Published November 10, 2016

Post–artificial insemination supplementation with calcium salts of soybean oil influences pregnancy establishment factors in *Bos indicus* beef cows[1]

R. S. Cipriano,* R. F. Cooke,†[2,3] A. D. Rodrigues,‡ L. G. T. Silva,‡ D. W. Bohnert,† R. S. Marques,† J. L. M. Vasconcelos,‡ A. V. Pires,§ and R. L. A. Cerri#

*Unisalesiano Auxilium, Araçatuba, SP, Brazil, 16016-500; †Eastern Oregon Agricultural Research Center – Oregon State University, Burns 97720; ‡Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu, SP, Brazil, 18618-970; §Departamento de Zootecnia, ESALQ – Universidade de São Paulo, Piracicaba, SP, Brazil, 13418-900; and #Faculty of Land and Food Systems – University of British Columbia, Vancouver, BC V6T 1Z4, Canada

ABSTRACT: The objective of this experiment was to compare hormonal, uterine, and conceptus factors associated with pregnancy establishment in *Bos indicus* beef cows supplemented or not with Ca salts of soybean oil (CSSO) for 21 d beginning after timed AI. One hundred lactating multiparous Nelore cows were allocated to 20 groups of 5 cows/group and timed inseminated on d 0 of the experiment. After AI, groups were randomly assigned to receive (as-fed basis) 100 g of protein–mineral mix + 100 g of ground corn per cow per day, in addition to 1) 100 g/cow daily of CSSO (n = 10) or 2) 100 g/cow daily of kaolin (CON; rumen-inert indigestible substance; n = 10). Groups were maintained in 4 *Panicum maximum* pastures (5 groups from the same treatment within each pasture) with ad libitum access to forage. Groups were segregated daily and individually offered treatments from d 0 to 21. Blood samples were collected and transrectal ultrasonography was performed to verify ovulation and corpus luteum (CL) volume immediately before AI (d 0) and on d 7 and 15. After ultrasonography on d 15, 60 cows (30 cows/treatment and 3 cows/group) diagnosed without the presence of a CL on d 0 but with a CL greater than 0.38 cm3 in volume on d 7 and 15 were assigned to conceptus collection via transcervical flushing with PBS followed by endometrial biopsy in the uterine horn ipsilateral to the CL. Additional blood samples were collected for whole-blood RNA extraction (d 20), and pregnancy status was verified by transrectal ultrasonography (d 30) in cows not assigned to conceptus collection. Cows receiving CSSO had greater ($P \leq 0.04$) mean plasma linoleic acid concentration, plasma linoleic:linolenic acid ratio, plasma progesterone (P4) concentration, and CL volume during the experiment compared with CON cows. Moreover, CSSO supplementation increased ($P \leq 0.04$) length and mRNA expression of *prostaglandin E synthase* and *interferon-tau* by the conceptus as well as blood mRNA expression of interferon-stimulated genes on d 20 in gestating cows. No treatment differences were detected ($P \geq 0.30$) for endometrial mRNA expression of *prostaglandin E synthase* and *cyclooxygenase-2*. In summary, post-AI CSSO supplementation to B. indicus beef cows increased plasma concentration of linoleic acid and enhanced pregnancy establishment factors, which included CL development and plasma P4 concentration, conceptus growth, and mRNA expression of *interferon-tau* as well as blood mRNA expression of interferon-stimulated genes.

Key words: beef cows, calcium salts of soybean oil, gene expression, interferon-tau, pregnancy, progesterone

J. Anim. Sci. 2016.94:4892–4902
doi:10.2527/jas2016-0916

[1]Financial support for this research was provided by Fundação de Amparo à Pesquisa do Estado de São Paulo (Brazil; grant number 2014/14136-4).

[2]Corresponding author: reinaldo.cooke@oregonstate.edu

[3]Reinaldo Cooke also is affiliated as graduate professor to the Programa de Pós-Graduação em Zootecnia/Faculdade de Medicina Veterinária e Zootecnia, UNESP – Univ. Estadual Paulista, Botucatu, SP, Brazil, 18618-970.

Received August 16, 2016.
Accepted September 7, 2016.

INTRODUCTION

Early embryonic loss is a major reproductive challenge in cow–calf systems and is defined as losses that occur from conception to d 27 of gestation (Humblot, 2001). Therefore, strategies to enhance early embryonic survival are warranted for optimal reproductive and overall efficiency of cow–calf operations. Our group reported that supplementation with Ca salts of soybean oil (**CSSO**) for 21 d beginning after AI—hence, through the period when maternal pregnancy recognition occurs (Spencer and Bazer, 2004)—increased pregnancy risk by 30% in *Bos indicus* beef cows (Lopes et al., 2009, 2011). This outcome was credited with enhancing early pregnancy maintenance and was later associated with incorporation of linoleic acid and its ω-6 derivatives into maternal and embryonic tissues (Cooke et al., 2014).

Earlier experiments, however, did not elucidate the mechanisms by which CSSO supplementation and subsequent tissue uptake of linoleic and ω-6 fatty acids benefit pregnancy maintenance in beef females (Lopes et al., 2009, 2011; Cooke et al., 2014). Linoleic acid serves as precursor for PGF2α synthesis, the hormone refor luteolysis that has been associated with reduced pregnancy rates in beef females (Hess et al., 2008). Moreover, linoleic and ω-6 fatty acids are also precursors of PGE2 (Schmitz and Ecker, 2008), which is a critical regulator of pregnancy establishment in sheep and cattle by promoting synthesis and endometrial activity of interferon-tau (**IFNt**; Erdem and Guzeloglu, 2010; Dorniak et al., 2011). Therefore, we hypothesized that CSSO supplementation during early gestation favors maternal and embryonic responses required for pregnancy establishment, including PGE2 synthesis and the IFNt-signaling cascade. To test this hypothesis, this experiment investigated the effects of CSSO supplementation during early gestation on hormonal, uterine, and conceptus factors associated with pregnancy establishment in *B. indicus* beef cows.

MATERIALS AND METHODS

This experiment was conducted from January through March 2016 at a commercial cow–calf operation located in Aruanã, Brazil. This work was determined to be exempt from review and approval by the Oregon State University Institutional Animal Care and Use Committee (EFIR17-06). Nevertheless, the animals utilized herein were cared for in accordance with the practices outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

Animals and Diets

One hundred lactating multiparous Nelore cows (430 ± 5 kg BW; BCS = 2.87 ± 0.02; age = 8.5 ± 0.2 yr; 152 ± 1 d postpartum) were ranked by BCS and days postpartum and allocated to 20 groups of 5 cows/group in a manner such that groups had equivalent BCS and days postpartum at the beginning of the experiment (d −11). To facilitate cattle management and sampling procedures, groups were randomly divided into 2 lots of 10 groups/lot. Lots (10 groups/lot and 5 cows/group) started the experiment over 2 consecutive days following the same experimental schedule (d −11 to 30). Groups were maintained in 4 *Panicum maximum* pastures (6 ha each) with ad libitum access to forage and water throughout the experimental period, with 5 groups from the same lot within each pasture. From d −11 to −1, groups were supplemented with (as-fed basis) 100 g of a protein–mineral mix (21.8% CP and 78% TDN, DM basis; Centrum Peso 20; MCassab Tecnologia Animal, São Paulo, Brazil) + 100 g of ground corn per cow daily. Supplement was provided in feed bunks placed within each pasture (1.0 m/cow of linear bunk space) and readily consumed by cows.

