Journal of Animal Science, 2020, Vol. 98, No. 5, 1–12

doi:10.1093/jas/skaa123
Advance Access publication April 20, 2020
Received: 13 March 2020 and **Accepted:** 16 April 2020
Fetal Programming

FETAL PROGRAMMING

Maternal supplementation of energy and protein, but not methionine hydroxy analog, enhanced postnatal growth and response to vaccination in *Bos indicus*-influenced beef offspring

Philipe Moriel,[†,1] Marcelo Vedovatto,[†] Elizabeth A. Palmer,[†] Rhaiza A. Oliveira,[†] Hiran Marcelo Silva,[†] Juliana Ranches,[‡] and Joao M. B. Vendramini[†]

[†]IFAS – Range Cattle Research and Education Center, University of Florida, Ona, FL 33865, [‡]Eastern Oregon Agricultural Research Center, Oregon State University, Burns, OR 97720

[1]Corresponding author: pmoriel@ufl.edu

ORCiD number: 0000-0002-9349-7575 (P. Moriel).

Abstract

A 2-yr study evaluated the growth and postvaccination immune response of beef calves born from heifers offered no supplementation or pre- and postpartum supplementation of sugarcane molasses + urea with or without methionine hydroxy analog (**MHA**). On day 0 of each year (57 ± 5 d prepartum), Brangus crossbred beef heifers (n = 36/yr; 20 to 22 mo of age) were stratified by their initial body weight (**BW**; 396 ± 24.1 kg) and body condition score (**BCS**; 5.6 ± 0.43) and randomly allocated into 1 of 12 bahiagrass (*Paspalum notatum*) pastures (3 heifers/pasture). Treatments were randomly assigned to pastures (4 pastures/treatment/yr) and consisted of no supplementation (**NOSUP**) and supplementation of sugarcane molasses + urea (7.2 kg of DM/heifer/wk) with (**MOL+**) or without (**MOL–**) fortification with 105 g/heifer/wk of MHA. Treatments were provided from 57 ± 5 d prepartum until 17 ± 5 d postpartum (day 0 to 74). On day 74, all heifer-calf pairs were combined and managed as a single group until the end of the breeding season (day 237). Calves were early weaned at 89 ± 5 d of age (day 147), limit-fed at 3.5% of BW (DM basis) in drylot until day 201, and vaccinated against respiratory disease pathogens on days 160 and 188. Prepartum BCS on day 44 did not differ (P = 0.26) between MOL+ and MOL– heifers but both groups had greater (P < 0.0001) BCS than NOSUP heifers. Plasma concentrations of L-methionine on day 44 were the greatest ($P \leq 0.04$) for MOL+ heifers and did not differ (P = 0.40) between NOSUP vs. MOL– heifers. Calf birth BW did not differ (P = 0.13) among treatments. Calf average daily gain (**ADG**) from birth to day 201 did not differ ($P \geq 0.17$) between MOL+ vs. MOL– calves, but both groups had greater ($P \leq 0.05$) ADG from birth to day 201 than NOSUP calves. Calf postvaccination plasma concentrations of glucose, cortisol, and haptoglobin did not differ among treatments ($P \geq 0.13$). However, plasma concentrations of IGF-1 on day 167 and the overall positive vaccine seroconversion did not differ ($P \geq 0.18$) between MOL– and MOL+ calves, but both were greater ($P \leq 0.04$) compared with NOSUP calves. Hence, maternal supplementation of sugarcane molasses + urea increased BCS at calving and offspring BW gain and response to vaccination against respiratory pathogens compared with no maternal supplementation. MHA inclusion into maternal supplements effectively increased maternal plasma L-methionine concentrations but did not enhance maternal BCS at calving and offspring growth and postvaccination immune response.

Key words: beef heifers, *Bos indicus*, methionine, offspring, prepartum, supplementation

Abbreviations

ADG	average daily gain
BCS	body condition score
BVDV	bovine viral diarrhea virus
BW	body weight
CP	crude protein
DM	dry matter
G:F	gain:feed
IGF-1	insulin-like growth factor 1
IGF-2	insulin-like growth factor 2
IVDOM	in vitro digestible organic matter
MAT1A	methionine adenosyltransferase 1A
MHA	methionine hydroxy analog
NEm	Net Energy for maintenance
PI-3	parainfluenza-3 virus
SAMS	adenosyl methionine

Introduction

Beef cattle in the Southeast United States rely primarily on warm-season forages of limited nutritive value, which may lead to energy and protein deficiency in late-gestating beef cows (NASEM, 2016). Maternal prepartum supplementation of protein (Kennedy et al., 2019), energy (Moriel et al., 2016), and methionine (Alharthi et al., 2018) offers an opportunity to modulate postnatal growth and immune response of beef and dairy calves. For instance, energy restriction in late-gestating *Bos taurus* beef cows weakened the offspring innate and humoral immune response to vaccination (Moriel et al., 2016), whereas maternal prepartum supplementation of methionine altered DNA methylation (Alharthi et al., 2018) and expression genes linked to hepatic gluconeogenesis and inflammation in *B. taurus* dairy calves (Jacometo et al., 2016).

Despite growing evidence in nonruminants and *B. taurus* cattle, few studies have addressed the role of maternal supplementation of protein, energy, and methionine during late gestation and their influence on postnatal development of *B. indicus*-influenced beef offspring. *Bos indicus* and *B. taurus* differ in reproductive physiology, nutritional requirements, social behavior, digestive system, and body composition (Cooke et al., 2020) and have been shown to elicit opposite outcomes following similar nutritional strategies (Piccolo et al., 2018; Moriel et al., 2019a). Our hypothesis was that energy and protein supplementation for growing, pregnant *B. indicus*-influenced beef heifers would enhance offspring postnatal growth and postvaccination immune response compared with no maternal supplementation. Also, supplement fortification with methionine hydroxy analog (**MHA**) would lead to the greatest improvements in offspring growth and immunity. Our objectives were to evaluate body weight (**BW**) growth and measurements of the innate and humoral immune response of beef calves born from heifers offered no supplementation or sugarcane molasses + urea supplementation with or without MHA during pre- and postpartum periods.

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 (RCREC), Ona, Florida (27°23′N and 81°56′W) from August to March of 2018 (year 1) and 2019 (year 2). All animals used in this experiment were cared for by practices approved by the University of Florida—Institute of Animal Care and Use Committee (protocol #201709982).

Animals and diets

Maternal management

On day 0 of years 1 and 2 (57 ± 5 d prepartum), Brangus crossbred beef heifers (n = 36/yr; 20 to 22 mo of age) were stratified by their initial BW and body condition score (**BCS**; mean ± SD here and throughout: 396 ± 24.1 kg and 5.6 ± 0.43, respectively) and then randomly allocated into 1 of 12 bahiagrass pastures (3 heifers/pasture/yr and 1.2 ha/pasture/yr). Treatments were randomly assigned to pastures (4 pastures/treatment/yr) and consisted of no supplementation of sugarcane (*Saccharum officinarum*) molasses + urea (**NOSUP**) and supplementation of sugarcane molasses + urea (7.2 kg of dry matter (**DM**)/heifer/wk; Westway Feed Products LLC, Clewiston, FL; Table 1) with (**MOL+**) or without (**MOL–**) fortification with 105 g/heifer/wk of MHA. Treatments were provided from day 0 (57 ± 5 d prepartum) until all heifers within each pasture calved (17 ± 5 d postpartum; day 74). Molasses + urea supplement was formulated to allow heifers to gain 0.5 BCS during late gestation (NASEM, 2016). The respective total weekly amount of MOL+ and MOL– supplements was divided into two equal quantities and offered in open tanks every Tuesday and Friday at 0800 hours. Feed bunks for MOL– and MOL+ supplementation were placed 1 m above ground to avoid calf consumption of maternal treatment supplements from birth until day 74. The MHA source utilized herein was a granular feed source of L-methionine precursor (2-hydroxy-4-methylthio butanoic acid), provided in Ca salt form (MFP, Novus International Inc., Romance, AR), and hand-mixed into supplements immediately before every supplementation event. The MHA dosage utilized herein was the highest dosage recommended by the company for growing beef heifers (15 g/d of MHA) and was 50% greater than the MHA amount that effectively increased the circulating concentrations of methionine equivalents in late-gestating *B. taurus* beef cows (Clements et al., 2017).

Offspring management

On day 74, all heifers and their calves were combined into a single bahiagrass pasture (14.4 ha) and offered free-choice access to long-stem stargrass (*Cynodon nlemfuensis*) hay (Table 1) and 12.7 kg of DM/heifer/wk of the molasses + urea supplementation without MHA fortification (Table 1). All calves were early weaned at 89 ± 5 d of age (day 147) and then transferred to a single drylot pen. Early weaning was implemented to improve the reproductive performance of primiparous *B. indicus*-influenced beef cows (Arthington and Kalmbacker, 2003) and also to simultaneously achieve similar calf feed DM intake among all treatments. Following calf early weaning, heifers remained on the same bahiagrass pasture and were placed with two Brangus bulls for a 90-d breeding season (day 147 to 237) and offered 12.7 kg of DM/heifer/wk of molasses + urea supplementation without MHA fortification until day 237. Early-weaned calves remained in a single partially covered drylot pen (20 × 40 m; 1.5 m of bunk space per calf) from day 147 to 154 to overcome the stress of weaning. During this 8-d period, calves were: (1) offered free-choice access to long-stem stargrass hay (Table 1) and water and 0.50 kg/d of a pelletized preconditioning supplement (guaranteed analysis, as fed: 14% CP, 1.0% fat, 18% fiber, 0.75% Ca, 0.40% P, and 0.40% NaCl; Land O'Lakes Purina Feed LLC, Gray Summit, MO) and (2) gradually adapted to a high concentrate-based diet (Table 1) by daily increasing the high-concentrate diet DM offered from 0% to 3.5% of BW (increments of 0.5% of BW daily; DM basis).

