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# Effects of calcium salts of soybean oil on factors that influence pregnancy establishment in *Bos indicus* beef cows[1]

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**ABSTRACT:** The objective of this experiment was to compare fatty acid (FA) concentrations in plasma and reproductive tissues as well as hormones and expression of genes associated with pregnancy establishment in beef cows supplemented or not with Ca salts of soybean oil (CSSO) beginning after timed AI. Ninety nonlactating multiparous Nelore (*Bos indicus*) cows were timed inseminated on d 0 of the experiment and divided into 18 groups of 5 cows/group. Groups were randomly assigned to receive (as-fed basis) 100 g of a protein–mineral mix plus 100 g of ground corn per cow daily in addition to 1) 100 g/cow daily of CSSO ($n = 9$) or 2) 100 g/cow daily of kaolin (CON; rumen-inert indigestible substance; $n = 9$). All groups were maintained in a single *Brachiaria brizanta* pasture (24 ha) with ad libitum access to forage and water. However, groups were segregated daily and offered treatments individually at the working facility during the experimental period (d 0 to 18). Blood samples were collected and transrectal ultrasonography was performed to verify ovulation and estimate corpus luteum (CL) volume immediately before AI (d 0) and on d 7 and 18 of the experiment. On d 19, 36 cows (18 cows/treatment; 2 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 18 were slaughtered for collection of conceptus, uterine luminal flushing, and tissue samples from the CL and endometrium. Cows receiving CSSO had greater concentrations of linoleic and other ω-6 FA in plasma ($P < 0.01$), endometrium ($P \leq 0.05$), CL ($P \leq 0.05$), and conceptus ($P \leq 0.08$) compared to CON. On d 7 of the experiment, CSSO-supplemented cows had greater plasma progesterone concentrations ($P < 0.01$) and CL volume ($P = 0.02$) compared to CON, whereas no treatment effects were detected ($P \geq 0.15$) for these parameters on d 18 (treatment × day interaction; $P < 0.01$). Cows receiving CSSO tended ($P = 0.09$) to have greater concentrations of interferon-tau in the uterine flushing media compared with CON. However, no treatment effects were detected for mRNA expression genes associated with pregnancy establishment in endometrial, CL, and conceptus samples ($P \geq 0.12$). In summary, supplementing beef cows with 100 g of CSSO beginning after AI favored incorporation of ω-6 FA into their circulation, reproductive tissues, and conceptus, without impacting expression of genes associated with pregnancy establishment on d 19 of gestation.

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



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## 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). Hence, strategies to enhance early embryonic survival are warranted for optimal reproductive and overall efficiency of cow–calf operations. Recently, our group reported that supplementation with Ca salts of soybean oil (**CSSO**) beginning after AI and through the

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period when luteolysis and maternal pregnancy recognition would occur (Spencer and Bazer, 2004) increased pregnancy rates in *Bos indicus* beef cows (Lopes et al., 2009). However, the biological mechanisms by which CSSO supplementation modulates reproductive function in beef cows during early gestation are still unknown but are likely associated with embryonic development and pregnancy establishment (Lopes et al., 2011).

The CSSO supplemented by Lopes et al. (2009, 2011) contained mostly PUFA. Supplemental PUFA has been shown to alter fatty acid (**FA**) profile in reproductive tissues of beef cows, including the corpus luteum (**CL**) and endometrium (Burns et al., 2003; White et al., 2012). Dietary PUFA are also known to modulate hormones associated with pregnancy establishment, such as PG and progesterone (**P4**; Hawkins et al., 1995; Grant et al., 2005). Based on these findings, we hypothesized that CSSO supplementation during early gestation increases incorporation of PUFA into maternal reproductive tissues and the conceptus. In turn, this incorporation favors the physiological mechanisms associated with pregnancy establishment, including antiluteolytic activities and pregnancy recognition by maternal tissues via the interferon-tau (**IFNt**) cascade (Thatcher et al., 1995). To test this hypothesis, this experiment compared FA concentrations in plasma and reproductive tissues as well as hormones and expression of genes associated with pregnancy establishment in *B. indicus* beef cows supplemented or not with CSSO beginning after AI.

## MATERIALS AND METHODS

This experiment was conducted from January to March at a commercial cow–calf operation located in Mineiros, Brazil. The animals used 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*

Ninety nonlactating multiparous Nelore cows (BCS = 5.46 ± 0.05; Wagner et al., 1988) were assigned to an estrus synchronization plus fixed-time AI protocol (Meneghetti et al., 2009) from d –11 to 0. All cows were inseminated on d 0 by the same technician, using semen from the same bull and batch. Immediately after AI, cows were ranked by BCS and divided into 18 groups of 5 cows/group. Groups were then randomly assigned to receive (as-fed basis) 100 g of a protein–mineral mix plus 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; $n$ = 9) or 2) 100 g/cow daily of kaolin (**CON**; rumen-inert indigestible substance; $n$ = 9). All groups were maintained in a single *Brachiaria brizanta* pasture (24 ha) with ad libitum access to forage and water. However, groups were segregated daily and offered treatments individually at the working facility during the experimental period (d 0 to 18; from 0800 to 1000 h), with bunk space of approximately 1.0 m/cow. Groups readily consumed treatments within 15 min after feeding. Nutritional and FA concentrations of all feedstuffs used herein are described in Table 1. Treatment composition was similar to that used by Lopes et al. (2011) and is described in Table 2.

**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, % | 88 | 78 | 192 | 56 |
| NEm,[4] Mcal/kg | 2.20 | 1.89 | 6.95 | 1.05 |
| NEg,[4] Mcal/kg | 1.52 | 1.25 | 5.30 | 0.51 |
| CP,[4] % | 8.7 | 21.8 | 0.8 | 7.8 |
| NDF,[4] % | 7.9 | 19.1 | 1.1 | 66.2 |
| Total identified fatty acids,[5] % | 3.9 | 2.9 | 87.2 | 2.6 |
| Palmitic acid (16:0), % | 0.86 | 2.19 | 15.26 | 0.54 |
| Stearic acid (18:0), % | 0.02 | 0.40 | 4.45 | 0.12 |
| Oleic acid (18:1), % | 0.29 | 0.00 | 27.64 | 0.12 |
| Linoleic acid (18:2), % | 2.64 | 0.03 | 35.58 | 0.51 |
| Linolenic acid (18:3), % | 0.07 | 0.09 | 2.88 | 1.09 |

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

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

[3]*Brachiaria brizanta* pasture (24 ha).

[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 equations (NRC, 1996): $NEm = 1.37\ ME - 0.138\ ME^2 + 0.0105\ ME^3 - 1.12$, given that ME = DE × 0.82 and 1 kg of TDN = 4.4 Mcal of DE.

[5]As a percentage of DM and analyzed according to the procedures described by Tripathy et al. (2010).

