Supplementing Ca salts of soybean oil after artificial insemination increases pregnancy success in *Bos taurus* beef cows[1]

Alice P. Brandão,[*,†] Reinaldo F. Cooke,[2,*] Kelsey M. Schubach,[*,†] Rodrigo S. Marques,[†] David W. Bohnert,[†] Rafael S. Carvalho,[‡] Nicholas W. Dias,[§] Claire L. Timlin,[§] Sherrie Clark-Deener,[#] John F. Currin,[#] Donald B. Jump,[‖] Ky G. Pohler,[*] Ronaldo L. A. Cerri,[¶] and Vitor R. G. Mercadante[§]

*Department of Animal Science, Texas A&M University, College Station, TX 77845; †Eastern Oregon Agricultural Research Center, Oregon State University, Burns, OR 97720; ‡São Paulo State University (UNESP), School of Veterinary Medicine and Animal Science, Botucatu, São Paulo 18168-000, Brazil; §Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061; #Department of Large Animal Clinical Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061; ‖Nutrition Program, School of Biological and Population Health Sciences, Linus Pauling Institute, Oregon State University, Corvallis, OR 97331; and ¶Faculty of Land and Food Systems, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada

ABSTRACT: Two experiments investigated the effects of supplementing Ca salts of soybean oil (CSSO) during early gestation on reproductive function and pregnancy rates to AI in *Bos taurus* beef cows. In Exp. 1, 771 suckled, lactating, multiparous Angus cows were divided into 22 groups of approximately 35 cows per group and timed inseminated on day 0. After AI, groups were assigned randomly to receive (as-fed basis) 100 g of ground corn + 100 g of soybean meal per cow/d, in addition to 1) 100 g/cow daily of CSSO (n = 11) or 2) 87 g of prilled saturated fat + 13 g of limestone per cow/d (CON; n = 11). Groups were maintained in individual tall fescue-dominated pastures and offered treatments from day 0 to 21. Pregnancy status was determined between days 45 and 55 via transrectal ultrasonography. Cows receiving CSSO had greater (P = 0.01) pregnancy rates to timed AI compared with CON (60.2 vs. 51.7%; SEM = 4.2). In Exp. 2, 90 suckled, lactating, multiparous Angus × Hereford cows housed in 18 drylot pens (5 cows per pen) were assigned to the same timed AI program and treatments from Exp. 1 (9 pens per treatment) and received 20 kg/d (DM basis) of grass–alfalfa hay. Transrectal ultrasonography was performed to verify ovulation and corpus luteum (CL) volume before AI (day 0), on days 7 and 15. After ultrasonography on day 15, cows diagnosed without a CL on day 0, but with a CL greater than 0.38 cm^3 in volume on days 7 and 15 (2 or 3 cows per pen; CSSO, n = 20; CON, n = 24), were assigned to conceptus collection via transcervical flushing and endometrial biopsy in the uterine horn ipsilateral to the CL. Blood samples were collected for FA analysis on days 0, 7, and 15. Blood was collected from cows not assigned to conceptus collection for whole-blood RNA extraction on day 20 and for pregnancy diagnosis on day 30 by measuring concentrations of pregnancy-associated glycoproteins. Cows receiving CSSO had greater ($P \leq 0.04$) mean plasma concentrations of linoleic acid and ω-6 FA compared with CON on days 7 and 15. Moreover, CSSO supplementation increased (P = 0.05) mRNA expression of *interferon-tau* by the conceptus and blood mRNA expression of *interferon-stimulated gene 15* and *20,50-oligoadenylate synthetase* on day 20 in gestating cows. Hence, post-AI CSSO supplementation to *B. taurus* beef cows improved pregnancy rates to timed AI, which can be associated with increased mRNA expression of *interferon-tau* by the conceptus when CSSO is supplemented during early gestation.

Key words: beef cows, *Bos taurus*, Ca salts of soybean oil, gene expression, interferon-tau, pregnancy.

[1]Financial support for this research was provided by Church & Dwight Co., Inc. (Princeton, NJ). A.P.B. is supported by CAPES, Brazil (#88881.128327/2016-01).

[2]Corresponding author: reinaldocooke@tamu.edu

Received March 27, 2018.
Accepted April 19, 2018.

J. Anim. Sci. 2018.96:2838–2850
doi: 10.1093/jas/sky156

INTRODUCTION

Early embryonic mortality is a major reproductive challenge in cow–calf systems and is defined as losses that occur from fertilization to day 27 of gestation (Humbolt, 2001). Strategies to enhance early embryonic survival are thus warranted for optimal reproductive and overall efficiency of cow–calf operations. Our research group reported that supplementation with Ca salts of soybean oil (**CSSO**) for 21 d beginning after AI increased pregnancy rates by 30% in *Bos indicus* beef cows (Lopes et al., 2009, 2011). This outcome was credited to enhanced early pregnancy maintenance (Spencer and Bazer, 2004) and later associated with incorporation of linoleic acid and its ω-6 derivatives into maternal and embryonic tissues (Cooke et al., 2014). Complementing these findings, Cipriano et al. (2016) reported that CSSO supplementation to *B. indicus* beef cows increased conceptus growth and mRNA expression of *interferon-tau* (**IFNt**) and *prostaglandin E synthase* on day 15 of gestation, which are critical regulators of pregnancy establishment in cattle (Spencer and Bazer, 2004; Erdem and Guzeloglu, 2010; Dorniak et al., 2011).

