4	0.30	–	311	307	3.4	0.39	–
Age at puberty[e], d	453	458	3.2	0.11	–	456	455	3.2	0.89	–

Injections of saline (5 mL 0.9% NaCl) or bST (6.14 mg/kg of $BW^{0.75}$) were administered subcutaneously in the neck during preweaning (days 0 and 10) and/or postweaning (days 167 and 177). Heifers were weaned on day 167 and limit-fed corn silage–based diets from days 191 to 380.

[a]*P*-value for comparison of treatment within day.

[b]Covariate-adjusted to BW and age on day 0 ($P \leq 0.002$), except for overall ADG from days 191 to 380 that was covariate-adjusted to BW on day 0 only ($P = 0.02$).

[c]Using a 1 to 9 scale according to Wagner et al. (1988).

[d]Assuming a mature cow BW of 450 kg.

[e]Body weight at puberty attainment was determined using the ADG, and initial and final BW measurements of the respective month when CL were detected [BW at puberty = initial BW of the respective month + (ADG of the respective month × number of days between the day at CL detection and initial BW collection)]. Age at puberty was set as the day when CL was detected (day 1 or 10 of each month from days 192 to 380).

Table 5. Preweaning and postweaning plasma IGF-1 concentrations of Nellore heifers administered, in a 2 × 2 factorial design, injections of saline solution or BST during preweaning (days 0 and 10) or postweaning phases (days 167 and 177)

	Preweaning injections				*P*-value	Postweaning injections				*P*-value
Item	Saline	BST	SEM	*P*-value[a]	PRE × day	Saline	BST	SEM	*P*-value[a]	POS × day
Plasma IGF-1, ng/mL										
Day 0	193.0	186.0	9.3	0.59	0.001	198.9	179.9	9.3	0.15	0.01
Day 10	159.7	211.4	9.3	0.0001		186.8	184.4	9.3	0.85	
Day 167	82.4	82.2	9.3	0.99		76.4	88.2	9.3	0.37	
Day 177	83.0	76.1	9.3	0.60		60.9	98.1	9.3	0.005	
Overall (days 259 to 380)[b]	197.7	210.8	7.1	0.19	–	216.0	192.5	7.1	0.03	–

Injections of saline (5 mL 0.9 % NaCl) or bST (6.14 mg/kg of $BW^{0.75}$) were administered subcutaneously in the neck during preweaning (days 0 and 10) and/or postweaning (days 167 and 177). Heifers were weaned on day 167 and limit-fed corn silage–based diets from days 191 to 380.

[a]*P*-value for comparison of treatment within a day.

[b]Average plasma IGF-1 concentrations of blood samples collected on days 259, 289, 319, 349, and 380.

plasma IGF-1 concentrations from days 259 to 380 compared with heifers administered postweaning injections of saline ($P = 0.03$; Table 5).

Interactions among day of the study, PRE, and POS, as well as main effects of preweaning injections were not detected ($P \geq 0.14$) for liver mRNA expression of *GHR-1A*. However, main effects of day of the study and postweaning injections were detected ($P \leq 0.03$). Liver mRNA expression of *GHR-1A* did not differ between days 0 and 10 (1.39 and 1.28 ± 0.210-fold increase, respectively; $P = 0.64$), decreased on day 167 (0.86 ± 0.210-fold increase; $P \leq 0.01$), achieved greatest values on day 177 (1.82 ± 0.210-fold increase, respectively; $P \leq 0.07$), and returned to baseline levels on day 380 (1.37 ± 0.210-fold increase, respectively; $P = 0.92$). Overall liver mRNA expression of *GHR-1A* was greater for heifers administered postweaning injections of bST vs. saline ($P = 0.03$; Table 6).

Liver mRNA expression of *GHR-1B* and *IGF-1* on day 0 was included as covariate ($P \leq 0.01$) in the analyses of liver mRNA expression of *GHR-1B* and *IGF-1*, respectively. Effects of POS × day of the study ($P = 0.02$), but not PRE, POS, and PRE × day of the study ($P \geq 0.16$), were detected for liver mRNA expression of *GHR-1B*. Heifers administered postweaning injections of bST had greater ($P = 0.02$) mRNA expression of *GHR-1B* on day 167, but tended to have less ($P = 0.08$) liver *GHR-1B* mRNA expression on day 177 compared with heifers administered postweaning injections of saline (Table 6). Interactions among day of the study, PRE, and POS, as well as main effects of PRE and POS were not detected ($P \geq 0.12$) for liver mRNA expression of *IGF-1* (Table 6). However, effect of day of the study was detected ($P < 0.0001$) for liver mRNA expression of *IGF-1*, which was greatest on day 10 (2.55 ± 0.170-fold increase; $P < 0.0001$), decreased on days 167 and 177 (0.84 and 1.03 ± 0.170-fold increase; $P \leq 0.01$), and were intermediate on day 380 (1.27 ± 0.170-fold increase, respectively).

Effects of PRE × day of the study, POS × day of the study, PRE, and POS were not detected ($P \geq 0.20$) for liver mRNA expression of *IGFBP-3* (Table 6). However, effect of day of the study was detected ($P < 0.0001$) for liver mRNA expression of *IGFBP-3*, which was greatest on day 10 (2.22 ± 0.209-fold increase; $P \leq 0.06$), decreased on days 0, 167, and 380 (0.95, 1.12, and 1.10 ± 0.209-fold increase, respectively), and were intermediate on day 177 (1.27 ± 0.170-fold increase, respectively; $P \leq 0.08$).