Table 1. Average nutritional composition[1] of sugarcane molasses + urea supplement offered to heifers from day 0 to 237 and long-stem stargrass (*Cynodon nlemfuensis*) hay and high-concentrate diet offered to calves from early weaning until drylot exit (day 147 to 201)

	Sugarcane molasses + urea supplement[2]		Stargrass hay		High-concentrate diet	
Item	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
DM, %	76.7	80.0	95.4	92.3	95.4	89.8
CP, %	21.3	22.9	5.60	9.00	26.1	28.3
Crude fat, %	2.10	1.30	—	—	—	—
ADF, %	—	—	43.4	45.8	28.1	19.3
NDF, %	—	—	79.7	75.1	37.1	37.7
TDN[3], %	74.0	76.0	52.0	55.0	75.0	75.0
NEm[4], Mcal/kg	1.80	1.85	0.90	1.01	1.81	1.81
NEg[4], Mcal/kg	1.17	1.21	0.35	0.46	1.19	1.17
Ca, %	0.89	0.91	0.19	0.25	1.16	1.69
P, %	0.10	0.11	0.14	0.19	0.52	0.62
Mg, %	0.39	0.33	0.31	0.14	0.25	0.30
K, %	4.80	5.25	0.55	1.16	1.39	1.51
Na, %	0.09	0.12	0.04	0.02	0.07	0.07
S, %	1.28	1.04	0.12	0.13	0.38	0.32
Fe, mg/kg	168	130	74.0	35.0	167	195
Zn, mg/kg	12.0	13.0	17.0	30.0	49.0	65.0
Cu, mg/kg	4.00	4.00	6.00	3.00	8.00	9.00
Mn, mg/kg	11.0	13.0	20.0	35.0	21.0	28.0
Mo, mg/kg	1.20	1.50	0.30	0.30	2.00	1.80

[1]Samples of sugarcane molasses + urea supplement were collected every 28 d, whereas samples of hay and high-concentrate diet were collected weekly throughout the study. All samples were pooled within each year and then sent in duplicates to a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY) for wet chemistry analyses.
[2]As-fed basis: 92% liquid sugarcane molasses, 4% urea, and 4% water.
[3]Calculated as described by Weiss et al. (1992).
[4]Calculated using the equations proposed by the NRC (2000).

On day 154, 24 calves/yr were randomly selected (4 steer and 4 heifer calves/treatment/yr) and transferred to individual concrete floor pens (3 × 6 m; 1.5 m of bunk space per calf) in a fully covered drylot facility (maximum drylot capacity = 24 individual pens) and individually fed the same high-concentrate diet (Table 1) once daily at 0800 hours until day 201. The remaining calves not assigned to drylot period were removed from the study. A high-concentrate diet was limit-fed to calves in drylot at 3.5% of BW (DM basis). From day 154 to 201, calves were also provided 1 kg/d of long-stem stargrass hay (Table 1) and free-choice access to water and a complete salt-based mineral supplement (Cattle Select Essentials Range, Lakeland Animal Nutrition, Lakeland, FL; 6.0%, 0.10%, 0.10%, 0.30%, 63%, and 1.0% of Ca, K, Mg, S, NaCl, and P, respectively, and 50, 1,500, 800, 210, 500, 40, and 3,000 mg/kg of Co, Cu, Fe, I, Mn, Se, and Zn, respectively). On day 160, all calves were administered oral drench of fenbendazole (5 mg of fenbendazole/kg of BW; Safe-Guard, Merck Animal Health, Summit, NJ) and vaccinated against infectious bovine rhinotracheitis, bovine viral diarrhea viruses (**BVDV**), parainfluenza-3 virus (**PI-3**), *Mannheimia haemolytica* (2 mL s.c.; Bovi Shield Gold One Shot; Zoetis Inc., New York, NY), and *Clostridium* (2 mL s.c.; Ultrabac 7, Zoetis Inc., New York, NY). Booster vaccinations with Bovi Shield Gold 5 (2 mL s.c.; Zoetis Inc.) and Ultrabac 7 (2 mL s.c.; Zoetis Inc.) were administered on day 188. This vaccination protocol was selected as our model to elicit an acute-phase response (Artioli et al., 2015; Moriel et al., 2016; Silva et al., 2018). Calf health was monitored daily by trained personal from birth to the end of the study. No signs of health problems were detected, and no calf was removed from the study.

Data and sample collection

Individual full BW and BCS of heifers were assessed on days 0, 44, and 147. Shrunk BW of pregnant heifers was not implemented to not disturb feeding behavior and avoid any unnecessary prenatal physiological stress response due to feed and water withdrawal (Marques et al., 2012), which could affect the postnatal evaluation of growth and immune response of the offspring (Littlejohn et al., 2016). Heifer BCS was performed by two trained technicians as described by Wagner et al. (1988). Blood samples (approximately 10 mL) were collected from all heifers, via jugular venipuncture into sodium heparin (158 USP)-containing tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ), 4 h after morning supplementation on days 0 and 44 to determine the plasma concentrations of glucose, insulin-like growth factors 1 and 2 (**IGF-1** and **IGF-2**), L-methionine, methionine adenosyltransferase 1A (**MAT1A**), and S-adenosyl methionine (**SAM**). Blood samples were collected 4 h after supplementation to correspond to the peak of ruminal fermentation and end products release after supplement intake (Artioli et al., 2015; Moriel et al., 2016; Silva et al., 2018). The percentage of heifers pregnant with their second calf crop was determined via rectal palpation by a trained veterinarian approximately 45 d after the end of the breeding season (day 282). Heifers were checked twice daily for calving. Individual calf birth BW obtained within 12 h of birth.

Shrunk BW of calves was recorded on days 147, 154, and 201, following 12 h of feed and water withdrawal. Blood samples (approximately 10 mL) were collected from all calves assigned to the drylot phase, via jugular venipuncture into sodium heparin (158 USP)-containing tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ), 4 h after high-concentrate diet supplementation on days 154, 160, 161, 163, 167, and 188 to determine the plasma concentrations of glucose, IGF-1, cortisol, and haptoglobin. Additional blood samples (10 mL) were collected from all calves, via jugular venipuncture into

tubes containing no additives (Vacutainer, Becton Dickinson), on days 160, 188, and 201 to determine serum titers against PI-3 and BVDV-1a. All blood samples (heifers and calves) were immediately placed on ice following collection and then centrifuged at 1,200 × *g* for 25 min at 4 °C. Serum and plasma samples were stored frozen at −20 °C until later laboratory analysis.

Herbage mass and hand-plucked samples of pastures to assess nutritive value (crude protein, **CP**; and in vitro digestible organic matter, **IVDOM**) were collected on days 0 and 44 using the double sampling technique (Gonzalez et al., 1990). Herbage allowance was calculated for each pasture as the herbage mass on days 0 and 44 divided by the respective total heifer BW on days 0 and 44 (Sollenberger et al., 2005). Hand-plucked samples of supplements offered to heifers were collected every 28 d from day 0 to 147, whereas samples of high-concentrate diet and hay offered to calves were collected every week from day 147 to 201. Immediately after collection, all forage and feed samples were dried in a forced-air oven at 56 °C for 72 h and ground in a Wiley mill (model 4, Thomas-Wiley Laboratory Mill, Thomas Scientific, Swedesboro, NJ) to pass a 4-mm stainless steel screen.

Supplement DM disappearance (% of initial DM offered) was calculated as the total number of hours to consume 100% of the initial supplement DM offered in each respective pasture and assessed on days 14, 28, and 44. Samples of supplements offered to heifers and hay and high-concentrate diet offered to calves were pooled within a year and analyzed in duplicates (Table 1) by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY) for concentrations of CP (method 984.13; AOAC, 2006), total digestible nutrients (**TDN**) (Weiss et al., 1992), Net Energy for maintenance (**NEm**), and Net Energy for gain (NRC, 2000). Nutritive value analyses of pastures samples (Table 2) were performed in duplicates at the University of Florida Forage Evaluation Support Laboratory using the micro-Kjeldahl technique for N (Gallaher et al., 1975) and the two-stage technique for IVDOM (Moore and Mott, 1974).

Laboratory analyses

Plasma concentrations of IGF-1 were assessed using a human-specific commercial ELISA kit (SG100; R&D Systems Inc., Minneapolis, MN) previously validated for bovine samples (Moriel et al., 2012). Commercial bovine ELISA kits were utilized to determine the plasma concentrations of IGF-2 (LS-F51244; LifeSpan BioSciences, Inc., Seattle, WA), MAT1A (MBS075055; MyBioSource, San Diego, CA), and SAM (EKU08443; Biomatik USA, LLC, Wilmington, DE). Intra-assay and inter-assay coefficient of variation (CV) for IGF-1, IGF-2, MAT1A, and SAM were 1.70% and 5.70%, 6.33% and 5.62%, 3.80% and 4.15%, and 6.8% and 7.2%, respectively. Plasma concentrations of L-methionine were assessed in a single assay by a commercial laboratory (Seventh Wave Laboratories, LLC, Maryland Heights, MO) using liquid chromatography-mass spectrometry techniques (Clements et al., 2017). Lower and upper limit quantitation were 0.5 and 50 μg/mL, respectively. Intra-assay CV for analysis of L-methionine was 4.50%.