Collectively, these outcomes provided evidence that supplementing CSSO to beef cows after timed AI enhances pregnancy establishment by increasing conceptus development and signaling via the IFNt cascade (Cipriano et al., 2016), whereas these effects are modulated by ω-6 FA (Cooke et al., 2014) and result in increased pregnancy rates to AI (Lopes et al., 2009, 2011). In contrast, the previously cited experiments were conducted with *B. indicus* cattle reared in tropical environments. Pregnancy establishment and overall reproductive physiology differ among *B. indicus* and *B. taurus* females (Carvalho et al., 2008; Mercadante et al., 2013), and FA composition differs among tropical and temperate feed ingredients. Hence, research is warranted to validate these outcomes in *B. taurus* cattle in typical U.S. operations. Based on this rationale, we hypothesized that CSSO supplementation after timed AI would increase ω-6 FA intake and absorption, favor embryonic responses required for pregnancy establishment including the IFNt-signaling cascade, and increase pregnancy rates to AI in *B. taurus* beef cows. To test this hypothesis, Exp. 1 compared pregnancy rates to timed AI, whereas Exp. 2 compared hormonal, uterine, and conceptus factors associated with pregnancy establishment in *B. taurus* beef cows supplemented or not with CSSO for 21 d after timed AI.

MATERIALS AND METHODS

All animals were managed in accordance with the practices outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010; Exp. 1), and experimental protocols were reviewed and approved by the Oregon State University Institutional Animal Care and Use Committee (Exp. 2; #4938).

Experiment 1

Animals, treatments, and sampling. This experiment (day −10 to 55) was conducted on cow–calf operations (n = 7) managed by the Virginia Department of Corrections, with a total of 771 suckled, lactating, multiparous, nonpregnant Angus cows [mean ± SE; age = 5.98 ± 0.11 yr, days postpartum = 65.2 ± 0.6 d, and BCS = 5.21 ± 0.03 according to Wagner et al., 1988]. Across locations, cows were ranked by BCS and days postpartum on day −10, and allocated to a total of 22 groups averaging 35 cows each (range = 22 to 50 cows per group) in a manner that average BCS and days postpartum were equivalent among groups. Groups were maintained in individual tall fescue-dominated pastures (*Festuca arundinacea*) with ad libitum access to forage, mineral supplement, and water throughout the experimental period. From day −10 to −1, groups were supplemented daily with (as-fed basis) 100 g of ground corn + 100 g of soybean meal per cow. Supplements were provided in feed bunks placed within each pasture (1.0 m/cow of linear bunk space) and readily consumed by cows within 15 min of feeding.

Groups were enrolled in an estrus-synchronization + fixed-time AI protocol (Larson et al., 2006) from day −10 to 0. More specifically, cows received 100 µg of gonadotropin-releasing hormone (Factrel; Zoetis, Florham Park, NJ) plus a controlled internal device release (**CIDR**) containing 1.38 g of progesterone (**P4**; Zoetis) on day −10, 25 mg of prostaglandin $F_{2\alpha}$ (Lutalyse; Zoetis) and CIDR removal on day −3, followed in 60 h by a second 100-µg injection of gonadotropin-releasing

hormone and AI (day 0). Multiple AI technicians (n = 11) and semen from different *B. taurus* sires (n = 13) were used across locations and groups, but balanced between treatments within each location. Estrus detection patches (Estrotect; Rockway Inc., Spring Valley, WI) were applied on day −3 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 patch (Thomas et al., 2014). Immediately after AI, groups within each location were assigned randomly to receive (as-fed basis) 100 g of ground corn + 100 g of soybean meal per cow daily, in addition to 1) 100 g/cow daily of CSSO (Essentiom; Church and Dwight Co., Inc., Princeton, NJ; n = 11) or 2) 87 g/cow daily of prilled saturated fat (EnergyBooster; Milk Specialties, Eden Prairie, MN) + 13 g/cow daily of limestone (**CON**, n = 11). Treatments were formulated to be isocaloric, isonitrogenous, and isolipidic, but differing in FA composition, and offered from day 0 to 21 in the same feed bunks used from day −10 to −1 (Tables 1 and 2). Limestone was added to CON to balance treatment Ca content (Table 2). Cows consumed treatments by 15 min after feeding, which prevented intake of treatments by calves. Cows were exposed to natural service ≥ 10 d after timed AI. Cow BCS (Wagner et al., 1988) and pregnancy rates to timed AI, assessed with transrectal ultrasonography by the presence of a viable fetus (5.0 MHz linear transducer, Ibex Pro, E.I. Medical Imaging, Loveland, CO), were determined between days 45 and 55 after AI. Pregnancy rates to bull breeding were beyond the scope of this project and thus not evaluated, whereas potential intake of supplements by bulls was accounted for by increasing treatment offer by 900 g (equaling 3 cows) per group.

Experiment 2

Animals and treatments. This experiment (day −10 to 30) was conducted at the Oregon State University—Eastern Oregon Agricultural Research Center (Union Station), with 90 suckled, lactating, multiparous, nonpregnant Angus × Hereford cows (mean ± SE; age = 6.81 ± 0.26 yr, days postpartum = 63.6 ± 1.2 d, BW = 572.5 ± 5.8 kg, and BCS = 5.11 ± 0.04 according to Wagner et al., 1988). Cows were ranked by BCS and days postpartum and allocated to 18 pens (5 cows per pen) in a manner that pens had equivalent BCS and days postpartum at the beginning of the experiment (day −10). To facilitate cattle management and sampling procedures, pens were divided randomly into 2 groups (group A = 10 pens, group B = 8 pens). Groups started the experiment over 2 consecutive days following the same experimental schedule (day −10 to 30). Throughout the experimental period, cows were maintained in drylot pens (450 m^2, with 2.0 m/cow of linear bunk space) with their respective calves, receiving 20 kg/cow daily (DM basis) of grass–alfalfa hay and ad libitum access to water and mineral mix.