A tendency for effects of PRE × day of the study ($P = 0.09$), but not POS × day of the study ($P = 0.18$), was detected for heifer puberty attainment. A greater percentage of heifers administered preweaning injections of saline attained puberty

Table 6. Preweaning and postweaning liver mRNA expression (fold increase[a]) of Nellore heifers administered, in a 2 × 2 factorial design, injections of saline solution or BST during preweaning (days 0 and 10) or postweaning phases (days 167 and 177)[b]

	Preweaning injections				*P*-value	Postweaning injections				*P*-value
	Saline	BST	SEM	*P*-value[c]	PRE × day	Saline	BST	SEM	*P*-value[c]	POS × day
Item	*Fold increase*					*Fold increase*				
GHR-1A[d]	1.37	1.32	0.174	0.78	0.98	1.17	1.52	0.171	0.03	0.87
IGF-1[d]	1.37	1.48	0.129	0.53	0.86	1.37	1.48	0.131	0.52	0.12
IGFBP-3	1.50	1.31	0.122	0.29	0.64	1.46	1.36	0.122	0.54	0.69
GHR-1B[d]										
Day 10	1.75	1.64	0.179	0.71	0.46	1.56	1.84	0.222	0.34	0.02
Day 167	1.14	1.12	0.179	0.93		0.77	1.49	0.222	0.02	
Day 177	1.36	0.79	0.179	0.07		1.35	0.81	0.222	0.08	
Day 380	1.31	1.31	0.179	0.99		1.14	1.47	0.222	0.28	

[a]Responses were quantified based on the threshold cycle (CT) and were normalized to average CT of cyclophilin and RSP9 (ΔCT) examined in the same sample and assessed at the same time as the targets. Within each target gene, results are expressed as relative fold change ($2^{-\Delta\Delta CT}$) using the average ΔCT of all samples as reference, as described by Ocón-Grove et al. (2008).

[b]Injections of saline (5 mL 0.9% NaCl) or bST (6.14 mg/kg of $BW^{0.75}$) were administered subcutaneously in the neck during preweaning (days 0 and 10) and/or postweaning (days 167 and 177). Heifers were weaned on day 167 and limit-fed corn silage–based diets from days 191 to 380.

[c]*P*-value represents the main effects of treatment for overall mRNA expression of *GHR-1A*, *IGF-1*, and *IGFBP-3* obtained on days 0, 10, 167, 177, and 380. For mRNA expression of *GHR-1B*, the *P*-value represents the comparison of treatment within day for.

[d]Liver mRNA expression of *IGF-1* and *GHR-1B* were covariate-adjusted to respective mRNA expression on day 0 ($P \leq 0.02$), whereas liver mRNA expression of *GHR-1A* was covariate-adjusted to BW on day 0 only ($P = 0.05$).

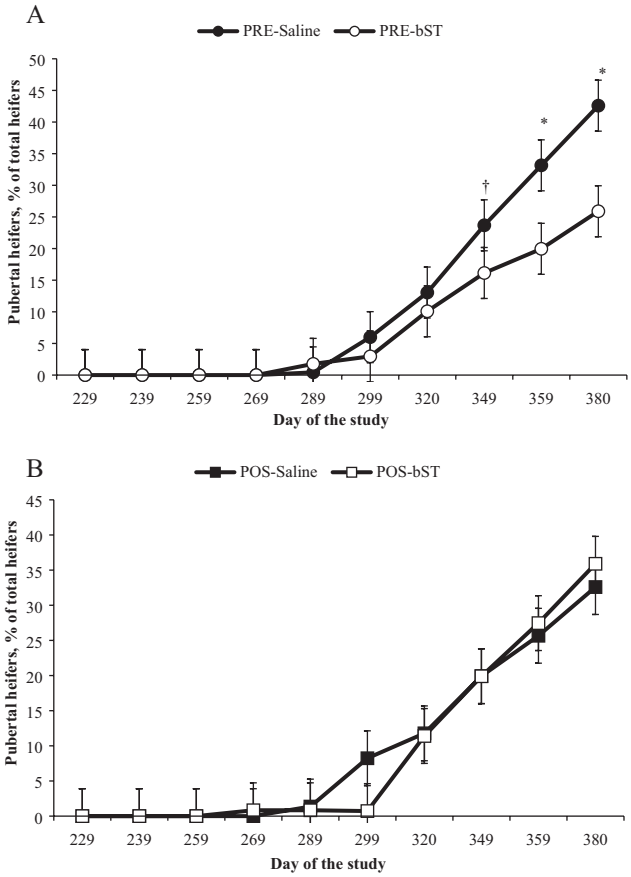

Figure 1. Puberty attainment of Nellore heifers administered, in a 2 × 2 factorial design, injections of saline solution or BST during preweaning (days 0 and 10; (a)) or postweaning phases (days 167 and 177; (b)). Effects of PRE × day of the study, but not POS × day of the study (P = 0.22), tended to be detected (P = 0.09) for puberty attainment during the study. *$P \leq 0.05$; †$0.05 < P \leq 0.10$.

on days 349 (P = 0.07), 359 (P = 0.002), and 380 (P = 0.0001) compared with heifers administered preweaning injections of bST (Figure 1a).