Plasma concentrations of glucose were determined using quantitative colorimetric kits (#G7521; Pointe Scientific Inc., Canton, MI). Plasma concentrations of haptoglobin were evaluated using a biochemical assay assessing haptoglobin–hemoglobin complex by the estimation of differences in peroxidase activity (Cooke and Arthington, 2013). Plasma concentrations of cortisol were determined using chemiluminescent enzyme immunoassays (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA; Silva et al., 2018). Intra-assay and inter-assay CV for glucose, haptoglobin, and cortisol were 2.26% and 2.34%, 1.56% and 2.05%, and 2.46% and 3.08%, respectively.

Serum antibody titers against BVDV-1a and PI-3 viruses were evaluated by the Oklahoma Animal Disease and Diagnostic Laboratory using a virus neutralization test (Rosenbaum et al., 1970). Serum PI-3 and BVDV-1a titers were reported as the $\log_2$ of the greatest dilution of serum that provided complete protection of the cells (lowest and greatest dilutions tested were 1:4 and 1:256, respectively). For the seroconversion analyses, serum samples with neutralization value of less than 4 were considered negative and assigned a value of 0, whereas samples with serum neutralization value equal or greater than 4 were considered positive and assigned a value of 1. Subsequently, the assigned values (0 or 1) were used to determine the percentage of calves with positive seroconversion (Artioli et al., 2015; Moriel et al., 2016; Silva et al., 2018).

Statistical analyses

Except for binary data, all data were analyzed as a complete randomized study using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA, version 9.4) with Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. Pasture was considered the experimental unit for all statistical analyses. Random effects in all statistical analyses were pasture (maternal supplementation × year) and heifer (pasture) or calf (pasture), except for herbage mass which included only pasture (maternal supplementation × year) as a random effect. Heifer BW and BCS, herbage mass and allowance, calf BW, and plasma data of heifers and calves were analyzed as repeated measures and tested for fixed effects of year, day of the study, maternal supplementation, and all resulting interactions. Heifer (pasture) or calf (pasture) was

Table 2. Average herbage mass and allowance, IVDOM, and CP of bahiagrass pastures (4 pastures/treatment/yr; 3 heifers and 1.2 ha/pasture)[1]

	Treatment[2]				P-value	
Item	NOSUP	MOL−	MOL+	SEM	Trt	Trt × day
Herbage mass, kg DM/ha	4,545	4,588	4,570	62.6	0.90	0.91
Herbage allowance, kg DM/kg BW	3.75	3.68	3.86	0.081	0.41	0.57
IVOMD, %	36.2	33.7	35.6	1.08	0.28	0.55
CP, % of DM	7.71	7.49	7.71	0.099	0.14	0.97

[1]Herbage mass and hand-plucked samples of pastures were collected on days 0 and 44 using the double sampling technique (Gonzalez et al., 1990). Herbage allowance was calculated as the herbage mass divided by the total heifer BW in each pasture (Sollenberger et al., 2005).
[2]Treatments were provided to heifers from 57 ± 5 d prepartum until 17 ± 5 d postpartum (day 0 to 74) and consisted of NOSUP, no supplementation; MOL-, sugarcane molasses and urea supplemented at 7.2 kg DM/heifer/wk; MOL+, sugarcane molasses and urea (7.2 kg DM/heifer/wk) fortified with 105 g MHA/heifer/wk.

included as subjects and the compound symmetry covariance structure included in all repeated measures analyses, as it generated the lowest Akaike information criterion (except for plasma concentrations of glucose and IGF-1 of heifers which utilized the autoregressive 1 covariance structure). Calf age at early weaning and sex were included as covariates into all statistical analyses of calf data but removed from the model if $P > 0.10$. Heifer supplement disappearance, calf birth BW, average daily gain (**ADG**), gain:feed (**G:F**), and DM intake were tested for fixed effects of maternal supplementation, year, and maternal supplementation × year. Heifer reproductive and calf seroconversion data were analyzed using the GLIMMIX procedure of SAS. Pregnancy and calving percentages of heifers and percentage of male calves at birth were tested for fixed effects of year, maternal supplementation, and maternal supplementation × year. Positive seroconversion was analyzed as repeated measures and tested for fixed effects of maternal supplementation, day of the study, year, and all resulting interactions. All results are reported as least-square means. Data were separated using PDIFF if a significant *F*-test was detected. Significance was set at $P \leq 0.05$, and tendencies were noted if $P > 0.05$ and ≤ 0.10.

Results

Maternal performance

Effects of maternal supplement × day of the study × year and maternal supplementation × year were not detected for any variable analyzed in the study ($P \geq 0.12$). Effects of day of the study, but not maternal supplementation and maternal supplementation × day of the study ($P \geq 0.14$), were detected ($P < 0.0001$) for herbage mass, herbage allowance, CP, and IVDOM (Table 2). Herbage mass and allowance increased from day 0 to 44 (4,041 vs. 5,104 ± 66 kg of DM/ha and 3.44 vs. 4.08 ± 0.054 kg of DM/kg of BW, respectively), whereas CP and IVDOM decreased from day 0 to 44 (8.18% vs. 7.09 ± 0.085% CP of DM and 63.2% vs. 55.4 ± 0.80 % IVDOM, respectively).

Effects of maternal supplementation were not detected ($P \geq 0.42$) for days on treatment during the pre- and postpartum periods (Table 3). Hence, heifers were provided their respective treatments for 57 ± 5 d prepartum and 17 ± 5 d postpartum. Effects of maternal supplementation × day of the study and day of the study were not detected ($P \geq 0.68$) for supplement disappearance, but overall hours for total supplement disappearance was greater ($P = 0.01$) for MOL+ vs. MOL− heifers (59 vs. 31 ± 5.7 h, respectively).

Effects of maternal supplementation × day of the study were detected ($P = 0.05$) for heifer BCS but not BW ($P = 0.60$; Table 3). Heifers offered MOL+ and MOL− supplementation had greater ($P \leq 0.006$) BCS gain from day 0 to 44 and BCS on day 44 compared with NOSUP heifers. Heifer BCS gain from day 0 to 44 and BCS on day 44 did not differ ($P \geq 0.26$) between MOL+ and MOL− heifers. However, MOL+ and MOL− heifers had a greater ($P \leq 0.05$) BCS loss from day 44 to 147 compared with NOSUP heifers. Heifer BCS on day 147 did not differ ($P \geq 0.17$) among treatments. Effects of maternal supplementation were not detected ($P \geq 0.12$) for the percentage of pregnant heifers on day 288, percentage of heifers calving, and calving date of their second calf (Table 3).

Effects of maternal supplementation × day of the study, maternal supplementation, and day of the study were not detected ($P \geq 0.39$) for plasma concentrations of SAM and glucose

Table 3. Growth and reproductive performance of beef heifers offered NOSUP, MOL+, or MOL− for 57 ± 5 d prepartum and 17 ± 5 d postpartum (day 0 to 74; 4 pastures/treatment/yr)

	Maternal supplementation[1]				P-value	
Item	NOSUP	MOL−	MOL+	SEM	Trt	Trt × day
Days on treatment						
Prepartum	59	57	55	5.1	0.85	—
Postpartum	18	15	18	4.6	0.42	—
Heifer BW, kg						
Day 0	396	397	396	5.2	0.25	0.60
Day 44	408	419	425	5.2		
Day 147	352	358	357	5.2		
Heifer BW change, kg						
Day 0 to 44	12[a]	23[ab]	30[b]	6.0	0.09	—
Day 44 to 147	−57	−61	−69	5.1	0.23	—
Heifer BCS						
Day 0	5.67	5.65	5.69	0.084	0.04	0.05
Day 44	5.77[a]	6.10[b]	6.17[b]			
Day 147	4.85	4.95	5.01			
Heifer BCS change						
Day 0 to 44	0.09[a]	0.42[b]	0.49[b]	0.081	0.002	—
Day 44 to 147	−0.93[a]	−1.16[b]	−1.17[b]	0.099	0.10	—
Pregnant 2nd calf crop[2], % of total	91.7	95.8	93.8	4.20	0.78	—
Calving 2nd calf crop[2], % of total	79.2	81.3	93.8	5.37	0.12	—
Calving date 2nd calf crop[2], day of the study	446	450	447	7.4	0.93	—

[1]Total weekly amount of MOL+ and MOL− supplementation was divided by 2 and offered every Tuesday and Friday. The MHA was provided in Ca salt form (MFP, Novus International Inc., Romance, AR) and hand-mixed into supplements immediately before supplementation. Heifers received 12.7 kg of DM/heifer/wk of molasses + urea from day 75 to 237.

[2]On day 147, all calves were weaned, and heifers were placed with two Brangus bulls until day 237. The percentage of heifers pregnant with their second calf crop was determined via rectal palpation by a trained veterinarian on day 282. Heifers calved their second calf crop around day 446 to 450 of the study.