From day −10 to −1, cows within pens were supplemented with (as-fed basis) 100 g of ground corn + 100 g of soybean meal per cow daily, which was offered separate from hay and readily consumed by cows within 15 min of feeding. On day −10, cows from all pens were enrolled in the same estrus-synchronization + fixed-time AI protocol (Larson et al., 2006) described in Exp. 1, including the use of estrus detection aids (Estrotect; Rockway, Inc.). All cows were inseminated on day 0 by the same technician, using semen from the same Angus bull and batch.

Table 1. Nutritional and fatty acid profile (DM basis) of feedstuffs[1]

Item	Corn	Soybean meal	Essentiom[2]	EnergyBooster[3]	Grass–alfalfa hay
Total digestible nutrients, %	89	81	190	218	58
Net energy for maintenance, Mcal/kg	2.17	2.32	4.86	5.79	1.33
Crude protein, %	8.92	50.9	0.72	0.42	17.4
Neutral detergent fiber, %	7.51	14.8	0.91	1.75	43.2
Fatty acids, %	3.74	2.71	82.5	96.1	2.09
Palmitic (16:0), %	0.49	0.44	26.5	31.1	0.44
Stearic (18:0), %	0.06	0.10	3.33	46.1	0.09
Oleic (18:1), %	1.03	0.37	22.6	6.84	0.11
Linoleic (18:2), %	2.07	1.47	25.4	0.80	0.42
Linolenic (18:3), %	0.07	0.24	2.57	0.01	0.59

[1]Corn, soybean meal, and grass–alfalfa hay samples collected from Exp. 2 only. Values obtained from a commercial laboratory wet chemistry analysis (Dairy One Forage Laboratory, Ithaca, NY). Total digestible nutrients were calculated according to the equations described by Weiss et al. (1992). Net energy for maintenance was calculated with equations described by the NRC (2000).

[2]Church and Dwight Co., Inc. (Princeton, NJ).

[3]Milk Specialties (Eden Prairie, MN).

Table 2. Composition and nutritional profile of treatments[1,2]

Item	CSSO	CON
Ingredients, g/day (as-fed)		
Ground corn	100	100
Soybean meal	100	100
Essentiom	100	0
EnergyBooster	0	87
Limestone	0	13
Nutrient profile, DM basis		
DM, %	92.5	93.1
Total digestible nutrients,[3] %	122	122
Net energy for maintenance,[4] Mcal/kg	3.16	3.23
Crude protein, %	19.9	19.6
Neutral detergent fiber, %	7.60	7.78
Ca, %	3.27	2.81
Fatty acids, %	30.9	31.7
Palmitic (16:0), %	9.58	9.87
Stearic (18:0), %	1.25	14.23
Oleic (18:1), %	8.35	2.56
Linoleic (18:2), %	10.0	1.39
Linolenic (18:3), %	0.99	0.10
Daily intake, DM basis		
DM, g	278	279
Total digestible nutrients,[3] g	338	341
Net energy for maintenance,[4] Mcal	8.77	9.03
Crude protein, g	55.2	54.9
Neutral detergent fiber, g	21.1	21.7
Ca, g	9.08	7.84
Fatty acids, g	85.84	82.69
Palmitic (16:0), g	26.6	27.6
Stearic (18:0), g	3.47	39.8
Oleic (18:1), g	23.2	7.14
Linoleic (18:2), g	27.9	3.88
Linolenic (18:3), g	2.77	0.28

[1]Based on ingredients collected in Exp. 2.

[2]CSSO = daily supplementation (per cow) with 100 g of a soybean meal + 100 g of ground corn + 100 g of Ca salts of soybean oil (Essentiom, Church and Dwight Co., Inc., Princeton, NJ); CON = daily supplementation (per cow) with 100 g of a soybean meal + 100 g of ground corn + 87 g of prilled saturated fat (Energy Booster 100, Milk Specialties, Eden Prairie, MN) + 13 g of limestone.

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

[4]Calculated with equations described by the NRC (2000).

Immediately, after AI, pens were assigned randomly to receive the same treatments described in Exp. 1 (n = 9 pens per treatment, with 5 pens per treatment in group A and 4 pens per treatment in group B). Treatments were offered from day 0 to 21 in the same feed bunks used from day −10 to −1, and cows consumed treatments by 15 min after feeding, which prevented intake of treatments by calves as in Exp. 1.

Sampling. Samples of hay and supplement ingredients were collected before the beginning of the experiment 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), NDF (Van Soest et al., 1991; modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp.), and FA concentrations using gas chromatography (Autosystem XL Gas Chromatograph, Perkin Elmer, Inc., Waltham, MA). Calculations for TDN used the equations proposed by Weiss et al. (1992), whereas NE_m was calculated with the equations proposed by the NRC (2000). Nutritional and FA concentrations of all feedstuffs utilized in Exp. 2 are described in Table 1, whereas composition and nutritional profile of dietary treatments are in Table 2. Corn and soybean meal collected from Exp. 2 were considered to have similar nutritional and FA profile as those used in Exp. 1, whereas the same source of CSSO and prilled saturated fat was used in both experiments.

Blood samples were collected immediately before AI (day 0) and on days 7 and 15 of the experiment from either the coccygeal vein or artery into blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing freeze-dried sodium heparin. Transrectal ultrasonography (7.5-MHz transducer; 500 V; Aloka, Wallingford, CT) was performed concurrently with blood sampling on days 0, 7, and 15 to verify diameter of the largest follicle (day 0) and estimate corpus luteum (**CL**) volume (days 7 and 15). Corpus luteum volume was estimated using the formula for volume of a sphere; volume = $4/3\pi \times (D/2)^3$, where D is the maximum luteal diameter (Cooke et al., 2009). When the CL had a cavity, the cavity volume also was calculated as a sphere and subtracted from the CL volume.