DISCUSSION

Day and Anderson (1998) proposed that the period from birth to puberty in beef heifers could be divided into infantile, developmental, static, and peripubertal periods. During the developmental phase (2 to 6 months of age), GnRH secretion and follicular growth increase with LH secretion and number of ovarian follicles peaking at 3 to 4 months of age. Enhancing heifer ADG and nutrient intake during this developmental phase led to hastened follicle growth (Gasser et al., 2006a, 2006b) and puberty attainment of beef heifers (Moriel et al., 2014). The exact nutrition-mediated mechanisms involved in this early activation of the reproductive axis in beef heifers are unknown. However, circulating IGF-I can affect gonadotropin secretion and activity required for the first ovulation and subsequent puberty achievement in beef heifers by influencing hypothalamic–pituitary secretory activity (Butler and Smith, 1989; Schillo et al., 1992) and augmenting the effects of gonadotropins in ovarian follicular cells (Spicer and Echternkamp, 1995). In agreement, heifer ADG and plasma IGF-1 concentrations from 70 to 160 days of age explained approximately 34% of the variability on age at puberty (Moriel et al., 2014). Thus, metabolic imprinting may be explored by identifying strategies to increase heifer ADG and plasma IGF-1 during the developmental phase leading to optimized future reproductive performance.

Systemic IGF-1 has been positively correlated with muscle skeletal growth (Jiang and Ge, 2014). Piccolo et al. (2018) reported that Brangus-crossbred heifers administered preweaning bST injections (250 mg every 14 days from 132 to 174 days of age) had an 8.6 ng/mL increase in plasma IGF-1 concentrations and 7.2% increase on ADG from days 0 to 42, relative to first injection, but no differences on ADG from first injection to weaning (167-d period) compared with saline heifers. Likewise, preweaning bST injections in the present study increased plasma IGF-1 concentrations by 51.7 ng/mL and ADG from days 0 to 10 by 35%, but did not affect ADG from days 0 to 177. The increase in BW gain and circulating IGF-1 concentrations following bST injections varied from 0% to 45% compared with control vehicles (Dalke et al., 1992; Houseknecht et al., 1992), and several factors, such as plane of nutrition, age, and size of treated animals may explain this large variation (Rausch et al., 2002). Also, multiple mechanisms may be involved in post-bST BW gain including repartitioning of nutrients toward muscle rather than adipose tissue deposition (Breier, 1999), and enhanced long-bone growth (Buskirk et al., 1996), nitrogen retention (Eiseman et al., 1986), and circulating IGF-1-induced protein synthesis of muscle (Jiang and Ge, 2014) and noncarcass tissues (Early et al., 1990). Multiple 14-day apart administrations of bST, during the postweaning phase, reduced subcutaneous fat thickness by 9.2% without affecting LM depth, marbling scores, and BW gain (Cooke et al., 2013). Although body composition was not evaluated in the present study, it is unlikely that only two 10-day apart injections of bST substantially affected body composition and nutrient requirements of heifers. Thus, our results perhaps indicate that the increment on bST-induced ADG from days 0 to 10 may be the result of increased feed intake and gut fill, as reported by Enright et al. (1990), or that muscle protein deposition from days

0 to 10 was not sufficient to affect heifer BW at weaning. Nevertheless, preweaning bST injections in the present study successfully increased plasma IGF-1 concentrations and ADG of heifers during the developmental phase of the reproductive axis in beef heifers (Day and Anderson, 1998).

In contrast, postweaning bST injections increased plasma IGF-1 concentrations by 37.2 ng/mL, but did not affect ADG during the injection period (days 167 to 177). Other studies also demonstrated that postweaning bST injections increased plasma IGF-1 concentrations (Cooke et al., 2013), but did not increase postweaning ADG of Angus × Holstein administered 500 mg of bST every 14 d from 6 to 10 months of age (Carstens et al., 1997) and Angus × Hereford heifers injected with 250 mg of bST every 14 d from 6 to 13 months of age (Cooke et al., 2013). Body weight gain and circulating IGF-1 responses to bST are influenced by cattle age and nutritional status (Rausch et al., 2002; Radcliff et al., 2004). Cattle somatotropic axis is functional at birth (Granz et al., 1997), and the response to bST begins as early as 1 day of age (Govoni et al., 2004), gradually increasing as age increases (Velayudhan et al., 2007). Likewise, plasma IGF-1 concentrations following bST injection were greater for Holstein heifers gaining 1.2 vs. 0.8 kg/d (Radcliff et al., 2004). In the current study, heifers were weaned on day 167 and provided free choice access to grass silage and pastures of relatively poor nutritional composition, which resulted in BW loss from days 167 to 177 and reduced ADG from days 177 to 191 compared with the preweaning period. Hence, despite the more advanced age at the time of bST injections, the lack of positive impacts of postweaning injections of bST on BW gain from days 167 to 177 and 177 to 191 may be related to the reduced magnitude of increase in plasma IGF-1 concentrations, which was likely suppressed by the weaning-induced physiological stress (Arthington et al., 2008) and the poorer nutritional status of heifers during postweaning vs. preweaning periods (Radcliff et al., 2004).