Samples of pasture and supplement ingredients were collected weekly, pooled across all weeks, 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 International, 2006), ADF (method 973.18 modified for use in an Ankom 200 fiber analyzer; Ankom Technology Corp., Fairport, NY; AOAC International, 2006), and NDF (Van Soest et al., 1991; modified for use in an Ankom 200 fiber analyzer; Ankom Technology Corp.). Samples were also analyzed for FA concentrations using gas chromatography (Agilent 7890; Agilent Technologies, Inc., Wilmington, DE) according to the procedures described by Tripathy et al. (2010). Only FA identified by the assay were recorded. Calculations for TDN used the equations proposed by Weiss et al. (1992), whereas NEm and NEg were calculated with the equations proposed by the NRC (1996).

**Table 2.** Composition and nutritional profile of supplements containing Ca salts of soybean oil (CSSO) or kaolin (CON) offered in the experiment[1]

| Item | Containing CSSO | CON |
|---|---|---|
| Ingredient, % 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, % | 92.8 | 94.5 |
| TDN,[2] % | 86.0 | 53.6 |
| NEm,[3] Mcal/kg | 3.47 | 1.25 |
| NEg,[3] Mcal/kg | 2.50 | 0.82 |
| CP, % | 10.4 | 9.9 |
| NDF, % | 9.34 | 8.81 |
| Total identified fatty acids,[4] % | 32.0 | 2.19 |
| Palmitic acid (16:0), % | 6.22 | 1.00 |
| Stearic acid (18:0), % | 1.66 | 0.14 |
| Oleic acid (18:1), % | 9.53 | 0.09 |
| Linoleic acid (18:2), % | 13.01 | 0.85 |
| Linolenic acid (18:3), % | 1.03 | 0.05 |
| Daily intake | | |
| DM, g | 278 | 283 |
| TDN,[2] g | 240 | 152 |
| NEm,[3] Mcal | 1.04 | 0.38 |
| NEg,[3] Mcal/kg | 0.75 | 0.25 |
| CP, g | 29.0 | 28.2 |
| NDF, g | 26.0 | 25.0 |
| Total identified fatty acids,[4] g | 89.1 | 6.2 |
| Palmitic acid (16:0), g | 17.33 | 2.82 |
| Stearic acid (18:0), g | 4.62 | 0.39 |
| Oleic acid (18:1), g | 26.55 | 0.26 |
| Linoleic acid (18:2), g | 36.23 | 2.40 |
| Linolenic acid (18:3), g | 2.88 | 0.14 |

[1]Containing CSSO = daily supplementation (per cow) with 100 g of a protein–mineral mix plus 100 g of ground corn per cow daily plus 100 g of CSSO (Megalac-E; Elanco Saúde Animal, São Paulo, Brazil; $n = 9$). CON = daily supplementation (per cow) with 100 g of a protein–mineral mix plus 100 g of ground corn per cow daily plus 100 g of kaolin (rumen-inert indigestible substance; $n = 9$).

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

[3]Calculated with the following equations (NRC, 1996): NEm = 1.37 ME – 0.138 $ME^2$ + 0.0105 $ME^3$ – 1.12 and NEg = 1.42 ME – 0.174 $ME^2$ + 0.0122 $ME^3$ – 0.165, given that ME = DE × 0.82 and 1 kg of TDN = 4.4 Mcal of DE.

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

### *Sampling*

Blood samples were collected immediately before AI (d 0) and on d 7 and 18 of the experiment via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing 158 United States Pharmacopeia units of freeze-dried sodium heparin. After collection, blood samples were placed immediately 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, 500V; Aloka, Wallingford, CT) was performed concurrently with blood sampling on d 0, 7, and 18 to verify dominant follicle diameter (d 0) and estimate CL volume (d 7 and 18). 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.

After the ultrasonography exam on d 18, 36 cows (18 cows/treatment; 2 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 18 were selected for slaughter at a local commercial packing facility (Marfrig Group, Mineiros, Brazil). 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). Selected cows, which received their respective treatments in the morning, were loaded into a commercial livestock trailer on d 18 (1700 h), transported for 30 miles to the packing facility where they remained overnight, and slaughtered on d 19 (0800 to 0900 h). Upon slaughter, reproductive tracts were immediately collected, placed on ice, and processed for collection of conceptus, uterine luminal flushing, and tissue samples from the CL and endometrium based on the procedures described by Bilby et al. (2004).

More specifically, the uterine horn ipsilateral to the CL was isolated from the reproductive tract, and the ovary containing the CL was removed. The CL was incised with a scalpel for collection of luteal tissue. Subsequently, 20 mL of saline were injected into the uterotuberal junction of the selected uterine horn, massaged gently, and exited through an incision at the tip of the uterine horn. Uterine luminal flushing media and the conceptus were recovered in a sterile 100 by 15 mm petri dish (CRAL Artigos para Laboratórios Ltda., São Paulo, Brazil). The conceptus was measured for length and weight, whereas the uterine luminal flushing was stored in a 15-mL sterile conical tube (Corning Life Sciences, Tewksbury, MA). The selected uterine horn was then cut along the mesometrial border, and samples of the endometrium were collected. After collection, the conceptus as well as luteal 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.

### *Laboratorial Analysis*

***Plasma.*** All samples were analyzed for FA concentrations using gas chromatography (Agilent 7890; Agilent Technologies, Inc.) according to the procedures

**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 |
|---|---|---|---|
| 3β-hydroxysteroid dehydrogenase | | | |
| Forward | TGTTGGTGGAGGAGAAGG | BC111203 | Pretheeban et al. (2010) |
| Reverse | GGCCGTCTTGGATGATCT | | |
| Steroidogenic acute regulatory protein | | | |
| Forward | CCTCTCTACAGCGACCAA | Y17259 | Hartung et al. (1995) |
| Reverse | TCGTGAGTGATGACCGTG | | |
| Oxytocin | | | |
| Forward | GTCTGCACCATGGCAGGTT | M25648.1 | Ye et al. (2012) |
| Reverse | CAGGGGGCAGTTCTGAATGT | | |
| Prostaglandin receptor | | | |
| Forward | TTAGAAGTCAGCAGCACAG | D17395 | Lee et al. (2009) |
| Reverse | ACTATCTGGGTGAGGGCTGATT | | |
| Cyclooxygenase-2 | | | |
| Forward | AGGTGTATGTATGAGTGTAGGA | NM_174445 | Sayre et al. (2000) |
| Reverse | GTGCTGGGCAAAGAATGCAA | | |
| Oxytocin receptor | | | |
| Forward | CCTGGGGACCCAAGGCCTAC | NM_174134.2 | Ivell et al. (1995) |
| Reverse | AAGAAGAAAGGCGTCCAGCACACGA | | |
| Interferon-tau | | | |
| Forward | GCCCTGGTGCTGGTCAGCTA | AF238612 | Rizos et al. (2003) |
| Reverse | CATCTTAGTCAGCGAGAGTC | | |
| Glyceraldehyde-3-phosphate dehydrogenase | | | |
| Forward | ACCCAGAAGACTGTGGATGG | NM_001034034 | Cerri et al. (2012) |
| Reverse | CAACAGACACGTTGGGAGTG | | |

described by Tripathy et al. (2010). Only FA identified by the assay were recorded. Plasma samples collected on d 7 and 18 from cows that did not have a CL on d 0 but with a CL greater than 0.38 $cm^3$ in volume concurrently with blood collection (Figueiredo et al., 1997) were analyzed for P4 concentrations in duplicates, using a Coat-A-Count solid phase $^{125}I$ RIA kit (Siemens Healthcare Diagnostics, Los Angeles, CA). The intra- and interassay CV for the P4 assay were, respectively, 3.46 and 1.25%, whereas the minimum detectable concentration of P4 was 0.07 ng/mL