[a,b]Within a row, means without a common superscript differ ($P \leq 0.05$).

Table 4. Plasma concentrations of L-methionine, MAT1A, SAM, IGF-1, IGF-2, and glucose of beef heifers offered NOSUP, MOL+, or MOL– for 57 ± 5 d prepartum and 17 ± 5 d postpartum (day 0 to 74; 4 pastures/treatment/yr)

	Maternal supplementation[1]				*P*-value	
Item	NOSUP	MOL–	MOL+	SEM	Trt	Trt × day
Plasma L-methionine, μg/mL						
Day 0	3.44[a]	3.41[a]	3.40[a]	0.117	0.16	0.06
Day 44	3.06[a]	3.22[a]	3.58[b]	0.117		
P[2]	0.06	0.27	0.08			
Plasma MAT1A, pg/mL						
Day 0	1,620[a]	1,558[a]	1,633[a]	111	0.09	0.08
Day 44	1,131[a]	1,484[b]	1,576[b]	115		
P[2]	0.002	0.62	0.72			
Plasma SAM, ng/mL						
Day 0	256	268	263	19.3	0.61	0.90
Day 44	236	249	260	19.8		
P[2]	0.48	0.52	0.90			
Plasma IGF-1, ng/mL						
Day 0	64.4[a]	63.9[a]	67.1[a]	2.76	0.52	0.05
Day 44	41.4[a]	46.7[ab]	50.3[b]	2.76		
P[2]	<0.0001	0.0006	0.0008			
Plasma IGF-2, ng/mL						
Day 0	227[a]	257[a]	255[a]	38.2	0.59	0.06
Day 44	186[a]	298[b]	274[b]	42.2		
P[2]	0.49	0.48	0.75			
Plasma glucose, ng/mL						
Day 0	66.8	66.1	66.8	1.82	0.87	0.70
Day 44	71.9	72.5	71.9	1.82		
P[2]	0.02	0.003	0.08			

[1]Total weekly amount of MOL+ and MOL– supplementation was divided by 2 and offered every Tuesday and Friday. The MHA was provided in Ca salt form (MFP, Novus International Inc., Romance, AR) and hand-mixed into supplements immediately before supplementation.
[2]P-value for the comparison of day of the study within each respective treatment.
[a,b]Within a row, means without a common superscript differ ($P \leq 0.05$).

(Table 4). Effects of maternal supplementation × day of the study were detected ($P = 0.05$) for heifer plasma concentrations of IGF-1 and tended to be detected for heifer plasma concentrations of L-methionine ($P = 0.06$), MAT1A ($P = 0.08$), and IGF-2 ($P = 0.06$; Table 4). Plasma concentrations of IGF-1, L-methionine, MAT1A, and IGF-2 did not differ ($P \geq 0.42$) among treatments on day 0. Plasma concentrations of IGF-1 on day 44 were greater ($P = 0.02$) for MOL+ vs. NOSUP heifers and intermediate ($P \geq 0.16$) for MOL– heifers. Plasma concentrations of L-methionine on day 44 were the greatest ($P \leq 0.04$) for MOL+ heifers and did not differ ($P = 0.40$) between NOSUP vs. MOL– heifers. Plasma concentrations of MAT1A and IGF-2 on day 44 were the least ($P \leq 0.04$) for NOSUP and did not differ ($P \geq 0.56$) between MOL– vs. MOL+ heifers.

Offspring performance

Percentage of male calves at birth and calf birth BW did not differ ($P \geq 0.13$) among treatments (Table 5). Effects of maternal supplementation × day of the study were detected ($P = 0.05$) for calf BW. Calf BW did not differ ($P \geq 0.13$) among treatments on day 147. Calves born from MOL+ and MOL– cows tended ($P = 0.07$) to have greater BW on day 154 and had greater ($P \leq 0.05$) BW on day 201 compared with NOSUP calves (Table 5). Calf BW and ADG did not differ ($P \geq 0.17$) between MOL+ vs. MOL– calves on any day of the study, but both groups had greater ($P \leq 0.05$) ADG from day 147 to 154, day 154 to 201, and birth to day 201 compared with NOSUP calves (Table 5). DM intake (hay, high-concentrate diet, and total) and G:F from day 154 to 201 did not differ ($P \geq 0.39$) among treatments (Table 5).

Effects of maternal supplementation were detected ($P = 0.0006$) for plasma IGF-1 concentrations of calves (Figure 1). Plasma concentrations of IGF-1 did not differ ($P \geq 0.31$) among treatments from day 154 to 163 but were greater ($P \leq 0.04$) for MOL+ and MOL– vs. NOSUP calves on day 167. Effects of maternal supplementation × day of the study and maternal supplementation were not detected ($P \geq 0.13$) for calf plasma concentrations of glucose, cortisol, haptoglobin, and serum titers against BVDV-1a and PI-3 (Table 6). However, effects of maternal supplementation were detected ($P \leq 0.02$) for positive seroconversion against BVDV-1a and PI3 (Table 6), which did not differ ($P \geq 0.18$) between MOL– and MOL+ calves, but both had greater ($P \leq 0.04$) seroconversion percentages compared with NOSUP calves.

Discussion

Maternal performance

Sugarcane molasses + urea supplementation can be provided infrequently and in large amounts due to its self-limiting and slow-rate intake characteristics (Moriel et al., 2019b). The addition of methionine to supplements decreased the supplement intake of growing calves (Hersom et al., 2009; Moriel and Arthington, 2013). Likewise, the addition of MHA to sugarcane molasses slowed the rate of sugarcane molasses + urea supplement disappearance by 28 h compared with MOL– supplement, without detrimental impacts to heifer growth performance. The addition of MHA to liquid supplements may be used to effectively limit daily supplement intake of grazing cattle.

In the current study, herbage mass and allowance did not differ among treatments and were in average 2.7-fold greater than the threshold of bahiagrass herbage allowance that limits

Table 5. Growth performance of calves born from beef heifers offered NOSUP, MOL+, or MOL– for 57 ± 5 d prepartum and 17 ± 5 d postpartum (day 0 to 74; 4 pastures/treatment/yr)

	Maternal supplementation[1]				P-value	
Item	NOSUP	MOL–	MOL+	SEM	Trt	Trt × day
Male calves at birth, %	43.9	72.2	55.8	11.20	0.24	—
Calf birth BW[1,2], kg	25.2	28.0	26.5	1.00	0.13	—
Calf age on day 147 (early weaning), d	87	91	91	6.0	0.85	—
Calf BW[2,3], kg						
Day 147 (early weaning)	79[a]	84[a]	86[a]	3.2	0.54	0.05
Day 154 (drylot entry)	81[x]	88[y]	89[y]	3.2		
Day 201 (drylot exit)	125[a]	134[b]	134[b]	3.2		
Calf ADG, kg/d						
Birth to day 147	0.58	0.57	0.62	0.029	0.48	—
Day 147 to 154	0.30[a]	0.54[b]	0.64[b]	0.109	0.05	—
Day 154 to 201[2]	0.83[a]	0.91[b]	0.98[b]	0.032	0.02	—
Birth to day 201	0.64[a]	0.72[b]	0.75[b]	0.037	0.05	—
DM intake, day 154 to 201[2], kg/d						
Hay	0.73	0.76	0.75	0.034	0.79	0.93
High-concentrate diet	3.00	3.16	3.17	0.096	0.39	0.97
Total	3.73	3.92	3.92	0.113	0.41	0.95
G:F, day 154 to 201[2,3,4]	0.236	0.243	0.246	0.0060	0.51	—

[1]Calves were born on day 57 ± 5 and early weaned on day 147. From day 147 to 154, calves offered ad libitum stargrass hay and gradually adapted to a high concentrate diet. From day 154 to 201, 4 steer and 4 heifer calves/treatment/yr were individually offered 1 kg/d of stargrass hay and high-concentrate diet at 3.5% of BW (DM basis).
[2]Covariate-adjusted for calf age ($P \leq 0.05$).
[3]Covariate-adjusted for calf sex ($P \leq 0.05$).
[4]Calculated by dividing total BW gain by total DM intake from day 154 to 201.
[a,b]Within a row, means without a common superscript differ ($P \leq 0.05$).
[x,y]Within a row, means without a common superscript tended to differ ($0.05 > P \leq 0.10$).