The binding of GH to GHR-1A stimulates hepatic synthesis of IGF-1 (Smith et al., 2002) and is highly correlated with the hepatic mRNA expression of *GHR-1A* and *IGF-1* (Lucy et al., 2001). Transcription of the growth hormone receptor (*GHR*) gene is initiated from multiple transcription start sites, generating *GHR-1A, -1B,* and *-1C* mRNA that differ in the 5′ untranslated region but still encode the same amino acid sequence (Jiang and Lucy, 2001). The *GHR-1A* mRNA is only expressed in the liver (Lucy et al., 1998), whereas *GHR-1B* and *-1C* mRNA are expressed in a wide array of tissues, including liver, skeletal muscle, adipose tissue, and mammary gland (Jiang et al., 1999; Jiang and Lucy, 2001). Hepatic synthesis of IGF-1 is regulated primarily at the transcriptional level (Thissen et al., 1994) and is the major source of circulating IGF-1 (Yakar et al., 1999), which is also responsible for stimulating the hepatic expression of *IGFBP-3* mRNA (Thissen et al., 1994). Thus, an increased hepatic expression of *GHR-1A* mRNA enhances the capacity for GH binding (Lapierre et al., 1992) and the hepatic synthesis of IGF-1 (Radcliff et al., 2004). Nutrient intake and BW gain positively affects the hepatic abundance of *GHR-1A*, *IGF-1,* and *IGFBP-3* (Thissen et al., 1994; Smith et al., 2002; Radcliff et al., 2004). Holstein heifers administered daily injections of bST (25 µg/kg of BW from 120 to 247 days of age, in average) had greater mRNA expression of *IGF-1*, but similar mRNA expression of *GHR-1A* and *IGFBP-3* (Radcliff et al., 2004).

Piccolo et al. (2018) demonstrated that three preweaning injections of bST did not affect liver mRNA expression of *GHR-1A* and *IGFBP-3* throughout the study, but increased liver mRNA expression of *GHR-1B* and *IGF-1* at approximately 220 days after the last preweaning injection of bST, suggestive of metabolic imprinting effects causing long-term changes to gene expression, despite the similar nutritional status of those heifers. In contrast, preweaning injections of bST did not affect the mRNA expression of any gene measured in the present study, whereas postweaning bST injections increased overall liver mRNA expression of *GHR-1*A and decreased liver *GHR-1B* mRNA on day 177. Several reasons may be responsible for this lack of effects of preweaning bST injections.

Sartori et al. (2016) reported that *B. indicus* cattle naturally have greater circulating IGF-I concentrations compared with *B. taurus* cohorts. Moreover, Mendonça et al. (2013) demonstrated that even under the same environment and diet, *B. taurus*–influenced dairy cows have less circulating concentrations of IGF-I compared with *B. indicus* cows, which might be related to the different organ sensitivity to IGF-1. Despite the lack of published evidence, it is possible that a breed × bST effect on liver gene mRNA expression exists, but further studies comparing effects of breed on physiological and growth responses with bST are warranted to confirm this rationale. Also, due to differences on nutritional status, liver mRNA expression of *IGF-1* and *GHR-1A* of all heifers was greater on days 0 vs. 167 (data not shown). Therefore, liver

mRNA expression of *GHR-1A* and *IGF-1* was perhaps at maximum during preweaning phase, which may have prevented further increments on mRNA expression of these genes. Plasma IGF-1 concentrations increase after 3 days, peak at approximately 7 to 8 days, and gradually return to baseline levels starting 12 days postinjection of bST (Bilby et al., 1999, 2004). Hence, it is possible that the timing of liver sample collection was not optimal to detect the peak expression of liver mRNA of *IGFBP-3*, *IGF-1*, *GHR-1B*, and *GHR-1A*. The detection of greater plasma IGF-1 concentrations on days 10 and 177, but similar liver mRNA expression of IGF-1 on those days between bST and saline heifers supports this rationale. Nevertheless, the greater overall mRNA expression of *GHR-1A* following postweaning bST injections provides evidence that even postweaning injections of bST can cause metabolic imprinting effects and alters long-term gene expression, despite the similar postweaning nutritional status.

The impact of bST injections on posttreatment circulating concentrations of IGF-1 and puberty attainment of beef heifers has been variable. Injections of bST (250 mg every 14 days from 120 to 232 days of age) did not affect posttreatment serum concentrations of IGF-1 and puberty attainment of Angus × Simmental crossbred heifers (Buskirk et al., 1996). Preweaning injections of bST (250 mg every 14 days from 132 to 174 days of age) hastened puberty attainment of Brangus heifers at the start of the breeding season compared with saline injections, despite their similar nutritional management, ADG, and BW during breeding season (Piccolo et al., 2018). Postweaning bST injections (250 mg of bST every 14 days from 6 to 13 months of age) increased puberty attainment of Angus × Hereford heifers at the start of breeding season (Cooke et al., 2013), but had no impact on puberty achievement of Angus heifers administered bST (350 mg every 14 days from 7 to 14.5 months of age) compared with vehicle-treated heifers (Hall et al., 1994).