After ultrasonography on day 15, cows diagnosed without the presence of a CL on day 0, but with a CL greater than 0.38 cm^3 in volume on days 7 and 15 (2 or 3 cows per pen; CSSO, n = 20; CON, n = 24), were assigned to conceptus collection and endometrial biopsy in the uterine horn ipsilateral to the CL, following the procedures described by Cipriano et al. (2016). Selection was performed randomly when pens had ≥4 cows that met the aforementioned criteria. Conceptus and endometrial samples were stored into 10-mL sterile tubes 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. After conceptus collection and endometrial biopsy on day 15, all cows returned to their respective pens. On day 20, blood samples were collected from the nonflushed cows (2 or 3 cows per pen; CSSO, n = 25; CON, n = 21) into PAXgene tubes (BD Diagnostics, Sparks, MD) for whole-blood RNA extraction. On day 21, treatment

administration and supplementation were terminated, whereas blood samples were collected from the nonflushed cows on day 30 for pregnancy evaluation.

Laboratorial analysis. Blood samples were placed immediately on ice after collection, centrifuged (2,500 × *g* for 30 min; 4 °C) for plasma harvest, and stored at −20 °C on the same day of collection. Samples collected on days 0, 7, and 15 were analyzed for FA concentrations using gas chromatography (Aligent 7890, Agilent Technologies, Inc.) using the procedures described by Tripathy et al. (2010). Samples collected on days 7 and 15 from cows that did not have a CL on day 0, but with a CL greater than 0.38 cm^3 in volume concurrently with blood collection (Cipriano et al., 2016), were analyzed for 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 2.1% and minimum detectable concentration of 0.1 ng/mL. Plasma samples collected on day 30 were analyzed for pregnancy-associated glycoproteins for evaluation of pregnancy status as reported by Pohler et al. (2016).

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). Reverse transcription of extracted RNA and real-time reverse transcription–PCR using gene-specific primers (20 pM each; Table 3) were completed as described by Cipriano et al. (2016). Responses from genes of interest 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 C_T responses from conceptus and endometrial genes of interest were normalized to the geometrical mean of C_T values of (Vandesompele et al., 2002), respectively, *glyceraldehyde-3-phosphate dehydrogenase* and *ribosomal protein L19*, and *suppressor of zeste 12 homolog* and *zinc finger protein 131*. The CV for the geometrical mean of *glyceraldehyde-3-phosphate dehydrogenase* and *ribosomal protein L19* C_T values across all conceptus samples was 4.9%. The CV for the geometrical mean of suppressor of *zeste 12 homolog* and *zinc finger protein 131* C_T values across all endometrial samples was 4.7%. Results are expressed as relative fold change ($2^{-\Delta\Delta CT}$), as described by Ocón-Grove et al. (2008).

Total RNA was extracted from whole-blood samples using the PAXgene Blood RNA Kit (Qiagen, Valencia, CA). Assessment of quantity and quality of isolated RNA, reverse transcription, and real-time reverse transcription–PCR with gene-specific primers (20 pM each; Table 3) was performed as in Cipriano et al. (2016). Responses from genes of interest were quantified based on C_T and normalized to the geometrical mean of C_T values from *β2-microglobulin* and *β-actin* (Vandesompele et al., 2002). The CV for the geometrical mean of *β2-microglobulin* and *β-actin* C_T values across all blood samples was 1.8%. Results are expressed as relative fold change ($2^{-\Delta\Delta CT}$) as in Ocón-Grove et al. (2008).

Statistical Analyses

Quantitative and binary data were analyzed, respectively, with the MIXED and GLIMMIX procedures of SAS (SAS Inst., Inc., Cary, NC) and Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. Data from Exp. 1 were analyzed using group as experimental unit, whereas model statements contained the effect of treatment and included group (treatment × location), cow (group), and location as random variables. Pregnancy rates to timed AI also included estrus expression as an independent covariate, as well as sire and AI technician as random variables. Data from Exp. 2 used pen as experimental unit, as well as pen (treatment × group), cow (pen), and group as random variables. The model statement used for diameter of the largest follicle, estrus expression, presence of conceptus, conceptus length, 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 day 30, and the resultant interaction. The model statement used for CL volume, plasma P4 and FA concentrations, and proportion of cows without a CL on day 0 but with CL greater than 0.38 cm^3 in volume on days 7 and 15 contained the effects of treatment, day, and the resultant interaction. Plasma FA concentrations were analyzed using values from day 0 as an independent covariate, whereas all reproductive variables included estrus expression as independent covariate. The specified term for the repeated-measures 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 least square or covariately

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

Target gene	Primer sequence	Accession number	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		

adjusted least square means when appropriate and 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 highest-order interaction detected.