Groups were enrolled in an estrus-synchronization + fixed-time AI protocol (Meneghetti et al., 2009) from d −11 to 0. Cows were inseminated on d 0 by the same technician, using semen from the same bull and batch. Estrus detection aids (Estrotect; Rockway Inc., Spring Valley, WI) were applied on d −2 to all cows, and occurrence of estrus was recorded at timed AI. Estrus was defined as removal of >50% of the rub-off coating on the Estrotect patch (Thomas et al., 2014). Immediately after AI, groups were randomly assigned to receive (as-fed basis) 100 g of a protein–mineral mix + 100 g of ground corn per cow daily, in addition to 1) 100 g/cow daily of CSSO (Megalac-E; Elanco Saúde Animal, São Paulo, Brazil; $n = 10$) or 2) 100 g/cow daily of kaolin (**CON**; rumen-inert indigestible substance; $n = 10$). On d 0, groups were reallocated in a manner such that each pasture had 5 groups from the same lot and treatment. Groups were segregated daily and individually offered treatments at the working facility from d 0 to 21 (0600 to 1200 h), with bunk space of approximately 1.0 m/cow. Cows readily consumed treatments within 15 min after feeding. Groups were rotated among pastures daily to account for potential effects of pasture on the variables evaluated herein. Nutritional and fatty acid concentrations of all feedstuffs used herein are described in Table 1. Composition and nutritional profile of dietary treatments were similar to that described earlier (Lopes et al., 2011; Cooke et al., 2014) and are listed in Table 2.

Samples of pastures and supplement ingredients were collected weekly during the experiment, pooled

Table 1. Nutritional and fatty acid profile (DM basis) of feedstuffs used in the experiment

Item	Corn	Protein–mineral mix[1]	Megalac-E[2]	Pasture[3]
TDN, %	86	78	193	59
NEm,[4] Mcal/kg	2.16	1.89	6.98	1.05
CP,[4] %	8.5	21.8	0.8	11.1
NDF,[4] %	8.1	19.1	1.2	64.7
Total identified fatty acids,[5] %	4.2	2.7	88.3	2.4
Palmitic acid (16:0), %	0.88	2.02	15.77	0.50
Stearic acid (18:0), %	0.03	0.37	4.54	0.13
Oleic acid (18:1), %	0.33	0.00	27.32	0.16
Linoleic acid (18:2), %	2.72	0.02	36.11	0.47
Linolenic acid (18:3), %	0.09	0.11	2.89	0.99

[1]Centrum Peso 20 (MCassab Tecnologia Animal, São Paulo, Brazil).

[2]Elanco Saúde Animal (São Paulo, Brazil).

[3]*Panicum maximum* pasture.

[4]Values obtained from a commercial laboratory wet chemistry analysis (Dairy One Forage Laboratory, Ithaca, NY). The TDN concentration was calculated according to the equations described by Weiss et al. (1992). The NEm concentration was calculated with the following equation (NRC, 2000): NEm = 1.37 ME − 0.138 ME2 + 0.0105 ME3 − 1.12, given that ME = DE × 0.82 and 1 kg TDN = 4.4 Mcal DE.

[5]As a percentage of DM (Tripathy et al., 2010).

within week, and analyzed for nutrient concentration by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). All samples were analyzed by wet chemistry procedures for concentrations of CP (method 984.13; AOAC, 2006), ADF (method 973.18 modified for use in an Ankom 200 fiber analyzer; Ankom Technology Corp., Fairport, NY; AOAC, 2006), and NDF (Van Soest et al., 1991; modified for use in an Ankom 200 fiber analyzer). Samples were also analyzed for fatty acid concentrations using gas chromatography (Agilent 7890; Agilent Technologies, Inc., Wilmington, DE) according to Tripathy et al. (2010). Only fatty acids identified by the assay were recorded. Calculations for TDN used the equations proposed by Weiss et al. (1992), whereas NEm was calculated with the equations proposed by the NRC (2000).

Sampling

Blood samples were collected immediately before AI (d 0) and on d 7 and 15 of the experiment from either the coccygeal vein or artery into blood collection tubes (Vacutainer, 10 mL; Becton, Dickinson and Company, Franklin Lakes, NJ) containing 158 US pharmacopeia units of freeze-dried sodium heparin. After collection, blood samples were immediately placed on ice, centrifuged (2,500 × *g* for 30 min at 4°C) for plasma harvest, and stored at −20°C on the same day of collection. Transrectal ultrasonography (7.5-MHz transducer, 500 V; Hitachi Aloka Medical America, Inc., Wallingford, CT) was performed concurrent with blood sampling on d 0, 7, and 15 to verify dominant follicle diameter (d 0) and estimate corpus luteum (**CL**) volume (d 7 and 15). Corpus luteum volume was estimated using the formula for volume of a sphere: volume = $4/3\pi \times (D/2)^3$, in which D is the maximum luteal diameter (Cooke et al., 2009). When the CL had a cavity, the cavity volume was also calculated as a sphere and subtracted from the CL volume.

Immediately after the ultrasonography exam on d 15, 60 cows (30 cows/treatment and 3 cows/group) diagnosed without the presence of a CL on d 0 but with a CL greater than 0.38 cm^3 in volume on d 7 and 15 were selected for conceptus collection and endometrial biopsy. This criterion was based on the smallest diameter of a functional CL detected in Nelore cows following induced ovulation, as reported by Figueiredo et al. (1997). Selection was randomly performed when groups had ≥4 cows that met the aforementioned criteria. Conceptus collection was performed via transcervical flushing of the uterine horn ipsilateral to the CL, according to the methods described by Ribeiro et al. (2016). Up to 5 flushings of 20 mL PBS each were performed if no conceptus tissue was recovered. The recovered conceptuses were transferred to a sterile 100 by 15 mm petri dish (CRAL Artigos para Laboratórios Ltda., São Paulo, Brazil) and measured for length. The uterine luminal media from the first PBS flush was deposited in a 50-mL sterile conical tube (Corning Life Sciences, Tewksbury, MA), placed on ice, stored at −20°C within 4 h for 5 d, and then stored at −80°C until further processing. Following conceptus collection, endometrial biopsy was performed in the uterine horn ipsilateral to the CL according to Cerri et al. (2011). Conceptus and endometrial samples were stored into 5-mL sterile cryogenic tubes (CRAL Artigos para Laboratórios Ltda.) containing 2 mL of RNA stabilization solution (RNAlater; Ambion Inc., Austin, TX), maintained at 4°C for 24 h, and stored at −20°C until further processing.