In the present study, preweaning injections of bST decreased postweaning growth performance compared with saline injections, whereas postweaning injections of bST did not affect postweaning growth performance of heifers. Age at puberty in cattle typically decreases as BW gain increases (Schillo et al., 1992). Hence, the similar postweaning ADG likely explains the similar puberty achievement of heifers given postweaning injections of bST and saline, whereas the decreased puberty attainment of heifers administered preweaning injections of bST vs. saline may be at least partially explained by the observed differences in postweaning growth rate and BW. The reason for the decreased postweaning growth performance of heifers following preweaning bST injections is unknown. Moriel et al. (2014) observed that during similar nutritional management, liver mRNA expression of *IGF-1* and puberty attainment at start of breeding season were greater for heifers early-weaned at 60 days of age and placed on high-concentrate diets until 150 days of age compared with heifers normally weaned at 270 days of age, suggestive of metabolic imprinting effects. Hence, it was expected that the long-term postweaning plasma concentrations of IGF-1 would be positively affected by preweaning rather than postweaning injections of bST due to the greater susceptibility of younger animals to metabolic imprinting effects. However, overall plasma IGF-1 concentrations collected from days 259 to 380 were decreased by postweaning injections of bST, and not affected by preweaning injections of bST. Regardless of the reasons for such contradiction, these results reinforce our rationale that the timing of bST injecting can have profound and diverse long-term impacts on growth, reproductive, and physiological parameters of beef heifers.

Additional factors beyond BW gain may have contributed to the variable responses on heifer growth performance and puberty achievement following bST treatment, including body composition, timing of injections, duration of injections, dosage, breed, and potentially the interactions among all of these factors. Although bST injections may stimulate lipolytic activity in adipose tissue (Lanna et al., 1995) and reduce backfat thickness in cattle (Vestergaard et al., 1993; Cooke et al., 2013), neither preweaning nor postweaning injections of bST altered heifer BCS throughout the postweaning phase, indicating that the preweaning bST-induced reduction in postweaning ADG was likely not be attributed to altered body composition and nutrient requirements. One could argue that the period duration of bST injections (two injections 10 days apart) may have been insufficient to affect puberty attainment of heifers. This rationale supports the observed results of postweaning, but not the preweaning bST-induced effects on puberty attainment. Despite the lack of evidence to support this rationale, potential candidates are the effects of breed or the interaction between breed and bST dosage. The bST dosage used in the present study (6.14 mg/kg$^{0.75}$) was obtained from previous work that successfully hastened percentage of pubertal heifers at start of breeding season following preweaning bST administration (Piccolo et al., 2018).

However, in that study, preweaning plasma IGF-1 concentrations increased by 8.6 ng/mL after bST injections (94.8 and 103.4 ng/mL for saline and bST heifers, respectively). In the present study, baseline plasma concentrations of IGF-1 were 186 and 88.2 ng/mL on days 0 and 167, respectively, and increased by 25.4 and 9.9 ng/mL on days 10 and 177, respectively, following first bST injection. Brahman (Simpson et al., 1997) and Nellore cows (Roberts et al., 2005) have greater circulating concentrations of IGF-1 than Angus cattle. Furthermore, circulating concentrations of IGF-1 in straight-bred Brahman cows were greater than in the crossbred cows (Roberts et al., 2005), which likely explains the greater baseline IGF-1 levels observed in the present study compared with Piccolo et al. (2018). However, it is also possible that the greater increment on plasma IGF-1 concentrations following bST injection, in combination with interval between bST injections, was detrimental to the development of the reproductive axis. Further studies investigating the effects of breed on ovarian activity and gene expression in reproductive tissue organs and brain, following bST injections, is warranted to confirm this rationale.

In conclusion, preweaning injections of bST, administered 10 days apart starting at approximately 2 to 3 months of age, successfully increased preweaning plasma concentrations of IGF-1 and growth performance of Nellore heifers during the period of bST treatment. However, preweaning injections of bST reduced postweaning growth and puberty attainment, whereas postweaning bST injections had less increments on plasma concentrations of IGF-1 following bST injections, did not affect postweaning growth, and failed to hasten puberty attainment of Nellore heifers. Therefore, preweaning and postweaning administrations of bST, at the dosage and intervals utilized in the present study, were not feasible strategies to hasten reproductive development of Nellore beef heifers.

LITERATURE CITED

AOAC. 2006. Official methods of analysis, 18th ed. Arlington (VA): AOAC Int.

Arthington, J. D., and L. R. Corah. 1995. Liver biopsy procedures for determining the trace mineral status in beef cows. Part II. (Video, AI 9134). Manhattan: Extension TV, Dep. Commun. Coop. Ext. Serv., Kansas State Univ.

Arthington, J. D., X. Qiu, R. F. Cooke, J. M. Vendramini, D. B. Araujo, C. C. Chase, Jr, and S. W. Coleman. 2008. Effects of preshipping management on measures of stress and performance of beef steers during feedlot receiving. J. Anim. Sci. 86:2016–2023. doi:10.2527/jas.2008-0968

Bilby, C. R., J. F. Bader, B. E. Salfen, R. S. Youngquist, C. N. Murphy, H. A. Garverick, B. A. Crooker, and M. C. Lucy. 1999. Plasma GH, IGF-I, and conception rate in cattle treated with low doses of recombinant bovine GH. Theriogenology 51:1285–1296. doi:10.1016/S0093-691X(99)00072-2

Bilby, T. R., A. Guzeloglu, S. Kamimura, S. M. Pancarci, F. Michel, H. H. Head, and W. W. Thatcher. 2004. Pregnancy and bovine somatotropin in nonlactating dairy cows: I. Ovarian, conceptus, and insulin-like growth factor system responses. J. Dairy Sci. 87:3256–3267. doi:10.3168/jds.S0022-0302(04)73462-1.