RESULTS AND DISCUSSION

Experiment 1

Cow age, days postpartum, and BCS on day −10 of the experiment did not differ ($P \geq 0.59$) between CSSO-supplemented and CON cows (Table 4). Moreover, all cows utilized herein were in adequate nutritional status according to their BCS and within the recommended voluntary waiting period for *B. taurus* cattle to optimize pregnancy rates to timed AI and maintain a 365-d calving interval (Short et al., 1990; Hess et al., 2005). In addition, no treatment differences were detected in estrus expression from day −3 to 0 according to activation of estrus detection patches (Thomas et al., 2014; Table 4), although treatment administration began after the period of estrus expression evaluation. Nevertheless, estrus expression affects pregnancy rates to timed AI in beef cows (Perry et al., 2005; Whittier et al., 2013; Thomas

Table 4. Performance and reproductive variables in beef cows supplemented with Ca salts of soybean oil (CSSO; $n = 11$) or prilled saturated fat (CON; $n = 11$) in Exp. 1[1,2]

Item	CSSO	CON	SEM	*P* value
Cow variable				
Age, yr	5.8	5.9	0.7	0.94
Days postpartum, d	66	67	3	0.86
BCS[3]				
Day −10	5.2	5.2	0.1	0.78
Day 30	5.3	5.3	0.2	0.81
Change	0.1	0.1	0.1	0.99
Reproductive variables				
Estrus detection patch, %				
Activated	43.9 (169/383)	40.9 (157/388)	3.7	0.59
Nonactivated	42.9 (165/383)	45.1 (176/388)	3.3	0.63
Lost	13.2 (49/383)	14.0 (55/388)	2.0	0.71
Pregnancy rate,[4] %	60.2 (226/383)	51.7 (193/388)	4.2	0.01

[1]Cows were enrolled in an estrus-synchronization + fixed-time AI protocol (Larson et al., 2006) from day −10 to 0. Estrus detection aids (Estrotect; Rockway Inc., Spring Valley, WI) were applied on day −7 to all cows, and occurrence of estrus was recorded at timed AI (day 0).

[2]CSSO = daily supplementation (per cow) with 100 g of a soybean meal + 100 g of ground corn + 100 g of Ca salts of soybean oil (Essentiom, Church and Dwight Co., Inc., Princeton, NJ); CON = daily supplementation (per cow) with 100 g of a soybean meal + 100 g of ground corn + 87 g of prilled saturated fat (Energy Booster 100, Milk Specialties, Eden Prairie, MN) + 13 g of limestone. Treatments were offered from day 0 (timed AI) to 21 of the experiment.

[3]According to Wagner et al. (1988).

[4]Values are least square means covariately adjusted to estrus detection patch status. Values within parenthesis represent number of pregnant cows divided by number total cows within each treatment. Pregnancy was determined by the presence of a viable fetus via transrectal ultrasonography (5.0 MHz linear transducer, Ibex Pro, E.I. Medical Imaging, Loveland, CO) between days 45 and 55 of gestation.

et al., 2014), and for this reason, it was included as independent covariate in the pregnancy analysis. Therefore, treatment effects reported herein for reproductive performance were not related to inherent differences in estrus expression between CSSO-supplemented and CON cows.

Cows supplemented with CSSO had greater ($P < 0.01$) pregnancy rates to timed AI compared with CON cows (Table 4), supporting our hypothesis and corroborating our previous research in *B. indicus* cattle (Lopes et al., 2009, 2011). Moreover, the treatments utilized herein were isocaloric, isonitrogenous, and isolipidic, whereas BCS change and BCS at pregnancy diagnosis did not differ ($P \geq 0.81$; Table 4) between CON and CSSO-supplemented cows. Collectively, these results corroborate that CSSO supplementation enhances pregnancy success in beef cattle beyond its contribution to energy and fat intake (Lopes et al., 2009, 2011).

Experiment 2: Plasma FA Concentrations

As in Exp. 1, cow age, days postpartum, BW, and BCS on day −10 of the experiment (Table 5) did not differ between CSSO and CON cows, indicating that any treatment effects reported herein were independent of these variables. Plasma concentrations of individual and total identified FA did not differ ($P \geq 0.18$; data not shown) between CSSO-supplemented and CON cows on day 0, indicating plasma FA concentrations and profile before timed AI did not differ between treatments. During the experimental period, CSSO-supplemented cows had greater ($P < 0.01$) mean concentrations of plasma linoleic, PUFA, linoleic:linolenic ratio, and ω-6 FA compared with CON cows (Table 5). In turn, CON cows had greater ($P \leq 0.02$) mean concentrations of plasma palmitoleic, oleic, linolenic, docosadienoic, and ω-3 FA and tended ($P = 0.09$) to have greater mean concentration of plasma mystiric acid compared with CSSO-supplemented cows (Table 5). No treatment difference was detected ($P = 0.24$) for total FA, given the isolipidic content of treatments. As in Cipriano et al. (2016), these results corroborate the FA content and profile of the CSSO treatment (Table 2; predominantly linoleic acid), given that plasma FA concentrations directly reflect intake and duodenal flow of FA (Lake et al., 2007; Scholljegerdes et al., 2007; Hess et al., 2008). Previous research similarly reported that CSSO supplementation increased plasma concentrations of linoleic acid, ω-6 FA, and total PUFA in beef cattle while reducing plasma concentrations of linolenic acid and ω-3 FA (Cooke et al., 2011, 2014). Hence, supplementing 100 g of CSSO to *B. taurus* beef cows herein effectively increased intake and circulating concentrations of linoleic and ω-6 FA, as previously observed in *B. indicus*

Table 5. Cow variables at the beginning of the experiment (day −11) and plasma fatty acid concentrations (μg/mL of plasma) in beef cows supplemented with Ca salts of soybean oil (CSSO; $n = 9$) or prilled saturated fat (CON; $n = 9$) in Exp. 2[1]