***Uterine Luminal Flushing.*** Samples from cows that had a conceptus were analyzed for IFNt concentrations using a bovine-specific commercial ELISA kit (MyBioSource LLC, San Diego, CA). This procedure was validated by pooling the uterine luminal flushing from cows used herein that did not have a conceptus, adding known concentrations of recombinant bovine IFNt (0, 50, and 100 pg/mL; PBL Assay Science, Piscataway, NJ), and including these samples into the assay. The IFNt concentrations obtained were 0.2, 56.4, and 109.2 pg/mL for, respectively, pools enriched with 0, 50, and 100 pg/mL of recombinant bovine IFNt. All samples were analyzed in duplicates within a single assay, with intra-assay CV of 4.45% and minimum detectable concentration of 0.1 pg/mL.

***Tissue Samples.*** All tissue samples were analyzed for FA concentrations using gas chromatography (Agilent 7890; Agilent Technologies, Inc.) according to the procedures described by Tripathy et al. (2010). Only FA identified by the assay were recorded. 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 2000; Thermo Fisher Scientific, Minneapolis, MN) at 260 nm and 260:280 nm ratio, respectively (Fleige and Pfaffl, 2006), and then stored at –80°C until further processing.

Extracted RNA from tissue samples (2.5 μg) were incubated at 37°C for 30 min in the presence of RNase-free DNase (New England Biolabs Inc., Ipswich, MA) to remove contaminant genomic DNA. After inactivating the desoxyribonuclease (75°C for 15 min), samples were reverse transcribed using the High Capacity cDNA Reverse Transcription Kit with random hexamers (Applied Biosystems, Foster City, CA). Quantity and quality of cDNA were again assessed via UV absorbance at 260 nm and 260:280 nm ratio, respectively (NanoDrop 2000; Thermo Fisher Scientific). Real-time PCR was completed using the Rotor-Gene SYBR Green PCR Kit (Qiagen Inc., Valencia, CA) and specific primer sets (25 ng/mL; Table 3), with a Rotor-Gene Q real-time PCR cycler (Qiagen Inc.) according to procedures de-

**Table 4.** Plasma fatty acid concentrations (mg/mL of plasma) of beef cows receiving a supplement containing 100 g of Ca salts of soybean oil (CSSO) or kaolin (CON)[1,2]

| Item[3] | Containing CSSO | CON | SEM | *P*-value |
|---|---|---|---|---|
| Mystiric acid (14:0) | 0.032 | 0.036 | 0.002 | 0.21 |
| Palmitic acid (16:0) | 0.421 | 0.413 | 0.009 | 0.59 |
| Stearic acid (18:0) | 0.599 | 0.570 | 0.016 | 0.21 |
| Oleic acid (18:1) | 0.216 | 0.233 | 0.008 | 0.16 |
| Linoleic acid (18:2) | 0.540 | 0.275 | 0.021 | <0.01 |
| Linolenic acid (18:3) | 0.136 | 0.143 | 0.005 | 0.41 |
| Arachidonic acid (20:4 n-6) | 0.025 | 0.023 | 0.004 | 0.74 |
| Total SFA | 1.051 | 1.019 | 0.027 | 0.41 |
| Total MUFA | 0.216 | 0.233 | 0.008 | 0.16 |
| Total PUFA | 0.701 | 0.439 | 0.025 | <0.01 |
| Total ω-6 | 0.565 | 0.296 | 0.022 | <0.01 |
| Total ω-3 | 0.136 | 0.143 | 0.005 | 0.41 |
| Ratio ω-6:ω-3 | 4.391 | 1.977 | 0.205 | <0.01 |
| Total identified fatty acids | 1.966 | 1.693 | 0.045 | <0.01 |

[1]Containing CSSO = daily supplementation (per cow) with 100 g of a protein–mineral mix plus 100 g of ground corn per cow daily plus 100 g of containing CSSO (Megalac-E; Elanco Saúde Animal, São Paulo, Brazil; $n$ = 9). CON = daily supplementation (per cow) with 100 g of a protein–mineral mix plus 100 g of ground corn per cow daily plus 100 g of kaolin (rumen-inert indigestible substance; $n$ = 9).

[2]Treatments were offered from d 0 to 18 of the experiment. Blood samples were collected from all cows ($n$ = 90, being 45 per treatment) on d 0 (before the first treatment application), 7, and 18. Values obtained on d 0 served as covariate; therefore, values reported are covariately adjusted means.

[3]SFA consist of mystiric, palmitic, and stearic acids; MUFA consists of oleic acid; PUFA consist of linoleic, linolenic, and arachidonic acids; ω-6 consists of linoleic and arachidonic acids; ω-3 consists of linolenic acid.

scribed by Yoganathan et al. (2012). At the end of each reverse transcription (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. All $C_T$ responses from genes of interest were normalized to glyceraldehyde-3-phosphate dehydrogenase $C_T$ examined in the same sample and assessed at the same time as the targets. Results are expressed as relative fold change ($2^{-\Delta\Delta CT}$), as described by Ocón-Grove et al. (2008).