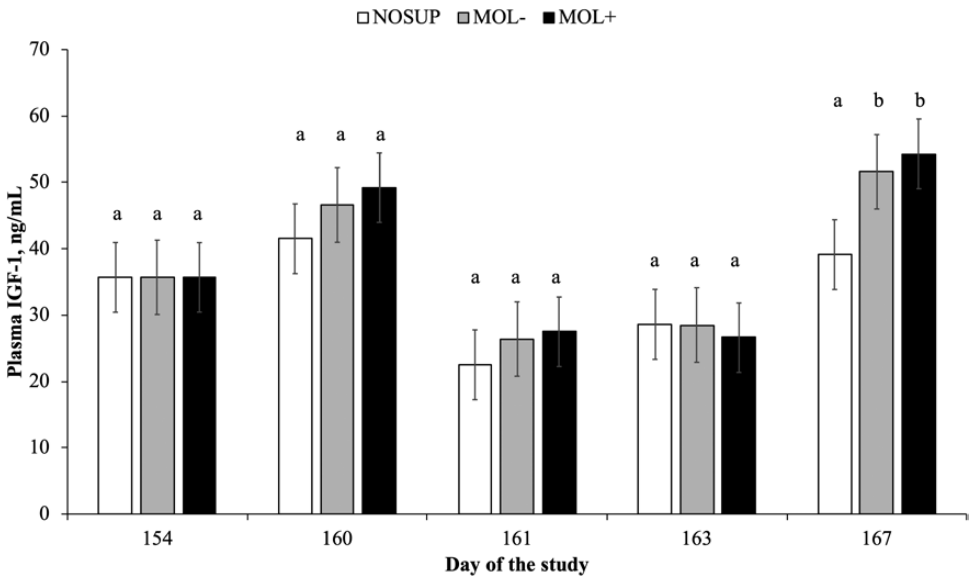

Figure 1. Plasma concentrations of IGF-1 of calves born from beef heifers offered NOSUP, MOL+, or MOL– for 57 ± 5 d prepartum and 17 ± 5 d postpartum (day 0 to 74; 4 pastures/treatment/yr). On day 160, calves received oral drench of fenbendazole (Safe-Guard, Merck Animal Health, Summit, NJ) and the first round of vaccinations (Bovi Shield Gold One Shot and Ultrabac 7; Zoetis Inc., New York, NY). Plasma IGF-1 concentrations on day 154 were included as covariate ($P = 0.01$). [a,b]Within day, means without a common superscript differ ($P \leq 0.04$).

forage DM intake of beef heifers (1.40 kg DM/kg of BW; Inyang et al., 2010). All heifers were provided free-choice access to stargrass hay from the day treatment supplementation was ceased (day 74) until the end of the breeding season. Thus, forage quantity did not limit heifer growth performance throughout the study. Nutritive value of pastures did not differ among treatments and was slightly above the energy and protein requirements of pregnant beef heifers (NASEM, 2016). According to the mechanistic model of NASEM (2016), late-gestating beef heifers offered no prepartum supplementation

Table 6. Plasma and serum measurements of calves born from beef heifers offered NOSUP, MOL+, or MOL– for 57 ± 5 d prepartum and 17 ± 5 d postpartum (day 0 to 74; 4 pastures/treatment/yr)[1]

	Maternal supplementation				P-value	
Item	NOSUP	MOL–	MOL+	SEM	Trt	Trt × day
Plasma glucose, mg/dL	89.0	90.2	90.4	1.13	0.66	0.72
Plasma cortisol, ug/dL	2.05	1.99	1.87	0.15	0.71	0.99
Plasma haptoglobin, mg/mL	0.56	0.51	0.50	0.044	0.56	0.33
Serum BVDV-1[2]						
Titers, $\log_2$	2.45	3.20	2.42	0.306	0.13	0.11
Positive seroconversion, %	56.1[a]	84.2[b]	78.7[b]	7.16	0.02	0.14
Serum PI-3[2]						
Titers, $\log_2$	4.72	4.67	4.74	0.266	0.99	0.22
Positive seroconversion, %	83.9[a]	100.0[b]	94.3[b]	4.15	0.01	0.30

[1]On day 160, calves received oral drench of fenbendazole (5 mg/kg of BW; Safe-Guard, Merck Animal Health, Summit, NJ) and vaccination against BVDV, PI-3, *Mannheimia haemolytica* (2 mL s.c.; Bovi Shield Gold One Shot; Zoetis Inc., New York, NY), and *Clostridium* (2 mL s.c.; Ultrabac 7, Zoetis Inc., New York, NY). Booster vaccinations with Bovi Shield Gold 5 (2 mL s.c.; Zoetis Inc.) and Ultrabac 7 (2 mL s.c.; Zoetis Inc.) were administered on day 188.
[2]Serum PI-3 and BVDV-1a titers were reported as the $\log_2$ of the greatest dilution of serum that provided complete protection of the cells. Positive seroconversion was determined as the percentage of calves with serum neutralization value of ≥4.
[a,b]Within a row, means without a common superscript differ ($P \leq 0.05$).

of energy and protein and grazing bahiagrass pastures (7.5% CP and 58% TDN of DM) under environmental conditions similar to those observed in the current study (in average 29 °C and 90% relative humidity) would achieve an ME-allowable ADG of 0.06 kd/d (2.6 kg of BW gain from day 0 to 44). In the current study, NOSUP heifers gained 12 kg from day 0 to 44, which is greater than estimated by NASEM (2016) but likely a result of gut fill effects and cattle ability to selectively graze parts of the forage with a nutritional value greater than hand-plucked samples.

Supplementation of protein and energy increased prepartum BCS gain of MOL– and MOL+ compared with NOSUP heifers, which is in agreement with others (Bohnert et al., 2013; Kennedy et al., 2019). Supplemental MHA did not further increase prepartum BCS gain of MOL+ vs. MOL– heifers, despite the fact that methionine can be the first-limiting amino acid in grazing and lactating beef cows (NASEM, 2016). However, our results agree with previous studies (Waterman et al., 2012; Clements et al., 2017). Rumen-protected DL-methionine supplementation (15 g/d of DL-MET starting at 58 ± 1 d prepartum) did not affect the BW and BCS of late-gestating, primiparous, winter-grazing beef heifers receiving wheat middling-based supplementation (Waterman et al., 2012). Prepartum BW and BCS did not differ between beef cows grazing cool-season grasses and offered 0.45 kg/d of wheat midd-based pellets with or without 10 g/d of MHA from 23 ± 7 d prepartum through 73 ± 7 d postpartum (Clements et al., 2017). Hence, the lack of positive effects of MHA supplementation on cow prepartum BW and BCS change may indicate that supplemental MHA amount was perhaps below optimal levels for BW and BCS change; a methionine deficiency during late gestation was nonexistent in those three studies; or an increased supplemental methionine uptake by the fetus as fetal growth is most rapid during late gestation leading to increased amino acid utilization (NASEM, 2016). Plasma concentrations of methionine decreased markedly during the last 3 wk of gestation in dairy cows (Zhou et al., 2016). In agreement, plasma L-methionine concentrations from day 0 to 44 tended to decrease in NOSUP heifers. The addition of 15 g/d of MHA into MOL+ supplements effectively increased plasma concentrations of L-methionine from day 0 to 44 in MOL+ and were greater compared with NOSUP and MOL– cows. Plasma methionine concentrations increased linearly in multiparous, late-gestating beef cows receiving increasing amounts of post-ruminal infusions of methionine (0, 5, 10, and 15 g/d of DL-methionine) starting at 84 ± 10 d prepartum (Waterman et al., 2007). In contrast, Clements et al. (2017) did not report an increase in serum concentrations of L-methionine at 73 ± 7 d postpartum (2.51 vs. 2.15 ± 0.207 μg/mL for MHA and no MHA supplementation, respectively) but observed greater serum concentrations of 2-hydroxy-4-methylthio butanoic acid (methionine precursor) for cows offered supplemental MHA. Multiple factors may explain the discrepancy on circulating concentrations of L-methionine among these studies, such as nutritional composition of forages, ruminal outflow of microbial and dietary protein, and breed and age of cattle. Discussing all factors is beyond the scope of this manuscript, but the most likely explanation for detecting greater plasma L-methionine concentrations for MOL+ heifers is that the current study provided 50% more MHA (15 vs. 10 g/d, respectively) compared with the study of Clements et al. (2017). Plasma Amino acid (**AA**) concentrations will remain low or static when below the requirement but will accumulate once the requirement is reached (Bergen, 1979). Therefore, the greater circulating concentrations of L-methionine indicate that MOL+ heifers successfully consumed the supplements, and perhaps that their prepartum methionine requirements were exceeded by MHA supplementation.

Maternal dietary methyl donors (i.e., methionine) are essential nutrients during pregnancy involved in DNA methylation and altered gene transcription (Alharthi et al., 2018). During its metabolism, methionine is converted into its active form (SAM) by MAT1A enzyme (Martinov et al., 2010). Placental concentrations of SAM are greatest when placental growth is most rapid (Wu et al., 2005). In the present study, plasma concentrations of MAT1A were greater for MOL+ vs. NOSUP likely to increase the conversion of methionine into SAM. However, plasma MAT1A concentrations did not differ between MOL+ vs. MOL– heifers, which were also greater compared with NOSUP heifers. The exact mechanism leading to greater plasma MAT1A concentrations in MOL– vs. NOSUP heifers is unknown but could be related to increased microbial synthesis and supply for intestinal absorption and methionine sparing following MOL– supplementation. Plasma concentrations of SAM on day 44 did

not differ among treatments and this outcome was unexpected considering that plasma concentrations of L-methionine and MAT1A were increased after MHA supplementation. Our results may have been masked by the transmethylation cycle (conversion of methionine into homocysteine and then back to methionine; Dasarathy et al., 2010) and the fact that circulating concentrations of SAM do not account for intracellular synthesis of SAM (Mentch et al., 2015).

Glucose is essential for fetal growth and its supply to the fetus is modulated by maternal glucose concentration (Baumann et al., 2002). Circulating concentrations of glucose are positively associated with energy and protein intake and rates of BW gain (Cappellozza et al., 2014). In the present study, plasma glucose concentrations did not differ among treatments, which may indicate that NOSUP heifers were capable of maintaining their plasma concentrations of glucose at the expense of other nutrients (McLean et al., 2018) or that excess glucose was mobilized by maternal tissues, placenta, and fetus. It is also possible that sugarcane molasses + urea supplementation did not provide the necessary substrate profile (i.e., propionate and glucogenic AA) for greater hepatic synthesis of glucose (Moriel et al., 2019b) due to the butyrate-induced inhibition on hepatic gluconeogenesis (Aiello et al., 1989). In agreement with Waterman et al. (2012) and Clements et al. (2017), supplemental MHA did not increase plasma glucose concentrations in the current study.