Item[3]	CSSO	CON	SEM	*P* value
Cow variables				
Age, yr	6.74	6.76	0.39	0.96
Days postpartum, d	64.9	61.9	2.4	0.25
BW, kg	582	577	9	0.71
BCS[2]	5.12	5.13	0.08	0.91
Plasma fatty acids[3,4]				
Mystiric (14:0)	3.17	3.50	0.12	0.09
Myristoleic (14:1)	4.36	3.87	0.65	0.61
Palmitic (16:0)	92.6	92.4	3.8	0.97
Palmitoleic (16:1)	5.60	6.62	0.22	<0.01
Stearic (18:0)	141	146	6	0.52
Oleic (18:1)	55.3	63.3	2.3	0.02
Linoleic (18:2, ω-6)	223	149	4.7	<0.01
Linolenic (18:3, ω-3)	94.5	114	3.3	<0.01
Dihomo-gamma-linolenic acid (20:3, ω-6)	10.1	9.49	0.37	0.29
Arachdonic (20:4, ω-6)	13.8	14.1	0.4	0.59
Docosadienoic (22:2, ω-6)	14.5	17.1	0.7	0.03
Docosapentaenoic (22:5, ω-3)	9.50	9.73	0.50	0.75
Total saturated fatty acids	249	255	10	0.69
Total monounsaturated fatty acids	64.2	69.8	3.9	0.33
Total polyunsaturated fatty acids	371	311	11	<0.01
ω-3	105	123	3	<0.01
ω-6	263	192	8	<0.01
Ratio linoleic:linolenic acid	2.53	1.56	0.03	<0.01
Total identified fatty acids	679	642	21	0.24

[1]CSSO = daily supplementation (per cow) with 100 g of a soybean meal + 100 g of ground corn + 100 g of Ca salts of soybean oil (Essentiom, Church and Dwight Co., Inc., Princeton, NJ); CON = daily supplementation (per cow) with 100 g of a soybean meal + 100 g of ground corn + 87 g of prilled saturated fat (Energy Booster 100, Milk Specialties, Eden Prairie, MN) + 13 g of limestone. Treatments were offered from day 0 (timed AI) to 21 of the experiment.

[2]According to Wagner et al. (1988).

[3]Blood samples were collected from all cows ($n = 90$, being 45 per treatment) on days 0 (before the first treatment application), 7, and 15. Values obtained on day 0 served as covariate; therefore, values reported are covariately adjusted means from days 7 and 15.

[4]Saturated fatty acids = mystiric, palmitic, and stearic acids; monounsaturated fatty acids = myristoleic, palmitoleic, and oleic acids; polyunsaturated fatty acids = linoleic, linolenic, dihomo-gamma-linolenic, arachidonic, docosadienoic, and docosapentaenoic acids.

beef cows reared in tropical environments with diets based on warm-season feed ingredients (Cooke et al., 2014; Cipriano et al., 2016).

Experiment 2: Ovarian Variables and Plasma P4 Concentration

None of the cows evaluated herein had a CL on day 0 of the experiment. The proportion of CSSO-supplemented and CON cows that had a CL greater than 0.38 cm^3 in volume on days 7 and 15 did not differ ($P \geq 0.65$; Table 6), indicating that treatment effects on plasma P4 concentration and CL volume were evaluated using a balanced dataset. The same ($P = 0.99$) proportion of CSSO-supplemented and CON cows expressed estrus from day −3 to 0 (Table 6), whereas estrus expression did not differ (data not shown) between treatments within cows assigned ($P = 0.62$) to conceptus flushing or within cows assigned to flushing that had a conceptus collected on day 15 ($P = 0.88$). Despite not differing between treatments and evaluated before treatment administration, estrus expression was included into all reproductive analyses as independent covariate because of its impacts on ovarian dynamics (Sá Filho et al., 2010), conceptus development, and expression of genes associated with pregnancy establishment in endometrial and conceptus tissues (Davoodi et al., 2016).

Diameter of the largest follicle on day 0 did not differ ($P = 0.51$) between CSSO-supplemented and CON cows (Table 5) and thus did not influence the impact of treatments on CL development and circulating P4 (Vasconcelos et al., 2001). In addition, no treatment differences were detected ($P \geq 0.73$) for plasma P4 concentration and CL volume during

Table 6. Ovarian and pregnancy variables in beef cows supplemented with Ca salts of soybean oil (CSSO) or prilled SFA source (CON) in Exp. 2[1,2]

Item	CSSO	CON	SEM	*P* value
Ovarian variables				
Largest follicle diameter (day 0), mm	16.6	15.7	0.44	0.14
Corpus luteum on days 7 and 15, %	86.1 (39/45)	92.7 (42/45)	7.4	0.29
Corpus luteum volume,[3] cm^3	7.11	7.00	0.35	0.83
Plasma progesterone,[3] ng/mL	4.20	4.35	0.32	0.73
Reproductive variables				
Estrus expression, %	38.0 (18/45)	38.0 (18/45)	21.2	0.97
Proportion of cows with conceptus,[4] %				
Day 15	57.5 (11/20)	39.0 (10/24)	11.2	0.26
Day 30	52.6 (12/25)	47.4 (11/21)	11.6	0.73
Conceptus length,[5] cm	11.3	11.4	3.1	0.97

[1]CSSO = daily supplementation (per cow) with 100 g of a soybean meal + 100 g of ground corn + 100 g of Ca salts of soybean oil (Essentiom, Church and Dwight Co., Inc., Princeton, NJ); CON = daily supplementation (per cow) with 100 g of a soybean meal + 100 g of ground corn + 87 g of prilled saturated fat (Energy Booster 100, Milk Specialties, Eden Prairie, MN) + 13 g of limestone. Treatments were offered from day 0 (timed AI) to 21 of the experiment. All results are covariately adjusted to estrus expression (Estrotect; Rockway Inc., Spring Valley, WI; Thomas et al., 2014) from day −3 to 0 of the experiment. Values within parenthesis represent number of cows with a positive response divided by number total cows within each treatment.