### *Statistical Analysis*

Quantitative and binary data were analyzed, respectively, with the MIXED and GLIMMIX procedures of SAS (SAS Inst., Inc., Cary, NC), using group as experimental unit, group(treatment) and cow(group) as random variables, and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. The model statement used for dominant follicle diameter, presence of conceptus at slaughter, conceptus length, weight, and FA concentrations as well as IFNt concentrations in the uterine flushing and all gene expression results contained the effect of treatment. The model statement used for endometrium and luteal FA concentrations contained the effects of treatment, presence of a conceptus, and the resultant interaction. The model statement used for CL volume, plasma P4 and FA 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 18 contained the effects of treatment, day, and the resultant interaction in addition to values obtained on d 0 as an independent covariate for the plasma FA analysis. 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 least square means, or covariately adjusted means for plasma FA concentrations, 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

### *Plasma and Tissue Fatty Acid Concentrations*

Plasma concentrations of individual and total identified FA on d 0 were significant covariates ($P \leq 0.04$) but did not differ ($P \geq 0.46$; data not shown) between CSSO-supplemented and CON cows, indicating that both treatment groups had similar plasma FA concentrations before treatment administration. During the experimental period, CSSO-supplemented cows had greater ($P < 0.01$) plasma concentrations of linoleic acid, PUFA, total ω-6, ω-6:ω-3 ratio, and total identified FA (Table 4) compared to CON cows. No treatment differences were detected ($P = 0.41$) for plasma linolenic acid and total ω-3 (Table 4). These results are in accordance with the FA profile of CSSO (predominantly linoleic acid), given that plasma FA concentrations directly reflects intake and duodenal flow of FA (Lake et al., 2007; Scholljegerdes et al., 2007; Hess et al., 2008). Accordingly, Cooke et al. (2011) reported that beef steers supplemented with 150 g of a CSSO source similar to that used herein had greater plasma concentrations of linoleic acid, PUFA, ω-6, and total FA but similar or reduced linolenic acid and ω-3 concentrations compared to nonsupplemented cohorts. Hence, supplementing 100 g of CSSO to beef cows effectively increased plasma concentrations of linoleic and ω-6 FA but not of linolenic and ω-3 FA.

**Table 5.** Concentrations of fatty acids (mg of fatty acids/g of tissue) in endometrial samples collected from from beef cows receiving a supplement containing 100 g of Ca salts of soybean oil (CSSO) or kaolin (CON)[1,2]

| Item[3] | Containing CSSO | CON | SEM | *P*-value |
|---|---|---|---|---|
| Mystiric acid (14:0) | 0.082 | 0.020 | 0.011 | <0.01 |
| Pentadecylic acid (15:0) | 0.202 | 0.141 | 0.025 | 0.10 |
| Palmitic acid (16:0) | 1.086 | 0.771 | 0.151 | 0.15 |
| Palmitoleic acid (16:1) | 0.007 | 0.011 | 0.002 | 0.16 |
| Margaric acid (17:0) | 0.068 | 0.045 | 0.009 | 0.08 |
| Stearic acid (18:0) | 1.318 | 0.912 | 0.181 | 0.12 |
| Oleic acid (18:1) | 1.141 | 0.895 | 0.176 | 0.33 |
| Vaccenic acid (18:1 trans-11) | 0.060 | 0.049 | 0.009 | 0.43 |
| Linoleic acid (18:2 n-6) | 0.358 | 0.144 | 0.043 | <0.01 |
| Gamma-linolenic acid (18:3 n-6) | 0.028 | 0.018 | 0.005 | 0.16 |
| Linolenic acid (18:3 n-3) | 0.016 | 0.014 | 0.002 | 0.53 |
| Arachidic acid (20:0) | 0.032 | 0.024 | 0.005 | 0.26 |
| Eicosadienoic acid (20:2 n-6) | 0.027 | 0.007 | 0.003 | <0.01 |
| Dihomo-gamma-linolenic acid (20:3 n-6) | 0.154 | 0.083 | 0.020 | 0.02 |
| Eicosatrienoic acid (20:3 n-3) | 0.407 | 0.262 | 0.095 | 0.14 |
| Arachidonic acid (20:4 n-6) | 0.266 | 0.241 | 0.061 | 0.77 |
| Eicosapentaenoic acid (20:5 n-3) | 0.008 | 0.013 | 0.003 | 0.29 |
| Behenic acid (22:0) | 0.608 | 0.365 | 0.095 | 0.08 |
| Adrenic acid (22:4 n-6) | 0.038 | 0.019 | 0.006 | 0.03 |
| Docosapentaenoic acid (22:5 n-3) | 0.090 | 0.059 | 0.014 | 0.13 |
| Docosapentaenoic acid (22:5 n-6) | 0.064 | 0.034 | 0.008 | 0.02 |
| Docosahexaenoic acid (22:6 n-3) | 0.114 | 0.085 | 0.016 | 0.23 |
| Total SFA | 3.398 | 2.282 | 0.460 | 0.09 |
| Total MUFA | 1.207 | 0.956 | 0.187 | 0.34 |
| Total PUFA | 1.576 | 0.983 | 0.221 | 0.05 |
| Total ω-6 | 0.938 | 0.549 | 0.136 | 0.05 |
| Total ω-3 | 0.637 | 0.433 | 0.090 | 0.12 |
| Ratio ω-6:ω-3 | 1.555 | 1.236 | 0.095 | 0.02 |
| Total identified fatty acids | 6.181 | 4.221 | 0.863 | 0.12 |

[1]Containing CSSO = daily supplementation (per cow) with 100 g of a protein–mineral mix × 100 g of ground corn per cow daily plus 100 g of CSSO (Megalac-E; Elanco Saúde Animal, São Paulo, Brazil; $n = 9$). CON = daily supplementation (per cow) with 100 g of a protein–mineral mix plus 100 g of ground corn per cow daily plus 100 g of kaolin (rumen-inert indigestible substance; $n = 9$).

[2]Treatments were offered from d 0 to 18 of the experiment. Cows were slaughtered on d 19, and endometrial samples were collected ($n = 36$, being 18 per treatment) based on the procedures described by Bilby et al. (2004).

[3]SFA consist of mystiric, pentadecylic, palmitic, margaric, stearic, arachidic, and behenic acids; MUFA consist of palmitoleic, oleic, and vaccenic acids; PUFA consist of linoleic, gamma-linoleic, linolenic, eicosadienoic, dihomo-gamma-linolenic, eicosatrienoic, arachidonic, eicosapentaenoic, adrenic, docosapentaenoic n-3 and n-6, and docosahexaenoic acids; ω-6 consists of linoleic, gamma-linoleic, eicosadienoic. dihomo-gamma-linolenic, arachidonic, adrenic, and docosapentaenoic n-6 acids; ω-3 consists of linolenic, eicosatrienoic, eicosapentaenoic, docosapentaenoic n-3, and docosahexaenoic acids.

Upon slaughter, CSSO-supplemented cows had greater ($P \leq 0.05$; Table 5) endometrial concentrations of linoleic acid and its ω-6 elongation/desaturation products such as eicosadienoic, dihomo-γ-linolenic, adrenic, and docosapentaenoic acids (Leonard et al., 2000) as well as total PUFA, total ω-6, and ω-6:ω-3 ratio compared with CON cows. No treatment differences were detected ($P \geq 0.12$) for linolenic and other ω-3 FA in endometrial samples (Table 5). Similarly, CSSO-supplemented cows had greater ($P \leq 0.05$; Table 6) luteal concentrations of linoleic, eicosadienoic, and adrenic acids, total PUFA, total ω-6, and ω-6:ω-3 ratio compared with CON cows. In regards to luteal ω-3 FA, no treatment effects were again detected ($P \geq 0.25$) for linolenic acid and total ω-3 concentrations, although CSSO-supplemented cows had greater ($P = 0.01$) concentrations of docosahexaenoic acid but reduced ($P < 0.01$) concentrations of eicosapentaenoic acid compared with CON (Table 6). These results agree with the treatment effects detected for plasma FA concentrations, given that circulating PUFA are incorporated, elongated, and desaturated in bovine reproductive tissues (Mattos et al., 2000; Wathes et al., 2007). Supporting our results, other research studies reported that supplemental PUFA, such as fish meal or safflower seeds, modulates PUFA concentrations in the endometrium (Burns et al., 2003; Scholljegerdes et al., 2007) and CL (White et al., 2012) of beef cows. Therefore, linoleic acid and its elongation/desaturation ω-6 products were the predominant PUFA being incorporated into the reproductive tissues of beef cows supplemented with 100 g of CSSO.