Breier, B. H. 1999. Regulation of protein and energy metabolism by the somatotropic axis. Domest. Anim. Endocrinol. 17:209–218. doi:10.1016/S0739-7240(99)00038-7

Buskirk, D. D., D. B. Faulkner, W. L. Hurley, D. J. Kesler, F. A. Ireland, T. G. Nash, J. C. Castree, and J. L. Vicini. 1996. Growth, reproductive performance, mammary development, and milk production of beef heifers as influenced by prepubertal dietary energy and administration of bovine somatotropin. J. Anim. Sci. 74:2649–2662. https://doi.org/10.2527/1996.74112649x

Bustin, S. A., V. Benes, J. A. Garson, J. Hellemans, J. Huggett, M. Kubista, R. Mueller, T. Nolan, M. W. Pfaffl, G. L. Shipley, et al. 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin. Chem. 55:611–622. doi:10.1373/clinchem.2008.112797

Butler, W. R., and R. D. Smith. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. J. Dairy Sci. 72:767–783. doi:10.3168/jds.S0022-0302(89)79169-4

Cappellozza, B. I., R. F. Cooke, M. M. Reis, P. Moriel, D. H. Keisler, and D. W. Bohnert. 2014. Supplementation based on protein or energy ingredients to beef cattle consuming low-quality cool-season forages: II. Performance, reproductive, and metabolic responses of replacement heifers. J. Anim. Sci. 92:2725–2734. doi:10.2527/jas.2013-7442

Carstens, G. E., D. E. Glaser, F. M. Byers, L. W. Greene, and D. K. Lunt. 1997. Effects of bovine somatotropin treatment and intermittent growth pattern on mammary gland development in heifers. J. Anim. Sci. 75:2378–2388. doi:10.2527/1997.7592378x

Cooke, R. F., J. D. Arthington, D. B. Araujo, G. C. Lamb, and A. D. Ealy. 2008. Effects of supplementation frequency on performance, reproductive, and metabolic responses of brahman-crossbred females. J. Anim. Sci. 86:2296–2309. doi:10.2527/jas.2008-0978

Cooke, R. F., D. W. Bohnert, C. L. Francisco, R. S. Marques, C. J. Mueller, and D. H. Keisler. 2013. Effects of bovine somatotropin administration on growth, physiological, and reproductive responses of replacement beef heifers. J. Anim. Sci. 91:2894–2901. doi:10.2527/jas.2012-6082

Coyne, G. S., D. A. Kenny, and S. M. Waters. 2011. Effect of dietary n-3 polyunsaturated fatty acid supplementation on bovine uterine endometrial and hepatic gene expression of the insulin-like growth factor system. Theriogenology 75:500–512. doi:10.1016/j.theriogenology.2010.09.018

Dalke, B. S., R. A. Roeder, T. R. Kasser, J. J. Veenhuizen, C. W. Hunt, D. D. Hinman, and G. T. Schelling. 1992. Dose-response effects of recombinant bovine somatotropin

implants on feedlot performance in steers. J. Anim. Sci. 70:2130–2137. doi:10.2527/1992.7072130x

Day, M. L., and L. H. Anderson. 1998. Current concepts on the control of puberty in cattle. J. Anim. Sci. 76:1–15. doi:10.2527/1998.76suppl_31x

Du, M., J. Tong, J. Zhao, K. R. Underwood, M. Zhu, S. P. Ford, and P. W. Nathanielsz. 2010. Fetal programming of skeletal muscle development in ruminant animals. J. Anim. Sci. 88 (13 Suppl):E51–E60. doi:10.2527/jas.2009-2311

Early, R. J., B. W. McBride, and R. O. Ball. 1990. Growth and metabolism in somatotropin-treated steers: II. Carcass and noncarcass tissue components and chemical composition. J. Anim. Sci. 68:4144–4152. doi:10.2527/1990.68124144x

Eiseman, J. H., A. C. Hammond, D. E. Bauman, P. J. Reynolds, S. N. McCutcheon, H. F. Tyrrell, and G. L. Haaland. 1986. Effect of bovine growth hormone administration on metabolism of growing Hereford heifers: dietary digestibility, energy and nitrogen balance. J. Nutr. 116:157–163. doi:10.1093/jn/116.1.157

Enright, W. J., J. F. Quirke, P. D. Gluckman, B. H. Breier, L. G. Kennedy, L. C. Hart, J. F. Roche, A. Coert, and P. Allen. 1990. Effects of long-term administration of pituitary-derived bovine growth hormone and estradiol on growth in steers. J. Anim. Sci. 68:2345–2356. doi:10.2527/1990.6882345x

FASS. 2010. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. 3rd ed. Champaign, IL: FASS.