[2]Transrectal ultrasonography (7.5-MHz transducer; 500 V; Aloka, Wallingford, CT) was performed on days 0, 7, and 15 of the experiment. Blood samples were collected for progesterone analysis on days 0, 7, and 15.

[3]Evaluated in cows without a corpus luteum on day 0, but with a corpus luteum greater than 0.38 cm^3 in volume on days 7 (n = 39 for CSSO and 42 for CON) and 15. Corpus luteum volume was calculated using the formula for volume of a sphere; $V = 4/3\pi \times (D/2)^3$, where D is the maximum luteal diameter (Cooke et al., 2009).

[4]On day 15, 44 cows (CSS0, n = 20; CON, n = 24) were assigned to transcervical flushing for conceptus collection (Cipriano et al., 2016). On day 30, pregnancy status of the nonflushed cows was evaluated by measuring pregnancy-associated glycoproteins in plasma (Pohler et al., 2016).

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

the experiment (Table 6), differing from Cooke et al. (2014) and Cipriano et al. (2016). Previous authors reported greater CL volume and plasma P4 concentrations by 15 d after timed AI in *B. indicus* cows supplemented with CSSO and related such outcomes to improved pregnancy success reported by Lopes et al. (2009, 2011). One could attribute these discrepant results to physiological differences among *B. taurus* and *B. indicus* breeds, including hastened CL development observed in *B. taurus* cattle (Carvalho et al., 2008). Alternatively, treatments evaluated herein were isolipidic and dietary lipid content modulates CL development and steroidogenesis (Hawkins et al., 1995). Lopes et al. (2009) also reported that *B. indicus* cows receiving 100 g/d of CSSO for 28 d after AI (day 0) had greater pregnancy rates on day 30 compared with cohorts receiving 100 g/d of Ca salts of saturated fat, whereas serum P4 concentrations on day 7 did not differ between these treatments. Collectively, these outcomes diverge from Cipriano et al. (2016) by providing evidence that CSSO supplementation improves reproductive function and performance (as in Exp. 1) in *B. taurus* and *B. indicus* cows without increasing circulating P4 concentrations during early gestation.

Experiment 2: Pregnancy Development and Establishment Factors

Experiment 2 was not designed to compare pregnancy rates to timed AI between treatments based on sample size and sampling schedule, although such outcomes are reported in Table 6 and did not differ ($P \leq 0.26$) between CSSO-supplemented and CON cows. No treatment differences were detected ($P = 0.97$) for conceptus length (Table 6), which contradicts Cipriano et al. (2016) where CSSO supplementation doubled the length of conceptus on day 15 of gestation. Indeed, ω-6 FA have been shown to hasten early embryonic development (Thangavelu et al., 2007) and play important roles in conceptus development by maintenance of cell metabolism, membrane fluidity, permeability, and conformation (Leroy et al., 2014; Ribeiro et al., 2016). Alternatively, average conceptus length across treatments was 11.4 ± 1.9 cm herein and 2.4 ± 0.5 cm in Cipriano et al. (2016). These suggest that on day 15 of gestation, *B. taurus* conceptus is at an advanced stage of elongation compared with *B. indicus* conceptus, and perhaps past the stage in which conceptus growth is enhanced by CSSO supplementation and ω-6 FA incorporation (Cooke

et al., 2014). Supporting this rationale, Cooke et al. (2014) reported that conceptuses length and weight did not differ between CSSO-supplemented and nonsupplemented *B. indicus* cows on day 19 of gestation.

A treatment effect was detected for mRNA expression of IFNt in the conceptus, which was greater (P = 0.05) in conceptuses from CSSO-supplemented vs. CON cows (Table 7). This result supports our hypothesis that CSSO supplementation enhances the IFNt-signaling cascade (Thatcher et al., 1995) and increases pregnancy success as in Exp. 1, corroborating with similar outcomes in *B. indicus* cattle consuming tropical feed ingredients (Lopes et al., 2009, 2011; Cipriano et al., 2016). These outcomes were independent of conceptus length and plasma P4 concentration, which may also increase IFNt synthesis by the conceptus (Bilby et al., 2004; Mann et al., 2006) but did not differ between treatments (Table 6) herein. In contrast, no treatment effects were detected (P = 0.30) for mRNA expression of *prostaglandin E synthase* (Table 7) in the conceptus, a rate-limiting enzyme in the synthesis of prostaglandin E_2 (Park et al., 2006). This prostaglandin is derived from ω-6 FA (Schmitz and Ecker, 2008) and produced by the conceptus and endometrium, and seems to be fundamental for conceptus development and pregnancy signaling to maternal tissues by modulating synthesis and endometrial activity of IFNt (Erdem and Guzeloglu, 2010; Dorniak et al., 2011). Cipriano et al. (2016) also reported that CSSO supplementation increased mRNA expression of *prostaglandin E synthase* in conceptuses collected on day 15 of gestation. As stated for conceptus length, perhaps conceptus collected herein (day 15; *B. taurus* conceptus) was beyond the elongation stage when CSSO supplementation modulates mRNA expression of *prostaglandin E synthase.* In endometrial samples, no treatment effects were detected for mRNA expression of *cyclooxygenase-2* and *prostaglandin E synthase* (Table 7), as in Cipriano et al. (2016) and Cooke et al. (2014). Hence, these results imply that CSSO supplementation to *B. taurus* beef cows increases expression of IFNt in the conceptus, without modulating expression of prostaglandin-related genes in conceptus and endometrial tissues on day 15 of gestation.