No treatment differences were detected for the proportion of cows that had a conceptus on slaughter ($P = 0.73$) as well as conceptus weight ($P = 0.93$) and length ($P = 0.78$; Table 7). It is important to note that the aim of the study was not to compare pregnancy rates between treatments, which was properly documented by Lopes et al. (2009, 2011). In addition, the lack of treatment effects for conceptus weight and length indicate that the reproductive benefits associated with CSSO supplementation reported by Lopes et al. (2009, 2011) may not be associated with embryo dimensional development. Nevertheless, conceptuses from CSSO-supplemented cows had greater ($P \leq 0.05$) concentrations of stearic acid, total SFA, and eicosadienoic, arachidonic, and adrenic acids and tended to have greater concentrations of linoleic acid, eicosapentaenoic acid, and ω-6:ω-3 ratio ($P = 0.08$) compared to conceptuses from CON cows (Table 8). As previously mentioned, eicosadienoic, arachidonic, and adrenic acids are originated from the elongation and desaturation of linoleic acid (Leonard et al., 2000) and eicosapentaenoic acid is originated from the elongation, desaturation, and peroxisomal β-oxidation of linolenic acid (Leonard et al., 2000), whereas linoleic and linolenic acids can be saturated into stearic acid in the rumen (Jenkins et al., 2008). Treatment effects for stearic acid can also be attributed to the stearic acid concentrations of CSSO, although stearic acid concentrations were similar between CSSO-supplemented and CON cows in plasma, endometrial, and CL samples. Therefore, supplementation with 100 g of CSSO pre-

**Table 6.** Concentrations of fatty acids (mg of fatty acids/g of tissue) in luteal samples collected from beef cows receiving a supplement containing 100 g of Ca salts of soybean oil (CSSO) or kaolin (CON)[1,2]

| Item[3] | Containing CSSO | CON | SEM | *P*-value |
|---|---|---|---|---|
| Mystiric acid (14:0) | 0.698 | 0.695 | 0.065 | 0.97 |
| Pentadecylic acid (15:0) | 0.272 | 0.294 | 0.023 | 0.51 |
| Palmitic acid (16:0) | 13.92 | 13.70 | 0.970 | 0.87 |
| Palmitoleic acid (16:1) | 0.298 | 0.355 | 0.023 | 0.10 |
| Margaric acid (17:0) | 0.640 | 0.687 | 0.045 | 0.47 |
| Stearic acid (18:0) | 9.314 | 8.239 | 0.479 | 0.12 |
| Oleic acid (18:1) | 8.525 | 9.672 | 0.511 | 0.12 |
| Vaccenic acid (18:1 trans-11) | 1.675 | 1.251 | 0.508 | 0.55 |
| Linoleic acid (18:2 n-6) | 7.035 | 3.935 | 0.543 | <0.01 |
| Gamma-linolenic acid (18:3 n-6) | 0.180 | 0.119 | 0.062 | 0.50 |
| Linolenic acid (18:3 n-3) | 0.615 | 0.729 | 0.070 | 0.25 |
| Arachidic acid (20:0) | 0.426 | 0.386 | 0.0488 | 0.57 |
| Eicosadienoic acid (20:2 n-6) | 1.519 | 0.692 | 0.152 | <0.01 |
| Dihomo-gamma-linolenic acid (20:3 n-6) | 1.697 | 1.462 | 0.172 | 0.34 |
| Eicosatrienoic acid (20:3 n-3) | 0.109 | 0.024 | 0.040 | 0.17 |
| Arachidonic acid (20:4 n-6) | 4.942 | 4.731 | 0.349 | 0.67 |
| Eicosapentaenoic acid (20:5 n-3) | 0.402 | 0.689 | 0.042 | <0.01 |
| Behenic acid (22:0) | 0.597 | 0.271 | 0.089 | 0.01 |
| Adrenic acid (22:4 n-6) | 1.972 | 1.348 | 0.203 | 0.03 |
| Docosapentaenoic acid (22:5 n-3) | 3.688 | 4.317 | 0.394 | 0.26 |
| Docosapentaenoic acid (22:5 n-6) | 0.351 | 0.378 | 0.035 | 0.58 |
| Docosahexaenoic acid (22:6 n-3) | 0.544 | 0.283 | 0.067 | 0.01 |
| Lignoceric acid (24:0) | 0.078 | 0.071 | 0.017 | 0.76 |
| Total SFA | 25.949 | 24.348 | 1.477 | 0.44 |
| Total MUFA | 10.499 | 11.278 | 0.886 | 0.53 |
| Total PUFA | 23.157 | 18.763 | 1.625 | 0.05 |
| Total ω-6 | 17.798 | 12.719 | 1.197 | <0.01 |
| Total ω-3 | 5.359 | 6.044 | 0.488 | 0.32 |
| Ratio ω-6:ω-3 | 3.643 | 2.347 | 0.289 | <0.01 |
| Total identified fatty acids | 59.605 | 54.389 | 3.320 | 0.27 |

[1]Containing CSSO = daily supplementation (per cow) with 100 g of a protein–mineral mix plus 100 g of ground corn per cow daily plus 100 g of CSSO (Megalac-E; Elanco Saúde Animal, São Paulo, Brazil; $n = 9$). CON = daily supplementation (per cow) with 100 g of a protein–mineral mix plus 100 g of ground corn per cow daily plus 100 g of kaolin (rumen-inert indigestible substance; $n = 9$).

[2]Treatments were offered from d 0 to 18 of the experiment. Cows were slaughtered on d 19, and samples from the corpus luteum were collected ($n = 36$, being 18 per treatment) based on the procedures described by Bilby et al. (2004).

[3]SFA consist of mystiric, pentadecylic, palmitic, margaric, stearic, arachidic, behenic, and lignoceric acids; MUFA consist of palmitoleic, oleic, and vaccenic acids; PUFA consist of linoleic, gamma-linoleic, linolenic, eicosadienoic, dihomo-gamma-linolenic, eicosatrienoic, arachidonic, eicosapentaenoic, adrenic, docosapentaenoic n-3 and n-6, and docosahexaenoic acids; ω-6 consists of linoleic, gamma-linoleic, eicosadienoic. dihomo-gamma-linolenic, arachidonic, adrenic, and docosapentaenoic n-6 acids; ω-3 consists of linolenic, eicosatrienoic, eicosapentaenoic, docosapentaenoic n-3, and docosahexaenoic acids.