Table 2. Composition and nutritional profile of treatments offered in the experiment[1]

Item	CSSO	CON
Ingredients, % as-fed		
Ground corn	33.3	33.3
Protein–mineral mix	33.3	33.3
Megalac-E	33.3	–
Kaolin	–	33.3
Nutrient profile, DM basis		
DM, %	91.7	93.0
TDN,[2] %	120.3	53.7
NEm,[3] Mcal/kg	3.74	1.32
CP, %	10.2	10.0
NDF, %	9.36	8.94
Total identified fatty acids,[4] %	32.8	2.25
Palmitic acid (16:0), %	6.40	0.95
Stearic acid (18:0), %	1.70	0.13
Oleic acid (18:1), %	9.54	0.11
Linoleic acid (18:2), %	13.36	0.89
Linolenic acid (18:3), %	1.06	0.07
Daily intake		
DM, g	275	279
TDN,[2] g	331	147
NEm,[3] Mcal	1.02	0.36
CP, g	28.1	27.4
NDF, g	25.7	24.6
Total identified fatty acids,[4] g	90.1	6.2
Palmitic acid (16:0), g	17.60	2.62
Stearic acid (18:0), g	4.67	0.36
Oleic acid (18:1), g	26.24	0.29
Linoleic acid (18:2), g	36.74	2.44
Linolenic acid (18:3), g	2.92	0.18

[1]Cows received (as-fed basis) 100 g of a protein-mineral mix + 100 g of ground corn per cow daily, in addition to: 1) 100 g/cow daily of Ca salts of soybean oil (CSSO; Megalac-E, Elanco Saúde Animal, São Paulo, Brazil; $n = 10$), or 2) 100 g/cow daily of kaolin (CON; rumen-inert indigestible substance; $n = 10$).

[2]Calculated according to the equations described by Weiss et al. (1992).

[3]Calculated with the following equation (NRC, 2000): NEm = 1.37 ME − 0.138 ME2 + 0.0105 ME3 − 1.12, given that ME = DE × 0.82 and 1 kg TDN = 4.4 Mcal DE.

[4]Estimated from the treatment consumption of each individual experimental unit.

After conceptus collection and endometrial biopsy on d 15, all cows were returned to their respective groups and pastures. On d 20, blood samples were collected from the nonflushed cows (20 cows/treatment and 2 cows/group) into PAXgene tubes (BD Diagnostics, Sparks, MD) for whole-blood RNA extraction. On d 21, treatment administration and supplementation were terminated, and pregnancy status of the nonflushed cows was evaluated via transrectal ultrasonography (5.0-MHz transducer, 500 V; Hitachi Aloka Medical America, Inc., Wallingford, CT) on d 30.

Laboratorial Analysis

Plasma Samples. All samples were analyzed for fatty acid concentrations using gas chromatography (Agilent 7890) with the same procedure used for feed samples (Tripathy et al., 2010). Plasma samples collected on d 7 and 15 from cows that did not have a CL on d 0 but with a CL greater than 0.38 cm^3 in volume concurrent with blood collection (Figueiredo et al., 1997) were analyzed for progesterone (**P4**) concentrations using a chemiluminescent enzyme immunoassay (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA). All plasma samples were analyzed for P4 within a single assay, with intra-assay CV of 5.6% and minimum detectable concentration of 0.1 ng/mL.

Uterine Luminal Flushing. The uterine luminal media from the first PBS flush collected from cows that had a conceptus was analyzed for IFNt concentrations using a bovine-specific commercial ELISA kit (MyBioSource LLC, San Diego, CA) validated by Cooke et al. (2014). All samples were analyzed within a single assay, with intra-assay CV of 2.29% and minimum detectable concentration of 0.1 pg/mL.

Tissue Samples. Total RNA was extracted only from tissue samples collected from cows that had a conceptus using the TRIzol Plus RNA Purification Kit (Invitrogen, Carlsbad, CA). Quantity and quality of isolated RNA were assessed via UV absorbance (NanoDrop Lite; Thermo Fisher Scientific, Wilmington, DE) at 260 nm and 260:280 nm ratio, respectively (Fleige and Pfaffl, 2006). Extracted RNA (100 ng for conceptus and 200 ng for endometrial samples) was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit with random hexamers (Applied Biosystems Inc., Foster City, CA). Real-time reverse transcription PCR (**RT-PCR**) was completed using the Fast SYBR Green Master Mix (Applied Biosystems Inc.) and gene-specific primers (20 p*M* each; Table 3) with the StepOne Real-time PCR system (Applied Biosystems Inc.), according to procedures described by Cooke et al. (2008). At the end of each RT-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. Responses were quantified based on the threshold cycle ($\mathbf{C_T}$), the number of PCR cycles required for target amplification to reach a predetermined threshold. The CT responses from conceptus and endometrial genes of interest were normalized to the geometrical mean of C_T values of (Vandesompele et al., 2002) *glyceraldehyde-3-phosphate dehydrogenase* and *ribosomal protein L19* and those of *suppressor of zeste 12 homolog* and *zinc finger protein 131*, respectively. The CV for the geometrical mean of *glyceraldehyde-3-phosphate dehydrogenase* and *ribosomal protein L19*

Table 3. Primer sequences, accession number, and reference for all gene transcripts analyzed by real-time reverse transcription PCR

Target gene	Primer sequence	Accession no.	Source
Genes of interest			
20,50-oligoadenylate synthetase			
Forward	ACCCTCTCCAGGAATCCAGT	NM 001040606	Fricke et al. (2016)
Reverse	GATTCTGGTCCCAGGTCTGA		
Cyclooxygenase-2			
Forward	TCCTGAAACCCACTCCCAACA	NM_174445	Takagi et al. (2008)
Reverse	TGGGCAGTCATCAGGCACAG		
Interferon-stimulated gene 15			
Forward	GGTATGAGCTGAAGCAGTT	NM_174366	Fricke et al. (2016)
Reverse	ACCTCCCTGCTGTCAAGGT		
Interferon-tau			
Forward	GCCCTGGTGCTGGTCAGCTA	AF238612	Rizos et al. (2003)
Reverse	CATCTTAGTCAGCGAGAGTC		
Myxovirus resistance 2			
Forward	CTTCAGAGACGCCTCAGTCG	NM 173941	Fricke et al. (2016)
Reverse	TGAAGCAGCCAGGAATAGTG		
Prostaglandin E synthase			
Forward	CGCTGCTGGTCATCAAAAT	NM_174443.2	Takagi et al. (2008)
Reverse	GGAAGGGGTAGATGGTCTCC		
Reference genes			
β-actin			
Forward	CTGGACTTCGAGCAGGAGAT	AY141970	Gifford et al. (2007)
Reverse	GGATGTCGACGTCACACTTC		
β2-microglobulin			
Forward	GGGCTGCTGTCGCTGTCT	NM_173893	Silva et al. (2008)
Reverse	TCTTCTGGTGGGTGTCTTGAGT		
Glyceraldehyde-3-phosphate dehydrogenase			
Forward	ACCCAGAAGACTGTGGATGG	NM_001034034	Cerri et al. (2012)
Reverse	CAACAGACACGTTGGGAGTG		
Ribosomal protein L19			
Forward	ATTGACCGCCACATGTATCA	NM_001040516	Monteiro et al. (2014)
Reverse	GCGTGCTTCCTTGGTCTTAG		
Suppressor of zeste 12 homolog			
Forward	GAACACCTATCACACACATTCTTGT	XM_582605	Walker et al. (2009)
Reverse	TAGAGGCGGTTGTGTCCACT		
Zinc finger protein 131			
Forward	AGAAAGAAGCTTTATGAATGTCAGG	NM_001101218	Walker et al. (2009)
Reverse	GTTTATCTCCAGTGTGTATCACCAG		

CT values across all conceptus samples was 1.7%. The CV for the geometrical mean of suppressor of *zeste 12 homolog* and *zinc finger protein 131* CT values across all endometrial samples was 2.4%. Results are expressed as relative fold change ($2^{-\Delta\Delta CT}$), as described by Ocón-Grove et al. (2008).