Gasser, C. L., C. R. Burke, M. L. Mussard, E. J. Behlke, D. E. Grum, J. E. Kinder, and M. L. Day. 2006b. Induction of precocious puberty in heifers II: advanced ovarian follicular development. J. Anim. Sci. 84:2042–2049. doi:10.2527/jas.2005-637

Gasser, C. L., D. E. Grum, M. L. Mussard, F. L. Fluharty, J. E. Kinder, and M. L. Day. 2006a. Induction of precocious puberty in heifers I: enhanced secretion of luteinizing hormone. J. Anim. Sci. 84:2035–2041. doi:10.2527/jas.2005-636

Govoni, K. E., T. A. Hoagland, and S. A. Zinn. 2004. The ontogeny of the somatotropic axis in hereford calves from birth to one year of age and its response to administration of exogenous bovine somatotropin. J. Anim. Sci. 82:1646–1655. doi:10.2527/2004.8261646x

Granz, S., F. Ellendorff, R. Grossmann, Y. Kato, E. Mühlbauer, and F. Elsaesser. 1997. Ontogeny of growth hormone and LH beta-, FSH beta- and alpha-subunit mRNA levels in the porcine fetal and neonatal anterior pituitary. J. Neuroendocrinol. 9:439–449. doi:10.1046/j.1365-2826.1997.00599.x

Hall, J. B., K. K. Schillo, B. P. Fitzgerald, and N. W. Bradley. 1994. Effects of recombinant bovine somatotropin and dietary energy intake on growth, secretion of luteinizing hormone, follicular development, and onset of puberty in beef heifers. J. Anim. Sci. 72:709–718. doi:10.2527/1994.723709x

Houseknecht, K. L., D. E. Bauman, D. G. Fox, and D. F. Smith. 1992. Abomasal infusion of casein enhances nitrogen retention in somatotropin-treated steers. J. Nutr. 122:1717–1725. doi:10.1093/jn/122.8.1717

Janovick-Guretzky, N. A., H. M. Dann, D. B. Carlson, M. R. Murphy, J. J. Loor, and J. K. Drackley. 2007. Housekeeping gene expression in bovine liver is affected by physiological state, feed intake, and dietary treatment. J. Dairy Sci. 90:2246–2252. doi:10.3168/jds.2006-640

Jiang, H., and X. Ge. 2014. Meat Science and Muscle Biology Symposium–mechanism of growth hormone stimulation of skeletal muscle growth in cattle. J. Anim. Sci. 92:21–29. doi:10.2527/jas.2013-7095

Jiang, H., and M. C. Lucy. 2001. Variants of the 5'-untranslated region of the bovine growth hormone receptor mRNA: isolation, expression and effects on translational efficiency. Gene 265:45–53. doi:10.1016/S0378-1119(01)00356-0

Jiang, H., C. S. Okamura, and M. C. Lucy. 1999. Isolation and characterization of a novel promoter for the bovine growth hormone receptor gene. J. Biol. Chem. 274:7893–7900. doi:10.1074/jbc.274.12.7893

Lanna, D. P., K. L. Houseknecht, D. M. Harris, and D. E. Bauman. 1995. Effect of somatotropin treatment on lipogenesis, lipolysis, and related cellular mechanisms in adipose tissue of lactating cows. J. Dairy Sci. 78:1703–1712. doi:10.3168/jds.S0022-0302(95)76795-9

Lapierre, H., C. K. Reynolds, T. H. Elsasser, P. Gaudreau, P. Brazeau, and H. F. Tyrrell. 1992. Effects of growth hormone-releasing factor and feed intake on energy metabolism in growing beef steers: net hormone metabolism by portal-drained viscera and liver. J. Anim. Sci. 70:742–751. doi:10.2527/1992.703742x

Lucas, A. 1991. Programming by early nutrition in man. Ciba Found. Symp. 156:38–50; discussion 50.

Lucy, M. C., C. K. Boyd, A. T. Koenigsfeld, and C. S. Okamura. 1998. Expression of somatotropin receptor messenger ribonucleic acid in bovine tissues. J. Dairy Sci. 81:1889–1895. doi:10.3168/jds.S0022-0302(98)75760-1

Lucy, M. C., H. Jiang, and Y. Kobayashi. 2001. Changes in the somatotrophic axis associated with the initiation of lactation. J. Dairy Sci. 84:E113–E119. doi:10.3168/jds.S0022-0302(01)70205-6

Mendonça, L. G., N. B. Litherland, M. C. Lucy, D. H. Keisler, M. A. Ballou, L. B. Hansen, and R. C. Chebel. 2013. Comparison of innate immune responses and somatotropic axis components of holstein and montbéliarde-sired crossbred dairy cows during the transition period. J. Dairy Sci. 96:3588–3598. doi:10.3168/jds.2012-5804

Moriel, P., R. F. Cooke, D. W. Bohnert, J. M. Vendramini, and J. D. Arthington. 2012. Effects of energy supplementation frequency and forage quality on performance, reproductive, and physiological responses of replacement beef heifers. J. Anim. Sci. 90:2371–2380. doi:10.2527/jas.2011-4958

Moriel, P., S. E. Johnson, J. M. Vendramini, V. R. Mercadante, M. J. Hersom, and J. D. Arthington. 2014. Effects of calf weaning age and subsequent management system on growth and reproductive performance of beef heifers. J. Anim. Sci. 92:3096–3107. doi:10.2527/jas.2013-7389

NRC. 2000. Nutrient requirements of beef cattle. Revised 7th ed. Washington, DC: Natl. Acad. Press.

NRC. 2016. Beef cattle nutrient requirements model. Washington, DC: National Academies Press.