**Table 7.** Proportion of pregnancies, conceptus dimension, concentrations of interferon-tau (IFNt) in the uterine flushing media as well as plasma progesterone and corpus luteum volume of beef cows receiving a supplement containing 100 g of Ca salts of soybean oil (CSSO) or kaolin (CON)[1,2]

| Item | Containing CSSO | CON | SEM | *P*-value |
|---|---|---|---|---|
| Proportion of cows with conceptus,[3] % | 66.7 (12/18) | 61.1 (11/18) | 11.6 | 0.73 |
| Conceptus dimension[4] | | | | |
| Length, cm | 33.4 | 34.7 | 3.3 | 0.78 |
| Weight, mg | 0.239 | 0.243 | 0.036 | 0.93 |
| IFNt in the uterine flushing media,[4] ng/mL | 10.95 | 7.29 | 1.47 | 0.09 |
| Plasma progesterone,[5] ng/mL | | | | |
| Day 7 | 5.69 | 3.91 | 0.38 | <0.01 |
| Day 18 | 3.94 | 3.92 | 0.44 | 0.98 |
| Corpus luteum volume,[5] $cm^3$ | | | | |
| Day 7 | 8.26 | 6.54 | 0.52 | 0.02 |
| Day 18 | 8.44 | 9.83 | 0.68 | 0.15 |

[1]Containing CSSO = daily supplementation (per cow) with 100 g of a protein–mineral mix plus 100 g of ground corn per cow daily plus 100 g of CSSO (Megalac-E; Elanco Saúde Animal, São Paulo, Brazil; $n = 9$). CON = daily supplementation (per cow) with 100 g of a protein–mineral mix plus 100 g of ground corn per cow daily plus 100 g of kaolin (rumen-inert indigestible substance; $n = 9$).

[2]Treatments were offered from d 0 to 18 of the experiment. Blood samples were collected and transrectal ultrasonography (7.5-MHz transducer, 500 V; Aloka, Wallingford, CT) was performed on d 7 and 18 of the experiment. Cows were slaughtered on d 19, whereas uterine flushing media and conceptuses were collected based on the procedures described by Bilby et al. (2004).

[3]At slaughter. Within parenthesis are the number of cows with conceptus/total slaughtered cows.

[4]Evaluated from cows that had a conceptus on slaughter.

[5]Evaluated from cows that did not have a corpus luteum on d 0 but with a corpus luteum greater than 0.38 $cm^3$ in volume on d 7 ($n = 39$ for CSSO-receiving and 37 for CON-receiving cows) and 18 ($n = 21$ for CSSO -receiving and 20 for CON-receiving). Corpus luteum volume was calculated using the formula for volume of a sphere; $V = 4/3\pi \times (D/2)^3$, in which D is the maximum luteal diameter (Cooke et al., 2009).

dominantly increased the incorporation of linoleic acid and its elongation/desaturation ω-6 products into the conceptus of beef cows during early gestation.

Collectively, treatment effects detected for plasma, endometrial, CL, and conceptus FA concentrations indicate that CSSO supplementation effectively increased intake and intestinal absorption of linoleic acid by beef cows, which in turn was incorporated, elongated, desaturated, and accumulated by their reproductive tissues, resulting in a greater passage of ω-6 FA to the conceptus. Hence, the reproductive benefits associated with CSSO supplementation reported by Lopes et al. (2009, 2011) may be attributed to the prevalent incorporation of ω-6 FA, and not ω-3 FA, into the reproductive tract of beef cows. It is well known that ω-6 FA, particularly arachidonic acid, are precursors for $PGF_{2\alpha}$ synthesis (Yaqoob and Calder, 2007), the hormone responsible for luteolysis and pregnancy termination (Senger, 2003). Conversely, ω-3 supplementation has been shown to inhibit $PGF_{2\alpha}$ synthesis (Yaqoob and Calder, 2007), delay luteolysis, and reduce pregnancy losses in cattle (Burke et al., 1997; Thatcher et al., 1997; Mattos et al., 2000). However, linoleic acid can

**Table 8.** Concentrations of fatty acids (mg of fatty acids/g of tissue) in conceptuses collected from beef cows receiving a supplement containing 100 g of Ca salts of soybean oil (CSSO) or kaolin (CON)[1,2]

| Item[3] | Containing CSSO | CON | SEM | *P*-value |
|---|---|---|---|---|
| Mystiric acid (14:0) | 0.448 | 0.432 | 0.055 | 0.84 |
| Pentadecylic acid (15:0) | 0.095 | 0.032 | 0.030 | 0.15 |
| Palmitic acid (16:0) | 2.163 | 1.582 | 0.253 | 0.13 |
| Palmitoleic acid (16:1) | 0.107 | 0.058 | 0.024 | 0.19 |
| Margaric acid (17:0) | 0.157 | 0.121 | 0.018 | 0.19 |
| Stearic acid (18:0) | 1.609 | 1.073 | 0.180 | 0.05 |
| Oleic acid (18:1) | 2.907 | 2.768 | 0.583 | 0.87 |
| Vaccenic acid (18:1 trans-11) | 0.778 | 0.304 | 0.385 | 0.39 |
| Linoleic acid (18:2 n-6) | 0.174 | 0.022 | 0.060 | 0.08 |
| Gamma-linolenic acid (18:3 n-6) | 0.017 | 0.016 | 0.005 | 0.81 |
| Linolenic acid (18:3 n-3) | 0.029 | 0.035 | 0.016 | 0.80 |
| Arachidic acid (20:0) | 0.423 | 0.119 | 0.080 | 0.01 |
| Eicosadienoic acid (20:2 n-6) | 0.169 | 0.002 | 0.052 | 0.04 |
| Dihomo-gamma-linolenic acid (20:3 n-6) | 0.505 | 0.029 | 0.279 | 0.24 |
| Eicosatrienoic acid (20:3 n-3) | 0.269 | 0.102 | 0.072 | 0.13 |
| Arachidonic acid (20:4 n-6) | 0.312 | 0.086 | 0.044 | <0.01 |
| Eicosapentaenoic acid (20:5 n-3) | 0.084 | 0.001 | 0.035 | 0.09 |
| Behenic acid (22:0) | 0.394 | 0.305 | 0.095 | 0.51 |
| Adrenic acid (22:4 n-6) | 0.063 | 0.000 | 0.021 | 0.04 |
| Docosapentaenoic acid (22:5 n-3) | 1.120 | 0.885 | 0.560 | 0.77 |
| Docosapentaenoic acid (22:5 n-6) | 0.726 | 0.219 | 0.385 | 0.37 |
| Docosahexaenoic acid (22:6 n-3) | 0.424 | 0.416 | 0.068 | 0.93 |
| Lignoceric acid (24:0) | 0.914 | 0.218 | 0.526 | 0.36 |
| Nervonic acid (24:1) | 0.159 | 0.047 | 0.028 | 0.01 |
| Total SFA | 6.183 | 3.878 | 0.710 | 0.03 |
| Total MUFA | 3.956 | 3.177 | 0.936 | 0.56 |
| Total PUFA | 3.967 | 1.824 | 0.950 | 0.13 |
| Total ω-6 | 2.045 | 0.384 | 0.755 | 0.13 |
| Total ω-3 | 1.923 | 1.439 | 0.578 | 0.56 |
| Ratio ω-6:ω-3 | 1.491 | 0.500 | 0.352 | 0.08 |
| Total identified fatty acids | 14.136 | 8.883 | 2.450 | 0.15 |