PAXgene Samples. Total RNA was extracted from all samples using the PAXgene Blood RNA Kit (Qiagen, Valencia, CA). Assessment of quantity and quality of isolated RNA, reverse transcription (120 ng of extracted RNA), and real-time RT-PCR with gene-specific primers (20 p*M* each; Table 3) were performed as described for tissue samples. Responses from genes of interest were quantified based on $C_{T\ a}$nd normalized to the geometrical mean of CT values from β*2-microglobulin* and β-*actin* (Vandesompele et al., 2002). The CV for the geometrical mean of β*2-microglobulin* and β-*actin* CT values across all conceptus samples was 2.1%. Results are expressed as relative fold change ($2^{-\Delta\Delta CT}$) as described by Ocón-Grove et al. (2008).

Statistical Analyses

Quantitative and binary data were analyzed with the MIXED and GLIMMIX procedures, respectively, of SAS (SAS Inst. Inc., Cary, NC) and with Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. All data were

analyzed using group as experimental unit as well as lot, group(treatment × lot), and cow(group) as random variables. The model statement used for dominant follicle diameter, estrus expression, presence of conceptus, conceptus length, IFNt concentrations in the uterine flushing, and all endometrial and conceptus gene expression results contained the effect of treatment. The model statement used for blood gene expression results contained the effects of treatment, pregnancy status on d 30, and the resultant interaction. The model statement used for CL volume, plasma P4 and fatty acid concentrations, and proportion of cows without a CL on d 0 but with CL greater than 0.38 cm^3 in volume on d 7 and 15 contained the effects of treatment, day, and the resultant interaction. Plasma fatty acid concentrations were analyzed using values from d 0 as an independent covariate, whereas all reproductive variables included estrus expression as an independent covariate. The specified term for the repeated measure analyses was day, cow(group) was the subject, and the covariance structure used was first-order autoregressive, which provided the smallest Akaike information criterion and, hence, the best fit for the variables analyzed. Results are reported as covariately adjusted least squares means and were separated using LSD. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and $P \leq 0.10$. Results are reported according to main treatment effect if no interaction containing the treatment effect was significant or according to the highest-order interaction detected.

RESULTS AND DISCUSSION

Plasma Fatty Acid Concentrations

Plasma concentrations of individual and total identified fatty acids did not differ ($P \geq 0.15$; data not shown) between CSSO-supplemented and CON cows on d 0, indicating that cows in both dietary treatments had similar plasma fatty acid concentrations and profiles before treatment administration. During the experimental period, CSSO-supplemented cows had greater ($P \leq 0.03$) plasma concentrations of palmitic, stearic, and linoleic acids; SFA and PUFA; linoleic:linolenic acid ratio; and total identified fatty acids and tended to have greater ($P \leq 0.07$) plasma concentrations of mystiric acid compared with CON cows (Table 4). A treatment × day interaction was detected ($P = 0.05$) for plasma concentration of linolenic acid, which was greater ($P < 0.01$) for CSSO-supplemented cows than for CON cows on d 7 (0.144 vs. 0.109 mg/mL of plasma, respectively; SEM 0.009) but similar ($P = 0.95$) between treatments on d 15 (0.122 vs. 0.123 mg/mL of plasma, respectively; SEM 0.009). These results corroborate the fatty acid content and profile of the CSSO treatment (Table 2; predominantly linoleic acid), given that plasma fatty acid concentrations directly reflect intake and duodenal flow of fatty acids (Lake et al., 2007; Scholljegerdes et al., 2007; Hess et al., 2008). Previous studies also reported that supplementing beef cattle with CSSO increased plasma concentrations of linoleic acid and total PUFA (Cooke et al. (2011, 2014), although these authors reported similar or reduced plasma linolenic acid concentrations in CSSO-supplemented vs. nonsupplemented cattle. Nevertheless, CSSO-supplemented cows had greater plasma concentrations of linolenic acid only on d 7, and the mean linoleic:linolenic acid ratio was nearly doubled compared with that in CON cows. Hence, supplementing 100 g of CSSO to beef cows in the present experiment favored intake and circulating concentrations of linoleic acid compared with linolenic acid as in Cooke et al. (2011, 2014), particularly on d 15 of the experiment, when maternal pregnancy recognition begins (Spencer and Bazer, 2004).

Table 4. Plasma fatty acid concentrations (mg/mL of plasma) of beef cows supplemented or not with 100 g of Ca salts of soybean oil[1,2]

Item[3]	CSSO	CON	SEM	*P*-value
Mystiric acid (14:0)	0.263	0.214	0.018	0.07
Myristoleic acid (14:1)	0.176	0.163	0.011	0.44
Palmitic acid (16:0)	0.219	0.188	0.013	0.02
Stearic acid (18:0)	0.347	0.285	0.020	<0.01
Oleic acid (18:1)	0.197	0.206	0.019	0.39
Linoleic acid (18:2)	0.386	0.199	0.025	<0.01
Linolenic acid (18:3)	0.133	0.116	0.06	0.06
Total SFA	0.964	0.783	0.054	0.03
Total MUFA	0.376	0.367	0.022	0.57
Total PUFA	0.523	0.315	0.033	<0.01
Linoleic:linolenic acid ratio	3.03	1.72	0.17	<0.01
Total identified fatty acids	1.863	1.465	0.073	<0.01

[1]Cows received (as-fed basis) 100 g of a protein-mineral mix + 100 g of ground corn per cow daily, in addition to: 1) 100 g/cow daily of Ca salts ofsoybean oil (CSSO; Megalac-E, Elanco Saúde Animal, São Paulo, Brazil; n = 10), or 2) 100 g/cow daily of kaolin (CON; rumen-inert indigestiblesubstance; n = 10). Treatments were offered from d 0 to 21 of the experiment.

[2]Blood samples were collected from all cows (n = 100; 50/treatment) on d 0 (before the first treatment application), 7, and 15. Values obtained on d 0 served as covariate; therefore, reported values are covariately adjusted means.

[3]SFA comprise mystiric, palmitic, and stearic acids; MUFA comprise oleic and myristoleic acids; PUFA comprise linoleic and linolenic acids.

Ovarian Variables and Plasma Progesterone Concentration

A similar ($P = 0.93$) proportion of CSSO-supplemented and CON cows expressed estrus from d −2 to 0 (42.0 vs. 44.0%, respectively [SEM 7.2%], which corresponds to 21 and 22 cows within 50 cows assigned to each treatment). In addition, no treatment differences

were detected (data not shown) for estrus expression within cows assigned ($P = 0.51$) or not ($P = 0.44$) to conceptus flushing or within cows assigned to flushing that had a conceptus collected on d 15 ($P = 0.56$). Treatment administration began on d 0, which is after the period of estrus expression evaluation. Nonetheless, estrus expression impacts ovarian dynamics (Sá Filho et al., 2010), conceptus development, and mRNA expression of genes associated with pregnancy establishment in endometrial and conceptus tissues (Davoodi et al., 2016), and for this reason, it was included in all reproductive analyses as an independent covariate. Therefore, all treatment effects reported herein for maternal and conceptus variables should not be associated with differences in estrus expression among treatments. A similar ($P \geq 0.65$) proportion of CSSO-supplemented cows and CON cows did not have a CL on d 0 but had a CL greater than 0.38 cm^3 in volume on d 7 (94.2 vs. 92.0%, respectively [SEM 7.3%], which corresponds to 47 and 46 cows within 50 cows assigned to each treatment) and 15 (92.0 vs. 90.0%, respectively [SEM 5.6%], which corresponds to 46 and 45 cows within 50 cows assigned to each treatment). The goal of the experiment was not to compare synchronization rate or to assess luteolysis between treatments. This information is being presented to demonstrate that treatment effects on plasma P4 concentration and CL volume were determined using a balanced data set as in Cooke et al. (2014).