Table 7. Expression of genes associated with pregnancy establishment in the endometrium, conceptus, and blood from beef cows supplemented with Ca salts of soybean oil (CSSO) or prilled SFA source (CON) in Exp. 2[1,2]

Item	CSSO	CON	SEM	*P* value
Endometrium[2]				
Cyclooxygenase-2	4.88	5.11	1.32	0.89
Prostaglandin E synthase	5.76	7.40	1.10	0.30
Conceptus[2]				
Interferon-tau	21.3	12.1	3.4	0.05
Prostaglandin E synthase	2.22	2.50	0.48	0.69
Blood cells[3]				
Interferon-stimulated gene 15				
Pregnant	43.1	29.8	4.6	0.04
Nonpregnant	1.87	3.57	5.48	0.81
Myxovirus resistance 2				
Pregnant	20.2	20.1	2.7	0.98
Nonpregnant	2.30	4.80	3.01	0.48
20,50-oligoadenylate synthetase				
Pregnant	26.8	18.3	2.7	0.03
Nonpregnant	1.67	2.64	3.33	0.83

[1]CSSO = daily supplementation (per cow) with 100 g of a soybean meal + 100 g of ground corn + 100 g of Ca salts of soybean oil (Essentiom, Church and Dwight Co., Inc., Princeton, NJ); CON = daily supplementation (per cow) with 100 g of a soybean meal + 100 g of ground corn + 87 g of prilled saturated fat (Energy Booster 100, Milk Specialties, Eden Prairie, MN) + 13 g of limestone. Treatments were offered from day 0 (timed AI) to 21 of the experiment. All results are covariately adjusted to estrus expression (Estrotect; Rockway Inc., Spring Valley, WI; Thomas et al., 2014) from day −3 to 0 of the experiment.

[2]Conceptus were collected via transcervical flushing, and endometrial biopsy was performed on day 15 from 44 cows (CSSO, n = 20; CON, n = 24). Only samples from cows with a retrieved conceptus were analyzed. Values are expressed as relative fold change compared with threshold cycle of reference genes analyzed within the same sample (Ocón-Grove et al., 2008).

[3]Blood samples collected from nonflushed cows (CSSO, n = 25; CON, n = 21) into PAXgene tubes (BD Diagnostics, Sparks, MD) for whole-blood RNA extraction on day 20 of the experiment and analyzed according to cow pregnancy status on day 30. Values are expressed as relative fold change compared with threshold cycle of reference genes analyzed within the same sample (Ocón-Grove et al., 2008).

Treatment × pregnancy status interactions were detected ($P \leq 0.01$) for blood mRNA expression of the interferon-stimulated genes (**ISG**) *interferon-stimulated gene 15* and *20,50-oligoadenylate synthetase* on day 20 of the experiment (Table 7). Expression of these ISG were greater ($P \leq 0.04$) for CSSO-supplemented cows compared with CON cows diagnosed as pregnant, but did not differ ($P \geq 0.27$) between treatments within cows diagnosed as nonpregnant on day 30. No treatment effects, however, were detected ($P \geq 0.48$) for blood mRNA expression of the ISG *myxovirus resistance 2*. Interferon-tau synthesis by the conceptus upregulates mRNA expression of ISGs in circulating blood leukocytes (Stevenson et al., 2007; Gifford et al., 2008; Green et al., 2010). For this reason, mRNA expression of ISGs in whole blood has been used to evaluate IFNt production and conceptus development from day 15 to 22 of gestation, as well as pregnancy diagnosis on day 18 of gestation (Fricke et al., 2016). Indeed, cows diagnosed as pregnant had greater ($P < 0.01$) expression of ISGs compared with cows diagnosed as nonpregnant within and across treatments (Table 7). Cipriano et al. (2016) also reported that CSSO supplementation increased mRNA expression of ISGs on day 20 of gestation in *B. indicus* beef cows, including *myxovirus resistance 2*. In this experiment, greater mRNA expression of *interferon-stimulated gene 15* and *20,50-oligoadenylate synthetase* in CSSO-supplemented cows on day 20 agrees with treatment effects detected for IFNt mRNA expression in the conceptus on day 15, despite lack of similar outcomes for *myxovirus resistance 2*. These outcomes provide further support that CSSO supplementation enhances IFNt synthesis by the conceptus during the pregnancy recognition period (Spencer and Bazer, 2004; Fricke et al., 2016) in *B. taurus* beef cows.

Overall Conclusions

In summary, supplementing *B. taurus* beef cows with 100 g of CSSO for 21 d after timed AI increased pregnancy rates compared with cohorts receiving an isocaloric, isonitrogenous, and isolipidic supplements based on prilled saturated fat (Exp. 1). Moreover, CSSO supplementation increased plasma concentrations of linoleic acid and ω-6 FA, and upregulated mRNA expression of IFNt by the conceptus on day 15 of gestation (Exp. 2), which likely facilitated the increase in pregnancy rates observed in CSSO-supplemented cows from Exp. 1. Collectively, these outcomes corroborate the reproductive benefits of CSSO supplementation to *B. indicus* beef cows previously reported by our research group (Lopes et al., 2009, 2011; Cooke et al., 2014; Cipriano et al., 2016). This experiment also provided novel insights into potential differences in conceptus development between subspecies, which may have contributed to the lack of CSSO supplementation effects on conceptus length and mRNA expression of *prostaglandin E synthase* herein. Collectively, these research efforts validate that supplementing CSSO for 21 d beginning at timed AI is an alternative to enhance pregnancy establishment and overall reproductive performance of *B. taurus* and *B. indicus* beef cows managed, respectively, in temperate and tropical environments.

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