[1]Containing CSSO = daily supplementation (per cow) with 100 g of a protein–mineral mix plus 100 g of ground corn per cow daily plus 100 g of CSSO (Megalac-E; Elanco Saúde Animal, São Paulo, Brazil; $n = 9$). CON = daily supplementation (per cow) with 100 g of a protein–mineral mix plus 100 g of ground corn per cow daily plus 100 g of kaolin (rumen-inert indigestible substance; $n = 9$).

[2]Treatments were offered from d 0 to 18 of the experiment. Cows were slaughtered on d 19, and conceptuses ($n$ = 12 for CSSO-receiving and 11 for CON-receiving cows) were collected based on the procedures described by Bilby et al. (2004).

[3]SFA consist of mystiric, pentadecylic, palmitic, margaric, stearic, arachidic, behenic, and lignoceric acids; MUFA consist of palmitoleic, oleic, vaccenic, and nervonic acids; PUFA consist of linoleic, gamma-linoleic, linolenic, eicosadienoic, dihomo-gamma-linolenic, eicosatrienoic, arachidonic, eicosapentaenoic, adrenic, docosapentaenoic n-3 and n-6, and docosahexaenoic acids; ω-6 consists of linoleic, gamma-linoleic, eicosadienoic. dihomo-gamma-linolenic, arachidonic, adrenic, and docosapentaenoic n-6 acids; ω-3 consists of linolenic, eicosatrienoic, eicosapentaenoic, docosapentaenoic n-3, and docosahexaenoic acids.

also be converted into eicosadienoic acid rather than arachidonic acid as reported herein, which is known to reduce $PGF_{2\alpha}$ synthesis (Staples et al., 1998; Cheng et al., 2001). In addition, recent research demonstrated that PG from the 2-series, such as $PGE_2$ and $PGI_2$ that are also originated from ω-6 FA (Schmitz and Ecker, 2008), are critical regulators of conceptus elongation and pregnancy establishment in sheep (Dorniak et al., 2011) and cattle (Erdem and Guzeloglu, 2010). More specifically, these PG are synthesized by the conceptus and endometrium, modulate synthesis and endometrial activity of IFNt, and appear to be fundamental for conceptus development and pregnancy signaling to maternal tissues (Erdem and Guzeloglu, 2010; Dorniak et al., 2011). These latter findings support our rationale that the increased pregnancy rates in cows supplemented with 100 g of CSSO reported by Lopes et al. (2009, 2011) may be attributed to a greater incorporation of ω-6 FA into their reproductive tissues and conceptus.

### *Plasma Progesterone, Corpus Luteum Volume, and Interferon-tau in the Uterine Flushing Media*

A similar ($P \geq 0.63$) proportion of CSSO-supplemented 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 (86.7 vs. 82.2%, respectively; SEM = 6.5%; which corresponds to 39 and 37 cows within 45 cows assigned to each treatment) and d 18 (46.7 vs. 44.4%, respectively; SEM = 6.5%; which corresponds to 21 and 20 cows within 45 cows assigned to each treatment). The goal of the experiment was not to compare synchronization rate or assess luteolysis between treatments; this information is being presented to demonstrate that treatment effects on plasma P4 and CL volume were determined using a balanced dataset.

A treatment × day interaction was detected ($P < 0.01$; Table 7) for plasma P4 and CL volume. On d 7 of the experiment, CSSO-supplemented cows had greater plasma P4 concentrations ($P < 0.01$) and CL volume ($P = 0.02$) compared to CON cows, whereas no treatment effects were detected ($P \geq 0.15$) for these parameters on d 18. It is important to note that diameter of the dominant follicle on d 0 was similar ($P = 0.47$) between CSSO-supplemented and CON cows (13.7 vs. 13.0 mm, respectively; SEM = 0.7). Hence, treatment differences reported for CL volume and P4 concentrations on d 7 were not related to size of the ovulatory follicle (Vasconcelos et al., 2001). Supporting our findings, Lopes et al. (2009, 2011) also reported that cows supplemented with 100 g of CSSO had greater serum P4 concentrations compared with nonsupplemented cohorts and attributed this outcome to increased P4 synthesis (Hawkins et al., 1995; Staples et al., 1998) and reduced hepatic catabolism of P4 (Sangsritavong et al., 2002). In the present experiment, the increase in plasma P4 concentration in CSSO-supplemented cows on d 7 appears to be directly related to their greater CL volume compared with CON cows, although this experiment did not appraise hepatic P4 catabolism as Lopes et al. (2009). These outcomes may

also be attributed to the increased intake and incorporation of ω-6 FA into luteal tissues of CSSO-supplemented cows (Table 6; Hawkins et al., 1995), although luteal FA concentrations was not evaluated on d 7 of the experiment. However, treatment effects were absent on d 18 of the experiment, which indicates that supplementation with 100 g of CSSO hastened initial CL development, without impacting final CL volume (Figueiredo et al., 1997). Circulating P4 concentrations after breeding have been positively associated with pregnancy rates in cattle (Robinson et al., 1989; Stronge et al., 2005), whereas Demetrio et al. (2007) reported a positive relationship among conception rates and serum P4 concentrations on d 7 following AI in dairy cows. Hence, treatment effects detected herein for plasma P4 on d 7 supports Lopes et al. (2009), which indicated that increased serum P4 concentrations is one of the mechanisms by which supplementing 100 g of CSSO after AI improve pregnancy rates in beef cows. However, Lopes et al. (2011) also suggested that the reproductive benefits of CSSO supplementation after AI are not entirely due to increased circulating P4 on d 7 after AI. These authors reported that cows receiving 100 g of CSSO from d 7 to 21 after AI had greater pregnancy rates compared to cows receiving 100 g of CSSO from d 0 to 14 after AI.