Cows supplemented with CSSO had a greater mean plasma P4 concentration ($P < 0.01$) and mean CL volume ($P \leq 0.04$) during the experiment compared with CON cows (Table 5). The diameter of the dominant follicle on d 0 was similar ($P = 0.51$) between CSSO-supplemented cows and CON cows (Table 5); hence, treatment differences reported for CL volume and plasma P4 concentration were not related to size of the ovulatory follicle (Vasconcelos et al., 2001). Supporting our findings, Cooke et al. (2014) reported that CSSO-supplemented cows had greater plasma P4 concentration and CL volume on d 7 after AI compared with nonsupplemented cows and attributed these outcomes to hastened CL development when CSSO was supplemented. Lopes et al. (2009, 2011) also reported that CSSO-supplemented cows had greater serum P4 concentrations compared with nonsupplemented cohorts on d 7 after AI because of increased P4 synthesis and reduced hepatic catabolism of P4 (Hawkins et al., 1995; Staples et al., 1998; Sangsritavong et al., 2002). The increase in plasma P4 concentration in CSSO-supplemented cows reported herein also seems to be directly related to their greater CL volume compared with CON cows. In turn, hastened CL development can be attributed to increased intake and incorporation of linoleic and ω-6 fatty acids into luteal tissues of CSSO-supplemented cows as suggested by Cooke et al. (2014), although this experiment did not evaluate hepatic P4 catabolism or luteal fatty acid concentrations. Nonetheless, circulating P4 concentrations after breeding have been positively associated with pregnancy rates in cattle (Robinson et al., 1989; Stronge et al., 2005). Therefore, results from this experiment corroborate that increased circulating P4 concentrations seems to be one of the mechanisms by which supplemental CSSO after AI improves pregnancy rates in beef cows (Lopes et al., 2009; Cooke et al., 2014).

Table 5. Ovarian and pregnancy factors in beef cows supplemented or not with 100 g of Ca salts of soybean oil[1,2]

Item	CSSO	CON	SEM	*P*-value
Ovarian factors				
Dominant follicle diameter (d 0), mm	13.2	13.0	0.5	0.51
Corpus luteum volume,[3] cm^3	3.25	2.76	0.19	0.04
Plasma progesterone,[3] ng/mL	4.58	3.89	0.29	<0.01
Pregnancy factors				
Proportion of cows with conceptus,[4] %				
Day 15	40.4	36.3	9.0	0.75
Day 30	43.5	40.1	12.0	0.85
Conceptus length[5]	2.58	1.15	0.59	0.04
IFNt[6] uterine flushing media,[5] ng/mL	19.3	15.6	3.7	0.49

[1]CSSO =Cows received (as-fed basis) 100 g of a protein-mineral mix + 100 g of ground corn per cow daily, in addition to: 1) 100 g/cow daily of Ca salts of soybean oil (CSSO; Megalac-E, Elanco Saúde Animal, São Paulo, Brazil; n = 10), or 2) 100 g/cow daily of kaolin (CON; rumen-inert indigestible substance; n = 10). Treatments were offered from d 0 to 21 of the experiment. Results are covariately adjusted to estrus expression (Estrotect; Rockway Inc., Spring Valley, WI; Thomas et al., 2014) from d −2 to 0 of the experiment.

[2]Transrectal ultrasonography (7.5-MHz transducer, 500 V; Hitachi Aloka Medical America, Inc., Wallingford, CT) was performed on d 0, 7, and 15 of the experiment. Blood samples were collected for progesterone analysis on d 7 and 15. Conceptus and uterine flushing media collected via transcervical flushing (Ribeiro et al., 2016) as well as endometrial biopsy (Cerri et al., 2011) were performed on d 15. Values reported are overall means originated from main treatment effects.

[3]Evaluated from cows that did not have a corpus luteum on d 0 but with a corpus luteum greater than 0.38 cm3 in volume on d 7 ($n = 47$ for CSSO and $n = 46$ for CON) and 15 ($n = 46$ for CSSO and $n = 45$ for CON). Corpus luteum volume was calculated using the formula for volume of a sphere: volume = $4/3\pi \times (D/2)3$, in which D is the maximum luteal diameter (Cooke et al., 2009).

[4]On d 15, 30 cows per treatment were assigned to transcervical flushing for conceptus collection. On d 30, pregnancy status of the nonflushed cows (20 cows/treatment) was evaluated via transrectal ultrasonography (5.0-MHz transducer, 500 V; Hitachi Aloka Medical America, Inc., Wallingford, CT).

[5]Evaluated from cows that had a conceptus collected via transcervical flushing.

[6]IFNt = interferon-tau.

Pregnancy Establishment Factors

No treatment differences ($P \geq 0.75$) were detected for the proportion of cows that had a conceptus on d 15 and 30 (Table 5), although the aim of the study was not to compare pregnancy risk between treatments

as in Lopes et al. (2009, 2011). Conceptuses collected from CSSO-supplemented cows had greater (P = 0.04) length compared with conceptuses from CON cows (Table 5). These results contradict Cooke et al. (2014), where conceptus length and weight were similar among CSSO-supplemented cows and nonsupplemented cows. In the present experiment, conceptuses were collected on d 15 of gestation, which corresponds to the beginning of the maternal pregnancy recognition period (Spencer and Bazer, 2004). Cooke et al. (2014) collected conceptuses on d 19—therefore, after the critical period for pregnancy recognition, which may have prevented proper assessment of early conceptus development. Nevertheless, Cooke et al. (2014) reported that post-AI CSSO supplementation to beef cows increased concentrations of ω-6 fatty acids in d 19 conceptuses. Essential fatty acids such as ω-6 have important roles on conceptus development through maintenance of cell metabolism and membrane fluidity, permeability, and conformation (Thangavelu et al., 2007; Leroy et al., 2014). Hence, results from this experiment combined with those of Cooke et al. (2014) suggest that CSSO supplementation and the resultant tissue incorporation of ω-6 fatty acids hastened conceptus development by d 15 of gestation.

Omega-6 fatty acids are precursors for $PGF_{2\alpha}$ synthesis (Yaqoob and Calder, 2007), the hormone responsible for luteolysis and pregnancy termination (Senger, 2003). In contrast, PGE2 also originates from ω-6 fatty acids (Schmitz and Ecker, 2008) and is a critical regulator of conceptus elongation and pregnancy establishment in livestock (Erdem and Guzeloglu, 2010; Dorniak et al., 2011). More specifically, PGE2 is synthesized by the conceptus and endometrium, modulates synthesis and endometrial activity of IFNt, and seems to be fundamental for conceptus development and pregnancy signaling to maternal tissues (Erdem and Guzeloglu, 2010; Dorniak et al., 2011). Therefore, mRNA expression of *prostaglandin E synthase* and IFNt were greater ($P \leq 0.03$) in conceptuses from CSSO-supplemented cows than those from CON cows (Table 6). These outcomes also can be attributed to CSSO-supplementation effects on conceptus development and plasma P4 concentration, given that IFNt synthesis by the conceptus has been positively associated with conceptus size and circulating P4 concentrations (Bilby et al., 2004; Mann et al., 2006). No treatment effects, however, were detected ($P \geq 0.30$) for mRNA expression of *cyclooxygenase-2* and *prostaglandin E synthase* in the endometrium (Table 6). Contrary to these outcomes, others have reported that ω-6 and ω-3 fatty acids modulate mRNA expression of *cyclooxygenase-2* and subsequent PG secretion by the endometrium (Mattos et al., 2003; Bilby et al., 2006; Leroy et al., 2014). Despite collecting endometrial samples 19 d after AI, Cooke et al. (2014) also reported similar *cyclooxygenase-2* mRNA expression between CSSO-supplemented cows and nonsupplemented cows. Hence, results from this experiment suggest that CSSO supplementation modulates expression of IFNt and *prostaglandin E synthase* expression in the conceptus but not expression of PG-related genes in endometrial tissues on d 15 of gestation.