IGFs are produced by the placenta and by maternal and fetal tissues (Gicquel and Le Bouc, 2006) and may modulate placental growth and function, substrate delivery to the fetus, and partitioning of nutrients between maternal tissues and conceptus (Sferruzzi-Perri et al., 2006). Plasma concentrations of IGF-1 and -2 are regulated by nutrient intake (Perry et al., 2002). Plasma concentrations of IGF-1 were greater for MOL+ vs. NOSUP and intermediate for MOL– heifers, whereas plasma concentrations of IGF-2 were greater for MOL+ and MOL– compared with NOSUP heifers. Likewise, daily supplementation of 1.4 kg of CP increased plasma IGF-1 concentrations of beef heifers at 179 and 271 d of gestation compared with 0.4 kg/d of CP supplementation (Sullivan et al., 2009). Plasma IGF-2 concentrations in beef heifers were reduced at lower CP concentrations during the second trimester of gestation (7% vs. 14% CP of DM; Perry et al., 2002). Supplemental MHA did not increase plasma concentrations of IGF-1 and -2 compared with MOL– supplementation. We are unaware of other studies evaluating the maternal plasma concentrations of IGF-2 following MHA supplementation. However, serum IGF-1 concentrations increased linearly in multiparous late-gestation beef cows administered increasing amounts of post-ruminal infusions of methionine (5, 10, and 15 g/d of DL-methionine; Waterman et al., 2007). In that study, however, serum concentrations of IGF-1 did not differ between cows supplemented with 0 and 15 g/d of methionine (Waterman et al., 2007).

The percentage of pregnant heifers on day 282 did not differ among treatments, which was likely masked as primiparous cows utilized herein achieved a relatively high reproductive success. The current study was not designed to evaluate the reproductive performance of beef cows due to the limited number of observations for statistical analyses of binary reproductive data. However, the reasons for the exceptional reproductive performance of all heifers are the BCS ≥ 5 near the time of calving and the use of calf early weaning for all treatments. Early-weaning calves on the first day of the breeding season have been shown to effectively decrease energy and protein requirements and increase the percentage of pregnant primiparous beef cows by 30% (Arthington and Kalmbacher, 2003; Arthington and Minton, 2004). In addition, prepartum BCS slightly increased for MOL+ and MOL– vs. NOSUP heifers, and such increment in prepartum BCS was lost before the start of the breeding season. In agreement with Clements et al. (2017), the reproductive performance of beef heifers was not impacted by MHA supplementation, despite effectively increasing prepartum plasma L-methionine concentrations than NOSUP and MOL– heifers.

Offspring performance

The effects of maternal protein and energy supplementation during late gestation on calf birth BW have been inconsistent, with some studies showing an increase (Bohnert et al., 2013; Kennedy et al., 2019) and others showing no influence on calf birth BW (Moriel et al., 2016; Hare et al., 2019). Although statistical differences were not detected in the present study, calf birth BW was numerically 2 kg greater for calves born from heifers offered prepartum supplementation of MOL+ and MOL– compared with NOSUP. Prepartum supplementation of dried distillers grains to multiparous beef cows increased calf birth BW by 1.5 (Bohnert et al., 2013) and 3.4 kg (Kennedy et al., 2019) compared with no prepartum supplementation. The potential reasons for the lack of consistency on calf birth BW among these studies are the differences in supplement type, amount and duration, and maternal BCS, age, and breed. The effects of maternal supplementation of methionine on offspring birth BW have also been variable. Birth BW did not differ between calves born from dams offered no supplementation and prepartum supplementation of MHA at 10 g/d (Clements et al., 2017) and 15 g/d (present study) or rumen-protected DL-methionine at 23.5 g/d (Waterman et al., 2012). In contrast, maternal supplementation of rumen-protected methionine during late gestation increased birth BW in Holstein steers (Alharthi et al., 2018). Reasons for the inconsistent results may be attributed to different cattle breed and source, amount, and duration of methionine supplementation. Also, protein deposition in growing cattle increases in response to amino acid supplementation until the requirement is met (Froidmont et al., 2000). Thus, methionine requirements in the current study were likely met by maternal supplementation of sugarcane molasses + urea and the supplemental MHA did not further increase calf birth BW. In support of this rationale, oversupplying metabolizable protein by 33% did not impact calf birth BW compared with meeting 100% of metabolizable protein requirements of late-gestating beef cows (Hare et al., 2019).

Prepartum supplementation of dried distillers grains (0.3% of BW; DM basis) of multiparous beef cows increased milk production on day 44 of lactation by 3.3 kg and calf weaning BW by 18 kg compared with no prepartum supplementation (Kennedy et al., 2019). In the present study, calf ADG from birth to early weaning did not differ among treatments, suggesting that maternal prepartum supplementation of MOL– and MOL+ may have not caused carryover effects on milk production (not evaluated herein). Likewise, Clements et al. (2017) reported that pre- and postpartum supplementation of 10 g/d of MHA did not increase cow milk production and calf preweaning BW growth compared with no pre- and postpartum supplementation of MHA, which supports our rationale of potential lack of treatments effects on maternal milk production, explaining why differences on calf ADG from birth to early weaning were not detected.

Calf ADG from early weaning to drylot entry (day 147 to 154) and from drylot entry until exit (day 154 to 201) were greater for MOL+ and MOL– compared with NOSUP calves. The lack of differences in total DM intake among treatments from day 154 to 201 indicates that prepartum supplementation of sugarcance molasses + urea potentially induced in utero programming effects leading to greater calf growth performance. Prepartum supplementation of dried distillers grains to multiparous beef cows increased calf weaning BW by 8 kg compared with no prepartum supplementation (Bohnert et al., 2013). Recent evidences indicate that such improvement on offspring growth performance was independent of cow BCS at calving and was a consequence of prepartum maternal BCS gain during late gestation (Marques et al., 2016). Calf weaning BW did not differ between calves born from cows that maintained and calved at BCS of 4 and 6 but increased by 13.5 kg for calves born from cows that increased BCS from 4 to 6 during the last trimester of gestation (Marques et al., 2016). The maternal BCS gain during prepartum supplementation of sugarcane molasses + urea observed in our study compared with non-supplemented cows supports this rationale that BCS gain rather than BCS at calving led to greater postnatal growth performance of MOL+ and MOL– compared with NOSUP calves.

In agreement with Clements et al. (2017), supplemental MHA did not impact calf growth performance in drylot. In contrast, rumen-protected methionine supplementation (0.9% of diet DM) during the last 28 d of gestation increased ADG of Holstein steers from 1 to 9 wk of age compared with no methionine supplementation (Alharthi et al., 2018). The rate of methionine transmethylation is higher during the third trimester of gestation compared with early pregnancy and the nonpregnant state, likely to meet the high demands for methyl donors by the fetus and placenta (Dasarathy et al., 2010). Thus, it is also possible that the supply of methionine to the fetus was insufficient to induce programming effects on calf postnatal growth. As discussed previously, the lack of methionine deficiency and differences in source, amount and duration of methionine supplementation, and maternal breed may explain the discrepancy among those studies.

Plasma glucose concentrations of calves were not impacted during the drylot phase by previous maternal supplementation treatment. Plasma concentrations of glucose during the first 2 mo of age also did not differ between calves born from beef cows offered prepartum supplementation of dried distillers grains at 0% or 0.3% of diet DM (Kennedy et al., 2019), beef calves born from cows offered 70% or 100% of NEm requirements during late gestation (Moriel et al., 2016), and dairy calves born from cows offered rumen-protected methionine at 0% vs. 0.9% of diet DM (Alharthi et al., 2018) and 0 vs. 10 g/d (Jacometo et al., 2016). Postvaccination plasma IGF-1 concentrations decreased on day 161 and 163 compared to day 160. Vaccination elicits an acute phase response (Moriel and Arthington, 2013; Artioli et al., 2015). Proinflammatory cytokines released during an acute phase response induce a state of IGF-1 resistance, which inhibits the anabolic effects of IGF-1 on muscle synthesis and facilitates energy and protein mobilization from body stores (O'Connor et al., 2008). Hence, the decrease in plasma concentrations of IGF-1 following vaccination indicates that nutrients were being partitioned to support the immune system rather than growth. After the peak of the acute-phase response, however, plasma IGF-1 concentrations on day 167 were greater for MOL+ and MOL– vs. NOSUP calves, suggestive that maternal prepartum supplementation of sugarcane molasses + urea led to altered postvaccination physiological responses related to growth and provide a partial explanation for the greater drylot growth performance of calves born from supplemented vs. non-supplemented heifers. The lack of MHA effects on calf plasma concentrations of IGF-1 supports the lack of differences in BW growth of MOL+ and MOL– calves throughout the study.