Ocón-Grove, O. M., F. N. Cooke, I. M. Alvarez, S. E. Johnson, T. L. Ott, and A. D. Ealy. 2008. Ovine endometrial expression of fibroblast growth factor (FGF) 2 and conceptus expression of FGF receptors during early pregnancy. Domest. Anim. Endocrinol. 34:135–145. doi:10.1016/j.domaniend.2006.12.002

Piccolo, M. B., J. D. Arthington, G. M. Silva, G. C. Lamb, R. F. Cooke, and P. Moriel. 2018. Preweaning injections of bovine ST enhanced reproductive performance of bos

indicus-influenced replacement beef heifers. J. Anim. Sci. 96:618–631. doi:10.1093/jas/sky016

Radcliff, R. P., M. J. VandeHaar, Y. Kobayashi, B. K. Sharma, H. A. Tucker, and M. C. Lucy. 2004. Effect of dietary energy and somatotropin on components of the somatotropic axis in holstein heifers. J. Dairy Sci. 87:1229–1235. doi:10.3168/jds.S0022-0302(04)73273-7

Rausch, M. I., M. W. Tripp, K. E. Govoni, W. Zang, W. J. Webert, B. A. Crooker, T. A. Hoagland, and S. A. Zinn. 2002. The influence of level of feeding on growth and serum insulin-like growth factor I and insulin-like growth factor-binding proteins in growing beef cattle supplemented with somatotropin. J. Anim. Sci. 80:94–100. doi:10.2527/2002.80194x

Roberts, A. J., J. Klindt, and T. G. Jenkins. 2005. Effects of varying energy intake and sire breed on duration of postpartum anestrus, insulin like growth factor-1, and growth hormone in mature crossbred cows. J. Anim. Sci. 83:1705–1714. doi:10.2527/2005.8371705x

Roberts, A. J., S. I. Paisley, T. W. Geary, E. E. Grings, R. C. Waterman, and M. D. MacNeil. 2007. Effects of restricted feeding of beef heifers during the postweaning period on growth, efficiency, and ultrasound carcass characteristics. J. Anim. Sci. 85:2740–2745. doi:10.2527/jas.2007-0141

Sartori, R., L. U. Gimenes, P. L. Monteiro, Jr, L. F. Melo, P. S. Baruselli, and M. R. Bastos. 2016. Metabolic and endocrine differences between *Bos taurus* and *Bos indicus* females that impact the interaction of nutrition with reproduction. Theriogenology 86:32–40. doi:10.1016/j.theriogenology.2016.04.016

Schillo, K. K., J. B. Hall, and S. M. Hileman. 1992. Effects of nutrition and season on the onset of puberty in the beef heifer. J. Anim. Sci. 70:3994–4005. doi:10.2527/1992.70123994x

Short, R. E., R. B. Staigmiller, R. A. Bellows, and R. C. Greer. 1994. Breeding heifers at one year of age: biological and economical considerations. In: M. J. Fields and R. S. Sand, editors, Factors affecting calf crop. Boca Raton (FL): CRC Press. p. 55–68.

Simpson, R. B., C. C. Chase, Jr, L. J. Spicer, J. A. Carroll, A. C. Hammond, and T. H. Welsh, Jr. 1997. Effect of exogenous estradiol on plasma concentrations of somatotropin, insulin-like growth factor-I, insulin-like growth factor binding protein activity, and metabolites in ovariectomized angus and brahman cows. Domest. Anim. Endocrinol. 14:367–380. doi:10.1016/S0739-7240(97)00042-8

Smith, J. M., M. E. Van Amburgh, M. C. Díaz, M. C. Lucy, and D. E. Bauman. 2002. Effect of nutrient intake on the development of the somatotropic axis and its responsiveness to GH in holstein bull calves. J. Anim. Sci. 80:1528–1537. doi:10.2527/2002.8061528x

Spicer, L. J., and S. E. Echternkamp. 1995. The ovarian insulin and insulin-like growth factor system with an emphasis on domestic animals. Domest. Anim. Endocrinol. 12:223–245. doi:10.1016/0739-7240(95)00021-6

Thissen, J. P., J. M. Ketelslegers, and L. E. Underwood. 1994. Nutritional regulation of the insulin-like growth factors. Endocr. Rev. 15:80–101. doi:10.1210/edrv-15-1-80

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2

Velayudhan, B. T., K. E. Govoni, T. A. Hoagland, and S. A. Zinn. 2007. Growth rate and changes of the somatotropic axis in beef cattle administered exogenous bovine somatotropin beginning at two hundred, two hundred fifty, and three hundred days of age. J. Anim. Sci. 85:2866–2872. doi:10.2527/jas.2007-0281

Vestergaard, M., K. Sejrsen, J. Foldager, S. Klastrup, and D. E. Bauman. 1993. The effect of bovine growth hormone on growth, carcass composition and meat quality of dairy heifers. Acta Agric. Scand., Sect. A 43:165–172. doi:10.1080/09064709309410161

Wagner, J. J., K. S. Lusby, J. W. Oltjen, J. Rakestraw, R. P. Wettemann, and L. E. Walters. 1988. Carcass composition in mature hereford cows: estimation and effect on daily metabolizable energy requirement during winter. J. Anim. Sci. 66:603–612. doi:10.2527/jas1988.663603x

Weiss, W. P., H. R. Conrad, and N. R. St. Pierre. 1992. A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. Anim. Feed Sci. Technol. 39:95–110. doi:10.1016/0377-8401(92)90034-4

Yakar, S., J. L. Liu, B. Stannard, A. Butler, D. Accili, B. Sauer, and D. LeRoith. 1999. Normal growth and development in the absence of hepatic insulin-like growth factor I. Proc. Natl. Acad. Sci. USA. 96:7324–7329.