Table 6. Expression of genes associated with pregnancy establishment in the endometrium, conceptus, and blood from beef cows supplemented or not with 100 g of Ca salts of soybean oil[1]

Item	CSSO	CON	SEM	*P*-value
Endometrium[2]				
Cyclooxygenase-2	6.90	4.89	1.50	0.37
Prostaglandin E synthase	5.34	4.21	0.76	0.30
Conceptus[3]				
Interferon-tau	21.0	5.1	5.0	0.03
Prostaglandin E synthase	7.89	2.99	1.49	0.02
Blood cells				
Interferon-stimulated gene 15				
Pregnant	33.8	23.7	2.6	<0.01
Nonpregnant	2.88	5.88	2.2	0.34
Myxovirus resistance 2				
Pregnant	47.1	27.6	4.2	<0.01
Nonpregnant	5.70	11.19	3.5	0.27
20,50-oligoadenylate synthetase				
Pregnant	48.1	35.2	3.8	0.02
Nonpregnant	5.38	9.80	3.2	0.34

[1]Cows received (as-fed basis) 100 g of a protein-mineral mix + 100 g of ground corn per cow daily, in addition to: 1) 100 g/cow daily of Ca salts of soybean oil (CSSO; Megalac-E, Elanco Saúde Animal, São Paulo, Brazil; n = 10), or 2) 100 g/cow daily of kaolin (CON; rumen-inert indigestible substance; n = 10). Treatments were offered from d 0 to 21 of the experiment. Results are covariately adjusted to estrus expression (Estrotect; Rockway Inc., Spring Valley, WI; Thomas et al., 2014) from d −2 to 0 of the experiment.

[2]Conceptus and uterine flushing media collected via transcervical flushing (Ribeiro et al., 2016) as well as endometrial biopsy (Cerri et al., 2011) were performed on d 15 (30 cows/treatment). Only samples from cows with a retrieved conceptus were analyzed. Values are expressed as relative fold change compared with the threshold cycle of reference genes analyzed within the same sample (Ocón-Grove et al., 2008).

[3]Blood samples collected from nonflushed cows (20 cows/treatment) into PAXgene tubes (BD Diagnostics, Sparks, MD) for whole-blood RNA extraction on d 20 of the experiment and analyzed according to cow pregnancy status on d 30. Values are expressed as relative fold change compared with the threshold cycle of reference genes analyzed within the same sample (Ocón-Grove et al., 2008).

No treatment effects were detected (P = 0.49) for concentrations of IFNt in the uterine flushing media (Table 5), despite treatment differences detected for IFNt mRNA expression in the conceptus (Table 6). The reason for this discrepancy may be associated with the time required for mRNA to be translated into the active protein (Clancy and Brown, 2008), whereas Cooke et al. (2014) reported that IFNt concentration in the uterine flushing media tended to be greater in

CSSO-supplemented cows than in nonsupplemented cows on d 19 of gestation. Synthesis of IFNt during maternal recognition of pregnancy upregulates mRNA expression of interferon-stimulated genes (**ISG**) in circulating blood leukocytes (Stevenson et al., 2007; Gifford et al., 2008; Green et al., 2010). Consequently, mRNA expression of ISG have been used to assess IFNt production and conceptus development from d 15 to 22 of gestation as well as pregnancy diagnosis on d 18 of gestation (Fricke et al., 2016). Accordingly, treatment × pregnancy status interactions were detected ($P \leq 0.01$) for blood mRNA expression of the ISG *interferon-stimulated gene 15*, *myxovirus resistance 2*, and *20,50-oligoadenylate synthetase* on d 20 of the experiment (Table 6). Expression of these genes were similar ($P \geq 0.27$) within cows diagnosed as nonpregnant on d 30 but greater ($P \leq 0.01$) for CSSO-supplemented cows than for CON cows diagnosed as pregnant. In addition, cows diagnosed as pregnant had greater ($P < 0.01$) expression of ISG compared with cows diagnosed as nonpregnant within and across treatments (Table 6). Therefore, the greater mRNA expression of ISG in CSSO-supplemented cows on d 20 corroborates with treatment effects detected for IFNt mRNA expression in the conceptus on d 15, suggesting that CSSO supplementation enhanced IFNt synthesis by the conceptus during the pregnancy recognition period (Spencer and Bazer, 2004; Fricke et al., 2016).

Overall Conclusions

In summary, supplementing *B. indicus* beef cows with 100 g of CSSO beginning after AI increased plasma concentration of linoleic acid, plasma linoleic:linolenic acid ratio, plasma P4 concentration, and CL volume during early gestation. Supplementing CSSO also increased conceptus length and mRNA expression of *prostaglandin E synthase* and IFNt by the conceptus on d 15 as well as blood mRNA expression of ISG on d 20 after AI in gestating cows. Collectively, results reported herein and by previous research from our group suggest that post-AI CSSO supplementation favors incorporation of linoleic acid and its ω-6 derivatives into maternal and embryonic tissues and increases plasma P4 concentration via hastened CL development during early gestation (Cooke et al., 2014). In turn, these outcomes enhance IFNt synthesis by the conceptus during the maternal pregnancy recognition period, which may facilitate the increased pregnancy rates in CSSO-supplemented cows reported by Lopes et al. (2009, 2011). It is important to note, however, that Cooke et al. (2014) evaluated nonlactating cows and did not take into account fatty acid uptake by the mammary gland (Lake et al., 2007). Hence, results from Cooke et al. (2014) may not fully elucidate fatty acid uptake by reproductive tissues and conceptus of the lactating cows used herein and by Lopes et al. (2009, 2011). Nevertheless, these research efforts validate that supplementing CSSO for 21 d beginning at AI is an alternative to enhance pregnancy establishment and overall reproductive performance of *B. indicus* beef cows

LITERATURE CITED

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

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: Ovarian, conceptus, and insulin-like growth factor system responses. J. Dairy Sci. 87:3256–3267. doi:10.3168/jds.S0022-0302(04)73462-1

Bilby, T. R., A. Guzeloglu, L. A. MacLaren, C. R. Staples, and W. W. Thatcher. 2006. Pregnancy, bovine somatotropin, and dietary *n*-3 fatty acids in lactating dairy cows: II. Endometrial gene expression related to maintenance of pregnancy. J. Dairy Sci. 89:3375–3385. doi:10.3168/jds.S0022-0302(06)72374-8

Cerri, R. L. A., R. C. Chebel, F. Rivera, C. D. Narciso, R. A. Oliveira, M. Amstalden, G. M. Baez-Sandoval, L. J. Oliveira, W. W. Thatcher, and J. E. P. Santos. 2011. Concentration of progesterone during the development of the ovulatory follicle: II. Ovarian and uterine responses. J. Dairy Sci. 94:3352–3365. doi:10.3168/jds.2010-3735

Cerri, R. L. A., I. M. Thompson, I. H. Kim, A. D. Ealy, P. J. Hansen, C. R. Staples, J. L. Li, J. E. P. Santos, and W. W. Thatcher. 2012. Effects of lactation and pregnancy on gene expression of endometrium of Holstein cows at day 17 of the estrous cycle or pregnancy. J. Dairy Sci. 95:5657–5675. doi:10.3168/jds.2011-5114

Clancy, S., and W. Brown. 2008. Translation: DNA to mRNA to protein. Nat. Educ. 1:101.