In the current study, plasma concentrations of cortisol and haptoglobin did not differ among treatments. These results contradict previous research demonstrating that beef calves born from cows provided 70% of their daily NEm requirements during the last 40 d of gestation had less postvaccination plasma concentrations of haptoglobin and cortisol compared with calves born from cows fed to meet NEm requirements (Moriel et al., 2016). The corticotropin-releasing hormone-induced plasma concentrations of cortisol were increased at 2 mo of age, but not at 5.5 and 10 mo of age, in lambs born from ewes provided 50% of global nutrient intake during the first 30 d of gestation compared with ewes provided 100% of nutrient requirement throughout gestation (Chadio et al., 2007). Perhaps calf age at the time of physiological stress may explain the inconsistent results observed for plasma concentrations of haptoglobin and cortisol among these studies. Maternal supplementation of MHA also did not impact calf plasma concentrations of haptoglobin and cortisol, which agrees with the findings of Jacometo et al. (2016).

Neutralizing serum antibody titers may be used as an indicator of immune protection, disease prevention, and vaccine efficacy in calves (Bolin, 1995). The ability of beef cattle to respond to vaccination differs from animal to animal and depends on multiple factors, such as timing of vaccination (Silva et al., 2018), metabolizable protein supply (Moriel and Arthington, 2013), frequency of supplementation (Artioli et al., 2015), and prenatal nutrition (Moriel et al., 2016). Maternal short-term energy restriction during late gestation reduced offspring serum BVDV1a titers following vaccination compared with cohorts born from cows fed to meet their energy requirements (Moriel et al., 2016). In that study, calves born from energy-restricted cows demonstrated downregulation of genes involved in the immune response following vaccination, such as killer cell lectin-like receptor 1, which is expressed on nearly all natural killer cells and stimulates their cytotoxicity and cytokine release, and antimicrobial peptide natural killer-lysin-like, an antimicrobial peptide stimulating natural killer cells cytotoxicity (Sanglard et al., 2018). Together, the findings of Moriel et al. (2016) and Sanglard et al. (2018) indicate that calves born from cows fed to meet their energy requirement during late gestation responded better to vaccination compared with calves born from energy-restricted cows, likely due to a better development of the immune system during the prenatal phase. Although NOSUP heifers in the present study did not lose BCS before calving, the positive prepartum nutritional status of MOL+ and MOL– heifers increased postvaccination seroconversion against BVDV-1a and PI-3 viruses of their offspring compared with calves born from NOSUP heifers. These responses were unexpected considering that plasma concentrations of haptoglobin and cortisol did not differ among treatments. However, maternal prepartum nutrition may alter the oxidative stress status in the young calf. A lower maternal energy density of diets provided during the last 21 d of gestation (5.25 vs. 6.48 MJ/kg of DM) altered antioxidant capability in dairy calves and resulted in lower total antioxidant capacity compared with a more energy-dense diet (Gao et al., 2012), whereas prepartum supplementation of rumen-protected methionine decreased circulating concentrations of reactive oxygen metabolites in dairy calves compared with no methionine supplementation (Jacometo et al., 2016). The exact

mechanism underlying the treatment effects observed for seroconversion cannot be determined at this point. However, it is possible that the communication between innate and humoral immunity and possibly other measurements of immune response (not evaluated herein) of the offspring were positively affected by maternal prepartum supplementation of MOL– and MOL+ vs. NOSUP.

In conclusion, supplementation of sugarcane molasses + urea from 57 ± 5 d prepartum until 17 ± 5 d postpartum increased plasma indicators of nutritional status (IGF-1 and -2) and BCS at calving of *B. indicus*-influenced beef heifers. Maternal supplementation of sugarcane molasses + urea also enhanced offspring post-weaning BW growth and measurements of immune response following vaccination against respiratory pathogens compared with no maternal supplementation. The addition of 105 g/wk of MHA in maternal pre- and postpartum supplementation effectively increased maternal prepartum plasma concentrations of L-methionine but did not impact maternal performance and offspring growth and postvaccination immune responses.

Acknowledgments

We thank Novus International (Romance, AR) for their donation of MHA and Zoetis Animal Health (Florham Park, NY) and Early Career Seed Fund from University of Florida – IFAS for their funding support.

Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

Literature Cited

Aiello, R. J., L. E. Armentano, S. J. Bertics, and A. T. Murphy. 1989. Volatile fatty acid uptake and propionate metabolism in ruminant hepatocytes. *J. Dairy Sci.* **72**:942–949. doi:10.3168/jds.S0022-0302(89)79187-6

Alharthi, A. S., F. Batistel, M. K. Abdelmegeid, G. Lascano, C. Parys, A. Helmbrecht, E. Trevisi, and J. J. Loor. 2018. Maternal supply of methionine during late- pregnancy enhances rate of Holstein calf development in utero and postnatal growth to a greater extent than colostrum source. *J. Anim. Sci. Biotechnol.* **9**:83. doi:10.1186/s40104-018-0298-1

AOAC. 2006. *Official methods of analysis*. 18th ed. Arlington (VA): AOAC International.

Arthington, J. D., and R. S. Kalmbacher. 2003. Effect of early weaning on the performance of three-year-old, first-calf beef heifers and calves reared in the subtropics. *J. Anim. Sci.* **81**:1136–1141. doi:10.2527/2003.8151136x

Arthington, J. D., and J. E. Minton. 2004. The effect of early calf weaning on feed intake, growth, and postpartum interval in thin, Brahman-crossbred primiparous cows. *Prof. Anim. Sci.* **20**:34–38. doi:10.15232/S1080-7446(15)31269-9

Artioli, L. F. A., P. Moriel, M. H. Poore, R. S. Marques, R. F. Cooke. 2015. Decreasing the frequency of energy supplementation from daily to three times weekly impairs growth and humoral immune response of preconditioning beef steers. *J. Anim. Sci.* **93**:5430–5441. doi:10.2527/jas.2015-9457

Baumann, M. U., S. Deborde, and N. P. Illsley. 2002. Placental glucose transfer and fetal growth. *Endocrine* **19**:13–22. doi:10.1385/ENDO:19:1:13

Bergen, W. G. 1979. Free amino acids in blood of ruminants–physiological and nutritional regulation. *J. Anim. Sci.* **49**:1577–1589. doi:10.2527/jas1979.4961577x

Bohnert, D. W., L. A. Stalker, R. R. Mills, A. Nyman, S. J. Falck, and R. F. Cooke. 2013. Late gestation supplementation of beef cows differing in body condition score: effects on cow and calf performance. *J. Anim. Sci.* **91**:5485–5491. doi:10.2527/jas.2013-6301

Bolin, S. R. 1995. Control of bovine viral diarrhea infection by use of vaccination. *Vet. Clin. North Am. Food Anim. Pract.* **11**:615–625. doi:10.1016/s0749-0720(15)30470-9

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

Chadio, S. E., B. Kotsampasi, G. Papadomichelakis, S. Deligeorgis, D. Kalogiannis, I. Menegatos, and G. Zervas. 2007. Impact of maternal undernutrition on the hypothalamic-pituitary-adrenal axis responsiveness in sheep at different ages postnatal. *J. Endocrinol.* **192**:495–503. doi:10.1677/JOE-06-0172

Clements, A. R., F. A. Ireland, T. Freitas, H. Tucker, and D. W. Shike. 2017. Effects of supplementing methionine hydroxy analog on beef cow performance, milk production, reproduction, and preweaning calf performance. *J. Anim. Sci.* **95**:5597–5605. doi:10.2527/jas2017.1828

Cooke, R. F., and J. D. Arthington. 2013. Concentrations of haptoglobin in bovine plasma determined by ELISA or a colorimetric method based on peroxidase activity. *J. Anim. Physiol. Anim. Nutr. (Berl).* **97**:531–536. doi:10.1111/j.1439-0396.2012.01298.x

Cooke, R. F., C. L. Daigle, P. Moriel, S. B. Smith, L. O. Tedeschi, and J. M. B. Vendramini. 2020. Cattle adapted to tropical and subtropical environments: social, nutritional, and carcass quality considerations. *J. Anim. Sci.* **98**: (in press). doi:10.1093/jas/skaa014

Dasarathy, J., L. L. Gruca, C. Bennett, P. S. Parimi, C. Duenas, S. Marczewski, J. L. Fierro, and S. C. Kalhan. 2010. Methionine metabolism in human pregnancy. *Am. J. Clin. Nutr.* **91**:357–365. doi:10.3945/ajcn.2009.28457

Froidmont, E., Y. Beckers, and A. Thewis. 2000. Determination of the methionine requirement of growing double-muscled Belgian blue bulls with a three-step method. *J. Anim. Sci.* **78**:233–241. doi:10.2527/2000.781233x

Gallaher, R. N., C. O. Weldon, and J. G. Futral. 1975. An aluminum block digester for plant and soil analysis. *Soil Sci. Soc. Am. J.* **39**:803–806. doi:10.2136/sssaj1975.03615995003900040052x

Gao, F., Y. C. Liu, Z. H. Zhang, C. Z. Zhang, H. W. Su, and S. L. Li. 2012. Effect of prepartum maternal energy density on the growth performance, immunity, and antioxidation capability of neonatal calves. *J. Dairy Sci.* **95**:4510–4518. doi:10.3168/jds.2011-5087

Gicquel, C., and Y. Le Bouc. 2006. Hormonal regulation of fetal growth. *Horm. Res.* **65 (Suppl 3)**:28–33. doi:10.1159/000091503