Cows receiving CSSO tended ($P$ = 0.09) to have greater concentrations of IFNt in the uterine flushing media compared with CON cows (Table 7), which is in accordance with the hypothesis of the present experiment and treatment effects detected for conceptus FA concentrations. More specifically, treatment effects detected for IFNt concentrations in the uterine flushing media may be associated with the greater incorporation of ω-6 FA in the conceptus of CSSO-supplemented cows (Table 8), which are precursors to PG shown to modulate IFNt synthesis by the conceptus in sheep (Dorniak et al., 2011). Therefore, results from the present experiment indicate that supplementing beef cows with 100 g of CSSO beginning after AI also improves pregnancy rates (Lopes et al., 2009, 2011) by enhancing pregnancy recognition by maternal tissues via the IFNt cascade (Thatcher et al., 1995).

### *Expression of Genes Associated with Pregnancy Establishment*

No treatment effects were detected ($P \geq 0.22$; Table 9) for luteal mRNA expression of 3β-hydroxysteroid dehydrogenase and steroidogenic acute regulatory protein, which agrees with the similar plasma P4 concentrations between treatments on d 18 given that these enzymes regulate luteal P4 synthesis (Rekawiecki et al., 2008). Hence, CSSO supplementation seemed to increase luteal P4 synthesis on d 7 of the present experiment by enhancing CL volume development rather than luteal steroidogenic activity. No treatment effects were also detected ($P \geq 0.12$) for expression of endometrial, luteal, and conceptus genes associated with pregnancy establishment (Table 9). As previously mentioned, ω-6 FA are known to modulate synthesis of PG associated with luteolysis ($PGF_{2\alpha}$; Staples et al., 1998; Cheng et al., 2001; Grant et al., 2005) and pregnancy establishment ($PGE_2$ and $PGI_2$; Dorniak et al., 2011) via cyclooxygenase-2 activity (Schmitz and Ecker, 2008), whereas $PGF_{2\alpha}$ simulates luteal oxytocin production (Skarzynski et al., 1997). However, CSSO-supplemented and CON cows had similar mRNA expression of luteal oxytocin and PG receptor ($P \geq 0.12$) as well as endometrial cyclooxygenase-2 and oxytocin receptor ($P \geq 0.24$) on d 19 after AI (Table 9), despite the greater concentration of ω-6 FA in luteal and endometrial tissues of CSSO-supplemented cows. In addition, conceptuses from CSSO-supplemented and CON cows had similar IFNt expression ($P$ = 0.68; Table 9), despite treatment differences detected for IFNt concentration in the uterine flushing media (Table 7).

It is important to note that cows were slaughtered on d 19 after AI to ensure that conceptuses would be elongated (Chang, 1952; Maddox-Hyttel et al., 2003) but not implanted into the endometrium (Chavatte-Palmer and Guillomot, 2007). This methodology was chosen to

**Table 9.** Expression of genes associated with pregnancy establishment in the endometrium, corpus luteum, or conceptus collected from beef cows receiving a supplement containing 100 g of Ca salts of soybean oil (CSSO) or kaolin (CON)[1,2]

| Item | Containing CSSO | CON | SEM | $P$-value |
|---|---|---|---|---|
| Corpus luteum | | | | |
| 3β-hydroxysteroid dehydrogenase | 2.45 | 2.65 | 0.30 | 0.63 |
| Steroidogenic acute regulatory protein | 1.68 | 1.90 | 0.12 | 0.22 |
| Oxytocin | 3.11 | 2.28 | 0.35 | 0.12 |
| PG receptor | 2.44 | 2.11 | 0.38 | 0.56 |
| Endometrium | | | | |
| Cyclooxygenase-2 | 23.97 | 40.98 | 9.95 | 0.24 |
| Oxytocin receptor | 23.98 | 31.56 | 7.01 | 0.45 |
| Conceptus | | | | |
| Interferon-tau | 2.74 | 2.93 | 0.33 | 0.68 |

[1]Containing CSSO = daily supplementation (per cow) with 100 g of a protein–mineral mix plus 100 g of ground corn per cow daily plus 100 g of CSSO (Megalac-E; Elanco Saúde Animal, São Paulo, Brazil; $n$ = 9). CON = daily supplementation (per cow) with 100 g of a protein–mineral mix plus 100 g of ground corn per cow daily plus 100 g of kaolin (rumen-inert indigestible substance; $n$ = 9). Values are expressed as relative fold change compared to threshold cycle of glyceraldehyde-3-phosphate dehydrogenase analyzed within the same sample (Ocón-Grove et al., 2008).

[2]Treatments were offered from d 0 to 18 of the experiment. Cows were slaughtered on d 19, whereas samples of corpus luteum, endometrium ($n$ = 36, being 18 per treatment), and conceptus ($n$ = 12 for CSSO-receiving and 11 for CON-receiving) were collected based on the procedures described by Bilby et al. (2004).

allow recovery of conceptuses that still expressed IFNt mRNA (Roberts et al., 1992) and provided enough tissue for both FA and reverse transcription-PCR assays. However, the physiological processes responsible for pregnancy signaling to maternal tissues, including maximal IFNt synthesis by the conceptus, occur around d 16 and 17 of gestation (Ealy et al., 2001; Senger, 2003). Hence, reproductive tissues and conceptuses may have been collected after the critical period for pregnancy recognition, which prevented proper assessment of treatment effects on expression of luteal, endometrial, and conceptus genes associated with pregnancy establishment. The reason to why IFNt concentrations in the uterine flushing media differed while IFNt mRNA expression in the conceptus was similar between CSSO-supplemented and CON cows is unknown. To address this question and further understand the mechanisms by which CSSO as well as ω-6 FA impact pregnancy establishment in beef cows, additional experiments collecting reproductive tissues and conceptus before or on d 16 of gestation are warranted.

In summary, supplementing beef cows with 100 g of CSSO beginning after AI increased plasma concentrations of linoleic and ω-6 FA, which in turn were the predominant FA being incorporated into their endometrium, CL, and conceptus during early gestation. In addition, supplementation with 100 g of CSSO beginning after AI increased plasma P4 concentrations and CL volume on d 7 after AI and tended to increase IFNt concentration in the uterine flushing media collected from cows slaughtered on d 19 after AI. No treatment effects were detected for expression of genes associated with pregnancy establishment in cows slaughtered on d 19 after AI, including IFNt mRNA in the conceptus. However, cows were slaughtered for tissue collection after the critical period for pregnancy recognition, which may have prevented proper expression assessment of pregnancy establishment genes. It is also important to note that cows used herein were nonlactating, whereas Lopes et al. (2009, 2011) evaluated postpartum lactating cows. Consequently, additional research is still required to further understand the reproductive benefits of CSSO supplementation during early gestation, such as assessment of hormones and genes associated with pregnancy establishment on d 16 of gestation in postpartum lactating cows. Nevertheless, the results reported in the present experiment indicates that the increase in pregnancy rates in cows supplemented with 100 g of CSSO beginning after AI reported by Lopes et al. (2009, 2011) may be attributed to the greater incorporation of ω-6 FA, and not ω-3 FA, into their reproductive tissues and conceptus.

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