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., J. D. Arthington, D. B. Araujo, and G. C. Lamb. 2009. Effects of acclimation to human interaction on performance, temperament, physiological responses, and pregnancy rates of Brahman-crossbred cows. J. Anim. Sci. 87:4125–4132. doi:10.2527/jas.2009-2021

Cooke, R. F., D. W. Bohnert, P. Moriel, B. W. Hess, and R. R. Mills. 2011. Effects of polyunsaturated fatty acid supplementation on forage digestibility, performance, and physiological responses of feeder cattle. J. Anim. Sci. 89:3677–3689. doi:10.2527/jas.2010-3515

Cooke, R. F., B. I. Cappellozza, T. A. Guarnieri Filho, D. W. Bohnert, C. M. Depner, K. A. Lytle, D. B. Jump, R. L. A. Cerri, and J. L. M. Vasconcelos. 2014. Effects of calcium salts of soybean oil on factors that influence pregnancy establishment in *Bos indicus* beef cows. J. Anim. Sci. 92:2239–2250. doi:10.2527/jas.2013-7422

Davoodi, S., R. F. Cooke, A. C. C. Fernandes, B. I. Cappellozza, J. L. M. Vasconcelos, and R. L. A. Cerri. 2016. Expression of estrus modifies the gene expression profile in reproductive tissues on day 19 of gestation in beef cows. Theriogenology 85:645–655. doi:10.1016/j.theriogenology.2015.10.002

Dorniak, P., F. W. Bazer, and T. E. Spencer. 2011. Prostaglandins regulate conceptus elongation and mediate effects of interferon-tau on the ovine uterine endometrium. Biol. Reprod. 84:1119–1127. doi:10.1095/biolreprod.110.089979

Erdem, H., and A. Guzeloglu. 2010. Effect of meloxicam treatment during early pregnancy in Holstein heifers. Reprod. Domest. Anim. 45:625–628.

FASS. 2010. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. (3rd ed.). Federation of Animal Science Societies, Savoy, IL.

Figueiredo, R. A., C. M. Barros, O. L. Pinheiro, and J. M. P. Soler. 1997. Ovarian follicular dynamics in Nelore breed (*Bos indicus*) cattle. Theriogenology 47:1489–1505. doi:10.1016/S0093-691X(97)00156-8

Fleige, S., and M. W. Pfaffl. 2006. RNA integrity and the effect on the real-time qRT-PCR performance. Mol. Aspects Med. 27:126–139. doi:10.1016/j.mam.2005.12.003

Fricke, P. M., P. D. Carvalho, M. C. Lucy, F. Curran, M. M. Herlihy, S. M. Waters, J. A. Larkin, M. A. Crowe, and S. T. Butler. 2016. Effect of manipulating progesterone before timed artificial insemination on reproductive and endocrine parameters in seasonal-calving, pasture-based Holstein-Friesian cows. J. Dairy Sci. 99:6780–6792. doi:10.3168/jds.2016-11229

Gifford, C. A., A. M. Assiri, M. C. Satterfield, T. E. Spencer, and T. L. Ott. 2008. Receptor transporter protein 4 (RTP4) in endometrium, ovary, and peripheral blood leukocytes of pregnant and cyclic ewes. Biol. Reprod. 79:518–524. doi:10.1095/biolreprod.108.069468

Gifford, C. A., K. Racicot, D. S. Clark, K. J. Austin, T. R. Hansen, M. C. Lucy, C. J. Davies, and T. L. Ott. 2007. Regulation of interferon-stimulated genes in peripheral blood leukocytes in pregnant and bred, nonpregnant dairy cows. J. Dairy Sci. 90:274–280. doi:10.3168/jds.S0022-0302(07)72628-0

Green, J. C., C. S. Okamura, S. E. Poock, and M. C. Lucy. 2010. Measurement of interferon-tau (IFN-τ) stimulated gene expression in blood leukocytes for pregnancy diagnosis within 18–20 d after insemination in dairy cattle. Anim. Reprod. Sci. 121:24–33. doi:10.1016/j.anireprosci.2010.05.010

Hawkins, D. E., K. D. Niswender, G. M. Oss, C. L. Moeller, K. G. Odde, H. R. Sawyer, and G. D. Niswender. 1995. An increase in serum lipids increases luteal lipid content and alters the disappearance rate of progesterone in cows. J. Anim. Sci. 73:541–545. doi:10.2527/1995.732541x

Hess, B. W., G. E. Moss, and D. C. Rule. 2008. A decade of developments in the area of fat supplementation research with beef cattle and sheep. J. Anim. Sci. 86(E. Suppl.):E188–E204.

Humblot, P. 2001. Use of pregnancy specific proteins and progesterone assays to monitor pregnancy and determine the timing, frequencies and sources of embryonic mortality in ruminants. Theriogenology 56:1417–1433. doi:10.1016/S0093-691X(01)00644-6

Lake, S. L., T. R. Weston, E. J. Scholljegerdes, C. M. Murrieta, B. M. Alexander, D. C. Rule, G. E. Moss, and B. W. Hess. 2007. Effects of postpartum dietary fat and body condition score at parturition on plasma, adipose tissue, and milk fatty acid composition of lactating beef cows. J. Anim. Sci. 85:717–730. doi:10.2527/jas.2006-353

Leroy, J. L., R. G. Sturmey, V. Van Hoeck, J. De Bie, P. J. McKeegan, and P. E. Bols. 2014. Dietary fat supplementation and the consequences for oocyte and embryo quality: Hype or significant benefit for dairy cow reproduction? Reprod. Domest. Anim. 49:353–361. doi:10.1111/rda.12308

Lopes, C. N., R. F. Cooke, M. M. Reis, R. F. G. Peres, and J. L. M. Vasconcelos. 2011. Strategic supplementation of rumen-protected polyunsaturated FA to enhance reproductive performance of *Bos indicus* beef cows. J. Anim. Sci. 89:3116–3124. doi:10.2527/jas.2011-3909

Lopes, C. N., A. B. Scarpa, B. I. Cappellozza, R. F. Cooke, and J. L. M. Vasconcelos. 2009. Effects of rumen-protected polyunsaturated fatty acid supplementation on reproductive performance of *Bos indicus* beef cows. J. Anim. Sci. 87:3935–3943. doi:10.2527/jas.2009-2201

Mann, G. E., M. D. Fray, and G. E. Lamming. 2006. Effects of time of progesterone supplementation on embryo development and interferon-tau production in the cow. Vet. J. 171:500–503. doi:10.1016/j.tvjl.2004.12.005