Gonzalez, M. A., M. A. Hussey, and B. E. Conrod. 1990. Plant height, disk and capacitance meters used to estimate bermudagrass herbage mass. *Agron. J.* **82**:861–864. doi:10.2134/agronj1990.00021962008200050002x

Hare, K. S., K. M. Wood, C. Fitzsimmons, and G. B. Penner. 2019. Oversupplying metabolizable protein in late gestation for beef cattle: effects on postpartum ruminal fermentation, blood metabolites, skeletal muscle catabolism, colostrum composition, milk yield and composition, and calf growth performance. *J. Anim. Sci.* **97**:437–455. doi:10.1093/jas/sky413

Hersom, M. J., M. Vázquez-Añón, K. P. Ladyman, M. S. Kerley, and J. D. Arthington. 2009. Effect of methionine source and level on performance of growing beef calves consuming forage-based diets. *Applied Anim. Sci.* **25**:465–474. doi:10.15232/S1080-7446(15)30745-2

Inyang, U., J. M. B. Vendramini, L. E. Sollenberger, B. Sellers, A. Adesogan, L. Paiva, and A. Lunpha. 2010. Effects of stocking rates on animal performance and herbage responses of Mulato and bahiagrass pastures. *Crop Sci.* **50**:179–185. doi:10.2135/cropsci2009.05.0267

Jacometo, C. B., Z. Zhou, D. Luchini, E. Trevisi, M. N. Corrêa, and J. J. Loor. 2016. Maternal rumen-protected methionine supplementation and its effect on blood and liver biomarkers of energy metabolism, inflammation, and oxidative stress in neonatal Holstein calves. *J. Dairy Sci.* **99**:6753–6763. doi:10.3168/jds.2016-11018

Kennedy, V. C., J. J. Gaspers, B. R. Mordhorst, G. L. Stokka, K. C. Swanson, M. L. Bauer, and K. A. Vonnahme. 2019. Late gestation supplementation of corn dried distiller's grains plus solubles to beef cows fed a low-quality forage: III. Effects on mammary gland blood flow, colostrum and milk production, and calf body weights. *J. Anim. Sci.* **97**:3337–3347. doi:10.1093/jas/skz201

Littlejohn, B. P., D. M. Price, J. P. Banta, A. W. Lewis, D. A. Neuendorff, J. A. Carroll, R. C. Vann, T. H. Welsh, and R. D. Randel. 2016. Prenatal transportation stress alters temperament and serum cortisol concentrations in suckling Brahman calves. *J. Anim. Sci.* **94**:602–609. doi:10.2527/jas.2015-9635

Marques, R. S., R. F. Cooke, C. L. Francisco, and D. W. Bohnert. 2012. Effects of twenty-four hour transport or twenty-four hour feed and water deprivation on physiologic and performance responses of feeder cattle. *J. Anim. Sci.* **90**:5040–5046. doi:10.2527/jas.2012-5425

Marques, R. S., R. F. Cooke, M. C. Rodrigues, P. Moriel, and D. W. Bohnert. 2016. Impacts of cow body condition score during gestation on weaning performance of the offspring. *Livest. Sci.* **191**;174–178. doi:10.1016/j.livsci.2016.08.007

Martinov, M. V., V. M. Vitvitsky, R. Banerjee, and F. I. Ataullakhanov. 2010. The logic of the hepatic methionine metabolic cycle. *Biochim. Biophys. Acta* **1804**:89–96. doi:10.1016/j.bbapap.2009.10.004

McLean, K. J., B. H. Boehmer, L. J. Spicer, and R. P. Wettemann. 2018. The effects of protein supplementation of fall calving beef cows on pre- and postpartum plasma insulin, glucose and IGF-I, and postnatal growth and plasma insulin and IGF-I of calves. *J. Anim. Sci.* **96**:2629–2639. doi:10.1093/jas/sky173

Mentch, S. J., M. Mehrmohamadi, L. Huang, X. Liu, D. Gupta, D. Mattocks, P. Gómez Padilla, G. Ables, M. M. Bamman, A. E. Thalacker-Mercer, *et al.* 2015. Histone methylation dynamics and gene regulation occur through the sensing of one-carbon metabolism. *Cell Metab.* **22**:861–873. doi:10.1016/j.cmet.2015.08.024

Moore, J. E., and G. O. Mott. 1974. Recovery of residual organic matter from "in vitro" digestion of forages. *J. Dairy Sci.* **57**:1258–1259. doi:10.3168/jds.S0022-0302(74)85048-4

Moriel, P., and J. D. Arthington. 2013. Effects of molasses-based creep-feeding supplementation on growth performance of pre- and post-weaned beef calves. *Lives. Sci.* **151**:171–178. doi:10.1016/j.livsci.2012.11.008

Moriel, P., L. F. A. Artioli, M. B. Piccolo, M. H. Poore, R. S. Marques, and R. F. Cooke. 2016. Short-term energy restriction during late gestation and subsequent effects on postnatal growth performance, and innate and humoral immune responses of beef calves. *J. Anim. Sci.* **94**:2542–2552. doi:10.2527/jas.2016-0426

Moriel, P., B. I. Cappellozza, M. B. Piccolo, R. F. Cooke, M. F. Miranda, L. F. D. Batista, R. S. Carvalho, E. A. Colombo, F. V. Santili, R. V. O. Filho, *et al.* 2019a. Pre- and post-weaning injections of bovine somatotropin to optimize puberty achievement of *Bos indicus* beef heifers. *Transl. Anim. Sci.* **3**:443–455. doi:10.1093/tas/txy125

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., J. M. B. Vendramini, C. Carnelos, M. B. Piccolo, and H. M. da Silva. 2019b. Effects of monensin on growth performance of beef heifers consuming warm-season perennial grass and supplemented with sugarcane molasses. *Trop. Anim. Health Prod.* **51**:339–344. doi:10.1007/s11250-018-1693-5

NASEM (National Academies of Sciences, Engineering, and Medicine). 2016. *Nutrient requirements of beef cattle*. 8th ed. *Animal Nutrition Series*. Washington (DC): The National Academies Press.

NRC. 2000. *Nutrient requirements of beef cattle*. 7th ed. Washington (DC): The National Academies Press.

O'Connor, J. C., R. H. McCusker, K. Strle, R. W. Johnson, R. Dantzer, and K. W. Kelley. 2008. Regulation of IGF-I function by proinflammatory cytokines: at the interface of immunology and endocrinology. *Cell Immunol.* **252**:91–110. doi:10.1016/j.cellimm.2007.09.010

Perry, V., S. Norman, R. Daniel, P. C. Owens, P. Grant, and V. Doogan. 2002. Insulin-like growth factor levels during pregnancy in the cow are affected by protein supplementation in the diet. *Anim. Reprod. Sci.* **72**:1–10. doi:10.1016/s0378-4320(02)00069-6

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

Rosenbaum, M. J., E. A. Edwards, and E. J. Sullivan. 1970. Micromethods for respiratory virus sero-epidemiology. *Health Lab. Sci.* **7**:42–52.

Sanglard, L. P., M. Nascimento, P. Moriel, J. Sommer, M. Ashwell, M. H. Poore, M. S. Duarte, and N. V. L. Serão. 2018. Impact of energy restriction during late gestation on the muscle and blood transcriptome of beef calves after preconditioning. *BMC Genomics*. **19**:702. doi:10.1186/s12864-018-5089-8

Sferruzzi-Perri, A. N., J. A. Owens, K. G. Pringle, J. S. Robinson, and C. T. Roberts. 2006. Maternal insulin-like growth factors-I and -II act via different pathways to promote fetal growth. *Endocrinology* **147**:3344–3355. doi:10.1210/en.2005-1328

Silva, G. M., M. H. Poore, J. Ranches, and P. Moriel. 2018. Effects of timing of vaccination relative to weaning and post-weaning frequency of energy supplementation on growth and immunity of beef calves. *J. Anim. Sci.* **96**:318–330. doi:10.1093/jas/skx021

Sollenberger, L. E., J. E. Moore, V. G. Allen, and C. G. S. Pedreira. 2005. Reporting forage allowance in grazing experiments. *Crop Sci.* **45**:896–900. doi:10.2135/cropsci2004.0216

Sullivan, T. M., G. C. Micke, N. Perkins, G. B. Martin, C. R. Wallace, K. L. Gatford, J. A. Owens, and V. E. A. Perry. 2009. Dietary protein during gestation affects maternal IGF, IGFBP, leptin concentrations, and fetal growth in heifers. *J. Anim. Sci.* **87**:3304–3316. doi:10.2527/jas.2008-1753

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

Waterman, R. C., C. A. Löest, W. D. Bryant, and M. K. Petersen. 2007. Supplemental methionine and urea for gestating beef cows consuming low quality forage diets. *J. Anim. Sci.* 2007. **85**:731–736. doi:10.2527/jas.2006–425

Waterman, R. C., V. L. Ujazdowski, and M. K. Petersen. 2012. Effects of rumen-protected methionine on plasma amino acid concentrations during a period of weight loss for late gestating beef heifers. *Amino Acids* **43**:2165–2177. doi:10.1007/s00726-012-1301-3

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

Wu, G., F. W. Bazer, J. Hu, G. A. Johnson, and T. E. Spencer. 2005. Polyamine synthesis from proline in the developing porcine placenta. *Biol. Reprod.* **72**:842–850. doi:10.1095/biolreprod.104.036293