Mattos, R., A. Guzeloglu, L. Badinga, C. R. Staples, and W. W. Thatcher. 2003. Polyunsaturated fatty acids and bovine interferon-τ modify phorbol ester-induced secretion of prostaglandinF2α and expression of prostaglandin endoperoxide synthase-2 and phospholipase-A2 in bovine endometrial cells. Biol. Reprod. 69:780–787. doi:10.1095/biolreprod.102.015057

Meneghetti, M., O. G. Sa Filho, R. F. G. Peres, G. C. Lamb, and J. L. M. Vasconcelos. 2009. Fixed-time artificial insemination with estradiol and progesterone for *Bos indicus* cattle: I. Basis for development of protocols. Theriogenology 72:179–189. doi:10.1016/j.theriogenology.2009.02.010

Monteiro, P. L. J., Jr., E. S. Ribeiro, R. P. Maciel, A. L. G. Dias, E. Solé Jr., F. S. Lima, R. S. Bisinotto, W. W. Thatcher, R. Sartori, and J. E. P. Santos. 2014. Effects of supplemental progesterone after artificial insemination on expression of interferon-stimulated genes and fertility in dairy cows. J. Dairy Sci. 97:4907–4921. doi:10.3168/jds.2013-7802

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

Ocón-Grove, O. M., F. N. T. 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

Ribeiro, E. S., A. P. A. Monteiro, R. S. Bisinotto, F. S. Lima, L. F. Greco, A. D. Ealy, W. W. Thatcher, and J. E. P. Santos. 2016. Conceptus development and transcriptome at preimplantation stages in lactating dairy cows of distinct genetic groups and estrous cyclic statuses. J. Dairy Sci. 99:4761–4777. doi:10.3168/jds.2015-10315

Rizos, D., A. Gutierrez-Adan, S. Perez-Garnelo, J. De La Fuente, M. P. Boland, and P. Lonergan. 2003. Bovine embryo culture in the presence or absence of serum: Implications for blastocyst development, cryotolerance, and messenger RNA expression. Biol. Reprod. 68:236–243. doi:10.1095/biolreprod.102.007799

Robinson, N. A., K. E. Leslie, and J. S. Walton. 1989. Effect of treatment with progesterone on pregnancy rate and plasma concentrations of progesterone in Holstein cows. J. Dairy Sci. 72:202–207. doi:10.3168/jds.S0022-0302(89)79098-6

Sá Filho, M. F., A. M. Crespilho, J. E. Santos, G. A. Perry, and P. S. Baruselli. 2010. Ovarian follicle diameter at timed insemination and estrous response influence likelihood of ovulation and pregnancy after estrous synchronization with progesterone or progestin-based protocols in suckled *Bos indicus* cows. Anim. Reprod. Sci. 120:23–30. doi:10.1016/j.anireprosci.2010.03.007

Sangsritavong, S., D. G. Mashek, A. Gumen, J. M. Haughian, R. R. Grummer, and M. C. Wiltbank. 2002. Metabolic clearance rate of progesterone and estradiol-17 β is decreased by fat. J. Anim. Sci. 80(Suppl. 1):142. (Abstr.)

Schmitz, G., and J. Ecker. 2008. The opposing effects of *n*-3 and *n*-6 fatty acids. Prog. Lipid Res. 47:147–155. doi:10.1016/j.plipres.2007.12.004

Scholljegerdes, E. J., S. L. Lake, T. R. Weston, D. C. Rule, G. E. Moss, T. M. Nett, and B. W. Hess. 2007. Fatty acid composition of plasma, medial basal hypothalamus, and uterine tissue in primiparous beef cows fed high-linoleate safflower seeds. J. Anim. Sci. 85:1555–1564. doi:10.2527/jas.2005-732

Senger, P. L. 2003. Pathways to pregnancy and parturition. 2nd ed. Current Conceptions, Inc. Pullman, WA.

Silva, E., M. Gaivão, S. Leitão, A. Amaro, L. Lopes da Costa, and L. Mateus. 2008. Blood COX-2 and PGES gene transcription during the peripartum period of dairy cows with normal puerperium or with uterine infection. Domest. Anim. Endocrinol. 35:314–323. doi:10.1016/j.domaniend.2008.07.001

Spencer, T. E., and F. W. Bazer. 2004. Conceptus signals for establishment and maintenance of pregnancy. Reprod. Biol. Endocrinol. 2:49–63. doi:10.1186/1477-7827-2-49

Staples, C. R., J. M. Burke, and W. W. Thatcher. 1998. Influence of supplemental fats on reproductive tissues and performance of lactating cows. J. Dairy Sci. 81:856–871. doi:10.3168/jds.S0022-0302(98)75644-9

Stevenson, J. L., J. C. Dalton, T. L. Ott, K. E. Racicot, and R. C. Chebel. 2007. Correlation between reproductive status and steady-state messenger ribonucleic acid levels of the Myxovirus resistance gene, MX2, in peripheral blood leukocytes of dairy heifers. J. Anim. Sci. 85:2163–2172. doi:10.2527/jas.2007-0014

Stronge, A., J. Sreenan, M. Diskin, J. Mee, D. Kenny, and D. Morris. 2005. Post-insemination milk progesterone concentration and embryo survival in dairy cows. Theriogenology 64:1212–1224. doi:10.1016/j.theriogenology.2005.02.007

Takagi, M., D. Yamamoto, S. Ogawa, T. Otoi, M. Ohtani, and A. Miyamoto. 2008. Messenger RNA expression of angiotensin-converting enzyme, endothelin, cyclooxigenase-2 and prostaglandin synthases in bovine placentomas during gestation and the postpartum period. Vet. J. 177:398–404. doi:10.1016/j.tvjl.2007.05.017

Thangavelu, G., M. G. Colazo, D. J. Ambrose, M. Oba, E. K. Okine, and M. K. Dyck. 2007. Diets enriched in unsaturated fatty acids enhance early embryonic development in lactating Holstein cows. Theriogenology 68:949–957. doi:10.1016/j.theriogenology.2007.07.002

Thomas, J. M., S. E. Poock, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2014. Delayed insemination of non-estrous heifers and cows when using conventional semen in timed artificial insemination. J. Anim. Sci. 92:4189–4197. doi:10.2527/jas.2014-7827

Tripathy, S., M. Torres-Gonzalez, and D. B. Jump. 2010. Elevated hepatic fatty acid elongase-5 activity corrects dietary fat-induced hyperglycemia in obese C57BL/6J mice. J. Lipid Res. 51:2642–2654. doi:10.1194/jlr.M006080

Vandesompele, J., K. De Preter, F. Pattyn, B. Poppe, N. Van Roy, A. De Paepe, and F. Speleman. 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 3:research0034.1–research0034.11. doi:10.1186/gb-2002-3-7-research0034

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 nutrition animal. J. Dairy Sci. 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2

Vasconcelos, J. L., R. Sartori, H. N. Oliveira, J. G. Guenther, and M. C. Wiltbank. 2001. Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. Theriogenology 56:307–314. doi:10.1016/S0093-691X(01)00565-9

Walker, C. G., S. Meier, M. D. Mitchell, J. R. Roche, and M. Littlejohn. 2009. Evaluation of real-time PCR endogenous control genes for analysis of gene expression in bovine endometrium. BMC Mol. Biol. 10:100–112. doi:10.1186/1471-2199-10-100

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

Yaqoob, P., and P. C. Calder. 2007. Fatty acids and immune function: New insights into mechanisms. Br. J. Nutr. 98:S41–S45. doi:10.1017/S0007114507832995